Charis M. Galanakis *Editor*

Food Bioactives and Health

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Preface

Over the past few years, food bioactives have gained attention due to their potential in reducing the risk of diseases, such as obesity, cardiovascular disease, diabetes, and cancer. This potential is attributed to the antitumor, anti-inflammatory, antihyperlipidemic, antioxidative, antihypertensive, and antiviral activities of bioactives, in addition to their essential nutritional functions. The effectiveness of food bioactives depends on different parameters such as bioactivity, bioavailability, metabolomics, nutrigenomics, and stability within the food matrix. For instance, bioactives' delivery via the oral route is restricted by gastrointestinal enzymes, harsh pH, the epithelium, and the mucus layer. Lately, researchers have investigated bioactive compounds, bioaccessibility, and functions in detail, whereas the development of nutraceutical applications has attracted considerable interest. Functional, "super," and "tailor-made" foods are generated after manufacturing typical or traditional food products with ingredients that modify their properties (e.g., by binding, changing structure, or interface) and provide health benefits to them.

The Food Waste Recovery Group provides insights into all scientific and technological aspects dealing with food and the environment. The group has published several books dealing with biobased products and industries, sustainable food systems, saving food, as well as technologies and applications (for commodities such as cereals, coffee, grape, olive, and meat) for food waste recovery. Others are handbooks that deal with innovations strategies in the food and environmental sectors, nonthermal processing, food shelf-life and quality, nutraceuticals, and food ingredients such as polyphenols, carotenoids, proteins, lipids, glucosinolates, and dietary fiber.

Following the above considerations, the book covers food bioactives' properties and health effects given the new trends in food science and technology. It aims at supporting the scientific community that aspires to understand the role of food bioactives in health and develop applications in personalized nutrition, in functional foods, nutraceuticals, and personalized nutrition.

The book consists of 10 chapters. **Chapter 1** describes the principal sources of polyphenols and then correlated them with their properties (health), particularly absorption (bioavailability), metabolism, gut flora, and chronic disease (cardiac

health, obesity, diabetes, cancer, among others). Polyphenols are a very diverse and multifunctional group of phytochemicals widely found throughout the plant kingdom. The main classes of polyphenols are tannins, lignans, phenolic acids, phenolic alcohols, flavonoids, stilbenes, coumarins, and chalcones. The remarkable chemical structure of polyphenols leads to their biological and physiological activities, mainly due to the antioxidant activity that allows them to be used as additives in food products, delaying the oxidation process.

Chapter 2 discusses the biochemistry and health properties of glucosinolates, their physiological significance, as well as the hydrolysis process in the plant response to different abiotic stresses. Glucosinolates are a group of sulfur- and nitrogen-containing glycosides found in plants such as broccoli, cabbage, radish, and cauliflower, among others. Their hydrolysis byproducts, namely isothiocyanates, are responsible for the distinct aroma and pungent taste of cruciferous species, most of which contain species-specific glucosinolates. They are considered as beneficial to human compounds with several confirmed health effects. At the same time, a significant amount of research work has been carried out recently to identify those mechanisms and synergisms that are responsible for the activities of glucosinolates, as well to reveal physiological aspects in the plant–environment interactions.

Chapter 3 reviews updated scientific reports about food-derived bioactive peptides and proteins and about their potential preventive or alleviating role in the deadliest noncommunicable diseases. Cardiovascular diseases, cancer, diabetes, neurodegenerative disorders, as well as oral cavity diseases as a predisposing factor to the development of other essential illnesses are addressed. The objective is to provide useful information to readers involved or interested in the fields of pharmacology and food technology, with the hope that it can serve as an introductory guide to recognize the immense potential of peptides and proteins as therapeutic agents.

Chapter 4 discusses the actual state of research concerning the effect of dietary fiber on health and the pathways by which this nutrient develops its action. In the last years, dietary fiber has gained attention as a bioactive due to its potential health benefits in reducing the risks for many diseases, such as cancer and cardiovascular ones. This effect is linked to its action against inflammation, oxidation, hyperlipidemia, and other physiological disorders. Although research in this area is extensive, the elucidation of the mechanisms involved in this bioactivity is not yet conclusive.

Chapter 5 provides information on substances of lipid origin that have had important effects on the treatment or prevention of diseases such as cancer, diabetes mellitus, cardiovascular disorders, and obesity, among others. Information associated with metabolites of plant origin, as well as lipids of animal origin and food lipids, that have demonstrated hypoglycemic, anti-inflammatory, antiproliferative, hypocholesterolemic, antihyperlipidemic, and antihypertensive effects is presented. The chapter also discusses topics dealing with the chemical structures of the reported lipids, their origin, synthesis, preclinical studies (in vitro, in situ), and clinical studies, detailing dosage, method of administration, biochemical, molecular, and genetic studies, and mechanisms of action.

Chapter 6 provides a brief review of marine bioactives, including peptides, proteins, vitamins, sterols, fatty acids, polyphenols, saccharides, amino acids, and minerals. It also discusses the bioactives derived from marine bacteria as well as different techniques used for marine bioactives recovery. Marine organisms are a rich source of bioactive compounds. Bioactive compounds are compounds with health-promoting effects. Consumption of these compounds may lower the risk of diseases such as heart diseases, cancer, diabetes, osteoporosis, and other complications. Recently, marine bioactives have attracted much attention due to their enormous health benefits.

Chapter 7 deals with food bioactives that reduce the risk of cardiovascular diseases. Bioactive peptides derived from fish, milk, meat, and plant derivatives demonstrated a significant antihypertensive and lipid-lowering effect in randomized clinical trials. Some polyphenols isolated from foods or plants exert anti-inflammatory and antioxidant activity, which could strengthen the prevention of chronic diseases. Furthermore, polyunsaturated fatty acids, lycopene, alliin, plant sterols, monacolin k, and berberine could be considered to support cardiovascular risk patients in clinical practice.

Chapter 8 discusses bioactives with neuronal and immune functions. Healthy diets are low in saturated fats and carbohydrates and high in fiber and antioxidants such as polyphenols and monounsaturated and omega-3 fatty acids, phytosterols, and probiotics. It has been shown that polyphenols are interfering with immune cell regulation, gene expression, and pro-inflammatory cytokines synthesis. As such, these molecules are associated with extended health benefits, playing an essential role in the prevention and treatment of various chronic conditions, such as neurological disorders. Omega-3 fatty acids are known for their positive health effects through their anti-inflammatory properties as well as for being essential in neuronal/brain functioning and its immunomodulatory properties. Intestinal immune stress associated with low omega-3 availability might also be involved in the development of neuroinflammation and the progression of related diseases.

Although many foods that are in the market are marked as functional foods, the problem with bioactive compounds, in and from food sources, is that the health claims and their bioavailability are still not fully explored. There are many examples of bioactive's functionalization health claims connected to their functional properties and their interactions in foods. **Chapter 9** leads the reader from the necessary steps of acquiring bioactive compounds to their bioavailability analysis, protection, and further improvement of their functional properties. The chapter also takes into account the fortification of foods with bioactive compounds as a strategy to reduce the occurrence of chronic illness as well as challenges that lie ahead for scientists dealing with all the aspects of bioactives, from processing to health claims.

Chapter 10 discusses the requirement and regulatory aspects of bioactive compounds from food for health claims. It also includes the fundamental processes on the health claims for bioactive compounds from vegetables, fruits, spices, nuts, cereals, herbal products, legumes, medicinal plants, probiotics, prebiotics as well as those from fungal, algal, and animal sources, and other natural antioxidants. These

requirements are meant to protect consumers from frauds perpetrated by producers/manufacturers on nutraceutical products. Bioactive compounds' requirements for health claims range from laboratory findings to systematic clinical trials to guarantee safety and provide bioavailability and efficacy of nutraceutical products.

It is hoped that this book will assist food chemists, food scientists, food technologists, nutritionists, and biochemists as well as researchers, academics, and professionals working in the food industry. It also concerns individuals and stakeholders in the food sector (including small startups) interested in developing nutrition-based products. Moreover, university libraries and institutes could use it as a textbook for undergraduates and postgraduate level multidiscipline courses dealing with food science, food chemistry, and food technology.

At this point, I would like to thank all the authors for their fruitful collaboration as well as for the fact that they remained dedicated to the timeline and editorial guidelines. I would also like to acknowledge the acquisition editor Daniel Falatko and the book manager Aravind M. Kumar, and all colleagues from Springer's production team, for their assistance during the preparation of this book. Finally, I have a message for all the readers: those collaborative efforts contain hundreds of thousands of words and thus may contain errors. Thus, constructive comments and even criticism are always welcome. In that case, please contact me to suggest any changes.

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Contents

Chapter 1
Polyphenols

Bianca Chieregato Maniglia, Evertan Antonio Rebelatto, Katia Suzana Andrade, Acácio Zielinski, and Cristiano José de Andrade

Abstract Polyphenols are a very diverse and multi-functional group of phytochemicals, widely found throughout the plant kingdom. Their basic monomer chemical structure comprises a phenolic ring—a benzene ring(s) with at least one hydroxyl group attached to it. The main classes of polyphenols are tannins, lignans, phenolic acids, phenolic alcohols, flavonoids, stilbenes, coumarins and chalcones. Flavonoids are the most plentiful classes of polyphenols, since they represent ≈4000 out of 8000 polyphenols already identified. Polyphenols are also classified, merely, as flavonoids and non-flavonoids. Flavonoids are chemically composed of backbone of two benzene rings linked by a 3 carbon atoms in a chain from the pyran ring. The oxidation state of central carbon can be used to subclassify them (flavonoids): flavanones, flavanols, flavonols, isoflavonoids, flavones, and anthocyanidins. Rich sources of phenolic compounds include grape pomace, apple, berries, oranges, pomegranate, tomatoes, coffee, tea, wine, olive oil, among others. The remarkable chemical structure of polyphenols leads to their biological and physiological activities, mainly due to their antioxidant activity. Regarding the effects of polyphenols on human health, the phenolics have many health-promoting benefits, including antimutagenic, antihypertensive, hypoglycemic and antihyperglycemic, anticancer and antiapoptotic, antimicrobial, and inflammatory effects. Furthermore, when the phenolic antioxidants are added in food products, they can delay the generation of toxic products (oxidation), to act as rancidity regulator and maintaining nutritional quality of foods, among others. This chapter describes the principal sources of poly-

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phenols and then correlated their properties (health), particularly absorption (bioavailability), metabolism, gut flora, chronic disease (cardiac health, obesity, diabetes, cancer, among others).

Keywords Polyphenols · Nutraceuticals · Flavonoids · Sources of polyphenols · Effect of polyphenols on human health

1.1 Introduction

The plants as fruits, vegetables, herbal teas, and seeds, are rich sources of polyphenols with a wide range of chemical structures (Abbas et al. 2017). These compounds are secondary metabolites which show a wide range of function such as protection, color and flavor in particular astringency and bitterness (Shahidi and Ambigaipalan 2015). Furthermore, many health-promoting benefits have been reported, including antioxidant, anticancer, antimicrobial, antihypertensive, hypoglycemic and antihyperglycemic effects (Teixeira et al. 2014; Gani et al. 2012).

It is worth noting that the plants and their processed products stand out as the main sources of polyphenols that are consumed by the population. Polyphenols are widely related to human health benefits. Currently, World Health Organization (WHO) has recommended ≈0.4 kg per day of vegetables and fruits (5 daily portions) (WHO 2019). In addition, the polyphenols also have been applied in food and pharmaceuticals products with the aim to supplement them mainly in their levels of antioxidants (Vuorela et al. 2004).

The chapter summarizes the classification and chemical structure of polyphenols, their main vegetable sources and effects on human health.

1.2 Polyphenols; Classification and Chemical Structure

1.2.1 Polyphenols

Phenolic compounds or polyphenols are natural biologically active compounds found in plant based-food and that show a wide range of complex structures (Abbas et al. 2017). In plants, they exhibit different functions as bio stimulating for plant growth or as defense compounds. These compounds are also acknowledged as strong natural antioxidants, and it was shown in the literature important biological and pharmacological properties such as anti-inflammatory, anticancer, antimicrobial, antiallergic, antiviral, antithrombotic, hepatoprotective, food additive, signaling molecules, etc. (Kumar and Goel 2019).

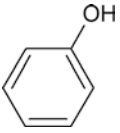
In plants, the most of polyphenols is chemically bounded to sugars, which is named glycosylated. Polyphenol skeletons can show carbohydrates and organic acids bound in different positions (Manach et al. 2004).

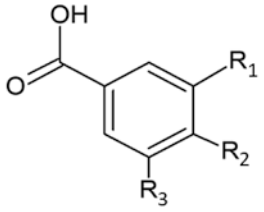
Polyphenols show as basic monomer a phenolic ring (structure in Fig. 1.1). Generally, these compounds are classified according to the structures shown as the number of phenolic rings, substituents linked to the rings, and the structural elements that bind these rings to each other. In this way, there are four main groups of polyphenols: phenolic acids, flavonoids, stilbenes, and lignans (Manach et al. 2004).

1.2.1.1 Phenolic Acids

Phenolic acids are related to phenolic compounds that have one carboxylic acid group and they are rarely found as free form, normally they are associated with amides, esters, and mainly glycosides (El Gharras 2009). Phenolic acids are widely found in food, in particular in cereals, herbs, vegetables, legumes, fruits, oilseeds, and beverages. These compounds show antioxidant capacity and it occurs by scavenging hydroxyl radical, several organic radicals, peroxyl radicals, superoxide radical anion, several organic radicals, singlet oxygen, and peroxynitrite. Moreover, phenolic acid can act as reducing agents, chain-breaking antioxidants, and they are important compounds to change cell signaling pathways (Chandrasekara 2019). There are two classes of phenolic acids: hydroxybenzoic acid (e.g. syringic acid, gallic acid, gentisic acid, and vanillic acid) and hydroxycinnamic acid (e.g. ferulic acid, caffeic acid, and *p*-coumaric acid) (Córdova and Medina 2014). Figure 1.2 shows the chemical structures of phenolic acids: hydroxybenzoic and hydroxycinnamic acids.

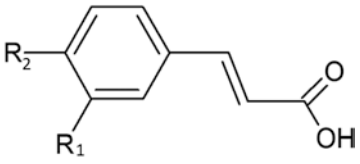
Fig. 1.1 Phenolic ring structure





Hydroxybenzoic acid

Galic acid => $R_1 = R_2 = R_3 = OH$
Protocatechuic acid => $R_1 = R_2 = OH$, $R_3 = H$:



Hydroxycinnamic acid

Coumaric acid => $R_2 = OH$
Caffeic acid => $R_1 = R_2 = OH$:
Ferulic acid => $R_1 = OCH_3$, $R_2 = OH$

Fig. 1.2 Chemical structures of phenolic acids

Generally, the content of hydroxybenzoic acid in edible plants is very low. However, some red fruits, onions, and black radish show higher concentrations (around tens of milligrams per kilogram fresh weight). In addition, complex structures such as hydrolysable tannins are composed of hydroxybenzoic acids (e.g. ellagitannins in red fruit such as raspberries, strawberries, and blackberries, and gallotannins in mangoes) (Manach et al. 2004).

According to Manach et al. (2004), hydroxycinnamic acid are more common than are the hydroxybenzoic acids, and it is represented, mainly, by *p*-coumaric, caffeic, ferulic, and sinapic acids.

In wine, there is one natural hydroxycinnamic acid present in an esterified form with tartaric acid, named tartaric *p*-coumaroyl ester (Salameh et al. 2008). Among the phenolic acid in fruits, caffeic acid (free and esterified form) is the most abundant compound present (75 until 100% of the total hydroxycinnamic acid content) (Cutrim and Cortez 2018).

In cereal grains, ferulic acid is the most abundant hydroxycinnamic acid found. For other side, ferulic acid can be found in free form in beer or tomatoes, and in this way, this compound is more efficiently absorbed (Bourne and Rice-Evans 1998; Bourne et al. 2000).

Spices, berry fruits, citrus, and vegetables show a bioavailable phytoconstituent named sinapic acid (Idehen et al. 2017). According to Vuorela et al. (2004), sinapic acid is becoming to be explored in the pharmaceutical, cosmetic, and food industries because of its inflammatory, preservative, antioxidant, and antimicrobial activities.

1.2.1.2 Flavonoids

Flavonoids show the structure composed of two aromatic rings (indicated as A and B in Fig. 1.3), linked by three carbon atoms and one oxygen, forming an oxygenated heterocycle (ring C in Fig. 1.3). The flavonoids can be classified according to the oxidation state of central carbon (C ring, Fig. 1.3) that is involved. In this way, there are six classes of flavonoids named: flavanones, flavanols, flavonols, isoflavones, flavones and anthocyanidins (Abbas et al. 2017).

1.2.1.2.1 Flavanones

Flavanones show the structure composed by a single bond in the positions of the C-ring, C_2 and C_3 with an oxygen atom in C_4 position, and a disaccharide in C7 (Fig. 1.4) (Liu et al. 2008). Flavanones are contained in citrus fruits, cherries, grapefruits, and tomatoes (Asakura and Kitahora 2018). Tomás-Navarro et al. (2014), reported that flavonoids show strong antioxidant capacity, and has been investigated for prevention of some cardiovascular disorders and certain kinds of cancer, and reduction of certain chronic diseases. These same authors showed that flavanones could also exhibit anti-inflammatory, antimicrobial, and antiviral activities, which can result in beneficial properties for the health human.

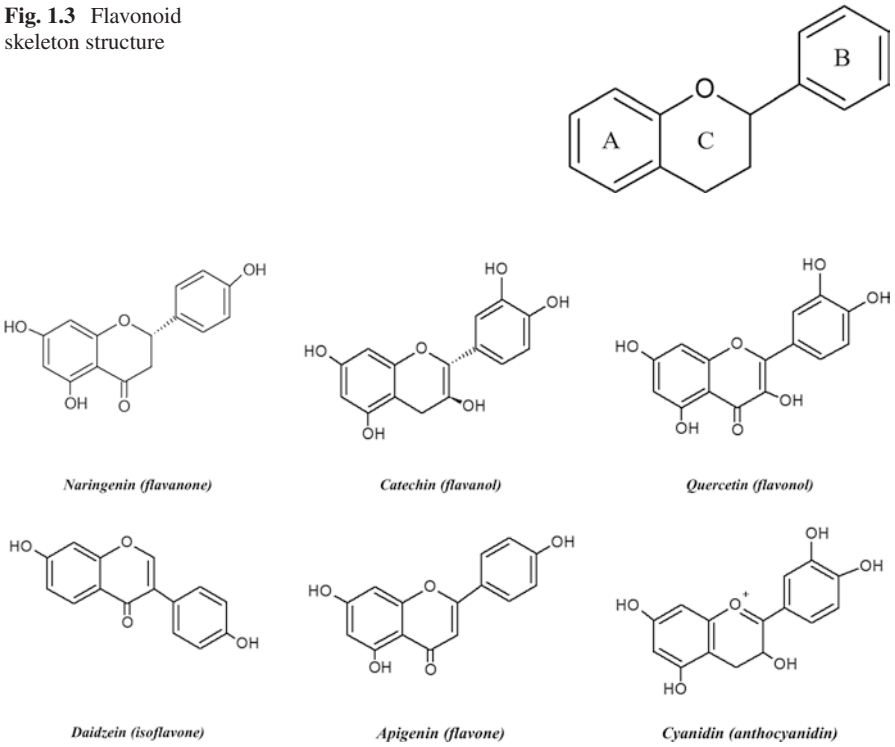


Fig. 1.3 Flavonoid skeleton structure

Fig. 1.4 Examples of flavanone, flavanol, flavonol, isoflavone, flavone, and anthocyanidin structures

1.2.1.2.2 Flavanols

Flavanols show a fully saturated heterocyclic ring with a hydroxyl substituent at position C_3 (Fig. 1.4). According to Bonetti et al. (2017), cocoa powder and chocolate, grapes, and teas show in it composition, flavanols and it polymerization products as epigallocatechin, catechin, epicatechin, gallocatechin, gallate derivatives, and proanthocyanidine.

1.2.1.2.3 Flavonols

Among the flavonoids, flavonols are the most found in foods, being kaempferol and quercetin the most representatives. Flavonols are present in glycosylated forms, they show 3-hydroxyflavone backbone, existing in the form of mono-, di-, or triglycosides *in vivo* (Stracke et al. 2007). Di Matteo et al. (2007) showed that the richest sources in flavonols: onions (up to 1.2 g/kg fresh weight), red wine and tea (contain up to 45 mg flavonols/L), leeks, curly kale, blueberries, and broccoli. In the litera-

ture (Kelsey et al. 2010; Mecocci et al. 2014) was reported that flavonols have shown antioxidant and anti-inflammatory properties.

1.2.1.2.4 Isoflavones

Isoflavones are compounds with the structure in the B-ring connected to the C-ring by the position C_3 (Figs. 1.3 and 1.4) (Liu et al. 2008). The most representative isoflavone is the daidzein (4′,7-dihydroxy-isoflavone) that is, mainly, found in food such as beans, apples, onions, and peas (Ying-Hui et al. 2017). According to Song et al. (2016), daidzein shows antioxidant, anti-inflammation, and antiestrogen functions. The authors also reported that the due the pharmacological activities of this isoflavone, daidzein has been applied in treating osteoporosis, autoimmune diseases, breast cancer, and cardiovascular disease.

1.2.1.2.5 Flavones

Within the flavonoids, flavones consist of one of the largest subgroups, it can be found in all parts of the plants as: leaves, stem, buds, heartwood, bark, thorns, rhizomes, roots, flowers, fruit, and seeds (Zuk et al. 2019). Flavones are synthesized from flavanones (direct biosynthetic precursor) in the branch point of the anthocyanidin/proanthocyanidin (Martens and Mithöfer 2005). Observing the Fig. 1.4, flavones differ from other flavonoids because show saturation of ring C which is named as c-pyrone (Atif et al. 2015).

Flavones show structures diversified, which guarantees a variety of functions, such as color control on vegetables and fruits to protect them from UV radiation and infectious attacks by microorganisms. (Harborne and Williams 2000). Flavones are also important for human nutrition and health, representing an abundant class of phytochemicals present in our daily diet (fruits, edible vegetables, seeds and nuts) (Martens and Mithöfer 2005). Rice-Evans et al. (1997) reported that polymethoxylated flavones, such as nobiletin and sinensetin can be found, mainly in citrus fruits as orange peel. Currently, flavone-containing food has attracted considerable scientific and therapeutic interest because of the beneficial effect for prevention of some human diseases. Agah et al. (2017) reported that flavones show structural features that make them among the strongest food-derived anti-inflammatory compounds. These authors observed that cereal derived flavones show strong synergistic interaction with derived flavonols against inflammation, and Yang et al. (2014) reported that flavones can also protect against estrogen-linked colon carcinogenesis.

1.2.1.2.6 Anthocyanidins

Anthocyanidins show structure with hydroxyl groups in the positions of C_3, C_5, and C_7 in the B ring (Fig. 1.4), however each structure may have its own characteristic hydroxyl or methoxyl groups (Swanson 2003). Anthocyanidins are mainly found conjugated with glucose moieties and they are found in large concentrations in wine, grapes and berries (Stalmach 2014).

The Fig. 1.4 shows some examples of flavanone, flavanol, flavonol, isoflavone, flavone, and anthocyanidin structures.

1.2.1.3 Stilbenes

Stilbenes are an important group of nonflavonoid phytochemicals of polyphenolic structure characterized by the presence of a 1,2-diphenylethylene nucleus (Sirerol et al. 2016). The Fig. 1.5 shows the stilbene skeleton.

According to Chong et al. (2009), the structures of common plant stilbenes showed the follow radicals (being OGlu: *O*-*β*-D-glucopyranoside):

- trans-resveratrol: R_1 = H, R_2 = OH, R_3 = OH, R_4 = OH;
- trans-piceid: R_1 = H, R_2 = OH, R_3 = OGlu, R_4 = OH;
- pinosylvin: R_1 = H, R_2 = H, R_3 = OH, R_4 = OH;
- piceatannol: R_1 = OH, R_2 = OH, R_3 = OH, R_4 = OH;
- pynosylvin monomethylether: R_1 = H, R_2 = H, R_3 = OCH_3, R_4 = OH;
- trans-pterostilbene: R_1 = H, R_2 = OH, R_3 = OCH_3, R_4 = OCH_3;
- astringin: R_1 = OH, R_2 = OH, R_3 = OGlu, R_4 = OH;
- rhapontin: R_1 = OH, R_2 = OCH_3, R_3 = OGlu, R_4 = OH.

Stilbenes are compounds naturally present in grapes and have gained a growing interest due to health-promoting properties reported (Segade et al. 2019). Raposo et al. (2018) reported in recent studies that stilbenes could act as compounds that help in the preservation of wine. Guerrero et al. (2020) explored this property, identifying the stilbene composition and concentration in wines as a quality marker.

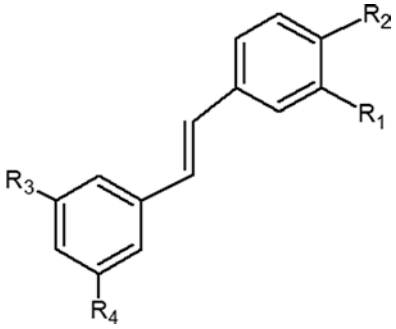


Fig. 1.5 Stilbene skeleton structures

1.2.1.4 Lignans

Lignans are a group of diphenolic compounds (two units of phenylpropane units) linked by a C-C bond between the central atoms of the respective side chains (position 8 or β), as we can see in the Fig. 1.6 (Linder et al. 2015). This type of polyphenol is concentrated in the bran layer of cereal grain (Higuchi 2014).

Observing the Fig. 1.7, a compound is considered a lignan if the two units of phenylpropane (in the dimericcase) are linked by a β-β' bond, subsequently denominated 8–8′ bond (Linder et al. 2015). However, according to Linder et al. (2015), we can found neolignans that consist in units of phenylpropane combined in other way.

According to Das and Devi (2019), we can classify lignans in 8 subgroups based on their carbon skeleton, cyclization pattern, and the way in which oxygen is incorporated in the molecule skeleton. The subgroups consist in: furans, furofurans, dibenzylbutanes, dibenzylbutyrolactones, dibenzocyclooctadienes, dibenzylbutyrolactols, aryltetralins and arylnaphthalenes (Das and Devi 2019). The Fig. 1.8 shows some generic of lignan skeleton structure.

In addition, according Linder et al. (2015), lignans are also classified into three categories in relation to oxygen position: lignans with oxygen at the 9(9′)-carbon, lignans without oxygen at the 9(9′)-carbon, lignans with dicarboxylic acid. There is possible to find some lignan in more than one category and/or there exist different cyclization patterns for a given type. Furan lignans is one example of this behavior, it is a lignin that occur with or without oxygen at the 9(9′)-carbon (Linder et al. 2015).

Foods rich in lignin (seeds, whole-grain cereals, and nuts) have been associated with biological activities such as cytotoxic (Huang et al. 2013), antioxidative (Duan et al. 2009), anti-bacterial (Tago et al. 2008), immunosuppressive (Park et al. 2007), anti-inflammatory (Zheng et al. 2014), anti-HIV (Chen et al. 1996), etc.

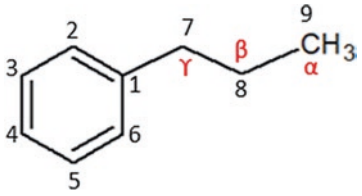


Fig. 1.6 Phenylpropane units

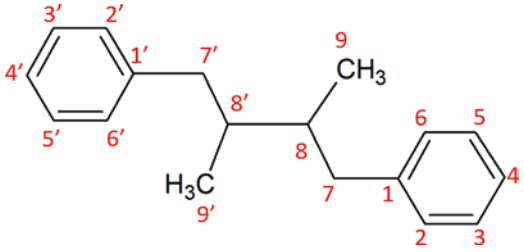


Fig. 1.7 Lignan skeleton

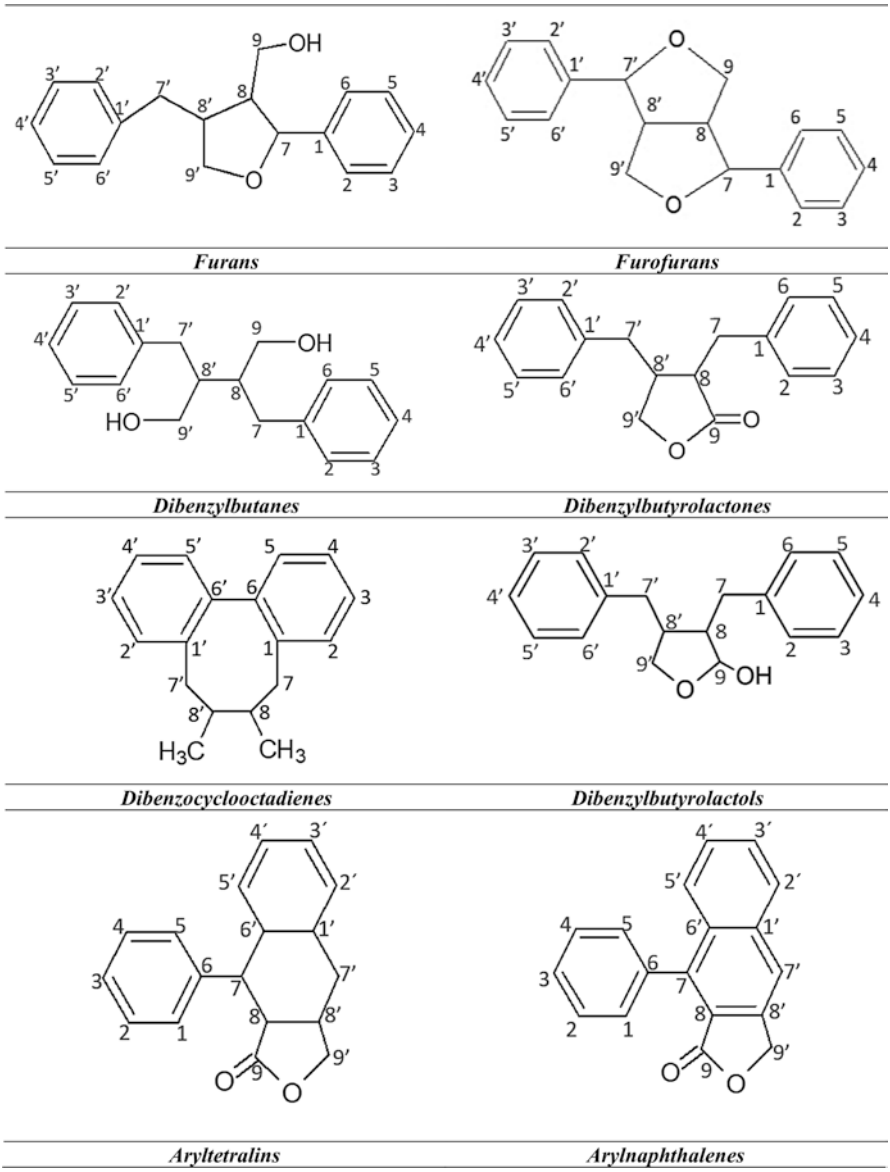


Fig. 1.8 Generic lignan skeletons

Lignans such as secoisolariciresinol and its precursor secoisolariciresinol diglucoside are the most abundant lignans found in the diet (Peirotén et al. 2019). Moreover, other lignans such as matairesinol, and the secoisolariciresinol precursors pinoresinol and lariciresinol, can also be found in some plant foods (Landete 2012).

Summing up, from the main structure surrounding the phenolic ring, there are highly diversified classes of secondary metabolites, named phenolic compounds, distributed widely in the plant kingdom. Moreover, the diversified structures show interesting and different properties that has attracted the attention of many sectors as biochemistry, physiology, human nutrition, and health.

1.3 Rich Sources of Polyphenols

1.3.1 Wine and Grape Pomace

The main sources of phenolic compounds in red wine are found in grape skins, pulp and seeds. During fermentation, important flavonoids (present in the rind, pulp and seeds) are transferred to the wine. Regarding white wine, the mixture is made from free running, without the mixture of grapes, i.e. without contact with the skin of the grape. Thus, when compared to red wine, white wines have lower polyphenol content and lower antioxidant properties (Fuhrman et al. 2001).

Flavonols are the main flavonoids present in wine. Among them, stand out quercetin, kaemppferol and myricetin. Also can be mentioned tannins, proanthocyanidins and flavanols, such as catechin and epicatechin (Shahidi and Ambigaipalan 2015). The concentration of phenolic compounds of red wines made from dark-skinned grapes usually contain about 3500 mg/L, in which the flavonoid portion corresponds to 1000–1800 mg/L (Di Lorenzo et al. 2016).

Wines and grapes also have phenolic acids and stilbenes in their composition. Phenolic acids can be found in both red and white wine. Among them can be mentioned quinic and shikimic and tartaric acid, present in their free form or glycosylated derivatives (Monagas et al. 2005).

Grape pomace is a low-cost source of phytochemicals. Different polyphenols are found in grape pomace. Among them, flavonols such as catechin, epicatechin and proanthocyanidins, as well phenolic acids, tannins and anthocyanins. There are several phenolic compounds found in grape skin, such as proanthocyanidins, ellagic acid, myricetin, prodelphinidins, kaempferol, quercetin and trans-resveratrol. In the grape seed there is catechin, epicatechin, gallic acid, proanthocyanidins and dimeric procyanidin (Brenes et al. 2016).

In grape seeds a higher concentration of phenolic compounds can be found than in grape skin. For example, in seed about up to 16.518 mg of catechin equivalents (EC)/100 g can be found. In the skin the value found was up to 1839 mg EC/100 g. Grape seed is abundant in flavonols (oligomeric and polymeric compounds) that

have high antioxidant capacity, while the skin is very rich in anthocyanins (289–935 mg/100 g) (Rockenbach et al. 2011). Flavonols (quercetin 3-*O*-glucuronide and 3-*O*-rutinoside-rutin) were found in grape stems, as well phenolic acids and dihydroflavonols like astribin (Karvela et al. 2009). There are several potential applications grape pomace, however grape pomace is mostly used for the production of animal feed (Celma et al. 2009).

1.3.2 Apple

Apple (*Malus domestica* Borkh) is a widely consumed fruit worldwide—the third largest production, 11.6 million tons (Bondonno et al. 2017; Rabetafika et al. 2014).

The main groups of polyphenols in apple are: phenolic acids, flavanols, anthocyanidins, flavonols, and dihydrochalcones. The major apple flavonoids are procyanidins, catechins, quercetin glycosides, dihydrochalcones, hydroxybenzoic acids and hydroxycinnamic acids and their derivatives (Bondonno et al. 2017; Kalinowska et al. 2014; Khanizadeh et al. 2008; Van Der Sluis et al. 2002).

The total phenolic content in the apple peel is significantly higher and in the tissue located just below the peel, than in the pulp, since apple skin contains ≈46% of the total phenolics in apples (Kalinowska et al. 2014; Kondo et al. 2002; McGhie et al. 2005).

A low concentration of are flavonoids found in apple juice. Regarding commercially available apple juice, the concentration of quercetin is 14 times lower than that found in apples fruits (Hertog et al. 1993).

Substantial fraction of apple fruit production, about 30%, it has used to manufacture processed foods, like beverages and desserts. After production, around 11% of the initial mass of the fruit is transformed into by-products (skin, pulp and seeds), generating annually, about three million tons of waste (Bondonno et al. 2017; Kammerer et al. 2014; Rana et al. 2015). In the apple pomace, there are several polyphenols including flavanols, flavonols and anthocyanins such as cyanidin-3-galactosides (Diñeiro García et al. 2009; Kammerer et al. 2014).

1.3.3 Berries

Among the berries black chokeberry, blackcurrant, black elderberry, blueberry, blackberry, raspberry, blackberry, strawberry and black grapes stand out due to high content of phenolic compounds (Kowalska et al. 2017; Skrovankova et al. 2015; Tylewicz et al. 2018).

One of the largest sources of polyphenols found is black chokeberry pomace. The production of chokeberry juice generates a larger amount of pomace. In addition, seed fractions, have high total dietary fiber content ≈75%, which are rich in proanthocyanidins (12,000 mg/100 g), anthocyanins (1200 mg/100 g) and amygda-

lin (7–185 mg/100 g), and can be used in the preparation of dietary fibers preparations and/or phenolic extracts (Sójka et al. 2013). Beyond that black elderberry contains a high amount of anthocyanins (813 mg/100 g), besides flavonols and cinnamic acid derivatives (Silva et al. 2017).

Blackberries contain severals polyphenols, in particular, stands out anthocyanins, like cyanidin-3-glucoside (Siriwoharn et al. 2004). Blackberries, raspberries, and strawberries have a similar amount of total phenolic compounds (215–260 mg/100 g) (Pérez-Jiménez et al. 2010). However, when compared to blueberries, blackberries, and raspberries; strawberries have a significant lower content of anthocyanins (Skrovankova et al. 2015).

The anthocyanins present in blueberries are mainly present in the skin. Many of these anthocyanins, exhibit excellent antioxidant activity, such as: delphinidin-3-*O*-galactoside, cyanidin-3-*O*-galactoside, delphinidin-3-*O*-arabinoside (Borges et al. 2010).

Likewise black currants and blueberries, cranberries contain high content of phenolics. Nevertheless cranberries have high content of flavonoids and the main phenolic compounds is ellagic acid (about 51% of the total) (Grace et al. 2014; Skrovankova et al. 2015; Tylewicz et al. 2018).

1.3.4 Orange, Guava and Pomegranate

Orange, including orange juice and their by-products have high levels of flavanones (hesperidin and narirutin) (Roowi et al. 2009).

The manufacture of orange juice leads to the production of various by-products such as seeds, pulp, leaves, peel and whole fruits (Rezzadori et al. 2012). After the juice is extracted, the solid residues of the orange industry represented by the peels, seeds and pulp, equivalent to about 50% of the weight of each fruit and with approximately 82% humidity, are transformed into pelletized bran. This bran is mainly used as a dietary supplement to cattle herds (Tienne et al. 2004). However, the most valuable byproduct of a citrus fruit is found in the orange peel (essential oil), being widely used as food and cosmetic ingredients (Rezzadori et al. 2012).

Guava fruits are rich in anthocyanins, flavonoids, proanthocyanidins and other phenolic classes including phenolic acids, flavonols and tannins (Gülçin 2012; Rojas-Garbanzo et al. 2017; Shi et al. 2005).

According to Rojas-Garbanzo et al. (2017), several polyphenols are reported, and 24 compounds were detected for the first time in *P. guajava*. Among them, phlorizin, nothofagin and astringin.

Pomegranate is a source of anthocyanins, ellagitannins and other phenolic substances with antioxidant and antitumor activities. Polyphenols are distributed in the peel, pulp and seeds, however in the peel has the highest polyphenol content (Fischer et al. 2011; Lansky and Newman 2007).

In pomegranate juice, a higher content of polyphenols can be found than in other fruit juices. The main class of polyphenols found is anthocyanins, such as

delphinidin-3-glucoside and cyanidin-3,5-diglucoside, followed by elagitanines and gallic and ellagic acids (Aviram and Rosenblat 2012; Bakkalbasi et al. 2009; Gil et al. 2000).

1.3.5 Potatoes, Sweat Potato, Cassava, Tomatoes, Onions and Cabbage

High flavonoid content was found for green and purple sweet potato leaves and onion leaves. In addition, sweet potato green leaves showed high antioxidant activity and reducing potential in comparasion with cabbage, spinach and potato (Chu et al. 2000).

Antioxidant activities have been found in several vegetables as perilla leaf, pepper and ginseng leaf, sweet potato leaf, chinese toon bud, loosestrife, cowpea, lotus root, soybean, that may be important for disease prevention caused by oxidative stress.

In these vegetables were identified phenolic compounds such as chlorogenic and gallic acids. Besides, a positive relationship was observed between antioxidant activity and total phenolic content (Deng et al. 2013).

According to FAO, in 2015, the potatoes represented the fifth largest harvest in the world (Tylewicz et al. 2018). The polyphenols in potatoes are present in flesh and skin. About 50% of the total polyphenol content was located in the tuber, whereas the remaining concentration decreases as it approaches the center of the tubers (Akyol et al. 2016; Friedman 1997).

Phenolic acids represent most of the polyphenols present in potatoes. Among these, chlorogenic acid is the most abundant, followed by caffeic acid, gallic acid, ferulic acid, among others (Akyol et al. 2016; Dao and Friedman 1992). However, the content of chlorogenic acid in potatoes can be reduced with food processing (e.g. heating)., which depends on the nature of the heat source used (Dao and Friedman 1992).

The second largest category of potato polyphenols is flavonoids. The main flavonoids in the tubers were flavanones, naringenin and eriodictyol, flavanols, catechin and epicatechin (Lewis et al. 1998).

The main phenolic compound found in potato peel extract is chlorogenic acid, and the phenolic content found is about 70.82 mg of CE/100 g. (Akyol et al. 2016; Kanatt et al. 2005).

Pigmented potatoes, such as red and purple ones are rich in anthocyanins, which may be used in the food industry, since the potato production cost is not as high compared to other crops. However, potatoes with high anthocyanin concentrations are required for the pigment extraction process (Ezekiel et al. 2013).

The main phenolic acid found in sweet potatoes is chlorogenic acid, and the highest content is present in a white pulp cultivar. Among the other phenolic acids present, can be highlighted 3,5-dicafeoylquinic acid, 3,4-dicafeoiliquinic,

4,5-dicafeoiliquinic and caffeic acids. The highest contents of these acids are found in a variety of purple pulp (Padda and Picha 2008).

Purple-fleshed sweet potatoes are also high in anthocyanins. About 39 anthocyanins have already been identified and they are dominated by cyanidin and peonidin aglycones (Gras et al. 2017; Oki et al. 2002).

Sweet potato leaves are considered processing residues, however, studies indicate that phenolic compounds such as 3,4,5-tri-*O*-caffeoylquinic acid can be found, and these compounds present a high antioxidant potential (Islam et al. 2002; Shahidi and Ambigaipalan 2015).

Regarding cassava, it has been found that polyphenol content in flours ranges from 2.1 to 120 mg/100 g. These polyphenols can form insoluble complexes, inactivating the thiamine enzyme, which will reduce starch digestibility. On the other hand, tannins and also catechins, have antioxidant and anticarcinogenic activities and are beneficial to the cardiovascular system (Chung et al. 1998; Wobeto et al. 2007).

The main polyphenols in tomatoes (range from 0.1 to 18.2 mg/100 g) are naringenin chalcone, rutin and quercetin. Anthocyanins such as delfidine and malvidine can also be found. (Martí et al. 2016; Tylewicz et al. 2018). The main phenolic acids identified in tomato peel are procatchoic and vanillic acid, with concentrations of 5.52 and 3.31 mg/100 g, respectively (Elbadrawy and Sello 2016).

Among the flavonols present in tomatoes, the main ones are quercetin conjugates; however, kaempferol amounts and traces of free aglycones were also found (Crozier et al. 1997).

In the pericarp and pulp of immature green tomatoes a high content of chlorogenic acid can be found. This acid level varies with fruit maturation as the fruit turns pink and then red (Shahidi and Ambigaipalan 2015; Toor and Savage 2005).

Tomato peels and seeds are usually removed during processing. Lyophilized tomato peel extracts showed a total polyphenol yield of 38.67 mg tannic acid equivalent/100 g peel (Sarkar and Kaul 2014).

Several flavonoids are found in onions, in particular quercetin, kaempferol, myricetin, and catechin (Pérez-Gregorio et al. 2014; Shahidi and Ambigaipalan 2015). In onions, monoglucoside quercetin and diglucoside quercetin represent 80% of the total flavonoids. Quercetin glucoside levels are much higher in onions than in other vegetables (Rhodes and Price 1996; Shahidi and Ambigaipalan 2015).

The total phenolic content in yellow onion ranges from 6.06 ± 0.24 to 22.32 ± 1.62 gallic acid equivalents (GAE) mg/g, and from 5.71 ± 0.20 to 18.58 ± 0.62 GAE mg/g dry weight in red onions (Cheng et al. 2013). In onions, low content of phenolic acids are bounded to cell walls., in which protocatechuic acid is the most (Ng et al. 2000). Anthocyanins are part of a lower proportion of flavonoids present in the edible portion of red onion. In this type of onion, the total flavonoid content is generally higher than in white or yellow onion bulbs (Rhodes and Price 1996; Shahidi and Ambigaipalan 2015).

A amount in the range of 600.72–2230.89 mg/100 g of quercetin can be found in onion bagasse, which varies with onion variety (Roldán et al. 2008; Tylewicz et al. 2018).

Cabbage is a good source of polyphenols, also rich in carbohydrates and vitamin C. Brassica vegetables, including all cabbage-like vegetables, are a genus of the Cruciferae family and contribute to the intake of glucosinolates (Chun et al. 2004; Shahidi and Ambigaipalan 2015).

1.3.6 Cereals

A variety of phytochemicals can be found in whole grains such as phenolic compounds, carotenoids, γ-oryzanol, dietary fibers and vitamin E (Okarter and Liu 2010). The main polyphenols found in whole grains are phenolic acids. Other classes of polyphenols are flavonoids and lignans. The ferulic acid is the major phenolic acid found in grains (mainly in the cortical layer). Other acids that may be cited are caffeic, oxalic and *p*-comuraic acids (Deng et al. 2012; Tian et al. 2019).

The phenolic content varies according to grain, for instance wheat (7.99 μg/g), oats (6.53 μg/g), and rice (5.56 μg/g) (Adom and Liu 2002; Tian et al. 2019).

A higher concentration of polyphenols can be found in whole grains when compared to grains that have been processed. In the case of rice for example, the phenolic portion is present mainly in the cortical layer of the grains. When the grain is polished, this part is removed, removing ferulic acid. For this reason, brown rice has more phenols than polished rice. Another factor that can be considered is that in smaller grains of rye, oat, millet and rice there is a higher availability of ferulic acid when compared to larger grains. This is because the acid is bound to the total fiber content (McCarty and Assanga 2018).

In cereal grains, there is no uniform distribution of phenolic compounds. The outer layers of the grain (bark, forehead, pericarp and aleurone) have a higher concentration of phenolics when compared to the endosperm. Usually, the outer layers are used for bran production, and the endosperm layer is used for refined flour production (Kaur et al. 2014; Tylewicz et al. 2018).

In wheat, the main phenolics are phenolic acids and flavonoids. These compounds are mainly found in the outer layer of the grain. There is a variation among wheat genotypes regarding the content of phenolic compounds, flavonoids, lignans and anthocyanins present (Žilić 2016). The main phenolic compounds present in wheat are ferulic acid and *p*-coumaric acid (Žilić et al. 2012).

There are several phenolic acids in wheat grains, such as hydroxybenzoic acids and hydroxycinnamic acids. Among them, ferulic acid is the main one, with concentrations around 1000 μg/g (Hernández et al. 2011). Leoncini et al. (2012) studied six varieties of wheat. The end result showed that the total flavonoid content varies depending on wheat cultivar. It was found in cultivar Rassineto the highest phenolic content (173.48 mg GAE/100 g of grain), which was similar to other cultivars (Andriolo, Gentil rosso, Inallettabile and Verna).

Phenolic compounds of oat are mainly found in the bran layer, although some are present in groats and hulls (Gangopadhyay et al. 2015; Ratnasari et al. 2017). Phenolic compounds in oat, as well in other cereals, are either in free or bound

forms (Naczk and Shahidi 2006). The main phenolic compounds in oat grain are phenolic acids, avenatramides and flavonoids. Among the phenolic acids, stand out the gallic, benzoic caffeic and ferulic acids. In the bound fraction, the phenolic concentration is higher, with ferulic acid being the main compound. The flavonoids found in the free fraction are as follows: catechin, rutin, quercetin, and tricin. However, the flavonoid found in the bound fraction is kaempferol (Hitayezu et al. 2015; Tylewicz et al. 2018; Verardo et al. 2011).

A phenolic compound that is only found in oats are avenantramides. It is an antipathogen produced by the plant itself in response to exposure to other pathogens such as fungi.

The avenanthramides are low-molecular-weight soluble phenolic compounds which are not present in other cereal grains, only in oats. These compounds are antipathogens (phytoalexins), which are produced by the plant in response to exposure to pathogens such as fungi. The avenanthramides 2c, 2p and 2f are the main ones found in oats (Hitayezu et al. 2015; Meydani 2009; Verardo et al. 2011).

The sorghum has a diversity of phytochemicals, especially the polyphenols. Several phenolic compounds are found in extracts obtained from white, red and brown sorghum grains. The main family of these compounds are phenolic acids, such as ferulic and caffeic acids (Chiremba et al. 2012; Stanisavljević et al. 2016). There are several flavoinoids found in sorghum, including: luteolin, apigenin, catechin and quercetin. As in other grains the outer layer of the grain is the richest in phenolic compounds (Moraes et al. 2015; Tylewicz et al. 2018).

In rice, various phenolic compounds are found, such as phenolic acids, anthocyanins and proanthocyanins. Phenolic acids include ferulic, *p*-coumaric, isoferulic and caffeic acids. Among them, ferulic acid is the most abundantly found. Proanthocyanidins in rice are usually type B, but recent research shows that type A and B coexist in red and black rice (Shao and Bao 2015).

Several anthocyanins were determined in colored rice grains. The main anthocyanin found in colored rice does cyanidin-3-glucoside, besides red and black rice also shows peonidin-3-glucoside, and in the black rice evidence of cyanidin-3-glucoside was found (Kapcum et al. 2016; Zhang et al. 2010).

In millets, besides micro and macronutrients, can also be found important phytochemicals, especially phenolic compounds. The main polyphenols present in millet are hydroxybenzoic (protocatechuic, phydroxybenzoic) and hydroxycinnamic (*p*-coumaric, ferulic syringic) acids, in addition to flavonoids and proanthocyanidins (Devi et al. 2014; Xiang et al. 2019). In finger millet free fractions, flavonoids such as catechin, epicatechin and quercetin are present. Phenolic acids are also present, but in lower concentration. Ferulic acid is also the major phenolic acid in millet, however *p*-coumaric, caffeic and protocatechuic acids are also present (Xiang et al. 2019). In finger millet of colored pericarp varieties, a higher concentration of phenolic compounds is found when compared to white pericarp varieties (Xiang et al. 2019).

In maize grains, the main phenolic compounds are phenolic acids, however, other phenolics such as anthocyanins, flavonols, and flavanols have been identified in colored maize grains (Salinas-Moreno et al. 2017). Several phenolic acids are

present in corn, such as caffeic, vanillic acids, among others. However the main ones are ferulic and *p*-coumaric acids present in soluble form, or attached to the cell wall (Salinas-Moreno et al. 2017). In the bound fraction of maize a higher concentration of phenolic compounds was found (150–300 mg/100 g), when compared to the free fraction (1–5 mg/100 g) (González-Muñoz et al. 2013). Other classes of phenolic compounds found in maize include quercetin, kaempferol, and isorhamnetin, which were found in purple corn. In colored corn cultivars, anthocyanins have been found, including elargonidin, cyanidin, and peonidin (Montilla et al. 2011; Paucar-Menacho et al. 2017; Tylewicz et al. 2018).

In barley, polyphenols may be present in bound, conjugated or free form. The main classes are flavonoids, lignans and phenolic acids (Fogarasi et al. 2015). The main phenolic acids in barley are benzoic and cinnamic acids. These acids are found in greater concentration in the bound form than in the conjugate and free form. The abundance of phenolic acids in barley indicates that it can serve as an excellent source of natural antioxidants (Idehen et al. 2017; Quinde-Axtell and Baik 2006; Zhao and Moghadasian 2008).

In the free form of barley, the concentration of phenolic acids varies between 4.6 and 23 mg/g, while in the conjugate form the value varies between 86 and 198 mg/g. In bound form, this value ranges from 133 to 523 mg/g. (Abdel-Aal et al. 2012; Holtekjølen et al. 2006). The major flavonoids in barley grains are flavanols, anthocyanins, which are located in the pericarp, mostly glycoside derivatives. Proanthocyanins are also present (Abdel-Aal et al. 2012; Idehen et al. 2017).

1.3.7 Coffee and Teas

Teas and coffees are two of the most popular beverages in the world. In both, polyphenols such as flavonoids are present and contribute to taste and health properties (Wang and Ho 2009).

Coffee is a beverage with stimulating power due to the presence of caffeine; however, other compounds are identified in this drink and many of them have health benefits, such as flavonoids, chlorogenic, caffeic, gallic and ferulic acid (Esquivel and Jiménez 2012; Meletis 2006).

Coffee flavor is strongly influenced by the presence of phenolic compounds, and 42 phenolics have been identified as being present in roasted coffee aroma. In coffee beverages, the main phenolic compounds are chlorogenic acids, in the form of various isomers, considered the most important and those present in greater quantities in green coffee beans. In coffee seeds, tannins, lignans and anthocyanins are another phenolic compounds present, but in smaller quantities. In coffee pulp, condensed tannins stands out as the main phenolic compounds (Clifford 1985; Farah and Donangelo 2006).

It was identified chlorogenic, gallic and protocatechuic acids in extracts obtained from spent coffee grounds and husks, suggesting the potential use of these residues in the recovery of phenolic compounds (Andrade et al. 2012).

Tea is a beverage produced from the tea plant (Camellia sinensis), that are rich in polyphenols (Tylewicz et al. 2018). The main polyphenols in tea leaves include flavonoids, particularly flavanols, and phenolic acids (Coe et al. 2013; Wang and Ho 2009).

Green tea is a minimally processed product obtained from freshly harvested leaves of the *Camellia sinensis* plant. Immediately after harvesting, tea leaves are heat treated to inactivate polyphenol oxidase, which preserves the freshness of the tea and its monomeric polyphenol profile (Bruno et al. 2014; Frei and Higdon 2003).

In green tea, about 42% of soluble solids are catechins such as epigallocatechin gallate, epigallocatechin, gallocatechin and epicatechin (Bradfield and Bate-Smith 1950; Graham 1992).

Black tea is a processed product obtained from the complete fermentation of fresh tea leaves and is characterized by the orange-brown color. This feature comes from the presence of teaflavins and thearubigins. In addition to color, these compounds are responsible for the flavor of black tea (Ferruzzi 2010). The polyphenols concentration in the black tea decreases during fermentation, then, the longer the processing time, the lower the polyphenols content in the tea (Astill et al. 2001).

Oolong teas are produced from the partial fermentation of tea leaves. The process is carried out in various ways and the products vary with respect to the degree of catechin oxidation that is observed. Because it is only partially fermented, it retains a considerable number of original polyphenols. Oolong tea composition is estimated to be intermediate between green and black teas (Graham 1992; Wang and Ho 2009).

1.3.8 Olive Oil

In olive oil, the main phenolic compounds are secoiridoids followed by phenolic alcohols, lignans and flavones (Bendini et al. 2007; Brenes et al. 2000).

The secoiridoids are only found in plants of the *Olearaceae* family. They are compounds produced by metabolism secondary of terpenes. One of the characteristics of these compounds is the presence of elenolic acid in their molecular structure (Bendini et al. 2007). The most abundant secoiridoids of virgin olive oil are the dialdehydic form of elenolic acid (Montedoro et al. 1992a, b, 1993). Tyrosol and hydroxytyrosol are the main phenyl alcohols found in olive oil (Oliveras-López et al. 2007).

The main phenolic acids present in olive oil are: protocatechuic, gallic, vanillic, caffeic acid, among others (Franco et al. 2014; Tylewicz et al. 2018).

In olives and virgin olive oil, natural lignans as (+) - pinoresinol and 1-acetoxypynoresinol are found. Pinoresinol (+) was found in other plants, however, 1-acetoxypynoresinol is often found only in olives. It is widely accepted that lignan consumption has beneficial health effects. Therefore, these two compounds are of great interest based on their properties (López-Biedma et al. 2016).

Flavonoids are important part in the polar fraction of olive oil. Among these flavonoids, luteolin, apigenin and diosmetine can be highlighted (Kelebek et al. 2017).

The main difference between olive leaves composition for olive oil can be considered the presence of oleuropein, as well ligstroside and several other flavonols in their glycoside form, that are not found in oil (Talhaoui et al. 2015).

As in olive oil, secoiridoids are the main class of phenolic compounds found in olive leaves. The component with the highest phenolic fraction in olive leaves is oleorupine (24.7 and 143.2 × 103 mg/kg). Olive leaves have a higher concentration of phenolic compounds (10,000–82,000 mg/k), when compared to olive oil (40–1000 mg/kg) (Bajoub et al. 2017; Loubiri et al. 2017; Talhaoui et al. 2014; Tylewicz et al. 2018).

1.4 Effect of Polyphenols on Human Health

Regarding nutraceuticals, polyphenols have been drawn attention, for instance Blackcurrant (*Ribes nigrum*) berrie have been named "superfruits" due to the presence of important sources of phytochemicals that have huge potential as immunomodulators, antimicrobials and anti-inflammatories, inhibiting low density lipoprotein and reducing cardiovascular disease. It has been cultivated for use in beverages and has a reputation for excellent health characteristics due to its high antioxidant content (Nour et al. 2013; Shahidi and Ambigaipalan 2015). Therefore, polyphenols consumption plays a fundamental role on human health, for instance antioxidant, anti-inflamatory, diabetes controller, microbiome modulator, anti-aging, antihypertensive and anticancer - briefly described below:

1.4.1 Antioxidant

Superoxide radical, peroxynitrite radical, nitric oxide, hydroxyl radical, and hydrogen peroxide, are ubiquitous molecules knows as reactive oxygen species, since reactive oxygen species are inherently produced by all living cells - metabolism. Reactive oxygen species are highly reactive molecules, short-lived derivatives of oxygen metabolism. Reactive oxygen species, at low concentrations, are essential to regular metabolism, more specifically intracellular communication, cell differentiation, apoptosis, antimicrobial and immunity properties. An oxidative stress condition occurs when the living cells have high reactive oxygen species rate and/or a depression of their antioxidant systems (unbalanced) (Roberts and Sindhu 2009).

Aerobic organisms produce, primarily, superoxide radical which is highly cytotoxic. Reactive oxygen species can react with biomolecules, for instance reactive oxygen species can damage DNA which may lead to chances on protein conformation; induce nucleic acid modifications or enhance lipid peroxidation. Oxidized and nitrated reactive oxygen species compounds usually affect cell signaling and basal

cellular functions. These disorders are related to health problems such as atherosclerosis and inflammation. Therefore, reactive oxygen species show harmful effects on human health, in particular metabolic syndrome, type 2 diabetes and cardiovascular diseases (coronary and hypertension) (Roberts and Sindhu 2009).

According to Huang et al. (2005), antioxidant activity is related to oxidation lipids, proteins, among other biomolecules that occurs by reducing the oxidative chain reactions, in particular propagation stage. Free radicals are directly scavenged by primary antioxidants, whereas secondary antioxidants act indirectly, restricting the production of free radicals by Fenton reactions. In this sense, polyphenols have remarkable antioxidant properties, since they are efficient scavengers of reactive oxygen species.

High intakes of polyunsaturated fatty acids lead to generation of toxic lipid oxidation species. Lamothe et al. (2019) investigated the effects of grape juice and tea (polyphenol-rich beverages) and milk on generation of toxic lipid oxidation species. Significant reductions of 4-hydroxyhexanal and 4-hydroxynonenal (toxic lipid oxidation species) were observed due to milk or polyphenol-rich beverages; 60% and 75% respectively.

Higher content of phenolic compounds with associated antioxidant activity was related to white guava (*P. guajava* L.) and red guava (*P. guajava* L.) leaves, when compared with other vegetables. On the other hand, between the white and red leaves of guava, the highest concentration of total phenolics is found in the pyrifera variety (Díaz-de-Cerio et al. 2016; Wang et al. 2007).

The antioxidant potential of cabbage was already widely reported in the literature. Red cabbage exhibits greater antioxidant capacity than white cabbage. In general, when compared to green cabbage, Chinese cabbage and Chinese white cabbage, red cabbage has the highest antioxidant activity (Abu-Ghannam and Jaiswal 2015; Amin and Lee 2005; Jaiswal et al. 2011).

In red cabbages, cyanidine glycosides are the main pigments found. Studies have shown that cyanidine made an excellent contribution to antioxidant capacity, and also to total flavonoid and phenolic content (Chun et al. 2004).

Oats have high concentration of β-glucan that are widely known for its health properties. Oats also have $\geq$20 exceptional (unique), for instance phenolic alkaloids (avenanthramides) (Meydani 2009).

Therefore, polyphenols are essential to balance antioxidant systems, that is, they are an excellent assistant for human health.

1.4.2 Anti-Inflammatory

Inflammation is a defense mechanism towards tissue imbalances. It is the immune system's response to harmful stimuli including pathogens, toxic compounds, lesions, osmotic stress, etc. Thus inflammation restores tissue homeostasis. It is worth noting that some diseases such as cardiovascular, cancer and chronic inflammatory are inflammation based diseases (Bollmann et al. 2014).

The antioxidant properties of polyphenols are widely known, nevertheless polyphenols have also anti-inflammatory properties, in particular those related to have been modulations of the arachidonic acid cascade. In this sense, according to Hartung et al. (2019), isoflavone genistein has potent 5-lipoxygenase inhibition in neutrophils (white blood cell). Then, the authors studied the effects of 5-lipoxygenase-inhibiting polyphenols on all branches arachidonic acid cascade. In addition, resveratrol inhibited the cyclooxygenase activity and also minimized lipoxygenase activity. Briefly, it was concluded that polyphenols have the ability to block 5-lipoxygenase activity.

The KH-type splicing regulatory protein is a regulator of multiple inherently unstable mRNAs in most cases related (coding) to pro-inflammatory intermediators such as TNFα and interleukin-8. Bollmann et al. (2014) used treated human cells, more specifically DLD-1 or Mono Mac 6, with polyphenol resveratrol. The authors observed a lower cytokine induced expression of TNFα, interleukin-8 and inducible nitric oxide synthase (effect of resveratrol).

García-Lafuente et al. (2009) reported a review on anti-inflammatory properties of polyphenols, which represents the state of art in this subject. The authors pointed out that most experiments are *in vitro* studies, thus there is a lack of *in vivo* data (models), which makes it difficult to draw deep conclusions about anti-inflammatory properties of polyphenols.

1.4.3 Diabetes Controller

Diabetes mellitus is a syndrome relates to improper fasting or postprandial hyperglycemia due to insulin deficiency and its consequent effects on fat and protein metabolisms. Type 2 diabetes is a gradual condition which insulin loses its activity and/or pancreas reduces insulin production. The incidence of Type 2 diabetes has increased since the last decade which leads to social and economic costs (Hartung et al. 2019).

Curcumin is a polyphenol that can be obtained from *Curcuma longa* (turmeric plant). Curcumin (0.2 mg of curcumin/kg diet) enhances insulin resistance in hamsters and mice. In addition, curcumin increases insulin content and decreases the blood concentration of triglyceride and glucose content. As a result, curcumin reduce body weight gain and vascular endothelial growth factor (Seo et al. 2008; Aryaeian et al. 2017). The effects of curcumin on human health were also investigated. Over 240 prediabetic adults have received, every day, 250 mg of curcumin or placebo, during the 9 months. The analysis of results indicated that curcumin prevented all type 2 diabetes cases (Chuengsamarn et al. 2012; Aryaeian et al. 2017).

Resveratrol, a non-flavonoid polyphenol, is widely found in grapes, peanuts, cranberries, and blueberries. 19 type 2 diabetic patients received orally 2 × 5 mg resveratrol or placebo for 28 days. Resveratrol reduced insulin resistance, on the other hand, the β-cell function was unaffected (Brasnyó et al. 2011; Aryaeian et al. 2017). Similarly, 14 type 2 diabetic patients have received 6 mg of cinnamon poly-

phenols. The authors conclude that cinnamon polyphenols decreased blood glucose levels (Hlebowicz et al. 2007; Aryaeian et al. 2017).

Costabile et al. (2018) studied the effects of red grape pomace consumption by human. The authors observed that of red grape pomace polyphenols have reduced the insulin secretion and increase its sensitivity, probably mediated by gallic acid. In addition, the dietary drink with a given dose of polyphenols (3 g of polyphenol per day) led to a significant increasing in the concentrations of glucose tolerance, insulin sensitivity and postprandial following a 4 month supplementation with flour rich in polyphenols in patients prone to having diabetes, heart disease or stroke. (cardiometabolic risk). An important finding has been demonstrated about the positive effects of polyphenols on glucose homeostasis, improving insulin sensitivity.

Thus, specific polyphenols as curcumin, resveratrol and red grape pomace polyphenols can positively affect sugar metabolism and preserve type 2 diabetic.

1.4.4 Microbiome Modulator

Microbiome is the microorganism community composed of bacteria (mostly), yeasts, virus and fungi, living in and on all vertebrates. Microbiome, in particular gut microbiome, is a key modulator of human health. The human gut microbiome is composed of trillions of bacteria. The relation between microbiome and health has been drawn attention, since it directly impact on human health. Specific compounds such as polyphenols can simultaneously favor some bacteria genera and inhibit other bacteria genera, which lead to unique microbiome architecture. Thus, it will change the gut microbiome and thus impact on human health.

Apples have high content of polyphenols. Trošt et al. (2018) described a study, in which 12 men and women consumed 0.25 L of apple juices (cloudy or enriched with 0.750 g of an apple polyphenol extract. Faecal samples were collected individually. The authors identified a very strong relation between gut microbiome and apple polyphenols. In addition, they speculated (since data were not statistically significant) that some metabolic produced from polyphenols are correlated to predominance of specific bacterial genera. Similarly, Queipo-Ortuño et al. (2012) investigated the effects of red wine intake (source of polyphenols) on select gut microbial groups. Experiments were carried out over 20 days (272 mL/d), which involved ten healthy adult men aged (≈48 years). The authors observed that dominant bacterial composition changed over experiments. The intake of red wine induce higher *Enterococcus*, *Prevotella*, *Bacteroides*, *Bifidobacterium*, *Bacteroides uniformis*, *Eggerthella lenta*, and *Blautia coccoides*. Thus, red wine modulates gut microbiota, in which prebiotic microorganisms as *Bifidobacterium* are benefited.

1.4.5 Anti-Aging

During the aging, there are degradation in the skin layers. Which provide changes the visual and physical aspects of the skin (Mukherjee et al. 2011).

In their study, Zhuang et al. (2017) verified that rambutan peel phenolic (RPP) extracts act in the protection of H_2O_2-induced HepG2 cells against oxidative stress. These inhibitory effects are due the extract capacity to inhibit the formation of intracellular ROS and provide an enhance on superoxide dismutase activity. The RPP also showed an increased in the *in vivo* anti-aging activity, and their histological evaluations showed that extracts decreased the liver and kidney damage.

Many plant-derived foods have in their composition proanthocyanidins (PAC). Jiao et al. (2017) investigated the use A-type and B-type proanthocyanidins from cranberry concentrate and grape seed extract against aging. Both products tested decreased the brain and hepatic thiobarbituric acid, plasma 8-isoprostane, further provided a reduction in the plasma and brain monoamine oxidases. According to authors cranberry concentrate increased by 42% the hepatic glutathione peroxidase activity, while that grape seed extract improved by 13% the hepatic superoxide dismutase activity. Based on the results, both extracts showed anti-aging activity.

1.4.6 Antihypertensive

Cardiovascular diseases have the hypertension as their main risk factor associated. Based on the causes reported, World Health Organization (WHO) has warned that healthy habits, such as diet and physical activity, can be reducing the hypertension incidence (Peñas et al. 2015). Therefore, the consumption of plant-based foods is associated the antihypertensive effects (Aguilera et al. 2016).

In their study, Shukor et al. (2013) investigated the inhibition ability in angiotensin-converting enzyme (ACE) of 22 phenolic compounds. According to results, tannic acid had the higher inhibition effect with a IC_{50} = 230 μM. While, others phenolic compounds tested showed lower inhibition varied from 0.41 to 9.3 mM. The main factor that contributes to ACE inhibition is the number of hydroxyl groups link on the benzene ring, while that methoxy groups into molecule reduce the activity.

Red raspberry fruit extracts were evaluated by Jia et al. (2011) against hypertensive effects on spontaneously hypertensive rats. The antihypertensive activity demonstrated by extracts depended of the amount managed in the hypertensive rats. Probably, the effect provided by extract is via antioxidation that increases NO activation and improvement of vascular dysfunction.

1.4.7 Anticancer

Cancer is a health problem that causes millions of deaths worldwide. The ill is associate the various endogenous causes which are inevitable, but also exogenous ones (e.g. tobacco consumption). For this, the phenolic antioxidants have been intensely investigated (Carocho and Ferreira 2013).

Rangel-huerta et al. (2015) showed that consumption of orange juice with at least 300 mg of flavonones over a period of 12 weeks improved the antioxidant defense system, reduced blood pressure in overweight and obese adults, protecting against DNA damage and lipid peroxidation.

Amongst all the urologic malignancies, renal cell carcinoma (RCC) stand out as one of the most harmful. As therapeutic intervention, green tea (*Camellia sinensis*) prevented the growth human renal cancer cell lines A-498 and 769-P, with an extract dose of 54 ± 10 and 129 ± 28 μg/mL (IC_{50} values), respectively (Carvalho et al. 2010). Furthermore, cervical cancer also deserves mention because it is the second higher cause of cancer death in women. In their study, Boeing et al. (2019) verified that *Butia odorata* fruit extracts, provided the preliminary evidences of their antitumor effects in SiHa and C33a cells.

Common beans are cultivated and consumed worldwide. In their study, López et al. (2013) studied the influence of boiling and germination processes of dark beans (*Phaseolus vulgaris* L.) on their anticancer activity. According to authors the phenolic composition of beans changed with the process used. The extract of raw beans was the most cytotoxic on TK-10 line. While, germinated beans extract showed a high cytotoxicity for breast adenocarcinoma and melanoma cell lines.

Among all fruits, apple is one of the most consumed. It has been reported that dihydrochalcones are the main flavonoids compound in *Malus domestica*. Xiao et al. (2017) tested in five cancer cell lines seven different dihydrochalcones from apples. The 3-hydroxyphlorizin and sieboldin compounds exhibited the higher anticancer ability than other dihydrochalcones tested. Their extract quantity varied from 30 to 80 μM.

1.5 Perspective

Regarding human metabolism, polyphenols are one of the most dynamic biological molecules. The wide range of sources, biological properties and chemical structures leads to nonconsensual understand on their mechanism of action - virtually infinite possibilities. The Table 1.1 shows the relation among source, biological property and polyphenols, which can be used to for further studies, in particular:

- Identification of compartments in the plant cell that contain high concentration of polyphenols;
- The metabolic effects of glycosylated polyphenols;
- *In vivo* assays using high purity polyphenols;
- To develop systems with increased polyphenols solubility in water (e.g. curcumin low).

Table 1.1 Phenolic compounds; sources and biological properties

Source		Biological property	Polyphenols	Notes	References
Grape	Pomace	Antioxidant Antitumor	Tannins; phenolic acids; falvonols; nthocyanins *Grape skin*: Proanthocyanidins; prodelphinidins; ellagic acid; myricetin; quercetin; kaempferol; transresveratrol *Grape seed*: Gallic acid; catechin; dimeric procyanidin; proanthocyanidins *Grape stems*: Flavanols; flavonol glycosides; quercetin 3-*O*-glucuronide; quercetin 3-*O*-rutinoside-rutin; dihydroflavonols (astribin); phenolic acids		Rockenbach et al. (2011), Karvela et al. (2009)
	Wine	Antioxidant, in particular against neuronal oxidative	Resveratrol; anthocyans; myricetin, quercetin; keretin-3-β-glucoside; caffeic acid; *p*-coumaric acid		Pérez-Serradilla and Luque de Castro (2011)
Apples		Antioxidant antitumor	Phlorizin; trilobatin; 3-hydroxyphlorizin; sieboldin; phloretin 2′-xyloglucoside	Sieboldin and 3-hydroxyphlorizin showed lower cytotoxicity rather than dihydrochalcone compounds	Xiao et al. (2017)

(continued)

Table 1.1 (continued)

Source		Biological property	Polyphenols	Notes	References
Berries	Cranberry	Anti-aging	Epicatechin, proanthocyanidins		Jiao et al. (2017)
	Red raspberry	Antihypertensive		Treatment based on raspberry fruit reduced the blood pressure	Jia et al. (2011)
	Blackberries	Antioxidant	Cyanidin 3-glucoside; cyanidin 3-rutinoside; malonic acid acylated cyanidin 3-glucoside		Siriwoharn et al. (2004).
	Blueberries	Antioxidant	Delphinidin-3-*O*-galactoside; cyanidin-3-*O*-galactoside; delphinidin-3-*O*-arabinoside; petunidin-3-*O*-galactoside; malvidin-3-*O*-galactoside; malvidin-3-*O*-arabinoside; 5-*O*-feruloylquinic		Borges et al. (2010)
Orange		Antioxidant Microbiome modulator	Flavanone glycoside; hesperitin; naringenin		Roowi et al. (2009)
Guava		Antioxidant	Anthocyanins; flavonoids; proanthocyanidins; phenolic acids; flavonols; tannins		Rojas-Garbanzo et al. (2017)
Pomegranate		Antioxidant Antitumor	Ellagic acid; punicalagin; punicalin; galagic acid		Seeram and Heber (2011)
Potatoes, sweat potato, cassava, tomatoes, onions and cabbage		Antioxidant	*Sweet potato*: 3,4,5-tri-*O*-caffeoylquinic acid; 4,5-di-*O*-caffeoylquinic acid *Green tomato*: Chlorogenic acid; tannic acid		Akyol et al. (2016), Shahidi and Ambigaipalan (2015); Tylewicz et al. (2018)
Cereals		Antioxidant Antitumor	Ferulic acid; oxalic acid; *p*-coumaric acid; caffeic acid		Tian et al. (2019)

Green tea	Antioxidant Anticancer Antitumor	Phenolic acids; catechins, flavonol glycosides	Green tea extract showed significant effect on renal cell carcinoma	Carvalho et al. (2010)
Olive oil	Anti-inflammatory Antioxidant Diabetes controller	Dialdehydic; tyrosol; hydroxytyrosol; protocatechuic; gallic; vanillic; caffeic; *p*-hydroxybenzoic; syringic; *p*- and *o*-coumaric; ferulic; cinnamic acid		Franco et al. (2014); Tylewicz et al. (2018)
Dark beans	Anticancer Antioxidant Neuroprotective	Phenolic acids; procyanidins; flavonols; flavanones; isoflavones; anthocyanins	In comparison among germination, boiling, and raw beans. Raw beans, rich in anthocyanins, showed the best neuroprotective and antitumoral	López et al. (2013)
Rambutan peel	Anti-aging	Phenolic acid; hydroxybenzoic acids; flavonols; flavonols; ellagic acid; hydrolyzable tannins; flavone	Rambutan peel phenolic extract effectively reduced liver and kidney tissue damage	Zhuang et al. (2017)
Butia odorata fruit	Antioxidant Antitumor	*p*-hydroxybenzoic acid; *p*-coumaric acid; sinapic acid; ferulic acid; ellagic acid; trans-resveratrol; quercetin; luteolin; naringenin; apigenin; catechin; epicatechin; chlorogenic acid; rutin	*B. odorata* fruit showed effective showed antitumor properties on cancer cell lines, in particular cervical cell C33a and SiHa	Boeing et al. (2019)

1.6 Conclusion

Polyphenols are natural biologically active compounds broadly found in plant based-food. Wine and grape pomace, apple, berries, tomatoes, coffee, teas and olive oils are well-known sources of polyphenols, whereas potatoes, cassava, onions and cabbage and cereals need deeper investigations. Polyphenols consumption plays a fundamental role on human health, in particular antioxidant, anti-inflamatory, diabetes controller, microbiome modulator, anti-aging, antihypertensive and anticancer. Thus, it is possible relates the source of polyphenol to biological property, for instance cranberry has epicatechin and proanthocyanidins that have anti-aging properties; green tea has phenolic acids; catechins, flavonol glycosides that have antioxidant, anticancer and antitumor properties; orange has flavanone glycoside, hesperitin and naringenin that have antioxidant and microbiome modulator properties; cereals that have ferulic acid, oxalic acid, *p*-coumaric acid and caffeic acid that have antioxidant and antitumoral properties, among others. The wide range of sources, biological properties and chemical structures leads to nonconsensual understand on their mechanism of action - virtually infinite possibilities. Thus, further investigations should be related to identification of compartments in the plant cell that contain high concentration of polyphenols (rich sources of polyphenols, including vegetable wastes); the metabolic effects of glycosylated polyphenols; *in vivo* assays using high purity polyphenols; and to develop systems with increased polyphenols solubility in water.

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Chapter 2
Glucosinolates

Francesco Di Gioia and Spyridon A. Petropoulos

Abstract Glucosinolates are a group of sulfur- and nitrogen-containing glycosides found in the plant order Brassicales which includes several important vegetable crops of the *Brassica* genus such as broccoli, cabbage, radish and cauliflower among others. Their hydrolysis byproducts, namely isothiocyanataes, are responsible for the distinct aroma and pungent taste of cruciferous species, most of which contain species-specific glucosinolates, hence the high number of individual compounds. They are considered as beneficial to human compounds with several confirmed health effects, while a significant amount of research work has been carried out recently to identify those mechanisms and synergisms that are responsible for the activities of glucosinolates, as well to reveal physiological aspects in the plant × environment interactions. This chapter discusses the biochemistry and health properties of glucosinolates, their physiological significance as well as the hydrolysis process in the plant response to different abiotic stresses.

Keywords Abiotic stress · Brassicaceae · Glucosinolates · Health effects · Isothiocyanates · Organosulphur compounds

2.1 Introduction

Glucosinolates (GSLs) or *β*-thioglucoside-N-hydroxisulfates are a distinctive class of phytochemicals derived from amino acids and constituted by glycosides containing sulfur and nitrogen (Grubb and Abel 2006; Mithen et al. 2010). The biosynthesis of GSLs is exclusive of plants belonging to the botanical families of the order *Brassicales* (formerly *Capparales*), among which the most representative to produce GSLs are the Brassicaceae and Moringaceae family (Mithen et al. 2010;

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Fahey et al. 2018). The majority of GLSs have been identified in Brassicaceae family and their occurrence is considered an important chemotaxonomic criterion for species classification (Holst and Fenwick 2003). The Brassicaceae family includes the model plant *Arabidopsis thaliana* and some very popular vegetable crops such as broccoli, cauliflower, cabbage, kale, kohlrabi, mustard, Brussel sprouts, radish, arugula, while many other less popular vegetables and wild plants are also part of the same family (Fahey et al. 2001; Petropoulos et al. 2017). Like other secondary metabolites synthesized by plants to face conditions of stress, GSLs are plant defense molecules and are characterized by a high level of variability and polymorphism that is strictly associated with the continuous coevolution of plants and pests (Newton et al. 2009). Since the first characterization of mustard seed extracts and the isolation of sinigrin and sinalbin as GSL structures (Ettlinger and Lundeen 1956; Wisniak 2013), over 130 different GSLs compounds have been isolated and documented so far, while several potential GSLs structures have been identified but not confirmed yet (Fahey et al. 2001; Clarke 2010; Agerbirk and Olsen 2012). More recently, reviewing all the GSLs structures claimed to be isolated from plant tissues based on the availability of both NMR spectroscopy and HPLC-MS evidence, Blažević et al. (2020) concluded that up to mid-2018, 88 GSL structures have been satisfactorily characterized, 47 more GSL structures have been partially characterized, while several structures claimed in previous studies have been discontinued due to insufficient evidence or characterization. GLSs can be found in all plant parts and several individual compounds are present in each species; however, three or four of them are usually the most abundant although the overall composition defines the bioactivities of each species (Holst and Fenwick 2003). Although a great number GLSs have been identified, not all of them are widely consumed since they are present in wild or in less common species or in non-edible plant parts (e.g. flowers and seeds) (Holst and Fenwick 2003). Therefore research interest has focused on those compounds that are present in commonly used vegetables such as species of *Brassica oleraceae* which are considered the most important dietary sources of GLSs (Kassie and Knasmüller 2004).

Accumulated and compartmentalized into specific cells (Koroleva et al. 2010), GSLs are part of an articulated two-component biological defense mechanism that is activated when, especially in case of damage or infection of the plant tissues, GSLs come in contact with specific hydrolytic enzymes called myrosinases (β-thioglucosidases), which constitute the second component of the defense mechanism referred also as the "mustard oil bomb"(Kissen et al. 2009). Coming in contact with myrosinase, GSLs are immediately degraded into bioactive compounds such as isothiocyanates (ITCs) and other metabolites that are deterrent or toxic for herbivores, insects, nematodes, bacteria, and plant pathogens (Textor and Gershenzon 2009; Pastorczyk and Bednarek 2016). Given their stability within plant cells, GSLs are therefore considered the storage form of their bioactive metabolites (Clarke 2010). ITCs and the other metabolites deriving from the hydrolysis of GSLs are in fact directly responsible for most of the biological properties credited to GSLs (Dinkova-Kostova and Kostov 2012; Burčul et al. 2018; Romeo et al. 2018). However, the chemical structure of ITCs defines their functionality and the side

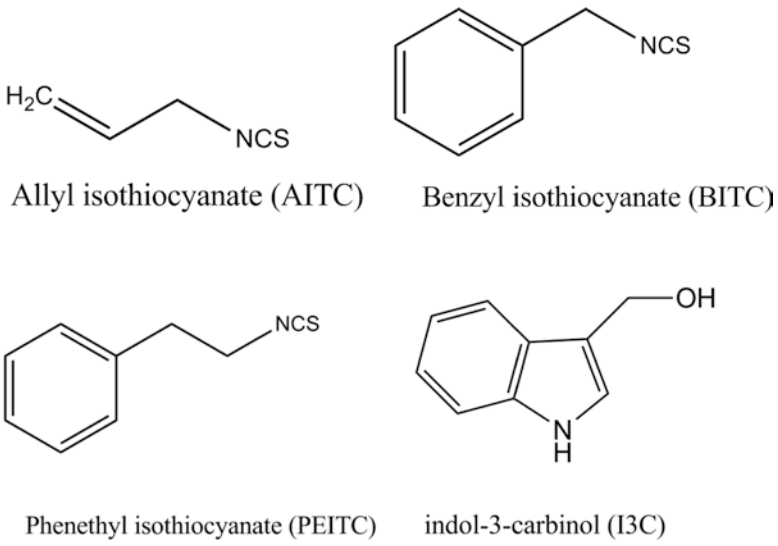

Fig. 2.1 The main isothiocyanates structures

chains with less than ten atoms of carbon are considered as more potent and beneficial for human health than longer side chains (Ishida et al. 2014). Moreover, the presence of aromatic rings and the oxidation state of sulfur atoms may affect the biological activities of ITCs, whereas double bonds had no significant effect (Pocasap et al. 2018). This factor has to be considered in breeding strategies aiming to increase the content of beneficial GSLs and minimize those that have antinutritional properties. The main ITCs derived from GSLs hydrolysis are presented in Fig. 2.1.

As volatile organosulfur compounds, ITCs are also the main determinants of the sulfurous aroma and pungent or sometimes bitter taste typical of cruciferous vegetables (Macleod and MacLeod 1990; Jirovetz et al. 2002; Bell et al. 2018; Di Gioia et al. 2018a) which is often disliked by consumers (Drewnowski and Gomez-Carneros 2000). Despite some consumers may lack appreciation toward the smell and taste of Brassicaceae, the interest for cole crops and their unique GSLs and derived hydrolysis products started rising since sulforaphane [1-isothiocyanato-(4R)-(methylsulfinyl)butane], a glucoraphanin-derived ITC isolated from broccoli, was identified as an inducer of phase 2 detoxication enzymes and as a potent anticancer compound (Zhang et al. 1992, 1994). Over the last decades, thanks to an extensive research effort, a significant amount of literature has been produced, greatly expanding our knowledge on the beneficial effects of phytochemicals such as GSLs and ITCs on human health as antioxidant, anticancer, anti-inflammatory, antibacterial, and protective molecules against a variety of chronic and inflammatory medical conditions (Dinkova-Kostova and Kostov 2012; Kumar et al. 2015; Moosavi et al. 2018; Palliyaguru et al. 2018). As research continues to disclose the biological activity and health-effects of GSLs and relative ITCs, there is also increasing interest toward understanding the physiological role of GSLs in the

plant response to biotic and abiotic stresses, as this knowledge can contribute to advance our ability to regulate the GSL profile of *Brassica* crops and develop products with enhanced content of specific beneficial GSLs through breeding or by implementing agronomic biofortification and other practices (Di Gioia et al. 2020).

After presenting the biochemistry of GSLs, this chapter provides an overview of the current knowledge on the physiological role of GSLs and hydrolysis-derived products in plant response to biotic and abiotic stress factors and provides an updated summary of the literature on the main health properties attributed to GSLs and ITCs.

2.2 Biochemistry of Glucosinolates

Given the great interest toward GSLs and their biological activity over the last decades and also considering the development of molecular biology, omics, bioinformatics, and novel analytical techniques, a number of studies focusing primarily on the model plant *A. thaliana*, have contributed to advance our understanding of the GSL biosynthetic pathway, transportation, storage, and overall metabolism within plants (Halkier 2016).

As plant defense phytochemicals, GSLs evolved from cyanogenic glucosides, another family of defense metabolites commonly present in the plant kingdom (Bolarinwa et al. 2016), which share with GSLs part of the biosynthetic pathway, as demonstrated by the presence of cytochrome P79 (CYP79) homologs and enzymes that catalyze the conversion of precursor amino acids to aldoximes in both pathways (Bak et al. 1998, 2001; Halkier and Gershenzon 2006). Compared to cyanogenic glucosides, derived only from valine, isoleucine, phenylalanine, and tyrosine amino acid precursors, GSLs are synthesized from a higher number of amino acids and from several amino acid-modified structures which contribute to the formation of a larger variety of GSLs (Møller 2010; Agerbirk and Olsen 2012).

From a structural standpoint, all GSLs share the same core structure consisting of a *β-D*-glucopyranose residue connected through a sulfur atom to a (*Z*)-N-hydroximinosulfate ester and to a side chain (R). The basic GSL structure is highly conserved in nature, whereas the amino acid-derived side chain could be subject to a series of structural changes that are associated with the biological properties of the final GSLs and hydrolysis metabolites (Blažević et al. 2020). In this perspective, considering that the biosynthetic process starts from amino acids, GSLs may be further classified based on the precursor amino acids and their structural characteristics (Table 2.1).

The GSLs biosynthetic process may be divided into three primary independent phases:

1. Chain-elongation by insertion of methylene groups of selected amino acids (methionine and phenylalanine);

Table 2.1 Amino acid precursor, glucosinolates (GSL), and relative hydrolysis products

Amino acid precursor	GSL number	GSL trivial and/or semi-systematic name	Main plant source of GSL	Isothiocyanate (ITC) and/or other hydrolysis products
Methionine	12	Gluconapin But-3-enyl GSL	*Brassica rapa* species and broccoli	3-butenyl ITC
	24R	Progoitrin (2R)-2-hydroxybut-3-enyl GSL	Bok choy, turnip, broccoli, cauliflower, Brussels sprouts	2-hydroxy-3-butenyl ITC, goitrin
	24S	Epiprogoitrin (2S)-2-hydroxybut-3-enyl GSL	Bok choy, turnip, broccoli, cauliflower, Brussels sprouts	(5R)-5-Vinyl-1,3-oxazolidine-2-thione, (2S)-1-cyano-2-hydroxy-3-butene, erythro-(2S)- and threo-(2S)-1-cyano-2-hydroxy-3,4-epithiobutanes
	63	Glucoraphenin (RS, 3E)-4-(Methylsulfinyl) but-3-enyl GSL	Radish	Sulforaphene
	64	Glucoraphanin (RS)-4-(Methylsulfinyl)butyl GSL	Broccoli, rocket	Sulforaphane (SFN)
	73	Glucoiberin (RS)-3-(Methylsulfinyl)propyl GSL	White and red cabbage, cauliflower and kale	Iberin
	83	(Glucoraphasatin or dehydroglucoerucin) 4-Methylsulfanyl-3-butenyl	*Raphanus sativus*	4-Methylthio-3-butenyl ITC, raphasatin
	84	Glucoerucin 4-(Methylsulfanyl)butyl GSL,	Rocket	Erucin
	101	Glucobrassicanapin Pent-4-enyl GSL	Chinese cabbage, turnip, turnip greens and swede	4-pentenyl-ITC
	107	Sinigrin Prop-2-enyl GSL	*Brassica nigra*, *B. juncea* and *B. oleracea*	Allyl ITC (AITC)
	126	6′-Benzoylglucoraphanin 6′-Benzoyl-4-(methylsulfinyl)butyl GSL	*Arabidopsis thaliana*	
	127	(6′-Benzoylglucoerucin) 6′-Benzoyl-4-(methylsulfanyl)butyl GSL	*Arabidopsis thaliana*	
	135	Diglucothiobeinin 4-(*β-D*-Glucopyranosyldisulfanyl) butyl GSL	Rocket	

(continued)

Table 2.1 (continued)

Amino acid precursor	GSL number	GSL trivial and/or semi-systematic name	Main plant source of GSL	Isothiocyanate (ITC) and/or other hydrolysis products
Alanine	51	(Glucocapparin) Methyl GSL	*Isomeris arborea*	Methyl ITC
Valine	9	(1R)-2-Benzoyloxy-1-methylethyl GSL (Glucobenzosisymbrin)	*Sisymbrium austriacum*	
	56	(Glucoputranjivin) 1-Methylethyl GSL	*Putranjiva roxburghii*	1-Methylethyl ITC
Leucine	52	3-Methylbut-3-enyl GSL	*Capparis linearis*	
	55	3-Methylbutyl GSL	*Armoracia lapathifolia*	3-Methylbutyl ITC
	59	4-Methylpentyl GSL	Radish	4-methylpentyl ITC
Isoleucine	7	(Glucobenzsisaustricin) (1R)-1-(Benzoyloxymethyl)propyl GSL	*Sisymbrium austriacum*	
	30	(Glucosisaustricin) (1R)-1-(Hydroxymethyl) propyl GSL	*Sisymbrium austriacum*	
	141	3-(Hydroxymethyl)pentyl GSL	*Cardamine pratensis*	
Phenylalanine	11	Glucotropaeolin Benzyl GSL	*Tropaeolum majus*	Benzyl ITC (BITC)
	23	Sinalbin 4-Hydroxybenzyl GSL	*Sinapis alba*	p-hydroxy benzyl-ITC
	105	Gluconasturtiin 2-Phenylethyl GSL	*Nasturtium officinale*	Phenethyl ITC (PEITC)
Phenylalanine - Tyrosine	110	(Glucomoringin) 4-(α-L-Rhamnopyranosyloxy) benzyl GSL	*Moringa oleifera*	Glucomoringin ITC
Tyrosine	152	(3,5- dimethoxysinalbin) 4-Hydroxy-3,5-dimethoxybenzyl GSL	*Lepidium densiflorum*	4-hydroxy-3,5-dimethoxy benzaldehyde
Tryptophan	43	(Glucobrassicin) 3-Indolylmethyl GSL	*Brassica oleracea*	indole-3-carbinol (I3C)
	47	(Neoglucobrassicin) 1-Methoxyindol-3-yl methyl GSL, N-Methoxyindol-3-ylmethyl GSL	*Brassica napus*	1-methoxyindol-3-yl methyl ITC
	48	(4-Methoxyglucobrassicin) 4-Methoxyindol-3-ylmethyl GSL	*Brassica oleracea*	

2. Formation of the core GLS structure through a multistep transformation of the amino acid or the chain-elongated derivate to form aldoxime, thiohydroximic acids, desulfo-GSLs, and the core GSL structure;
3. Side-chain modification in which GSLs are subject to secondary modification of the amino acid side chain including oxygenations, hydroxylations, alkenylations, and methoxylations.

Nevertheless, each of the three phases includes several steps and overall the biosynthesis of GSLs is quite complex and involves over 40 genes regulated at the transcriptional level to produce the existing variety of GSLs (Kopriva and Gigolashvili 2016). A number of comprehensive review articles have illustrated the GSL biosynthetic process describing the biochemistry and the genes involved in each step especially for methionine and phenylalanine derived GSLs (Mithen 2001; Sønderby et al. 2010; Ishida et al. 2014; Velasco et al. 2016; Sánchez-Pujante et al. 2017).

In the chain elongation phase reserved only to methionine and phenylalanine, branched-chain amino acid aminotransferase (BCATs) enzymes catalyze the amino acid deamination to form the relative 2-oxo acids with the involvement of the gene BCAT4 induced by wounding and identified as responsible for producing BCATs in *Arabidopsis* (Schuster et al. 2006). The 2-oxo acids are then subjected to further transformations which specifically in *Brassica oleraceae* cultivars are catalyzed by the genes BoGSL-ELONG and BoGSL-PRO, homologous of the methylthioalkylmate synthase (AtMAM) genes (Li and Quiros 2002; Gao et al. 2006). Finally, isopropylmalate isomerises (IPMIs) and isopropylmalate dehydrogenases (IPMDHs) catalyze the isomerization and decarboxylation of the 2-alkylmalic acid-generating chain-elongated amino acid derivatives (Sawada et al. 2009; He et al. 2009, 2010).

In the second phase, as described in detail by Halkier and Gershenzon (Halkier and Gershenzon 2006), the synthesis of the core GSL structure starts with the oxidation of the amino acid derivatives to the relative aldoxime mediated by cytochrome P450 mono-oxygenases belonging to the CYP79 family (Wittstock and Halkier 2002). The aldoxime is further oxidized by CYP83 enzymes producing unstable aci-nitro compounds that are conjugated with cysteine to form S-alkylthiohydroximates and converted to thiohydroximate acids through enzymatic reaction mediated by glutathione S-transferases and carbon-sulfur lyases (SUR1) (Hansen et al. 2001; Mikkelsen et al. 2004). The thiohydroximate acids are finally converted to desulfoglucosinolates and to GSLs through the action of uridine diphosphate glycotransferase (UGT74) and sulfotransferases (ST) (Grubb et al. 2004; Piotrowski et al. 2004).

The biosynthesis of GSLs seems to be regulated at the transcriptional level by the availability of different minerals which may have also interactive effects. Although deriving from amino acids, GSLs constitute a fundamental component of the sulfur metabolism. Each GLSs contains, in fact, two or three sulfur atoms and limited availability of sulfur surely lead to a reduced accumulation of GSLs. Sulfur deficiency has been associated with downregulation of the genes associated with GSL biosynthesis and the simultaneous upregulation of genes involved in the synthesis

of myrosinases and thus in the hydrolysis of GSLs (Hirai et al. 2005; Kopriva and Gigolashvili 2016). Using transcriptomics and metabolomics technologies (Bielecka et al. 2015) found that MYB29, a transcription factor controlling GSL biosynthetic genes, is downregulated under sulfate starvation and is restored with re-supply of sulfate, and following this pattern GSL content is reduced upon sulfate starvation and increases upon re-supply, suggesting that in presence of limited availability of sulfur GSLs may be metabolized to support the primary sulfur metabolism. Total GSL content and GSLs profile are also influenced by the total availability and form of nitrogen, which may influence also the effect of sulfur on GSL biosynthesis (Petropoulos et al. 2017). Higher levels of nitrogen have been associated with lower levels of GSLs and the prevalence of indole GSLs (Zhao et al. 1994; Rosen et al. 2005; Chun et al. 2017). Comparing the effect of ammonium versus nitrate nitrogen, increased accumulation of GSLs and myrosinase activity was observed in *A. thaliana* under exclusive ammonium nutrition, considered a condition of stress, and the same results were confirmed in broccoli (Marino et al. 2016). The deficiency of phosphorus has also been associated with increased accumulation of GSLs (Pant et al. 2015), while there are contrasting evidence on the effect of potassium (Troufflard et al. 2010; Almuziny et al. 2017; Chun et al. 2017).

Apart from dietary GSLs and ITCs, there has been also great research interest during the last decades for synthetic compounds with several approaches being suggested (Di Cesare et al. 2017). Recently, Eschliman and Bossmann (2019) who gathered the related information in the literature suggested several approaches to synthesize ITCs including the desulfurization of dithiocarbamate salts, the synthesis of ITCs from hydroximoyl chlorides or elemental sulfur, the micro-wave assisted synthesis or via the tandem Staudinger/aza-Wittig reactions.

2.3 The Role of Glucosinolates in Plant Physiology

GSLs are considered the stronghold in the plant defense system where through the "mustard oil bomb" reaction they can deter pest and pathogens attacks (Vig et al. 2009). Unlike most defense compounds of the plant, GSLs are not toxic per se and a hydrolysis reaction through the involvement or myrosinase must precede to produce ITCs and other biologically active compounds (Kuchernig et al. 2011; Winde and Wittstock 2011; Agerbirk and Olsen 2012). Although the whole concept is simply a more complex system that exists with genotype and environmental conditions specificities involved in the overall plant defense system and several species-specific GSLs being identified so far (Brown et al. 2002; Farnham et al. 2004). Recent studies comparing the GSL biosynthesis and profile of cabbage lines susceptible and resistant to ringspot and white mold caused by *Mycosphaerella brassicicola* and *Sclerotinia sclerotiorum*, respectively, reported that both fungal infections induced the expression of genes associated with the biosynthesis of specific GSLs and their increase was associated with the resistance to white mold (Abuyusuf et al. 2018a, b). Moreover, the plant × pathogen system is under con-

tinuous evolution and various pests and pathogens develop evading and/or tolerance pathways against plant defensive compounds (Winde and Wittstock 2011; Humphrey et al. 2016).

GSLs are not only involved in defense mechanisms against biotic stressors, but are also considered as major protectants against unfavorable abiotic conditions, such as high salinity, water shortage and temperature extremities (Radovich et al. (2005); Yuan et al. 2010; Justen et al. 2013; Esfandiari et al. 2017). The defensive role of GSLs against abiotic stressors is corroborated by the allocation and distribution of these compounds in the affected plant tissues and organs (Del Carmen et al. 2013). For example, under high salinity conditions total GSLs content increased for osmoprotective purposes and it was higher in the florets than in young leaves due to either higher de novo biosynthetic rates or to preferable transportation via the phloem (Del Carmen et al. 2013). Comparing the effect of moderate salinity stress on broccoli at different crop stages it was observed that exposure to salinity stress in the first vegetative growth phase determined an increase of glucobrassicin and neoglucobrassicin, significantly affecting the GSL profile (Di Gioia et al. 2018b). Similarly, for drought stress, an increase of secondary metabolites content and GSLs, in particular, has been also reported (Schreiner et al. 2009). However, contradictory results exist in the literature indicating that water stress intensity and duration and the plant developmental stage are key factors that determine whether GSLs will be increased or not compared to control conditions (Robbins et al. 2005; Del Carmen et al. 2013). Elevated temperatures, both in soil and air, are associated with high GSLs content in several *Brassica* species (Charron and Sams 2004; Charron et al. 2005), however thermal sensitivity differences among the various classes of GSLs may also affect GSLs profile (Bones and Rossiter 2006; Bohinc and Trdan 2012). Differences in GSLs composition between plant parts are also reported under storage conditions. For example, the most abundant compounds in the leaves Brassicoraphanus 'BB1', an inter-generic hybrid of *Brassica rapa* L. cv. 'Bulam 3' (Chinese cabbage) and (*Raphanus sativus* L. cv 'Taebaek' (radish), were sulforaphene and raphasatin, while the roots were rich in raphasatin and PEITC (Han et al. 2019b).

Considering the correlation of GSLs content in plant tissues with various stressors, eliciting of plant secondary metabolism through exogenous application of stress conditions has been suggested as an effective agronomic practice to biofortify cruciferous species and increase their GSLs and the overall phytochemicals content (Robbins et al. 2005; Hassini et al. 2019). So far, several studies have reported the beneficial effect of various elicitors on GSLs content which could increase the dietary value of food products highlighting the great research interest (Augustine and Bisht 2015; Trolove et al. 2018; Banerjee et al. 2019; Dall'Acqua et al. 2019). With the rising interest towards sprouts, microgreens, and baby-leaf as functional vegetables increasingly grown using soilless systems (Kyriacou et al. 2016; Di Gioia et al. 2017a, b), a number of studies have suggested the opportunity to increase the content of GSLs and ITCs by modifying the nutrient solution increasing the level of sulfur, salinity, or by modulating other eliciting factors (Kopsell and Sams 2013; Yang et al. 2015; Kyriacou et al. 2016; Yang et al. 2016d; Di Gioia et al.

2018a; Petretto et al. 2019). Working on broccoli sprouts, (Yang et al. 2015) found that compared to other sources of sulfur, $ZnSO_4$ improved sulforaphane formation inducing stress. Similarly, (Esfandiari et al. 2017) observed that in broccoli sprouts high salinity stress (160 mM of NaCl) decreased the content of some GSLs and did not affect the content of glucoraphanin, but increased the content of sulforaphane by six-times increasing the transcript of the gene MYROSINASE (BoMYO) and its cofactor EPITHIOSPECIFIER MODIFIER1 (BoESM1) which directs the enzyme myrosinase to hydrolize GSLs producing ITCs rather than nitrile products. These studies suggest that myrosinase activity plays a key role in determining the functional properties of biofortified vegetables. Selenium exogenous application on cruciferous plants has been suggested as a very effective elicitor of GSLs biosynthesis, while at the same time the increased Se content in plant tissues presents further health benefits to consumers (Bachiega et al. 2016; Schiavon et al. 2016; Wiesner-Reinhold et al. 2017).

Apart from their defensive role against stress factors, GLS is also very important from a physiological point of view since they can function as sulfur and nitrogen pools in plant biosynthetic processes although re-distribution of sulfur in plants under deprivation conditions needs to be confirmed (Aghajanzadeh et al. 2014).

2.4 Health Effects of Glucosinolates and Their Hydrolysis By-Products

The importance of GSLs for human health is pivotal when considering that most of these compounds have been associated with many beneficial effects, including activities against cancer, diabetes, heart diseases, obesity, bacteria, and fungi, and antioxidant and antimutagenic properties (Vig et al. 2009; Citi et al. 2014; Giacoppo et al. 2015; Raiola et al. 2018). Excluding a few exceptions (Abdull Razis et al. 2011), most of the biological effects attributed to GSLs, which can be beneficial or not for human health, are exerted by their hydrolysis metabolites, namely ITCs (Xiao et al. 2003; Gründemann and Huber 2018). Since Zhang et al. (1992, 1994) demonstrated that sulforaphane, an ITC isolated in broccoli and derived from the myrosinase-induced hydrolysis of glucoraphanin, is an inducer of phase 2 detoxication enzymes and thus a potent natural anticancer, sulforaphane and other ITCs' bioactivity have been the focus of hundreds of clinical studies. Moreover, the precursor of sulforaphane, namely glucoraphanin was effective against skin aging in senescence-accelerated mouse prone 1 after the dietary administration of glucoraphanin-enriched kale (Chawalitpong et al. 2019). Apart from sulforaphane, the most studied ITCs include allyl ITC (AITC), benzyl ITC (BITC), phenylethyl ITC (PEITC), indole-3-carbinol (I3C), erucin, iberin, sulforaphene, and goitrin with potent bioactive properties (Mithen et al. 2003; La Marca et al. 2012; Felker et al. 2016; Baenas et al. 2017; Romeo et al. 2018). Numerous in vitro and in vivo clinical studies conducted over the last decades have contributed and continue to highlight

the multiple beneficial health effects of other ITCs which include chemoprotective and anticancer effects, antioxidant and anti-inflammatory activities and other biological properties that may contribute to ameliorate a series of chronic disorders such as obesity, diabetes, and hypertension (Table 2.2).

Regarding anticancer activities of GSL hydrolysates, various mechanisms of action have been identified so far with various types of cancer being studied (Cavell et al. 2011; Li et al. 2016, 2018; Mitsiogianni et al. 2018; Lachance et al. 2020), while there is great interest from the pharmaceutical industry for using synthetic and dietary ITCs as anticancer and chemopreventive agents (Jiang et al. 2016; Li et al. 2016; Gründemann and Huber 2018; Rajakumar et al. 2018a; Crowley et al.

Table 2.2 Biological activity and effects on human health of the most studied isothiocyanates

Isothiocyanates	Health effect	Specific biological activity	Reference
Allyl isothiocyanate (AITC)	Anticancer	In vitro cytotoxic effect on androgen-insensitive human prostate cancer (AIPC) PC-3 and DU 145 cells by inducing apoptosis and cell cycle arrest.	Xiao (2003), Núñez-Iglesias et al. (2019)
		inhibited LPS-induced NF-κB-luciferase activations in human HT-29 colon cancer cells	Jeong et al. (2004)
		Inhibited cell viability by inducing the apoptosis of human cervical cancer HeLa cells	Qin et al. (2018)
		Decreased the expression of NF-κB p65, TNF-α, and IL-6 in mammary tissues and inhibits phase I and induction of phase II detoxification enzymes by modulating AhR/Nrf2 signaling pathway in mammary carcinogenesis	Rajakumar et al. (2018a, b)
		Inhibited the growth of human bladder cancer cells HT1376 by 90%	Chang et al. (2019)
		Inhibit the growth of A549 lung cancer cells	Rakariyatham et al. (2019)
		Inhibition of cell growth in malignant melanoma	Mitsiogianni et al. (2019)
		Anti-estrogenic and anti-proliferative effect against mammary carcinogenesis	Thangarasu et al. (2019)
		Cytotoxic activity against bladder cancer UM-UC-3 and glioblastoma LN229 cell lines	Blažević et al. (2019)
		Inhibition of renal carcinoma GRC-1 cell line proliferation	Jiang et al. (2016)

(continued)

Table 2.2 (continued)

Isothiocyanates	Health effect	Specific biological activity	Reference
	Antioxidant and anti-inflammatory	Ameliorates hepatic steatosis and inflammation by activating the Sirt1/AMPK pathway and inhibiting the NF-κB pathway	Li et al. (2019)
		Decreased tumor necrosis factor α mRNA levels and its secretion in LPS stimulated RAW264.7 macrophages, downregulated pro-inflammatory markers such as interleukin-1β and inducible nitric oxide synthase. Decreased nuclear p65 protein levels, a subunit of the transcription factor NF-κB.	Wagner et al. (2012)
		Reduced oxidative stress and inflammation by modulating Nrf2/HO-1 and NF-κB pathways in traumatic brain injury in mice	Caglayan et al. (2019)
		Reduced liver fibrosis by regulating Kupffer cell activation	Kim et al. (2018a)
	Anti-obesity	Increased basal and epinephrine-induced lipolysis in adipocytes and intensified hydrolysis of triacylglycerols in the blood serum	Okulicz (2010)
		Inhibited adipocyte differentiation by suppressing galectin-12 levels in 3T3L1 cells and has anti-obesity effects in high fat diet-fed mice	Lo et al. (2018)
		Reduced blood glucose, total cholesterol, triglycerides, and creatinine levels, and increased total antioxidant capacity	Sahin et al. (2019)
	Anti-diabetic	Inhibited the hyperglycemia and hyperinsulinemia induced by the consumption of a high-fat diet	Ahn et al. (2014)
		Suppression of oleic acid-induced lipid accumulation and lipogenesis in hepatocytes	Kim et al. (2015)
		Increased carbohydrate oxidation by enhancing insulin secretion via transient receptor potential (TRP) V1	Mori et al. (2018)
	Anti-bacterial, anti-fungal	Cytotoxic effect against several bacterial and fungi	Blažević et al. (2019)
		Reduced biofilm growth and virulence factors of C. albicans	Raut et al. (2017)

(continued)

Table 2.2 (continued)

Isothiocyanates	Health effect	Specific biological activity	Reference
Benzyl isothiocyanate (BITC)	Antimicrobial activity	Inhibition of the growth of oral pathogens higher than sulforaphane	Ko et al. (2016)
		Reduced the motility of *E. coli* O157:H7 and *Salmonella* and killed *Salmonella* by disrupting bacterial cell membrane and decreased shiga toxin production by *E. coli* O157:H7	Patel et al. (2020)
	Anticancer activity	Inhibited the growth of 3 different human lung cancer cell lines A549 (adenocarcinoma), H661 (large cell carcinoma) and SK-MES-1 (squamous cell carcinoma)	Zhang et al. (2017)
		Increased miR-99a expression through ERK/AP-1-dependent pathway showing antitumor properties in bladder cancer cells	Tsai et al. (2020)
		Suppressed cancer cell proliferation through the post-transcriptional regulation of the kinetochore protein Mis12	Abe-Kanoh et al. (2019)
Erucin	Anticancer	Induced apoptosis in human hepatoma (HepG2) cells	Lamy and Mersch-Sundermann (2009), Pocasap et al. (2018)
		Modulation of key enzymes in carcinogen metabolism in rat lung slices	Abdull Razis et al. (2011)
		Inhibition of PC3 cell proliferation by increasing p21 protein expression and ERK1/2 phosphorylation	Melchini et al. (2013)
		Inhibition of breast cancer proliferation acting at various levels	Wang et al. (2005), Bo et al. (2016), Prełowska et al. (2017)
		Inhibition of histone deacetylase (HDAC) activity in human bladder cancer cells	Abbaoui et al. (2017)
		Release of hydrogen sulfide (H_2S) in pancreatic adenocarcinoma cells (AsPC-1) and inhibition of AsPC-1 cell viability and migration	Citi et al. (2019)

(continued)

Table 2.2 (continued)

Isothiocyanates	Health effect	Specific biological activity	Reference
	Anti-inflammatory	Inhibition of pro-inflammatory enzymes and cytokines, through inhibition of NFκB signaling in RAW 264.7 murine macrophages and 12-O-tetradecanoylphorbol-13-acetate-treated mouse skin	Cho et al. (2013)
	Neuroprotective	Activation of the transcriptional nuclear factor (erythroid-derived 2)-like 2 (Nrf2) in in vitro and in vivo models of Parkinson's disease	Morroni et al. (2018)
		Neuroprotective effects in human neuronal cells	Sestito et al. (2019)
	Anti-hypertension and vasorelaxing	Release of H_2S in human aortic smooth muscle (HASMCs) cells and inhibition of noradrenaline-induced vasoconstriction	Martelli et al. (2019)
	Antimicrobial activity	Inhibition of the growth of oral pathogens higher than sulforaphane	Ko et al. (2016)
Goitrin	Antithyroid	Inhibit the uptake and organification of iodine by the thyroid glands limiting the formation of thyroid hormone	Gaitan (1990), Felker et al. (2016)
Iberin	Anticancer activities	Anticancer activities against prostate, breast and colon cancer and leukemia	Jakubikova et al. (2005, 2006), Sarikamiş (2009), Núñez-Iglesias et al. (2019)
		Anticancer activities against hepatocellular carcinoma cell HepG2 line through the increase of intracellular reactive oxygen species and the inhibition of tubulin depolymerization	Pocasap et al. (2019)
		Inhibition of carcinogens in hepatocytes	La Marca et al. (2012)
		Growth inhibition and apoptosis in lung cancer A549 cells	Wang et al. (2016)
		Induction of cycle arrest and apoptosis of human neuroblastoma SK-N-AS, SK-N-SH and SK-N-BE(2) cell lines	Jadhav et al. (2007)
	Antimicrobial activities	Antimicrobial activities against oral and food borne pathogens and *Pseudomonas aeurugunosa*	Jakobsen et al. (2012), Wilson et al. (2013), Tan et al. (2014), Ko et al. (2016)

(continued)

Table 2.2 (continued)

Isothiocyanates	Health effect	Specific biological activity	Reference
Indole-3 carbinol (I3C)	Anticancer activities	Management of biochemically recurrent prostate cancer through the downregulation of signal transduction pathways	Van Die et al. (2016), Wu et al. (2019), Núñez-Iglesias et al. (2019)
		Inhibition of cervical cancer, human breast cancer (T47D), and hepatocellular carcinoma (SK-Hep-1, SNU-449 and Huh-7) cells through the upregulation of phosphatase and tensin homologue (PTEN)	Meng et al. (2000), Qi et al. (2005), Aronchik et al. (2014), Wang et al. (2015), Jiang et al. (2019), Mokbel and Mokbel (2019)
	Antioxidant activity	Showed dopamine-like antioxidant activity mainly preventing the oxidative degradation of lipids	Vo et al. (2019)
	Antimicrobial activity	Potent inhibition of the growth of oral pathogens	Ko et al. (2016)
Phenethyl isothiocyanate (PEITC)	Anticancer activities	Activities against human prostate cancer PC-3 and DU 145 cell lines	Aggarwal et al. (2019), Núñez-Iglesias et al. (2019)
		Activities against human colon carcinoma cell line HT29 through the synergism with Laccaic acid	Gupta et al. (2019)
	Antiatherogenic activity	Protective effects against atherogenesis and thrombosis	Chuang et al. (2013), Huang et al. (2013), Jayakumar et al. (2013)
		Anticancer activities against human colon cancer cell lines DLD-1 and SW480 through the suppression of Wnt/β-catenin pathway	Chen et al. (2018b)
	Antiobesity activity	Antiobesity effects through the reduction of adipocyte differentiation and the induction of cell cycle	Chuang et al. (2019)
	Neuroprotective activity	In vitro and in vivo effects against neurodegenerative diseases	Jaafaru et al. (2018a)
	Antimicrobial activity	Inhibit of bacterial conjugation of pathogen microorganisms	Kwapong et al. (2019)
		Inhibition of the growth of oral pathogens, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Escherichia coli*	Jang et al. (2010), Ko et al. (2016), Kaiser et al. (2017), Yang et al. (2020)

(continued)

Table 2.2 (continued)

Isothiocyanates	Health effect	Specific biological activity	Reference
Sulforaphane (SFN)	Anticarcinogenic activity	Induction of phase II detoxication enzymes	Zhang et al. (1992, 1994)
	Antioxidant	Upregulation of genes that protect aerobic cells against oxidative stress, inflammation, and DNA-damage associated with autism spectrum disorder.	Singh et al. (2014)
		Protection against nitrative stress and inflammation by downregulating oxidative stress and inflammation by blocking NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway in autistic children.	Nadeem et al. (2020)
	Antimicrobial activity	Inhibition of the growth of oral pathogens	Ko et al. (2016)
Sulforaphene	Anticancer activity	Induction of apoptosis of hepatocarcinoma HepG2 cells	Pocasap and Weerapreeyakul (2016), Yang et al. (2016a), Kntayya et al. (2018)
		Growth inhibition of human breast MCF-7 and SUM159 cells	Bao et al. (2016), Pawlik et al. (2017)
		Activities against lung cancer through the inhibition of the PI3K-AKT signaling pathway	Yang et al. (2016c)
		Induction of apoptosis and inhibition of migration of gastric cancer AGS cells	Mondal et al. (2016)
		Induces apoptosis of cervical cancer (HeLa cell line)	Rhee et al. (2017)
		Suppression of growth of human colon cancer cell lines (HCT116, HT-29, KM12, SNU-1040, DLD-1)	Byun et al. (2016)
		Induction of apoptosis and inhibition of the invasion of esophageal cancer cells through the inhibition of the MSK2–CREB–Bcl2 and cadherin pathways	Zhang et al. (2019a)
	Antiobesity activity	Antiobesity activities through the activation of the Hedgehog (Hh) signaling pathway	Chen et al. (2018a)

2019). For example, dietary AITC is considered as a potent cancer chemopreventive agent with high bioavailability and low degree of side effects due to cytotoxicity and genotoxicity (Zhang 2010), although synergistic effects with other hydrolysis by-products and conventional drugs should be considered (Chatterjee et al. 2016; Rakariyatham et al. 2019). Moreover, Blažević et al. (2019) who compared the bioactive properties of hydrodistillates and extracts of *Lepidium latifolium* L. with pure AITC reported similar cytotoxic effects against bladder cancer UM-UC-3 and glioblastoma LN229 cell lines. Other researchers have reported the epigenetic effects of AITC against malignant melanoma through the regulation of lysine acetylation and methylation marks (Mitsiogianni et al. 2019). However, the volatile nature of AITCs inhibits their use in food products with enhanced bioactive properties and other forms should be considered. Therefore, Chang et al. (2019) studied the potential of encapsulating AITCs in nanoparticles and reported significant effectiveness against HT1376 bladder cancer cells proliferation, as well as anti-inflammatory activity against macrophage cell RAW 264.7, while Encinas-Basurto et al. (2017, 2018) suggested the increased delivery of AITC-loaded polylactic-co-glycolic acid (PLGA) nanoparticles (NPs) against epithelial squamous carcinoma cells. Apart from AITC, other GSL hydrolysates such as iberin, PEITC, I3C, 4-pentenyl-ITC (4PI) and SFN showed a dose- and time dependent effectiveness against two cell lines of androgen-insensitive human prostate cancer, namely PC-3 and DU 145 (Núñez-Iglesias et al. 2019). In the study of Zhang et al. (2017), the in vitro and in vivo growth inhibition of lung cancer cells (A549 (adenocarcinoma), H661 (large cell carcinoma) and SK-MES-1 (squamous cell carcinoma)) was also reported for BITC, with a concomitant induction of autophagy for the tested cancer cells. The same compound was also effective against bladder cancer cells through the upregulation of miR-99a expression (Tsai et al. 2020), induced apoptosis of gastric cancer AGS cells (Han et al. 2019a) and leukemia U937 cells (Stasiłojć et al. 2019), while 3,4-dimethoxybenzyl ITC (dMBITC) increased doxorubicin efficacy against resistant colon cancer cell lines (LoVoDX) and ameliorated its toxic effects (Psurski et al. 2019). Other types of cancer affected by BITC include breast cancer (Roy et al. 2019; Xie et al. 2019; Kim and Singh 2019), pancreatic adenocarcinoma (Si et al. 2019), liver and prostate cancer (Crowley et al. 2019), human brain glioblastoma (Ma et al. 2018b), and human melanoma A375.S2 cells among others (Ma et al. 2017). The suggested mechanisms of action of ITCs include the cell cycle arrest and cell apoptosis, the inhibition of angiogenesis and metastasis, the modulation of detoxifying enzymes, and the inhibition of phase I and the induction of phase II enzymes (Mitsiogianni et al. 2018; Di Gioia et al. 2020).

AITC dietary intake is also associated with antidiabetic, anti-inflammatory and antioxidant activities through the increase of glucose transporter-2, peroxisome proliferator-activated receptor-gamma, p-insulin receptor substrate-1, and nuclear factor erythroid-derived 2 and the reduction of nuclear factor-kappa B in kidney and liver tissues of Wistar rats (Sahin et al. 2019). In another recent study, Lo et al. (2018) attributed anti-obesity effects to AITC since its administration inhibited adipocyte differentiation through the suppression of galectin-12, while Subedi et al. (2017) highlighted the neuroprotective activities against microglia-induced toxicity

in neuroblastoma cells. Sulforaphane is in the focus of several clinical studies which evaluate its effect against diabetes and cardiometabolic disorders and promising results have been reported suggesting as possible mechanisms of action the induction of nuclear factor erythroid 2-related factor 2 (Nrf2) and the modulation of pro-inflammatory and metabolic signaling pathways (Patel et al. 2018). Antidiabetic effects of sulforaphane were also associated with the amelioration of insulin responsiveness and the lipid profile in male Wistar rats (De Souza et al. 2016). Other beneficial effects of sulforaphane for the cardiovascular system include the reverse of abnormal angiotensin II-induced migration of human vascular smooth muscle cells (Zhang et al. 2019b), the activation of Nrf2 (Bai et al. 2015), the downregulation of expression of intracellular adhesion molecule-1 in TNF-α-induced ECV 304 endothelial cells (Hung et al. 2014), as well as the attenuation of cardiotoxicity in breast cancer patients treated with doxorubicin (Bose et al. 2018). This potent compound may also exhibit antiobesity effects, since it can inhibit adipocyte differentiation and promote lipolysis in both in vitro and in vivo studies (Choi et al. 2012, 2014b; Martins et al. 2018).

Indole-3 carbinol (I3C) is another potent phytochemical which is derived from glucobrassicin hydrolysis. Several studies have reported its anticancer properties against various types of cancer, including recurrent prostate cancer, cervical cancer, human breast cancer, and hepatocellular carcinoma (Meng et al. 2000; Qi et al. 2005; Aronchik et al. 2014; Wang et al. 2015; Van Die et al. 2016; Lee et al. 2018; Tian et al. 2019). I3C also exhibited antimicrobial activities against a broad spectrum of bacteria (Ko et al. 2016; Vale et al. 2019), as well as anti-inflammatory and anti-arthritic properties (Hasan et al. 2018) and hepatoprotective effects (Choi et al. 2018). An I3C digestion byproduct, namely 3,3′-diindolylmethane has been found to be effective against hyperglycemia and diabetic nephropathy through the increased uptake of glucose, the inhibition of PKC-α expression and the activation of insulin signaling in 3T3-L1 adipocytes (Choi and Yoo 2018, 2019), as well as against neurodegenerative diseases (Lee et al. 2020) and obesity (Yang et al. 2017). This byproduct is an effective anticancer agent with several studies to confirm this (Tian et al. 2019; Ahmad et al. 2019), while it exhibited anti-ischemic effects through the inhibition of hypoxia-induced inflammation and apoptosis and the induction of cardiomyocyte autophagy (Liang et al. 2017).

GSL degradation byproducts such as AITC, SFN, PEITC, and 4-methoxyphenyl ITC may also inhibit bacterial conjugation which is responsible for the resistance of pathogenic microorganisms against antimicrobial agents (Kwapong et al. 2019). Moreover, according to Kaiser et al. (2017) natural ITCs (AITC, BITC, PEITC) isolated from *Tropaeolum majus* (nasturtium) and *Armoracia rusticana* (horseradish) may exhibit therapeutic properties against infections from the multi-drug resistant and biofilm-forming Gram-negative bacterium *Pseudomonas aeruginosa*. Similar results were reported for the effectiveness of AITC against *Candida albicans* biofilms (Raut et al. 2017). Several other studies have reported the antimicrobial properties of synthetic or natural ITCs against a broad spectrum of activity against both human affecting Gram-negative and Gram-positive bacteria with various mechanisms of action being suggested (Jang et al. 2010; Lu et al. 2016; Nowicki

et al. 2016, 2019; Saleh et al. 2017; Romeo et al. 2018). According to Ko et al. (2016), structural differences of ITCs have a significant effect on antimicrobial efficiency where the number of double bonds, the presence of thiol groups or the length of the side-chain defines ITCs activities. In particular, BITC was the most effective against *Escherichia coli* O157:H7 and *Salmonella enterica* among seven GSL hydrolysis products (butyl ITC, ethyl ITC, isopropyl ITC, methyl ITC, phenethyl ITC and allyl ITC), since it inhibited the bacteria motility and the production of Shiga toxin (Patel et al. 2020). Moreover, BITC and PEITC showed the highest activity against *Bacillus cereus* compared to 3-butenyl ITC and 4-pentenyl ITC, while they were effective against several other Gram-positive and Gram-negative bacteria (Jang et al. 2010). Recently, Yang et al. (2020) reported that antimicrobial activities of BITC and PEITC against *E. coli* (enterotoxigenic and Shiga-producing strains) are related to the down-regulation of virulence genes.

Other health effects include the attenuation of oxidative stress and anti-inflammatory activities of AITC against oxidative stress and inflammation caused after traumatic brain injury through the modulation of nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor kappa B (NF-κB) (Caglayan et al. 2019). The same compound was effective against inflammatory bowel disease by ameliorating the severity of colitis symptoms in mice models (Kim et al. 2018b). GSLs and various ITCs (SFN, PEITC, erucin, 6-(methylsulfinyl) hexyl ITC) showed promising in vitro and in vivo effects against neurodegenerative diseases mostly associated with their anti-amyloidogenic, antioxidant, and anti-inflammatory properties (Jaafaru et al. 2018a). Other suggested mechanisms for the protective effects of ITCs against neurodegenerative diseases include the cholinesterase inhibition, with phenyl ITC and 3-methoxyphenyl ITC showing the most promising results as cholinesterase inhibitors and anti-inflammatory agents (Burčul et al. 2018). According to Kim et al. (2018a), AITC produced from sinigrin hydrolysis mitigated hepatic fibrosis in carbon tetrachloride-induced hepatotoxicity in rats, while as a possible mechanism of action it was suggested the regulation of Kupffer cell and the activation of monocytes. The hepatoprotective activity of AITC has been also confirmed in vivo studies with carbon tetrachloride treated Sprague Dawley rats and the possible mechanism of action was suggested being the lipid peroxidation inhibition, the increased activity of antioxidant enzymes and the suppression of macrophages and Kupffer cells (Ahn et al. 2016). Moreover, AITC, BITC and 3-butenyl ITC exhibited significant antimutagenic activity against various mutagens (4-nitro-o-phenylenediamine, sodium azide and 2-aminofluorene) (Rampal et al. 2017), while SFN, BITC, and PEITC showed protective effects against atherogenesis and thrombosis through various mechanisms of action (Chuang et al. 2013; Huang et al. 2013; Jayakumar et al. 2013).

Regarding the health effect of other less studied ITCs, 4-carboxy phenyl-ITC (4CPI) acted as a hydrogen sulfide donor and decreased ischemia/reperfusion-induced tissue injury after acute myocardial infarction in rats (Testai et al. 2016). Moreover, 4CPI and phenyl ITC exhibited promising effects against hypertension, since they acted as hydrogen sulfide release agents which has vasorelaxing and hypotensive properties (Martelli et al. 2014). Two other ITCs, namely 4-[(α-L-

rhamnosyloxy)benzyl] ITC and 4-[(4′-O-acetyl-α-L-rhamnosyloxy)benzyl] ITC were also identified as potent indirect antioxidants through the induction of NAD(P)H quinone oxidoreductase 1 (NQO1) activity in Hepa1c1c7 cells (Tumer et al. 2015). Glucomoringin ITC (4-(α-L-rhamnosyloxy)benzyl ITC)) was also effective against resistant pathogens affecting long-term hospital patients (*Staphylococcus aureus*, *Enterococcus casseliflavus*, and *Candida albicans*) (Galuppo et al. 2013), as well as against human neuroblastoma SH-SY5Y cells (Cirmi et al. 2019; Jaafaru et al. 2019), human prostate adenocarcinoma (PC-3) cells (Jaafaru et al. 2018b) and human astrocytoma grade IV CCF-STTG1 cells (Rajan et al. 2016).

Iberin, an aliphatic ITC derived from glucoiberin hydrolysis, is associated with antimicrobial activities against oral and foodborne pathogens (Wilson et al. 2013; Ko et al. 2016) and anticancer activity against various types of cancer (Sarikamiş 2009; Wang et al. 2016; Pocasap et al. 2019; Núñez-Iglesias et al. 2019).

Raphasatin (4-Methylthio-3-butenyl ITC) which is the hydrolysis product of glucoraphasatin is a potent detoxifier and inducer of rat hepatic phase II enzymes and a potential chemopreventive agent against esophageal carcinogenesis and pancreatic carcinogenesis (Scholl et al. 2011; Abdull Razis et al. 2012; Okamura et al. 2013; Suzuki et al. 2016), without showing toxicity to urinary bladder (Suzuki et al. 2017). According to Ibrahim et al. (2018), this ITC is responsible for the apoptosis and cell cycle arrest of human breast adenocarcinoma MCF-7 cells, while its combined administration along with two other food components (vitexin-2-O-xyloside and (–)-epigallocatechin-3-gallate) inhibited the growth and induced the apoptosis of colon cancer LoVo and CaCo-2 lines (Papi et al. 2013). Moreover, La Marca et al. (2012) who studied the dose-effect of raphasatin and sulforaphane suggested that low doses of both ITCs may exhibit anti-aging activities and reduce chemotherapy-induced oxidative stress, whereas at high doses they may act synergistically with anticancer drugs and induce cell DNA damage (Zanichelli et al. 2012). Raphasatin and sulforaphene were detected in aqueous extracts of Spanish black radish vegetative portions and exhibited significant antioxidant properties by inducing detoxification enzymes in HepG2 cells; however, raphasatin content was significantly reduced within the first hour after extraction compared to sulforaphene (Hanlon et al. 2009). Regarding sulforaphene, which is derived from glucoraphanin and has been detected in various plants parts (Hanlon et al. 2009; Lim et al. 2016; Zhang et al. 2016), it may induce apoptosis in hepatocarcinoma HepG2 cells and growth inhibition in human breast adenocarcinoma MCF-7 cells and human HT-29 and HCT116 colon cancer cells (Byun et al. 2016; Pocasap and Weerapreeyakul 2016; Yang et al. 2016b; Bao et al. 2016; Pawlik et al. 2017; Kntayya et al. 2018), as well as in esophageal cancer cells (Zhang et al. 2019a). Other health effects include antiobesity activities (Chen et al. 2018a), as well anti-cancer properties against various types of cancer e.g. lung cancer and gastric cancer (Mondal et al. 2016; Yang et al. 2016c), and cervical cancer (Rhee et al. 2017).

While multiple beneficial health effects are attributed to most of the ITCs deriving from the myrosinase-mediated degradation of GSLs, some of the GLS degradation products may have harmful effects on human health and are considered antinutrients (Kupke et al. 2016; Di Gioia et al. 2020). Goitrin and thiocyanates

deriving from the hydrolysis of progoitrin and indole GSLs have antithyroid activity by inhibiting the uptake and organification of iodine by the thyroid glands limiting the formation of thyroid hormone, causing the enlargement of the thyroid with the development of a condition known as goiter (Gaitan 1990; Felker et al. 2016). Examining the concentration of goitrin and thiocyanate in human plasma upon ingestion of *Brassica* vegetables containing progoitrin and indole GSLs which are responsible for the formation of goitrogenic thiocyanates, Felker et al. concluded that, the consumption of regular serving size broccoli, broccoli rabe, bok choy, and Chinese cabbage results in plasma concentration levels of progoitrin and goitrogens-generating indole-GSLs that are well below the levels that may affect thyroid activity (Felker et al. 2016). On the other hand, excessive and continuous consumption of raw Russian kale, collards, and Brussel sprouts characterized by high levels of progoitrin may limit iodine uptake in the thyroid and cause hypothyroidism (Choi et al. 2014a; Felker et al. 2016).

Several other studies have indicated toxic effects of ITCs, such as goitrogenic and mutagenic ones (Wiesner et al. 2014; Eisenbrand and Peter 2016), while adverse activities have been also appointed to other byproducts of myrosinase-induced hydrolysis, e.g. nitriles, thiocyanates, goitrins, epithionitriles and cyanides (Cipollini and Gruner 2007; Kupke et al. 2016; Felker et al. 2016). There is also a particular species, *Carica papaya*, which contains both beneficial (glucotropaeolin) and toxic (cyanogenic glucosides) compounds (Bennett et al. 1997; Olafsdottir et al. 2002; Williams et al. 2013; Bolarinwa et al. 2016), while degradation byproducts of specific GSLs may exhibit either beneficial or adverse effects. A perfect example is the case of epithionitriles which may have toxic effects on mammals' liver and kidney (Kupke et al. 2016), or present cancer-preventive/therapeutic properties (Hanschen et al. 2015).

Despite the whatsoever limited negative effects, scarce evidence from epidemiological studies on humans exists, while limited data from toxicological studies are available to formulate safety regulations and recommend average daily intake amounts (Spcijers 1995; Latté and Appel 2011). Recently, a cohort study conducted by Ma et al. (2018a) between 1984–2013 associated dietary GSL intake with an increased risk of type 2 diabetes in US adults. A recent review paper, (Fimognari et al. 2012) stressed out the genotoxic potential of ITCs which may result in gene mutations and chromosomal aberrations, however, they suggested that further toxicological studies are required to evaluate the toxicity of ITCs and recommend safe daily intake allowance.

2.5 Conclusion Remarks

GSLs represent an important group of phytochemicals with great significance in plant physiology and defense system. Apart from that, several beneficial health effects have been confirmed with in vitro and in vivo studies during the last decades which are associated with their hydrolysis products, namely ITCs, and triggered the

current research interest of the scientific community. The numerous GSLs identified in various species of the Brassicales order exhibit a great structural diversity and originates a large number of byproducts which further results in a broad spectrum of bioactive properties, including anticancer, antimicrobial, antidiabetic and beneficial to cardiovascular system activities among others. The recent analytical techniques allowed researchers to identify the mechanisms of action behind the activities of many GSLs, as well as their bioavailability and bioaccessibility after ingestion in the human body. Moreover, considering the already confirmed positive health effects future research should focus on agronomic practices and breeding efforts that would increase GSLs content in the final products and improve their dietary value. However, despite the beneficial effects, there are also reports and clinical studies that highlight possible negative effects which need further consideration in order to define safe consumption limits.

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Chapter 3
Peptides and Proteins

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Abstract Non-communicable diseases are among the top causes of death worldwide. In the following decades, the number of people affected by non-communicable diseases will increase due to aging, and so the demand for medicines. Many of the treatments available to alleviate these diseases have adverse side effects, whereas others are costly, so there is an urgent need for alternatives. Currently, there is an increasing pharmacological interest for peptides and proteins as therapeutic agents because of advantages such as biocompatibility, high potency, high selectivity, and low risk of drug interactions. This chapter reviews updated scientific reports about food-derived bioactive peptides and proteins, about their potential preventive or alleviating role on the deadliest non-communicable diseases. Cardiovascular disease, cancer disease, diabetes, neurodegenerative disorders, as well as oral cavity diseases as a predisposing factor to the development of other essential illnesses, are addressed. The objective is to provide useful information to readers involved or interested in the fields of pharmacology and food technology, with the hope that it can serve as an introductory guide to recognize the immense potential of peptides and proteins as therapeutic agents.

Keywords Non-communicable diseases · Bioactive peptides and proteins · Alternatives to synthetic drugs · Protein technology · Drug development

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3.1 Introduction

According to the World Health Organization (WHO 2018), in 2016, deaths worldwide amounted to 56.9 million. The most prevalent noncommunicable diseases accounted for a combined 29.6 million deaths from which cardiovascular diseases represented 55%, a set of others, diabetes among them, 31%; and cancer, 14% (Fig. 3.1). Percentages vary between regions, income level, age, and sex, being the income level the most important factor since, in developing countries, infectious diseases represent the leading cause of death. Although some risk factors are well identified, which serves the health authorities to plan public health strategies, projections are not very encouraging. It is thought that by 2030, the sum of deaths due to noncommunicable diseases will rise to 52 million (WHO 2008). If this is added to the appearance of new diseases and the resurgence of others that were believed already eradicated, it results in a growing need for the population for the use of medications.

Evidence of the importance of the pharmaceutical sector is the world drug market, which had revenues of 1,204.8 billion US dollars (1.2 trillion) in 2018, from

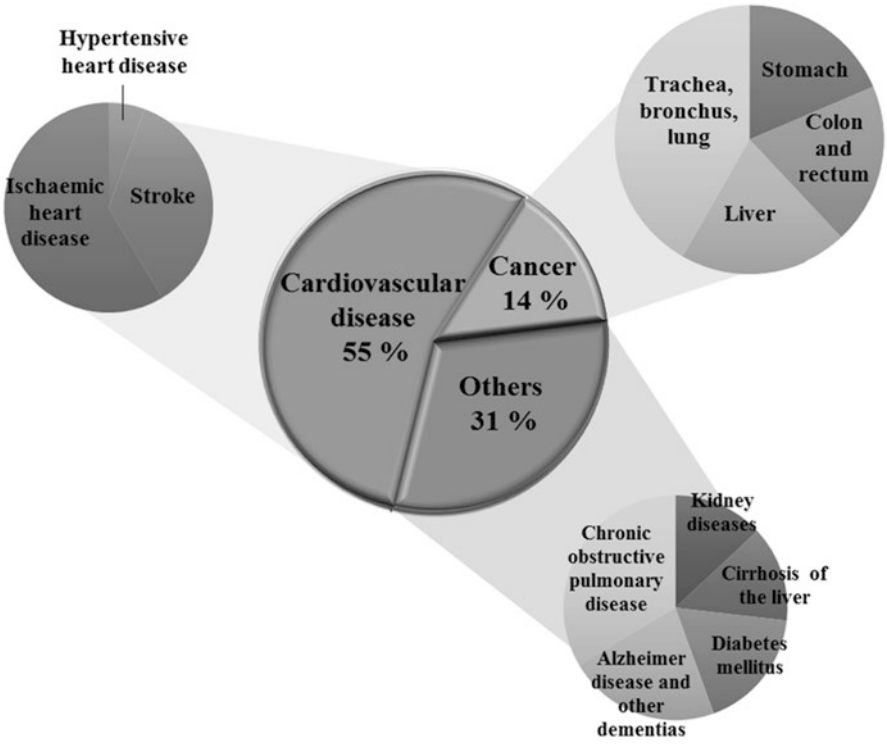

Fig. 3.1 Contribution of the most prevalent noncommunicable diseases to total worlwide deaths in 2016. Calculated with data of the World Health Organization (WHO 2018)

which 8.26% corresponded to cancer drugs. Cancer is not the most prevalent disease or the one that causes the most deaths, but anticancer drugs have the highest prices (Mikulic 2019). In this context, in addition to the fact that many pharmaceutical medications are unreachable for people in need, there are adverse effects of the same, which even end in a fatality (Karimi et al. 2015). Thus, a growing interest of people to use natural alternatives exists, representing an opportunity for the expansion of their market. However, something "natural" is not always free from undesirable side effects, so strict protocols must be followed before placing natural-derived drugs in the market. Such is the case of peptides and proteins used as therapeutic agents.

Bioactive peptides (BP) are those displaying some kind of biological activity, which goes beyond the nutritional one and has a positive impact on human health (Kitts and Weiler 2003). In general, BP has current or potential uses as nutraceuticals, food preservatives, or drugs, depending on the amino acid sequence. Bioactive peptides are usually encrypted in many proteins, which are part of the human diet. This is a latent form of BP, and becomes active after releasing by proteolysis, either chemical or enzymatic (Bhat et al. 2015a; Karami and Akbari-Adergani 2019). After a BP with determined biological activity is identified, its production at a high scale is frequently assisted by chemical synthesis. Another strategy is the use of recombinant technology to obtain the source protein, from which the BP of interest are subsequently released (Rasmussen 2018). On the other hand, bioactive proteins are those who have therapeutic properties as well, but unlike BP, their number of amino acid residues is higher than 50 (Dimitrov 2012).

There are several reviews on the subjects of BP, addressing either general or particular issues (Acquah et al. 2019; Belović et al. 2011; Boparai and Sharma 2020; Karami and Akbari-Adergani 2019; Lau and Dunn 2018; Pandit et al. 2020; Sánchez and Vázquez 2017). This chapter reviews updated scientific evidence on the bioactivity of BP derived from food proteins in terms of their potential impacts on the primary non-communicable diseases. A general view on drug design is presented, as a background, to discuss the research on peptides and proteins in a pharmacological context.

3.2 Generalities on Drug Design

Drug design is a challenging task. Commercialization of a particular drug is preceded by years of intense work and involves the participation of a myriad of specialists trained in a variety of disciplines. Typically, drug design consists of two major stages: drug discovery and drug development; each of them subdivided into many minor tasks (Fig. 3.2). The goal of drug discovery is the selection of one compound, among thousands, with the potential to be clinically relevant by demonstrating in vivo efficacy in animal models. The second primary stage, drug development, has the goal of placing the drug in the market. It starts by requesting permissions for clinical trials in humans and is progressed through several studies until it is approved

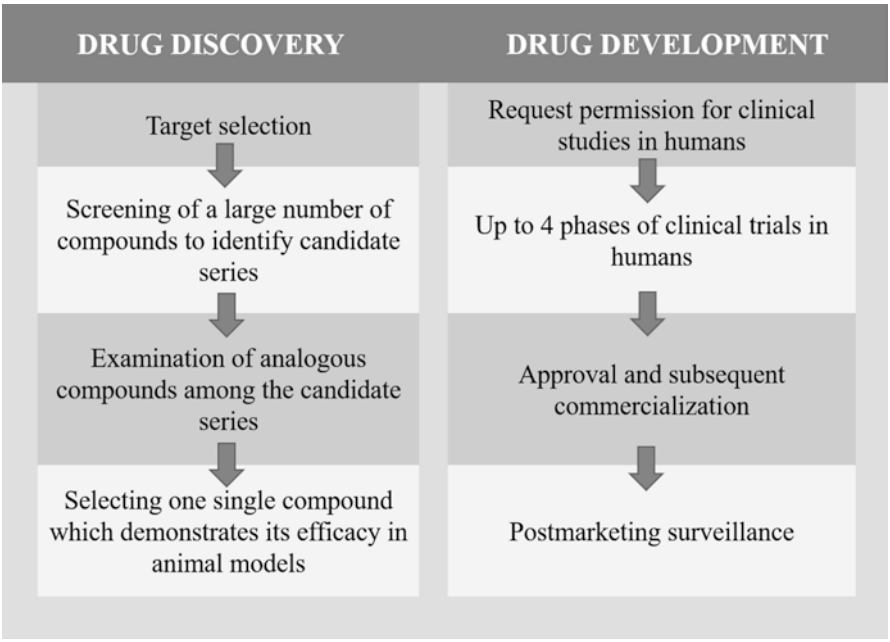

Fig. 3.2 Simplified diagram of the drug development process. Adapted from Blass (2015)

by the appropriate regulatory agencies. Then, post-market surveillance is followed (Blass 2015).

Since millions of chemical compounds exist, the starting point of drug discovery could be extremely laborious. Modern drug design is supported by computational and biological approaches to reduce costs and time (Xue et al. 2018; Zhou and Zhong 2017). However, physicochemical properties such as solubility and permeation across membranes, which are particularly crucial for drugs designed for oral administration, are not accurately predicted. As a result, physical measurements would be necessary, with the disadvantages that this conveys. In this regard, researchers have adopted the Lipinski's rule of five (Lipinski et al. 2001) to define drugability, i.e., whether a chemical compound meets the characteristics of a drug. This rule, proposed in 1997 and to date very useful (Benet et al. 2016), is based on experimental and computational approaches. It establishes that poor absorption or permeation of a compound is more likely if:

- There are more than 5 H-bond donors (expressed as the sum of OH and NH);
- The molecular weight is over 500;
- The Log P (octanol-water partition coefficient) is over 5;
- There are more than 10 H-bond acceptors (expressed as the sum of N and O)

In principle, peptides and proteins do not accomplish Lipinski's rule to be considered as candidates for drug development. However, their high potency and

selectivity have prompted researchers to develop strategies for enhancing their pharmacokinetic properties.

3.3 Bioactive Peptides in the Pharmaceutical Context

As with any other bioactive substance, the biological effects of peptides must be measurable at a physiological level and affect the health positively to be considered as BP (Möller et al. 2008). Demonstrated bioactivity of peptides includes the antioxidant, antimicrobial, antihypertensive, antithrombotic, anti-inflammatory, hypoglycemic, immunomodulatory, anticancer, and opioid, among others (Boparai and Sharma 2020). Non-communicable diseases are of high interest to the pharmaceutical industry, and the BP development is highly concentrated in such areas, too (Lau and Dunn 2018). Bioactive peptides are encrypted in proteins, whose sources are of both animal and plant origin. However, most of the bioactive peptides come from a food of animal origin, such as bovine milk, cheese, and other dairy products. Plant-based sources of BP include cereals such as wheat, corn, rice, and sorghum, in addition to soy, mushrooms, squash, and amaranth, being the latter a pseudocereal (Sánchez and Vázquez 2017).

Bioactivity of peptides is specific and depends on the amino acid sequence; however, they share some general characteristics:

- The length of the peptide chain is between 2 and 20 amino acid residues (although there are BP which have 20 or more amino acids);
- Molecular mass less than 6000 Da;
- BP contain hydrophobic amino acids, in addition to Pro, Lys, or Arg;
- BP is resistant to proteolysis by digestive peptidases (Karami and Akbari-Adergani 2019; Sánchez and Vázquez 2017)

Advantages of BP for their use in pharmacology include:

- High potency;
- High selectivity;
- Low potential for toxicity;
- Low risk of drug-drug interaction (Morimoto 2017)

Despite so many benefits, BP has the significant disadvantage of instability in biological matrices due to their susceptibility to degradation by proteases. Also, cellular absorption is low because of the molecular size and the polar nature of the peptide bond (Di 2015). Thus, since peptides violate each and every point of Lipinski´s rule of five, their pharmacological properties are enhanced through PEGylation, lipidation, glycosylation, cyclization, or non-natural amino acid substitution (Morimoto 2017). Purification of BP after hydrolysis is another challenging task (Acquah et al. 2019), and becomes more critical when it comes to taking advantage of agroindustrial wastes as sources of BP (Lemes et al. 2016). Efforts have been made to overcome such difficulties (Adhikari et al. 2020; Fosgerau and

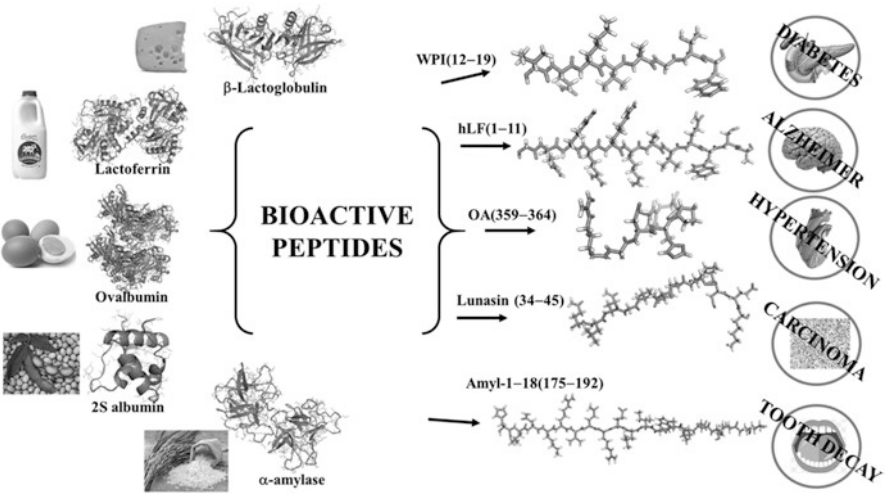

Fig. 3.3 Most prevalent non-communicable diseases into which the effects of bioactive peptides, encrypted in proteins from different sources have been investigated

Hoffmann 2015; Kapoor et al. 2020; Morimoto 2017; Raza et al. 2018). However, there is still much work to do in this regard, representing additional opportunity areas to the industrial sector.

The use of proteins and peptides due to their hormone- or drug-like activity is not emergent. The insulin hormone is a peptide isolated from the animal pancreas in 1922 (Karamitsos 2011). Since then, a crescent number of scientific publications regarding the bioactivity of peptides and proteins can be encountered. Contemporary interest for BP is due to their preventive and alleviating dualistic role in some medical conditions. At present, nearly 20 new peptide-based clinical trials are done annually; more than 60 peptide drugs have been discovered and approved for clinical use in the United States, Europe, and Japan; around 140 are under evaluation by clinical trials, and more than 500 are in the preclinical development (Lee et al. 2019; Wang et al. 2018). In Fig. 3.3 are schematized the most prevalent non-communicable diseases into which effects of BP have been investigated.

3.4 Bioactive Peptides and Cardiovascular Diseases

Cardiovascular diseases (CVD) represent the most significant public health problem in the world since atherosclerosis, stroke, or myocardial infarction affect a third of the adults (Yusuf et al. 2015). Atherosclerotic and thrombolytic processes are associated with the development of CVD, where the high levels of cholesterol, dyslipidemia, high blood pressure, obesity, and diabetes represent the key predisposing risk factors. In recent years, an essential preventive strategy to reduce these risks has

focused on dietary compounds that may contribute to improving cardiovascular health. The study of proteins and peptides with biological activity had gained an interest in preventive medicine due to their beneficial health effects, playing a significant role in reducing risks associated with CVD.

Several food proteins and their hydrolysates/peptides have shown diverse biological activities with beneficial effects in metabolic disorders such as hypertension, hypercholesterolemia, dyslipidemia, diabetes type 2, and thrombosis. Research on their beneficial effects are based on the inhibition or activation of key enzymes associated with the biological process of metabolic disorders such as hypertension (e.g., ACE, angiotensin I-converting enzyme; ECE, endothelin-converting enzyme and renin); diabetes (e.g., dipeptidyl peptidase-IV (DPP-IV) and α-glucosidase); atherosclerosis (e.g., platelet-activating factor-acetyl hydrolase (PAF-AH) and thrombin inhibition), and others (Gallego et al. 2019; Yoshikawa 2015). Diverse studies in vitro, in silico, ex vivo, and in vivo, have indicated that food proteins and their derived hydrolysates/peptides represent an attractive option for the development of nutraceutical and functional foods with potential use in non-pharmacological therapies to prevent or reduce risks associated to CVD.

3.4.1 Antihypertensive Peptides for Prevention of CVD

Hypertension or continuously high blood pressure can produce damage in vital organs such as kidney and heart. In 2015, 25% of men and 20% of women (representing 1.13 billion people worldwide), suffered hypertension, leading to the primary cause of premature death worldwide (WHO 2019). Blood pressure is regulated by a process known as the renin-angiotensin system and has been the focus of most research on bioactive peptides and functional foods. Briefly, the enzyme renin converts the angiotensinogen to angiotensin I, which is then hydrolyzed by the ACE, releasing the octapeptide angiotensin II (a potent vasoconstrictor). Thus, inhibition of renin or ACE activity plays a significant role in lowering blood pressure during hypertension. Most protein hydrolysates/peptides with potential antihypertensive properties are evaluated according to its capacity to inhibit ACE-activity in vitro, and then its effectivity confirmed in vivo by blood pressure reduction in spontaneously hypertense rats (SHR) (Howard and Udenigwe 2013; Mazorra-Manzano et al. 2018).

Many scientific reports indicate that several food proteins, either from animal or vegetal origin, contain peptide sequences that can inhibit ACE activity. Some proteins can exhibit bioactivity in their intact form after consumption; however, others require to be hydrolyzed to release their bioactive sequences by digestive, fermentative, or hydrolytic processes, using specific proteases. For example, undigested spinach leaf protein (rubisco, ribulose bisphosphate carboxylase/oxygenase, a major leaf protein), did not show any antihypertensive effect when was evaluated in HSR; however, their hydrolysates, prepared with pepsin or pepsin-pancreatin enzymes (ACE IC_{50} 56 and 120 μg/mL respectively), were adequate to reduce blood

pressure after oral ingestion at a minimum dose of 0.25 and 0.5 g/kg, respectively (Yang et al. 2004).

Bioactive peptide sequences can exhibit a beneficial effect if they are absorbed and reach the target site. Therefore, they must resist the digestive process occurring after its ingestion and be absorbed their bioactive form (or its fragments), which will depend on its structure. Permeability of two potential antihypertensives (ECA-Inhibition) peptides (KPLLCS and KPLL), obtained from the digestion of chicken breast, were evaluated ex vivo using the Caco-2 cell model system. The KPLLCS peptide (ECA IC_{50} 0.37 μM) was degraded during digestion, while KPLL (ECA IC_{50} 11.8 μM) was highly permeable and only partially degraded. The released peptide fragments (KP and LL) showed ECA-inhibitory activity but in a lower potency (ECA IC_{50} 8037 and 7870 μM, respectively) (Sangsawad et al. 2018).

A peptide fraction <3 kDa of chicken skin (IC_{50} 130 μg/mL) hydrolysate was produced with a mixture of endo- and exo-peptidases and showed significant suppression of increased blood pressure in SHR. The identified collagen-derived sequences with ACE-inhibitory activity were GAHGLHGP (IC_{50} 29.4 μg/mL) from collagen α1, and GIHGERGPVGPSG (IC_{50} 43.4 μg/mL), GAHGPAGPGGIHGERG (IC_{50} 45.6 μg/mL), and GLHGSRGERGLHG (IC_{50} 60.8 μg/mL) from collagen α2 (Saiga et al. 2008).

It has been well documented that milk proteins are an excellent source of peptides with antihypertensive properties. Casein-derived peptides such as VPP and IPP possess the highest ACE-inhibitory activity of food protein-derived peptides reported until now (ECA IC_{50} of 9 and 5 μM, respectively). The antihypertensive properties shown by fermented milk and by protein hydrolysates from fish, meat, soy, amaranth, chickpeas, and other protein sources, have increased the interest in the production and commercialization of functional foods and nutraceutical products. Some antihypertensive commercialized products include the fermented milk Calpis® and Evolus®, and the capsules petACE® and Vasotensin® from bonito fish hydrolysates (Mazorra-Manzano et al. 2018; Nakamura et al. 1995).

3.4.2 Hypoglycemic Peptides in Diabetes and CVD

CVD is the leading cause of death in adults with diabetes. Type 2 diabetes is characterized by increased glucose in the blood (hyperglycemia) as well as postprandial hyperglycemia. Typically, in response to food ingestion, the gastrointestinal incretins GIP (glucose-dependent insulinotropic polypeptide) and GLP-1 (glucagon-like peptide-1) are secreted into circulation, enhancing the secretion of insulin to control the glucose levels in the blood and inducing satiety by a food intake-modulating effect. These incretins have very short half-lives since they are renal degraded and hydrolyzed by the enzyme DPP-IV. Peptides sequences with the capacity to inhibit DPP-IV decrease blood glucose, increase glucose uptake, and stimulate insulin secretion. Then, bioactive peptides with DPP-IV-inhibitory capacity can lead to obesity and type 2 diabetes treatment (Baggio and Drucker 2007).

Protein hydrolysates containing peptide sequences with DPP-IV inhibitory activity have been the target of recent studies, and several peptide sequences have been reported. They have relatively high potency to inhibit DPP-IV in vitro; however, studies in vivo are still scarce (Lacroix and Li-Chan 2016; Nongonierma and Fitz Gerald 2016). The reduction of plasm DPP-IV activity in diabetic rats after the administration of protein hydrolysates (e.g., milk proteins, fish gelatin, and zein protein) has confirmed its anti-diabetic properties in vivo, observing a reduction of plasm glucose and increased insulin levels (Korish et al. 2020; Nongonierma and Fitz Gerald 2016).

The most potent food protein-derived hypoglycemic peptides reported until now include LPQNIPPL (β-CAS $_{f70\text{-}77}$), LKPTPEGDL (β-Lg $_{f46\text{-}54}$) and GPGA (from Atlantic salmon skin gelatin) with DPP-IV IC_{50} values in the range of 42-45 μM (Lacroix and Li-Chan 2014; Li-Chan et al. 2012; Uenishi et al. 2012). It is essential to mention that some peptides also have shown multifunctional properties (Mazorra-Manzano et al. 2018; Meisel 2004). For example, IQKVAGTW, VLDTDY, and LKALPMH from β-lactoglobulin and WLAHKAL from α-lactalbumin can inhibit ACE and DPP-IV activity, showing a possible beneficial effect in diabetes and hypertension (Lacroix and Li-Chan 2014, 2016). In other studies, milk fermented by a specific strain of *Lactococcus lactis* with ACE-inhibitory activity in vitro showed antihypertensive activity in SHR, enhanced nitric oxide production and reduced the oxidative stress index (i.e., lipid peroxidation and the enhancement of antioxidant enzymes activity SOD and CAT) (Beltrán-Barrientos et al. 2018).

It is widely supported that food proteins from different sources contain several peptides sequences that can exhibit more than one biological property. Antioxidant, antihypertensive, anticholesterolemic, antithrombotic, and antidiabetic peptides have been identified in milk, soybean, amaranth, chickpea, lupin, and cowpea proteins (Boachie et al. 2018; Lacroix and Li-Chan 2016; Sabbione et al. 2016; Zhang 2016). Peptides usually differ in structure, composition, length, and potency, thus exhibiting a different action mechanism. For example, camel milk hydrolysates are more hypoglycemic than bovine milk hydrolysates; however, bovine milk was more effective as antiplatelet/antithrombotic agent in streptozotocin-induced diabetic rats (Korish et al. 2020).

3.4.3 Bioactive Peptides in the Control of Dyslipidemia, Hypercholesterolemia, and Thrombosis

Protein hydrolysates or peptides derived from animal (e.g., milk, chicken, pork, and fish) and plant (e.g., soybean, rapeseed, peanut, and amaranth) proteins, have shown beneficial bioactive properties in CVD by regulating lipid metabolism, reducing absorption and synthesis of cholesterol, inhibiting thrombin and platelet aggregations, reducing oxidative stress of cells and inflammation (Rendon-Rosales et al. 2019; Rodríguez-Figueroa et al. 2013; Sabbione et al. 2016; Saiga et al. 2008; Yang et al. 2004; Yoshikawa 2015).

The platelet-activating factor (PAF), which is catalyzed by PAF-AH, is a pro-inflammatory phospholipid mediator that participates in several inflammatory and vascular diseases. Recent works have focused on the identification of food-derived peptides showing PAF-AH inhibition. These peptides have been considered promising therapeutic targets for the prevention of atherosclerotic lesions. Seven peptides were isolated from the seaweed *Palmaria palmata,* finding that NIGK was the most potent sequence to inhibit PAF-AH (50.74% inhibition at 1 mg/mL) (Fitzgerald et al. 2013). More recently, peptides released from dry-cured ham bones were also able to inhibit PAF-AH. The sequences identified were derived from collagen and hemoglobin and inhibited PAF-AH even after heating and simulated digestion. These treatments released additional bioactive peptides that could block activities of ACE, DPP-IV, and ECE, indicating a possibly beneficial effect on cardiovascular health (Gallego et al. 2019).

Thrombin is a serine protease vitally important during blood clotting, where it converts its soluble substrate fibrinogen into insoluble fibrin. Peptides with thrombin-inhibitory activity (antithrombotic) prevent the proteolysis of fibrinogen and formation of the fibrin clot. However, it can also occur that peptides, binding with the already formed fibrin monomers, prevent its polymerization (Tu et al. 2017; Zhang 2016). Peptides released from glycomacropeptide (k-CAS $_{f106\text{-}169}$) and lactoferrin have demonstrated platelet aggregation inhibition while other casein-derived peptide sequences have also shown excellent antithrombotic properties (thrombin inhibitors) (Rendon-Rosales et al. 2019). On the other hand, a peptide fraction from peanut protein hydrolysate (produced with alcalase) showed 65% of inhibition of thrombin activity (antithrombotic) at the same concentration (0.2 mg/mL) of antithrombotic heparin drugs. Sequences identified in the active fraction were SWAGL, GNHEAGE, and CFNEYG (Zhang 2016). An amaranth protein hydrolysate (produced by autolysis) showed antithrombotic activity in vitro (IC_{50} 5.6 mg/mL) and higher antioxidant activity than its protein isolate (IC_{50}, ORAC 0.1 vs. 0.05; ABTS 5.4 vs. 2.1 mg/mL) (Sabbione et al. 2016). Peptides sequences SSGE and DEE derived from soy protein also showed antithrombotic activity by inhibiting ADP-induced platelet aggregation of rats' blood in vitro (Lee and Kim 2005).

Dyslipidemia or abnormal levels of lipids in the blood occurs when low-density lipoproteins (LDL) and triglycerides are found in high levels (or HDL at deficient levels), thus increasing the risk of developing atherosclerosis. This last event develops when fatty deposits called plaques accumulate in blood vessels, making it difficult for the blood to flow, causing major circulation problems, thus promoting heart attacks and strokes. Different approaches have been used to decrease these disorders, such as cholesterol-lowering, hypolipidemic, and antithrombotic agents. Anticholesterolemic peptides can bind bile acids, inhibit cholesterol micellar solubility, or show statin-like activity (HMGCoAR, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) by interacting as sterol regulatory element-binding protein (Boachie et al. 2018). Several food proteins have been suggested to be hypocholesterolemic such as milk and soy proteins and its hydrolysates/peptides through the reduction of cholesterol biosynthesis, its uptake, and secretion and by

decreasing its micellar solubility. The hypocholesterolemic effect of amaranth flour (AF) was compared with amaranth isolate (AI), observing that flour shows the displacement of cholesterol in model micelles (in vitro), and increased the cholesterol excretion through faces with higher efficiency than its protein isolate (108% vs. 23%). However, the reduction in hepatic cholesterol accumulation in vivo was inversed (53% vs. 93%). Besides, their digested products (AFD and AID) do not increase the displacement of cholesterol in vitro (IC_{50} 0.1 vs. 0.71 and 0.2 vs. 2.1 for AF vs. AFD and AI vs. AID, respectively). The hypocholesterolemic effect of AF and AI indicate that protein and the presence of fiber influence cholesterol metabolism (Sisti et al. 2019). On the other hand, four peptides from soy (glycinin and β-conglycinin) and two from lupin-protein shown HMGCoAR-inhibitory activity. It was observed that the peptide lupin-protein derived LTFPGSAED (IC_{50} 68 μM) had a higher potency and was also transported across the Caco-2 cells (Boachie et al. 2018).

Protein hydrolysates from different food sources have also shown a hypolipidemic effect in animal studies by decreasing serum and hepatic TC and LDL + VLDL or by increasing fecal excretion of lipids and bile acids. Several peptides derived from soy (mainly soy glycinin and conglycinin) and milk proteins (i.e., β-lactoglobulin) have shown a hypolipidemic effect in vivo (i.e., rats) and ex vivo (i.e., cultured Caco-2 and HepT9A4 cells) (Howard and Udenigwe 2013). Milk fermented by *Lactococcus lactis* strains with antihypertensive properties were also able to reduce plasma low-density lipoprotein cholesterol and triglyceride contents in SHR (Rodríguez-Figueroa et al. 2013). Inhibition of thrombin-induced fibrin polymerization, anticoagulant activity and the inhibition of the micellar solubility of cholesterol and its bile acid-binding capacity, indicate that some lactic acid bacteria strains can release peptides with both properties with possible cardiovascular health benefits (Beltrán-Barrientos et al. 2018; Rendon-Rosales et al. 2019).

3.4.4 Structural Features of Bioactive Peptides Related to CVD

Bioactive peptides usually are in the range of 2–20 amino acids length with IC_{50} values in the range of μg-mg/mL (mM-μM), depending on its properties with IC_{50} values significantly higher than drugs used for the same purpose. Structure-function relationship of bioactive peptides has been studied recently, determining that depending on their structure, the sequence of amino acids, and charge, they could exhibit some specific biological function. For example, peptides containing hydrophobic (aromatic or branched side chain) amino acid residues at three C-terminal positions possess a vigorous ACE-inhibitory activity, where Pro is preferred (e.g., IPP and VPP). In addition, the positively charged amino acids Arg and Lys residues at the C-terminus contribute to the ACE-inhibitory activity of peptides. On the other hand, the presence of His in high amounts and hydrophobic amino acids in peptides can contribute to their antioxidant potency, such as the one showed by PHH with higher antioxidant activity among several peptides sequences evaluated (Erdmann

et al., 2008; Wang and Gonzalez-de-Mejia, 2005). Negatively charged amino acids in peptides may influence their antithrombotic potency. Docking studies about the interaction between enzymes and inhibitors have predicted that Trp and Pro at N-terminal position 2, show relatively potent inhibition of DPP-IV, which is in concordance with diprotin (Ile-Pro-Ile), a well-known DPP-IV inhibitor (Lacroix and Li-Chan 2015). The structural features of peptides such as length, charge, and amino acid sequence are the most critical factors that determine its biological activity to inhibit enzymes related with metabolic disorders in CVD. Therefore, structural characteristics of peptides should be more deeply investigated (Nongonierma and Fitz Gerald 2014).

3.5 Bioactive Peptides and Diabetes

Diabetes mellitus is a metabolic disorder of global importance due to its high prevalence and progressive increase in recent years (Kehinde and Sharma 2020). There are two types of diabetes mellitus. Type I derives from the failure of the pancreas to secrete insulin due to the destruction of beta cells (that synthesize and secrete insulin) and has a prevalence of around 5–10%. On the other hand, type II diabetes mellitus is characterized by insulin secretion deficiency and the inability of the body to use insulin (insulin resistance). As a result, tissues cannot use blood glucose for energy, and long-term high plasma glucose concentrations lead to severe consequences such as renal impairment, diabetic neuropathy, blindness due to retinopathy, and cardiovascular disease (Ramadhan et al. 2017). Type II diabetes mellitus represents 90–95% of the cases and has shown a gradual increase worldwide, rising from 4.7% of world's population (108 million) in 1980 to 8.5% (422 million) in 2014; current estimations indicate that the number of cases may reach 592 million cases by 2035 (Kehinde and Sharma 2020; Lee et al. 2016).

Sedentarism, high body mass index, aging, and inheritance are well-known factors for type II diabetes mellitus development (Lauritano and Ianora 2016). Therefore, a lifestyle change, including proper eating habits, regular exercise, and medication, is required to prevent or reduce short and long-term effects of diabetes and hyperglycemia (Yu et al. 2011). Pharmacologic therapies for diabetes mellitus type II comprise biguanides, GLP-1 receptor agonists, meglitinides, sulphonylureas, thiazolidinediones, gliflozins (SGLT-2 inhibitors), as well as inhibitors of enzymes involved in the regulation of postprandial hyperglycemia as α-amylase, α-glucosidase, and DPP-IV (Kalita et al. 2018; Wang et al. 2019a). Several of these therapies have shown toxicity and severe side effects such as the increased risk of kidney injury, vascular disease, pancreas infection, and bladder cancer (Chaudhury et al. 2017; Qaseem et al. 2017).

Bioactive proteins, protein hydrolysates, and peptides obtained from conventional and non-conventional food sources have demonstrated the ability to provide a natural replacement or complement to pharmaceutical approaches in diabetes therapy, having minor side effects based on their natural origin. Inhibition (in vitro)

of α-amylase, α-glucosidase, and DPP-IV is the conventional approach to evaluate the antidiabetic potential of proteins, hydrolysates, and peptides by determining their half-maximal inhibitory concentration (IC_{50}) or the percentage of inhibition on such enzymes (Kehinde and Sharma 2020; Li-Chan 2015). When administrated orally or intravenously, complementary determinations of the antidiabetic potential of bioactive proteins, hydrolysates, and peptides, include the estimation by in vivo studies with humans and laboratory animals, of the increase in insulin production, enhanced insulin sensitivity, and hypoglycemic effect, among others (Kehinde and Sharma 2020).

3.5.1 Antidiabetic Bioactive Peptides from Milk

Milk, cheese, whey protein, and specific protein (α-lactalbumin, β-lactoglobulin, lactoferrin, and casein) hydrolysates obtained by enzymatic digestion with several enzymes for example trypsin, pepsin, chymotrypsin, and pancreatin, among others, have shown in vitro (mainly DPP-IV and α-glucosidase inhibition) antidiabetic potential (Jan et al. 2016; Lacroix and Li-Chan 2012). In vivo experiments have shown mainly reduction in blood plasma glucose and DPP-IV inhibition (Lacroix and Li-Chan 2013; Uchida et al. 2011). Interestingly, oral intake of both milk protein and milk protein hydrolysate by diabetic rats reduced the plasmatic glucose and lipid levels so that milk protein hydrolysate could be used as an antidiabetic agent (El-Sayed et al. 2016). Enzymatic digestion of egg yolk and egg white protein also render peptides with in vitro antidiabetic activity (α-amylase, α-glucosidase, and DPP-IV inhibitors) (Yu et al. 2011; Zambrowicz et al. 2015).

3.5.2 Antidiabetic Bioactive Peptides from Marine Organisms

A few fish protein hydrolysates have shown to stimulate glucose uptake in vivo (Cheung et al. 2015). Enzymatic digestion of fish collagen and fish skin gelatin has shown to inhibit DPP-IV (in vivo and in vitro), to enhance both GLP-1 and insulin secretion in vivo (Wang et al. 2015), and to decrease blood fasting glucose and insulin levels in diabetic patients (Zhu et al. 2010).

3.5.3 Antidiabetic Bioactive Peptides from Plant Origin

Bioactive antidiabetic peptides are also obtained from many plants, being cereals and pseudocereals well-recognized sources. Rice, amaranth, and quinoa enzymatic hydrolysates have shown α-amylase, α-glucosidase, and DPP-IV inhibitory activities (Kehinde and Sharma 2020). Enzymatic hydrolysis of oats protein and cumin

seeds also generates interesting antidiabetic peptides (Yan et al. 2019; Zhang et al. 2015b). Legumes are a rich source of antidiabetic peptides. Several common bean varieties and soybean have shown to release antidiabetic peptides after enzymatic digestion and microbial fermentation, showing α-amylase, α-glucosidase, and DPP-IV inhibition; additionally, these bioactive peptides have shown to reduce blood glucose levels, and to enhance insulin secretion and glucose uptake in vivo (Kehinde and Sharma 2020; Yan et al. 2019).

Peptides and hydrolysates from fruits like walnut and watermelon seeds have shown to have anti-diabetic properties (Kehinde and Sharma 2020). Antidiabetic peptides have also been obtained by enzymatic hydrolysates from rapeseed napin, cannabis seed protein, and seaweed protein (Admassu et al. 2018; Ren et al. 2016; Xu et al. 2019). Five novel antidiabetic peptides were obtained from an unconventional food source: the Chinese giant salamander muscle. Such peptides strongly inhibited α-glucosidase and α-amylase, and, interestingly, one of the peptides inhibited both enzymes (Ramadhan et al. 2017). There are many unexplored food and plant sources for antidiabetic peptides. In this sense, byproducts of fruit industrialization and plant oil refining industry represent a sparsely exploded source of bioactive peptides (Balandrán-Quintana et al. 2019), among them antidiabetic ones.

Recently, a purified nitric oxide-generating protein from garlic was injected into diabetic mice and significantly reduced the blood sugar and increased insulin level in the animals. The protein also increased insulin-release, Glut-4 synthesis, and glucose uptake in the liver and β-cells of diabetic animals (Bhattacharya et al. 2019).

3.6 Effects of Bioactive Peptides on Cancer Disease

Cancer is a group of diseases identified by uncontrolled growth and spread of abnormal cells that may be induced by external factors like radiation, chemicals, and infectious organisms, or by internal factors such as mutations and altered hormonal and immune states (Tanaka 1997). Cancers are among the top causes of deaths worldwide, and the number of cases is expected to increase by approximately 70% over the next 20 years (González-Montoya et al. 2017). Cancer is mainly treated by invasive surgical methods and with radiotherapy and chemotherapy (Hubenak et al. 2014). Nevertheless, traditional chemotherapeutical drugs are not specific to target (tumor) cells and produce many side effects. Furthermore, chemotherapy also fails because of multidrug resistance (Huang et al. 2014).

Under such panorama, peptide-based drug therapies have received more attention because of their specificity, low toxicity, small size, tumor penetrating specificity, and easy modification (Barras and Widmann 2011). Peptides used in cancer therapy can bind to specific molecular targets on tumor cells and regulate the biosynthesis of malignant cells, they can serve as a drug delivery system or can induce specific immunological (T cell) responses to tumor cells (González et al. 2014; Xiao et al. 2015). Some peptide-based cancer therapies have shown promising results when tested in vivo and in vitro. Though, some of the clinical trials have shown

reduced effectiveness. Thus, novel methods like the combination of peptides with nanomaterials, personal peptide vaccination, and improved delivery systems have been tested in clinical trials with favorable results (Xiao et al. 2015).

Numerous studies have shown that terrestrial plants and animals, as well as organisms from marine environments, are relevant sources of bioactive proteins and BP with anti-cancer activity. Plant sources include cereals like wheat, barley, corn, and rice (Díaz-Gómez et al. 2017; Malaguti et al. 2014); pseudocereals like amaranth and quinoa (Huerta-Ocampo and Barba de la Rosa 2011; Vilcacundo et al. 2018), as well as legumes like soybean, bean, chickpea, pea, fava bean, and lentil (González-Montoya et al. 2017). Plant BP are usually generated by enzymatic hydrolysis and fermentation. However, germination, a natural hydrolytic process, has shown to improve the anti-proliferative effect of soybean protein on cervical cancer cells (Mora-Escobedo et al. 2009). Some dietary proteins (lectins) from legumes and soybean are resistant to the passage through the gastrointestinal tract and can enter the systemic circulation. Soybean agglutinin demonstrated to inhibit tumor growth in rats and improve life expectancy in mice (Malaguti et al. 2014). Whereas, lectins from Tepary bean displayed differential antiproliferative effect on non-transformed cell and different cancer cell lines (García-Gasca et al. 2012).

It has been described that higher consumption of legumes can considerably decrease the risk of colorectal adenoma (Wang et al. 2013b), whereas BP obtained from legumes has been reported to have productive anticancer activity (Mora-Escobedo et al. 2009). Bowman-Birk inhibitors isolated from *Glycine max*, *Pea sativum*, *Vicia faba,* and *Lens culinaris*, have shown anticancer effects in HT and HepG2 cells (Caccialupi et al. 2010; Clemente et al. 2012; Fang et al. 2011). Different peptides from chickpea and distinct common bean cultivars demonstrated anticancer activity on MCF-7, HCT-116, CNE-1, HNE-2, HepG2 and MDA-MB-231 cell lines (Fang et al. 2010; Lam and Ng 2011; Luna Vital et al. 2014; Xue et al. 2015). Additionally, the anticancer peptide X-MLPSYSPY and Lunasin, a 43 amino acid residues peptide isolated from soybean and other legumes, has shown to inhibit chemical carcinogen-induced transformation and selectively induction of apoptosis in transfected cells but not in non-transformed cells (de Mejia and Dia 2009; Galvez et al. 2001; Kim et al. 2000). Lunasin was also detected in cereals and pseudocereals, and bioactive properties of lunasin have been attributed to the capacity to inhibit histone acetylation, to arrest cell division in cancer cells, and to protect DNA from oxidative damage (Malaguti et al. 2014). Besides legumes, cereals, and pseudocereals, many other plants can be a source of proteins and peptides with anticancer activity. Walnut protein hydrolysates and proteins extracted from *Gynura procumbens* have shown interesting anticancer properties (Hew et al. 2013; Jahanbani et al. 2016), just to mention a couple of examples.

Bioactive peptides released from milk proteins have shown anti-cancer properties. Furthermore, this type of peptides can be isolated from fermented milk and milk products (Sah et al. 2015). Some casein derived peptides have shown to induce necrosis of leukemic cell lines (Otani and Suzuki 2003), have demonstrated cytotoxic activity against melanoma cells (Azevedo et al. 2012), and have inhibited proliferation of human ovarian cancer cells (Wang et al. 2013a). Additionally,

hydrolyzed casein has a β-glucuronidase inhibitory activity (Gourley et al. 1997). Lactoferrin digestion released peptides that exerted cytotoxic activity against fibrosarcoma, melanoma, and colon carcinoma cell lines (Eliassen et al. 2006), induced apoptosis in breast cancer cell cultures (Furlong et al. 2006), and displayed the capacity to inhibit angiogenesis in vitro and in vivo (Mader et al. 2006). Peptides released from fermented milk, peptides extracted from high-speed centrifugation of yogurt and isoelectric extraction of kefir, exhibited antioxidant, and antimutagenic activity (Sah et al. 2015).

Fish byproducts (viscera, heads, bone, and skin) are not marketable but can be recycled after processing. Production of a fish protein hydrolysate is the most common approach to use fish byproducts, and the type of peptides released is highly dependent on hydrolysis conditions: proteases, temperature, pH, chemicals, and time of hydrolysis (Nurdiani et al. 2017). Peptides isolated from dark tuna muscle treated with papain showed an antiproliferative effect on human breast cancer cells (Hsu et al. 2011). Snow crab byproducts treated with Protamex® produced peptides with toxicity against colon, breast, prostate, and lung cancer cell lines (Doyen et al. 2011). Sepia ink oligopeptides produced by trypsin digestion inhibited proliferation of DU-145 (Human prostate cancer) cells (Ding et al. 2011). Peptides released from lobster and shrimp shells demonstrated to inhibit the growth of colon (Caco-2) and liver (HepG2) cancer cells (Kannan et al. 2011). Similarly, backbones, skin gelatin, and fresh fileting byproducts from fishes subjected to different enzymatic treatments have shown antiproliferative effects on HepG2, hFOB 1.19 (osteoblastic) and breast cancer cell lines (Nurdiani et al. 2017). However, despite that the peptides derived from fish byproducts have demonstrated anticancer activities, cytotoxicity of such peptides on healthy cells is rarely discussed (Nurdiani et al. 2017).

Discovery of bioactive peptides and proteins with anti-cancer properties in terrestrial (plants and animals) and marine sources, as well as in their byproducts, is expected to lead to a broader market in the food-based therapies against cancer. Therefore, the discovery of new peptides with anticancer properties, and the formulation of functional foods based on bioactive proteins or their hydrolysates, demand rigorous tests to guarantee the effectiveness and safety of these formulations.

3.7 Bioactive Peptides and Degenerative Neurological Disorders

Degenerative neurological disorders (DND) are diseases that destroy neurons and neural communication. While the etiology associated with these disorders remains poorly understood, the incidence of neurodegeneration will convert into a public health problem in a few years due to the aging population (Gaugler et al. 2016). Therefore, it is necessary for a greater understanding of each disorder's etiology to develop, timely diagnosis, and effective treatments.

3.7.1 Bioactive Peptides and Alzheimer

Alzheimer's neurodegenerative disease (AD) is a brain disorder that causes 60–70% of cases of dementia. It is the principal cause of disability in later life (Gaugler et al. 2016). AD is irreversibly and progressively damages brain cells causing memory loss, thinking skills, and subsequent premature death 3–9 years following diagnosis (Scheltens et al. 2016). The causes of Alzheimer's are unknown. The most accepted hypotheses are the amyloidogenic ($_{\beta}$AH), and the tau proteins phosphorylation (Folch et al. 2018). A typical characteristic of AD is the extracellular accumulation of plaques between neurons formed by β-amyloid peptides. According to $_{\beta}$AH, this peptide accumulation (in particular, Aβ4, peptide) interferes with essential processes for neurons such as communication, repair capacity, metabolism, and neurogenesis leading to the death of nerve cells and subsequent behavioral/psychiatric changes (Mucke and Selkoe 2012). The tau protein hypothesis proposes that the leading cause of AD is the hyperphosphorylation of the 3R and 4R tau proteins (Folch et al. 2018). Tau proteins are involved in the microtubule stabilization of nerve cells, which is destabilized by the post-translational modification (hyperphosphorylation) of these proteins, causing cytoskeletal abnormalities (Zhang et al. 2015a). In this context, research has been focused on anti-amyloid Aβ42 production and anti-tau protein hyperphosphorylation for AD treatment. However, these treatments have not been effective in stopping the disease progression because of the multifactorial AD etiology (Folch et al. 2018).

Recent studies confirm that the complexity of AD pathophysiology is greater than the transformation of amyloid peptides and tau proteins. Metabolic alterations (insulin resistance, cholesterol homeostasis), chronic brain inflammation, oxidative stress, dendritic neuropathology, and influence of bacteria such as *P. gingivalis* have also been observed (Cochran et al. 2014; De Felice 2013; Ferreira et al. 2014; Ide et al. 2016). To develop more effective treatments is necessary to consider these new findings. According to the United Nations, the number of people with AD and other dementias will reach 152 million by 2050, if adequate therapies are not discovered (Patterson 2018).

The four approved drugs for the treatment of AD act (1) as inhibitors of acetylcholinesterase that increases cholinergic transmission in neuronal synapses (AChEI)., or (2) by blocking receptors for N-methyl-D aspartate (NMDAR antagonists) that decrease brain excitotoxicity (Folch et al. 2018). There are currently no approved drugs based on peptides or natural proteins. However, some of these molecules are being studied due to their neuroprotective activity. BP may occur naturally in foods or can be found encrypted in plant and animal proteins. In the last case, it can be released either by enzymatic hydrolysis or by microbial fermentation (Chakrabarti et al. 2018).

Apoptosis inhibition helps to reduce neuronal damage in neurodegenerative diseases (Balez et al. 2016). It has been shown that several peptides of animal origin can block some specific elements of the apoptotic signaling. For example, peptide MQIPVLTLTG from venison muscle, decrease the population of cells positive to

Annexin V, suppress the Cytochrome C release, and regulate the expression of apoptosis-related genes like those encoding to produce caspases 3 (Kim et al. 2010). PAYCS and CVGSY peptides obtained by hydrolysis of anchovy muscle using papain, pancreatin, and alcalase, also inhibit apoptosis (Zhao et al. 2017). Neural death in AD can also be reduced by decreasing oxidative stress. Overproduction and long-time exposure of reactive oxide species (ROS) cause an antioxidant disbalance leading to synapse loss, mitochondrial disfunction, receptor cell trafficking, communication perturbation, and disbalance in cellular homeostasis accompanied by a disfavored antioxidant status (Tönnies and Trushina 2017). ROS and other molecules of oxidative stress (nitric oxide, peroxynitrite) also alter the function of cellular and mitochondrial DNA, lipids, proteins, and energy production leading to neuron death (Huang et al. 2016). Whey protein hydrolysates, DWMH peptide from walnut, and PAYCS and CVGSY from anchovy show antioxidant capacity and neuroprotective activity (Chen et al. 2015a; Zhao et al. 2017).

Experimental and clinical evidence indicates that peptides inhibitors of DPP-IV may reduce ROS formation, mitochondrial dysfunction, and neuroinflammation, and also control tau protein hyperphosphorylation and amyloid plaque aggregation (Kosaraju et al. 2013a; Kosaraju et al. 2013b). Many investigations have demonstrated the DPP-IV inhibitor capacity of peptides from food origin (Table 3.1). For example, PGVGGPLGPIGPCYE, CAYQWQRPVDRIR, and PACGGFWISGRPG peptides obtained from tuna cooking juice hydrolysates showed DPP-IV inhibitor activity in a dose-dependent manner (Huang et al. 2012). Other inhibitor peptides obtained from casein (LPQNIPPL), salmon skin gelatin (GPAE), and rice protein (LP and IP) have been reported (Hatanaka et al. 2012; Li-Chan et al. 2012; Uenishi et al. 2012).

Additionally, peptides from food origin have shown different bioactivities associated with possible treatment for AD, like inhibition of acetylcholinesterase (AChE), or anti-inflammation activity (Table 3.1). However, there are different challenges to overcome so that these peptides can be used commercially. Some of these challenges are isolation and purification, large scale production, quality aspects, taste, and transfer through the blood-brain barrier (BBB) (Chakrabarti et al. 2018).

A promising protein related to AD and other neurodegenerative diseases treatments is lactoferrin (Lf). Lf is a non-heme iron-binding mammalian glycoprotein (~80 kDa, ~700 aa), secreted mainly in milk, saliva and tears. It is industrially produced by cow milk and used as a health-promoting protein (Wakabayashi et al. 2018). Lactoferrin supplementation to three-day-old male piglets induced the expression of genes related to:

- Neural development and cognition;
- Organization of brain cell structure (cytoskeleton, microtubule dynamics, the formation of cytoplasm projections, neurites formation);
- Diminution of anxiety (Chen et al. 2015b).

Mohamed et al. (2019) conducted a pilot study to determine the role of 3-month supplementation of bovine Lf in patients with AD. After supplementation, patients showed a decrease in many AD-related markers (Table 3.2). This and other studies

Table 3.1 Neuroprotective peptides isolated from food-origin proteins

Source	Peptide	Obtention	Model	Effects	Researchers
Venison muscle	MQIPVLTLTG	Papain; pH 6.0, 37 °C, 8 h	PC-12 cells neuroprotection model	↓Apoptosis by gen regulation; ↓Nitric Oxid production. ↑antioxidant enzyme activities	Kim et al. (2010)
Porcine (*Sus scrofa*) myofibrillar protein	DSGVT; IEAEGE DAQEKLE; EELDNALN VPSIDDQEELM	Papain in water pH 7.0, at 37 °C for 24 h with 1/100 (w/w)	In vitro measurement of: Hydroperoxides, DPPH radical scavenging and metal ion chelating activities	High antioxidant activity in a linolenic acid peroxidation system induced by Fe(2+). Metal ion chelating activity	Saiga et al. (2003)
Porcine hide gelatin	Hydrolysate; 50 mg/mL; 1000–3000 Da	Pepsin and papain, pH 7.0 (phosphate buffer, 20 mM), 37 °C	SH-SY5Y cells. Cell survival was evaluated by the ability to reduce 3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide (MTT)	Improvement of cell viability; neuroprotective effect	Wang et al. (2008)
Anchovy (*Coilia mystus*)	PAYCS; CVGSY	Papain, pancreatin, pH 7.0, 8 h, 55 °C	PC12 cell line	AChE inhibitory activity; Reduction of: lactate dehydrogenase release, malondialdehyde content, reactive oxygen species production, and the ratio of Bax/Bcl-2 of glutamate-induced apoptosis	Zhao et al. (2017)
Walnut (*Juglans regia* L.)	Protein hydrolysates	Papain pH7.0, 5 h, T 50 °C	PC12cell line; Wild-type AB strain of zebrafish; ICR mice (weighing 18–22 g)	Protection to cultured PC12 cells against H_2O_2-induced oxidative stress; Neuroprotective effect in zebrafish model; amelioration of learning and memory impairments in mice model	Liu et al. (2019)

(continued)

Table 3.1 (continued)

Source	Peptide	Obtention	Model	Effects	Researchers
Nile tilapia (*Oreochromis niloticus*) skin gelatin	GIV, GAP*GF, GFA*GPA, SGNIGFP*GPK, GIPGPIGPP*GRP	Alcalase pH 8, 50 °C, 60 min	In vitro Angiotensin I-converting enzyme (ACE)-inhibitory activity	ACE-inhibitory activity (IC_{50}) of 1.2 mg/m	Thuanthong et al. (2017)
Shrimp (*Pandalopsis dispar*) waste hydrolysates	DVLFH	Protamex® (*Bacillus amyloliquefaciens* and *Bacillus licheniformis*, 1.5 AU/g) 50 °C, 4 h	In vitro commercially available fluorogenic substrate, MCA–EVKMDAEFK–(DNP)–NH_2	β–secretase inhibitory activity, IC_{50} 92.70 μM	Li-Chan et al. (2016)
Grass carp (*Ctenopharyngodon idella*) skin	PYSK, GFGPEL, VGGRP	Alcalase; enzyme/substrate ratio 6.3%, 52 °C, pH 8.5; 115 min	In vitro antioxidant activity	High scavenging activity on DPPH radical, hydroxyl radical, and ABTS radical in a dose-dependent manner	Cai et al. (2015)

Table 3.2 Lactoferrin effect in *neurodegenerative diseases models*

Via	Model	Effect	References
Holo-Lf and Apo-Lf, 5–15 mg/kg, for 7 days	Male C57BL/6 mice PD model, aged 9–10 weeks $n = 120$	Protection against iron dysregulation, oxidative stress, and apoptosis with apo-Lf showing greater efficacy	Liu et al. (2020)
Oral administration of 250 mg/day for 3 months	Fifty AD patients (Men $n = 28$ and woman $n = 22$)	Alleviation the AD pathological cascade and cognitive decline via modulation of the p-Akt/PTEN pathway	Mohamed et al. (2019)
Nutraceutical product based on lactoferrin liposomes	*Caenorhabditis elegans* wild and transgenic type AD models	Protection against acute oxidative stress and extended lifespan of *C. elegans*; Paralysis of transgenic *C. elegans* strain CL4176, caused by Aβ1-42 aggregates, was clearly ameliorated by treatment	Martorell et al. (2016)
Orally 500 mg/kg/day via intragastric tube for 12 weeks	Male albino rats; aged 12–16 weeks old, $n = 30$	Alleviation of memory impairment induced by lipopolysaccharide; antioxidant activity	Madi and El-Saka (2018)
Daily injection of 100 mg/kg for 15 days	Adult male Wistar rats weighing 180–200 g	Antihyperalgesic and antiallodynic effects in neuropathic rats	Madi and Saka (2018)
Holo-Lf and Apo-Lf (human recombinant) Intraperitoneal injection	Rat MS model and Rat PD model	Apo-Lf induced the synthesis of neuroprotective molecules like erythropoietin and Nrf2 signal pathway	Zakharova et al. (2018)
Intraperitoneal injection of deferasirox-Lf conjugates	Rat model of AD	Attenuation of learning deficits	Kamalinia et al. (2013)
Intranasal human lactoferrin (hLf) 2–6 mg/kg/day for 3 months	Male APP/PS1 mice AD model (six months year old) $n = 24$	HLf enhanced the non-amyloidogenic metabolism of amyloid precursor protein; reduction of oxidative stress and neuroinflammation	Guo et al. (2017)

AD Alzheimer disease, *MS* multiple sclerosis, *PD* Parkinson disease

showed evidence about the protective effect of Lf supplementation in AD (Table 3.2). Possible mechanisms are iron sequester and antioxidant effect. However, additional studies over higher point immune functions are necessary.

Lactoferrin has also been proposed as a non-invasive biomarker for the detection and monitoring of AD in saliva (EP3171174A1; EPO patent). A significant increase in the concentration of Lf in saliva has been observed in patients with AD. This increase could be related to the neuroprotective, anti-inflammatory, and anti-oxidant effects of Lf and its ability as a chelator of iron deposited in the brain of patients with AD (Carro et al. 2017). Another possible application of Lf in AD is like brain-target-ligand conjugated with nanocarriers for the delivery of drugs and bioactive (Babazadeh et al. 2020). Lf can penetrate the BBB via receptor-mediated

transcytosis. This drug delivery system would take advantage of the significant increase in Lf-receptors observed in the brains of neurodegenerative disease patients (Wang et al. 2019b).

3.7.2 Bioactive Peptides and Multiple Sclerosis

Epidemiological studies indicate that approximately 2.5 million people were affected by multiple sclerosis (MS) around the world in the past decade (McFarland and Martin 2007). MS is a chronic autoimmune neurological and degenerative disease in which the immune system mistakenly attacks proteins of the myelin sheath surrounding nerve cells of the central nervous system (CNS). This leads to chronic inflammation of the CNC, breakdown of the BBB, axon damage by demyelination and lesion formation along the nerves, in the brain and spinal cord that decrease or impede the conduction of nerve stimuli (Dobson and Giovannoni 2019).

Symptoms of MS are unpredictable as they can vary significantly between patients and change or fluctuate throughout the disease (McFarland and Martin 2007). Among the common symptoms are weakness, fatigue, tremor, vision loss, seizures, vertigo, spasticity, depression, cognitive changes, pain syndromes, and speech, swallowing, breathing, bladder, bowel, and walking problems (Dobson and Giovannoni 2019).

At present, there is no cure for MS. However, several immunosuppressive agents are used as therapy for relapse and brain injury prevention (Badawi and Siahaan 2012). This kind of therapy increases the risk of opportunistic infections. Hence it is necessary to develop more specific therapeutic agents and look for bioactive molecules that help to reduce the symptoms of MS. Cyclotides are disulfide-rich cyclic peptides (27–37 amino acid long, including 6 Cys) produced by plants (Huang et al. 2019). These highly stable molecules can be found in flowers, leaves, stems, and roots of Fabaceae, Cucurbitaceae, Rubiaceae, Solanaceae, and Violaceae family plants (Craik and Du 2017). Cyclotide [T20K]kB1 derived from cyclotide kalata B1 purified from *Oldenlandia affinis* DC (Rubiaceae), inhibits T_H17 proliferation in an MS mouse model experimental autoimmune encephalomyelitis (Thell et al. 2016). T_H17 is an autoreactive T lymphocyte subset that causes demyelination, inflammatory cell influx into the CNS, axonal damage, and neuronal degradation (McFarland and Martin 2007). In vivo activity of [T20K]kB1 is sequence-specific, producing a significant reduction of demyelination and inflammation in the MS mouse model. In addition, oral treatment with daily lower doses was effective in preventing disease prevention. Consequently, [T20K]kB1 oral activity represents a promising alternative for the treatment of MS (Thell et al. 2016). However, more studies are needed to understand better the mechanisms of action of cyclotides in MS treatment.

Oral administration of the iron-binding glycoprotein lactoferrin (Lf) accelerates the recovery of Lewis rats in an experimental autoimmune encephalomyelitis MS

model. In addition, Lf reduced serum pro-inflammatory TGFβ and TNF-α cytokines associated with the progression of MS disease, and also decreases inflammation in the spinal cord of the treated rats (Zimecki et al. 2007). Other studies showed that prolonged administration of bovine Lf (bLf) decreases neuropathic pain in adult male Wistar rats (Onal et al. 2010). Fifteen days injection of bLF (50–100 mg/kg/day), also decreased c-Fos (a neural marker of pain) and NADPH-d immunoreactivity and TNF-α and nitric oxide expressions (Onal et al. 2010). These results confirm the immune modulator and anti-inflammatory activity of LF associated with neurogenerative disease (Kruzel et al. 2017). Moreover, Lf could serve as an essential element to direct drugs to the BBB of patients with MS. Targeting delivery of drugs into the brain is physically restricted by the BBB, but Lf can penetrate the BBB via receptor-mediated transcytosis (Wang et al. 2019b), indicating the opportunity of Lf as a brain-targeting ligand (Chen et al. 2010). Yu et al. (2012), developed a brain drug delivery system based on biodegradable PEG-PLGA polymersomes conjugated with 101 Lf molecules (Lf101-POS) and loaded with S14G-humanin peptides. Lf101-POS not only acted as a carrier for the S14G-humanin peptides but also protected them from protease attack. Under these conditions, S14G-humanin peptides could be successfully internalized into the brain, producing a neuroprotective effect in murine animal models and controlling the overexpression of brain cell apoptotic promoters. These findings position Lf101-POS as a promising brain drug delivery system for the treatment of neurodegenerative disease. In addition, several investigations are being carried out for the synthesis of other brain drug carriers (dendrimers, liposomes, nanoparticles) that include Lf as a brain-targeting ligand (Chen et al. 2010; Gao et al. 2010; Gao 2016; Huang et al. 2013; Liu et al. 2018; Su et al. 2014).

Other important neurodegenerative diseases are Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, frontotemporal dementia, and the spinocerebellar ataxias. All of them share similar symptoms to AD and MS, like degeneration of CNS, oxidative stress, permanent inflammation, damage of neuron axon, and destruction of the myelin sheath (Dugger and Dickson 2017). Since the main effect of naturally occurring peptides and proteins is a neuroprotective effect, it is possible to expect that these molecules can also be useful in relieving symptoms of neurodegenerative diseases other than AD and MS.

Available evidence suggests that peptides from food-origin can exert neuroprotective in DND models. However, some challenges must be overcome for the use of these peptides in approved treatments, such as large-scale production. An alternative could be the synthesis of peptides with sequences equal to those that have been effective. Before this occurs, more studies are necessary to understand the mechanisms of action of each peptide on DND. In addition, human studies should be conducted to confirm the neuroprotective effect of selected peptides. Lactoferrin is a promising protein for the diagnosis and alleviation of DND. In addition, its ability to cross the BBB makes Lf, an excellent candidate to be used as a targeting signal for brain delivery devices loaded with peptides, bioactive, or drug treatments.

3.8 Effects of Bioactive Peptides on Diseases of the Oral Cavity

Dental caries and periodontal conditions are the most prevalent oral cavity infectious diseases in humans and represent a first-rate public health problem that affects all countries of the world. The WHO reports that more than 530 million children suffer from dental caries of primary teeth, and 2.3 billion people suffer from caries of permanent teeth, while severe periodontal diseases that lead to tooth loss affect 10% of the global population (WHO 2020). Caries is a condition that starts with the formation of a polymicrobial biofilm on the tooth surface, known as dental plaque, and in advanced stages, it causes the dissolution of the enamel and the dentin of the teeth (Levine 2011). Periodontal diseases (gingivitis, periodontitis) are chronic inflammations that affect the supporting tissues of the teeth (Dashper et al. 2007).

In recent decades epidemiological associations have been reported between dental caries or periodontal conditions with systemic diseases (Seymour et al. 2007). On the one hand, caries is frequently related to the development of endocarditis (Leishman et al. 2010). On the other hand, periodontal conditions predispose to the development of atherosclerosis, Alzheimer's disease, adverse pregnancy outcomes, and different types of cancer that include the mouth, head, neck, gastrointestinal, and colorectal. Recent worldwide statistics reveal that the oral squamous cell carcinoma (mouth, head, neck) has increased alarmingly and represents 90% of all cancers (Bui et al. 2019; Chattopadhyay et al. 2019; Zhang et al. 2018). Likewise, oral infections significantly increase the risk of complications in individuals suffering from chronic diseases such as diabetes, respiratory diseases, and even osteoporosis (Seymour et al. 2007). In this situation, the WHO has recommended that all countries promote global efforts and develop strategies to prevent oral infections from improving the general health of the population and reducing public health expenses.

Among the strategies addressed by scientists to prevent oral diseases, the use of bioactive peptides stands out. To date, some peptides of animal and plant origin have been identified, which could affect different stages in the caries formation and periodontal infections, as well as the oral squamous cell carcinoma.

3.8.1 Peptides Derived from Proteins of Milk and Cheese

Proteins of milk and dairy products, particularly of bovine origin, are currently the primary source of bioactive peptides, among which are distinguished for their multiple anticariogenic functions, the caseinophosphopeptides (CPP) and glycomacropeptide (GMP) (Aimutis 2004). CPP are phosphorylated peptides produced during the digestion of α- and β-caseins, the family of proteins predominant in milk (80% of total protein). GMP represents one of the glycosylated forms of the caseinomacropeptidos (k-casein) and is a significant component of cheese whey protein (15–20% of total protein) (Eigel et al. 1984; Schlimme and Meisel 1995). CPP (resi-

dues 30–37 and 195–208 of α_{S1}-casein) and GMP (residues 106-169 of k-casein) can intervene in the initial and more advanced stages of dental caries formation. Both peptides have antimicrobial effects since they disrupt the membrane structure of opportunistic pathogenic bacterial species, such as the *Streptococcus mutans* (primary causative agent of caries), *Streptococcus sangius,* and *Streptococcus sobrinus* (Dashper et al. 2007). In the same way, CPP and GMP bind directly to the cell wall of these bacteria preventing them from adhering to saliva-coated hydroxyapatite (the main component of the tooth surface), and as a consequence, the cariogenic biofilm weakens (Neeser et al. 1994; Reynolds 1995). In more advanced stages of caries, CPP complexed with amorphous calcium phosphate (ACP) provide a reservoir of calcium and phosphate ions, which acts as a buffer system that controls the demineralization/remineralization process, preventing dental lesions (Reynolds 1995). CPP responsible for the property of binding minerals is a mixture of peptides from 1.4 to 9.6 kDa, from which 50% maintain the sequence SerP-SerP-SerP-Glu-Glu (Sgarbieri 2017). Due to their various anti-cariogenic functions, CPP, ACP, and GMP have currently been incorporated as a nano complex into toothpaste to test their antibacterial and remineralization potential (Elgamily et al. 2019). Similarly, CPP and ACP have been incorporated into chewing-gums as a source of calcium and phosphorus to maintain the remineralization in the whole dentition for a prolonged period (Dewani et al. 2019).

A casein macropeptide called kappacin, which represents the analogous phosphorylated form of GMP (glycosylated), is very efficient in inhibiting the growth of *Porphyromonas gingivalis*, the primary bacterium causing periodontal diseases (Sgarbieri 2017). Some peptides derived from milk globular glycoproteins also have bioactive effects. For example, LfcinB$(20–25)_4$, a tetrameric cationic peptide based on the core sequence RRWQWR of bovine milk lactoferricin, was efficient for the treatment of the oral squamous cell carcinoma (Solarte et al. 2017). Several hypotheses have been postulated about that the metabolic by-products of certain bacteria, among them *Porphyromonas gingivalis*, may induce permanent genetic alterations and chronic inflammation in epithelial cells of the oral cavity of the host, which contribute to the development of oral squamous cell carcinoma (Chattopadhyay et al. 2019).

3.8.2 Peptides Derived from Fish

Pardaxin is a polypeptide isolated from the marine fish Red Sea Moses sole (*Pardachirus marmoratus*) characterized by its cytotoxicity against cancer oral squamous cell. Its structure includes 33-aminoacids with the following sequence (H-GFFALIPKIISSPLFKTLLSAVGSALSSSGGQE-OH). Anticancer activity of pardaxin is mediated by apoptosis, the elevation of caspase-3/7 activities, disruption of the mitochondrial membrane potential, and accumulation of ROS. It is essential to mention that pardaxin belongs to a large family of antimicrobial peptides, which has shown effectiveness against various species of bacteria (Han et al. 2015;

Pangestuti and Kim 2017). However, pardaxin activity against the pathogenic bacteria that cause infectious diseases of the oral cavity has not been studied, so it would be worthwhile to research in this context.

3.8.3 Peptides Derived from Egg

Among the multiple proteins and peptides produced from the hen's egg, cystatin a protein contained in the egg white and a peptide of approximately 13 kDa derived from it, which is called L_7LGA_{10}, were shown to be inhibitors of *Porphyromonas gingivalis*. The antibacterial activity of cystatin and its peptides is attributed to the inhibition of essential microbial proteases. In the case of *Porhyromonas gingivalis* it refers to some forms of the enzymes gingipains and gingivains (Bhat et al. 2015b; Blankenvoorde et al. 1996).

3.8.4 Peptides Derived from Rice

Two powerful cationic peptides that selectively inhibit the growth of *Porphyromonas gingivalis* have been produced from proteins of rice (*Oryza sativa* L. *japonica*). One of them, a dodecapeptide derived from a region (residues 14–25) near the N-terminus of the enzyme cyanate lyase can inhibit the growth of *Porphyromona gingivalis* following different pathways. This dodecapeptide (CL(14-25)), which has the sequence RRLMAAKAESRK, contains three Arg and two Lys residues that might be important to disrupte the *Porphyromonas gingivalis* membranes in a detergent-like manner. Another way in which the dodecapeptide acts against *Porphyromonas gingivalis* is through the inhibition of the enzymatic activity of Arg-gingipains and Lys-gingipains, Both enzymes represent the main virulence factor of *Porphyromonas gingivalis*, they are capable of degrading a wide range of proteins and stimulating the expression and activity of the matrix metalloproteinases, which together degrade collagen, fibronectin and laminin, destroying periodontal tissue (Leishman et al. 2010; Taniguchi and Ochiai 2017).

The second peptide is derived from heat shock protein70. It is an octadecapeptide constituted by the residues Hsp70(241–258) with the sequence DNRMVNHFVQEFKRKHKK, which includes four Lys, two Arg, and two His residues that could participate in the disruption of the bacterial membrane. The antimicrobial activity of Hsp70(241–258) against *Porphyromonas gingivalis* is approximately sixfold than that of CL(14–25) (Taniguchi and Ochiai 2017).

A third octadecapeptide that is powerful, but not selective, has been isolated from the enzyme α-amylase in rice and is made up of residues Amyl-1-18(175-192) with the sequence HLNKRVQRELIGWLDWLK. In its action against *Porphyromona gingivalis*, AmyI-1–18 is approximately 26-fold and five fold higher than those of CL(14–25) and Hsp70(241–258), respectively. However, this octadecapeptide also

shows moderate to low inhibitory activity toward *Streptococcus mutans* and other bacteria, for example, *Propionibacterium acnes*, *Aggregatibacter actinomycetemcomitans*, *Pseudomonas aeruginosa*, *Candida albicans*, *Fusobacterium nucleatum*, *Escherichia coli*, and *Staphylococcus aureus* (Taniguchi and Ochiai 2017). Regardless of selectivity, cationic peptides are more powerful antimicrobials because they bind more strongly to negatively charged surfaces in lipid membranes of bacteria, and also are more useful than other peptides that have specific activity in the promotion of health and the treatment of diseases (Taniguchi and Ochiai 2017).

3.8.5 Other Peptides

Recently a novel bioactive peptide was developed from an endopeptide that is produced naturally by the human parotid and submandibular glands. Histatin 5 (H5) was modified by applying a graft based on phosphoserine (Sp) moiety onto the N-terminus of H5, leading to the formation of a bioactive peptide phosphoserine-histatin 5, whose sequence is Sp-H5 (phosphoserine-DSHAKRHHGYKRKFHEKHHSHRGY). This molecule has a higher binding affinity to the tooth surface, and therefore prevents the adhesión of *Streptococcus mutans* to hydroxyapatite, avoiding the formation of the biofilm; also serves as a nucleus to suppress demineralization and to initiate mineralization (Zhou et al. 2020).

Based on the information shown above, it is suggested that many proteins, especially those of plant origin, still need to be investigated to identify diet-derived bioactive peptides with possible pharmacological applications in the prevention of oral diseases. This area is promising for bioactive peptides since due to their chemical structures, local applications in the oral cavity could be highly advantageous since in this way they avoid exposure to peptidases and intestinal absorption difficulties. Furthermore, bioactive peptides with the double potential of preventing systemic diseases by controlling diseases of the oral cavity, are extensively sought-after.

3.9 Concluding Remarks

The world's population is threatened by an imminent increase in non-communicable diseases, which are currently the ones that take the most lives. In this scenario, the aggressiveness and expensiveness of the available medicinal treatments force to investigate cheaper and less risky alternatives. Bioactive proteins and peptides are becoming increasingly popular as preventive and therapeutic agents due to advantages such as biocompatibility, high selectivity, high potency, and low possibility of drug interactions. The downside is their poor pharmacokinetic properties, but these can be improved by chemical manipulation without further risks. Bioactive peptides have been found in many of the proteins present in the human diet. It is only

necessary to release them in their active form by hydrolysis. Agro-industrial waste represents another source of bioactive peptides in the context of sustainability. Scientific evidence shows the immense potential of peptides for the treatment and prevention of diseases such as cardiovascular diseases, cancer, diabetes, and dementias, that put humanity in check in the modern era. However, there are areas of opportunity to exploit this potential fully. For example, in many cases, clinical evidence is needed to extrapolate what has been observed in in vitro analyzes or animal models. It is also necessary to improve the purification and large-scale production processes of peptides and proteins that have already passed clinical tests. Collaborative work between government authorities, industry, and academia will make it possible to face these challenges.

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Chapter 4
Dietary Fibre

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Abstract In the last years, dietary fibre has gained attention as a bioactive due to its potential health benefits in reducing the risks for many diseases, such as cancer and cardiovascular ones. This effect is linked to its action against inflammation, oxidation, hyperlipidemia and other physiological disorders. The research in this area had been extensive but elucidation of the mechanisms involved in this bioactivity is not yet conclusive.

In this chapter, it will be analyzed the actual state of research concerning the effect of dietary fibre on health and the pathways by which this nutrient develops its action.

Keywords Dietary fibre · Nutrient · Health benefits · Gut microbiota · Immunity

4.1 Dietary Fibre

4.1.1 Definition: Chemical Components

The term "dietary fibre" was introduced in 1953 (Dai and Chau 2017). Early, the concept of fibre corresponded to an indigestible moiety which was quantified and named as "crude fibre". It was referred to as the residue of plant-based food left after extraction with solvent, dilute acid, and dilute alkali. According to Thompson and Brick (2016), the CODEX Alimentarius (2010) indicated that the carbohydrate polymers of plants consumed in the human diet that cannot be hydrolyzed by the

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endogenous enzymes in the small intestine are referred to as dietary fibre. A validated, integrated method of dietary fibre analysis that complies with that definition has been developed, which is the Association of Analytical Chemists' (AOAC) 2011.25 method (McCleary et al. 2012).

Nowadays, the precise definition of dietary fibre is evolving. For example, scientific research has initiated the expansion of the dietary fibre concept to include indigestible oligosaccharides with their DP between 3 and 9 (Dai and Chau 2017). Benítez-Paez et al. (2016) reported that dietary fibre is generally defined by the EFSA NDA Panel (2010) as non-digestible carbohydrates plus lignin. They are structurally different components including non-starch polysaccharides, resistant oligosaccharides (e.g. fructooligosaccharides or FOS, galacto-oligosaccharides or GOS) and resistant starch. According to de Vries et al. (2019), dietary fibre is made up of carbohydrate polymers with three or more monomeric units that are neither digested nor absorbed in the human intestine.

In the European Commission (2019), EU regulation 1169/2011, on the provision of food information to consumers, defines fibre as "carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:

– edible carbohydrate polymers naturally occurring in the food as consumed,
– edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence,
– edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence."

Similar to the EU, the United States (US) Food and Drug Administration (FDA) definition (FDA 2016) refers to "non-digestible soluble and insoluble carbohydrates (with 3 or more monomeric units), and lignin that are intrinsic and intact in plants; isolated or synthetic non-digestible carbohydrates (with 3 or more monomeric units) determined by the FDA to have physiological effects that are beneficial to human health".

The EU and US definitions differ from that of the Codex Alimentarius (FAO 2009) on the number of monomers that constitute the carbohydrate polymer; while the EU and US include three or more monomeric units, the Codex definition specifies ten or more, leaving national authorities to decide whether to include as fibre also carbohydrates with 3–9 monomers.

Dietary fibre is often referred to as non-starch polysaccharides' fibre or as AOAC fibre. Non-starch polysaccharides' fibre only includes polysaccharides of the plant cell wall components characteristic of plant foods, such as wholegrain cereals, fruits and vegetables. AOAC fibre comprises the total amount of non-digestible polysaccharides, and includes e.g. lignin and resistant starches, measured with a set of methods developed by the AOAC (BNF British Nutrition Foundation 2019). In effect, AOAC fibre includes non-starch polysaccharides' fibre, but in addition it also includes non-digestible carbohydrates (naturally present and isolated from foods and/or synthesized) that can be added as ingredients to foods.

Why are most of the carbohydrates non-digestible or non-hydrolysable (and then called dietary fibre)? Because of the inability of the enzymes found along the human digestive tract for hydrolyzing saccharides present in the bolus. The human genome encodes 97 glycoside hydrolases and no polysaccharide lyases, while gut microbiota have both types of enzymes. Eight of the human glycoside hydrolases can be directly linked to digestion, and nine of them are possibly digestive, while the rest act in reactions not associated with food digestion (El Kaoutari et al. 2013). Just in the mouth, the α-amylase comes into contact with food and finally impregnates the bolus, where it is able only to hydrolyze native and some modified starches (α-1,4 and α-1,6 bonds) of plants and the multibranched glycogen (α-1,4 and α-1,6 bonds), the readily mobilized storage form of glucose dispersed in the cytoplasm of animal cells. In the stomach, the high acidic pH kills the bacteria present in the chyme. Just into the lumen of the duodenum, the chyme is neutralized by the concentrated bicarbonate buffer and attacked by the enzymes, all of them secreted by the exocrine pancreas' cells through the hepatopancreatic (Oddi) sphincter. These enzymes include the pancreatic α-amylase able to hydrolyze glycogen and starches (>100 kDa), producing di-, tri-, and oligosaccharides' products. And also all the brush-border membrane enzymes that hydrolyze specific disaccharides: sucrase-isomaltase, lactase (β-glycosidase) and trehalase for hydrolysis of sucrose (β-D-fructofuranosyl α-D-glucopyranoside; α-1,2 bond), lactose (β-D-galactopyranosyl-(1→4)-D-glucopyranose; β-1,4 bond) and trehalose (α-1,1 bond) disaccharides, respectively. Uncommon in the American diet, the trehalose is found in algae, young mushrooms, and other fungi and may cause gastrointestinal distress if consumed by individuals without adequate quantities of intestinal trehalase (Goodman 2010). The ability to digest lactose varies across the populations and lactase activity can decrease with the age (Holscher 2017).

Mouth and pancreatic α-amylases are endosaccharidases specific for internal α-1,4 glycosidic bonds. They have no effect on α-1,6 glycosidic bonds or on α-1,4 bonds of glucose molecules at the branch points or at the ends. The mentioned complex carbohydrates are broken down into maltose, maltotriose (isomaltose), trisaccharides, larger oligosaccharides, and α-limit dextrins (oligosaccharides with branch points). The maltase, a β-glucoamylase, splits maltoses, while the isomaltase does it with the isomaltoses. Only monosaccharides (D-glucose, D-fructose) are then absorbed by the intestinal cells in order to reach the capillary blood of the enterohepatic cycle (Goodman 2010; Crawley et al. 2014).

Dietary fibre is essentially, constituted by the components of vegetables' and fruits' cell walls consumed by humans and animals in a normal balanced diet, which include pectins, cellulose, and hemicelluloses such as arabinoxylans and also (1,3;1,4)-β-D-glucans, which are distributed in the cell walls of the Poaceae family, whose economically important members are cereals and grasses (Scheller and Ulvskov 2010). In oat and barley, the β-glucans are specially located in the cell walls of the endosperm and aleurone (Kurek et al. 2018). β-glucans can contribute up to 70% by weight of the walls in barley, rye, and oats (Fincher and Stone 2004). Resistant starch is also being considered a dietary fibre, but it is located in the cellular cytoplasm, as part of the starch granules. Based on its digestive rate, starch is

actually divided in three fractions that comprise rapidly digesting starch, slowly digesting starch, and resistant starch. Resistant starch includes a wide range of materials, and it is divided into four types: physical inaccessible starch, ungelatinized starch granules, retrograded starch, and chemically modified starch (Dai and Chau 2017). Inulin or β-fructans, which like starch are storage carbohydrates present in the cellular cytoplasm of temperate and cool zone grasses, are composed of five types of fructans, all with β-linkage: inulin β2-1, levan β2-6, branched β2-1 and 2-6, inulin neoseries β2-1, and levan neoseries β2-6. Oligomeric fructans (DP 3-9), usually called oligofructose or FOS, are mostly obtained by inulin hydrolysis or enzymatic synthesis from the sucrose obtained from beet or cane (De Vries et al. 2019). On the other hand, lignin, which constitutes the secondary cell walls and it is then part of the fibre fraction of the diet, is the chemical exception because it is a phenolic polymer and not a carbohydrate (Brett and Waldron 1996; Broekaert et al. 2011; Qi et al. 2018). Lignin is the second most abundant natural polymer after cellulose, playing an important role in plants, providing rigidity to strengthen the structures of cell walls and resistance to microbial attack. Chemical structure of lignin consists of three monolignols' kinds of phenylpropane units: *p*-hydroxyphenyl unit (H unit, from *p*-coumaryl alcohol), guaiacyl unit (G unit, from coniferyl alcohol), and syringyl unit (S unit, from sinapyl alcohol). The content of each monolignol in lignin depends on the plant species. Through radical coupling reactions, the monolignols are linked together to form lignin, a complex three-dimensional structure (Wang et al. 2019). Bunzel et al. (2005) determined the insoluble fibre lignins in fruits and vegetables. They were classified as G-rich lignins (G/S ratio >3; carrot, spinach, kiwi, curly kale, radish, and asparagus), S-rich lignins (S/G ratio >3; rhubarb), or balanced lignins (0.3 < G/S ratio < 3; pear, apple, small radish, and kohlrabi).

Gums and mucilages are polysaccharides habitually considered as dietary fibre. They are derived not only from plant exudates and seeds, but also from seaweeds. Some of them such as gum arabic, karaya, tragacanth, and carob are obtained as exudates from trees or shrubs. Guar gum and locust bean gum are extracted from seeds. Xanthan gum, curdlan and gellan are produced by microbial fermentation (Huffman 2003; Qi et al. 2018). Other soluble fibres also represented by certain hydrocolloids habitually used in food formulations such as agar, alginate, and carrageenan are obtained from seaweeds, while carboxymethylcellulose and hydroxypropylmethyl cellulose are chemical derivatives of cellulose produced for obtaining water soluble cellulose. Chemically modified starches and xanthan gum are also used (Qi et al. 2018).

4.1.2 Classification

Based on the chemistry, i.e., in the character of the individual monomers, DP, and type of linkage (α or β, axial or equatorial), the following form of primary classification of dietary carbohydrates is considered (Cummings and Stephen 2007). Non-digestible carbohydrates with a DP of 2–10 (or 3–9 according to some conven-

tions), known as oligosaccharides, are also dietary fibre molecules, though they are often treated differently by the regulatory authorities (Qi et al. 2018). In general, carbohydrate chains with a number of carbon atoms up to nine are water-soluble. Many oligosaccharides are naturally found in vegetables. Raffinose, stachyose, and verbascose are galactooligosaccharides (GOS) found in legumes. They consist of a terminal sucrose to which one (raffinose), two (stachyose), or three (verbascose) galactose monomers are linked. Other oligosaccharides such as those derived from β-glucans, mannan oligosaccharides (MOS), GOS, oligofructans, xylan-oligosaccharides (XOS), arabinoxilan-oligosaccharides (AXOS), dextrins, and short pectins can be also found in some specific vegetables like mushrooms (Gerschenson et al. 2017). Moreover, they can be also liberated into the colon by the enzymatic battery of the microflora (e.g. endo-β1,4-xylanases and xylosidases) acting on the non-digested dietary fibre polysaccharides (e.g. arabynoxylans) (Broekaert et al. 2011; El Kaoutari et al. 2013).

Based on the different physiological effects, dietary fibre is classified in soluble (oligosaccharides of DP < 10, pectins, inulin of lower DP, soluble hemicelluloses, gums and mucilages) and insoluble (debranched hemicelluloses, cellulose, lignin, resistant starch) in the aqueous fluids. However, over the years a good amount of scientific research has shown that solubility is not necessarily the determinant of physiological effect. Therefore FAO/WHO in 1998 proposed to no longer use this classification (FAO/WHO 1998). In spite of this observation, the solubility of the dietary fibre determines the site of the colon where it is fermented and absorbed (Holscher 2017).

Based on the dietary fibre solubility, soluble fibre can interfere with the absorption of dietary fat and cholesterol. This, in turn, can help to lower low-density lipoprotein (LDL) cholesterol levels in the blood. Soluble fibre also slows digestion and the rate at which carbohydrates and other nutrients are absorbed into the bloodstream. This can help control the level of plasma glucose by preventing rapid increase in blood glucose following a meal. On the other hand, insoluble fibre provides "bulk" for stool formation and speeds up the movement of food and waste through the digestive system, which can help prevent constipation. Diets higher in dietary fibre promote intestinal regularity due to the stimulation of intestinal peristalsis. Simultaneous to this important mechanical effect, fibre can reduce the risk of developing cardiovascular disease, as well (Cadden 1987; FDA 2019).

According to Watson (2019), the FDA classifies dietary fibres into three groups:

1. Non-digestible soluble and insoluble carbohydrates (with three or more monomeric units), and lignin that are intrinsic and intact in plants: these don't need FDA pre-approval and automatically meet the definition.
2. Isolated or synthetic non-digestible carbohydrates (with three or more monomeric units) determined by FDA to have physiological effects that are beneficial to human health. The FDA initially approved six: β-glucan soluble fibre, psyllium husk, cellulose, guar gum, pectin, locust bean gum, and hydroxypropylmethylcellulose, but required suppliers of many others to submit citizen's petitions.

3. Isolated and synthetic non-digestible carbohydrates. The FDA has already approved:

    - Mixed plant cell wall fibres
    - Arabinoxylan
    - Alginate
    - Inulin and inulin-type fructans
    - High amylose starch (resistant starch 2)
    - Galactooligosaccharides
    - Polydextrose
    - Resistant maltodextrin/dextrin

In this scheme, the called mixed plant cell wall fibres imply a broad category that includes barley fibres, cocoa fibres, sugar cane fibre, apple fibre, sugar beet fibre, corn hull fibre, potato fibre, oat hull fibre, pea fibre (hull and cotyledon), bamboo fibre, cottonseed fibre, rice bran and hull fibre, soy fibre (cotyledon and hull), citrus fibre, and wheat fibre. It is defined by the FDA as "Ingredients that contain two or more of the following plant cell wall fibres in varying proportions: cellulose, pectin, lignin, β-glucan, and arabinoxylan", and mentions that "Examples of mixed plant cell wall fibres that we intend to consider enforcement discretion for as a dietary fibre are those obtained from whole or parts of fruits, vegetables, grains, legumes, pulses, nuts, and other plants that undergo processing methods".

As explained by Watson (2019), regarding the FDA decisions about which isolated or synthetic non-digestible carbohydrates should be classified as "dietary fibre" on the Nutrition Facts panel, actually the FDA did not approve or reject anything. The FDA granted and denied certain petitions and made determinations about whether the 26 non-digestible carbohydrates from the 2016 science review meet the criteria to be considered a dietary fibre as laid out in this guidance.

4.1.3 Recommended Daily Intake

According to the FDA (2019), the daily intake value recommended for fibre is 25 g, based on a 2000 calorie diet. According to Li and Komarek (2017), the National Center for Health Statistics (NCHS) conducted a study in adults aged 18 years and above within the 1999–2008 period, to estimate the daily fibre intake of individuals and compared it with recommended intakes. It was determined that the mean daily intake of dietary fibre is stagnant at the level of 15–16 g/day per person and, hence, individuals do not meet the recommendation in the US, considering that the recommended levels for total fibre intake by age and gender are 38 g/day for men aged 19–50 years, 30 g/day for men older than 50 years, 25 g/day for women aged 19–50 years, and 21 g/day for women older than 50 years.

Dietary fibre intake in most countries around the world is far below recommended levels. The gap between dietary fibre recommendations and intakes is so

extreme that the U.S. Dietary Guidelines Advisory Committee listed dietary fibre as one of five "nutrients of concern" (Miller 2014).

European Commission (2019) recommended amounts of dietary fibre for promotion of adequate laxation and for prevention of chronic diseases such as diabetes type 2, colorectal cancer, CVD or of overweight and obesity range from 25 to 38 g/day in adults. In children, recommended amounts vary according to the energy requirements of the different age groups. Recommended intake values are expressed in the majority of the cases as adequate intakes of AOAC fibre unless differently stated. Some public health organizations also recommend fibre intakes on the basis of energy requirements (grams fibre per Joules or grams per 1000 kcal).

Regarding the role of dietary fibre, recent reviews outline the benefits of ancestral diets and high fibre diets to maintain a rich and diverse gut microbiome and related health benefits. In light of these data, some studies propose that dietary fibre intake would at least reach 50 g/day, whereas the current recommendations are around 30 g/day in the adult, as above mentioned (Delzenne et al. 2019).

4.1.4 Nutritional and Functional Properties of Dietary Fibre

In his medical research into the occurrence and distribution of cancer in Africa, Denis Parsons Burkitt became convinced of the dietary fibre importance and, using his surgical knowledge, expertise in the geography of diseases and some experimental work, he showed that lack of fibre was a determinant of bowel cancer risk. Other conditions including diverticular disease, irritable bowel syndrome, appendicitis, varicose veins, haemorrhoids, diabetes, obesity, atherosclerosis and dental caries were added to the Burkitt's fibre hypothesis, as the non-communicable diseases of Western culture (Cummings and Engineer 2018).

A diet rich in fibre is usually lower in fat and contains fruit and vegetables. High intakes of dietary fibre may reduce absorption of some minerals from food as they can be bound by the fibre in insoluble complexes. However, fermentation of the fibre in the large intestine can release some of the bound minerals (e.g. calcium) and, hence, they can be absorbed. The amount of vitamins and minerals lost through eating a diet rich in fibre is not likely to be significant unless an individual's diet is already poor. The health risks of a low fibre diet are potentially much greater than those of a very high fibre diet (BNF British Nutrition Foundation 2019).

From a labeling perspective, the format and contents of which is set by EU law, fibre provides 2 kcal/g of energy (BNF British Nutrition Foundation 2019).

European regulations on nutrition and health claims state that a product claiming to be a "source" of fibre should contain at least 3 g of fibre per 100 g or at least 1.5 g of fibre per 100 kcal. A product claiming to be "high fibre" should contain at least 6 g of fibre per 100 g or at least 3 g of fibre per 100 kcal (The European Parliament and the Council of the European Union 2007).

As reported by FAO (2019), even when a great number of scientific investigations were stimulated by the Burkitt's hypothesis, it is still early to assign clear

health claims to dietary fibre. This difficulty derived from the fact that dietary fibre includes many complex substances, each having unique chemical structure and physical properties. In this sense, dietary fibre is often intimately associated in the plant cell structure with other bioactive organic compounds, such as vitamins, isoflavons (phytoestrogens), phenolics, etc., which display their own biological activity.

The functional properties of dietary fibre can be strongly associated to the biological effects, and comprise the hydration properties of the fibre (swelling, water-holding and water retention capacities), thickening, gelling and antioxidant effects, as well as, the effect of fibre on gut microbiota. Functional properties depend basically on the chemical composition of the fibre biopolymers, but physical properties derived from the matrix microstructure developed after drying and milling can also influence greatly, for a given chemical composition.

4.1.4.1 Dietary Fibre and Antioxidant (AOX) Effect

In the case of extraction of fibre enriched fractions from vegetables and fruits by-products and leftovers, this fact can be in part overcome by applying a sequential process for the extraction with solvents of decreasing polarity, and different pH and concentrations in the case of solvent mixtures. For example, only water soluble and ethanol insoluble biopolymers and associated substances like phenolics but not hydrophobic substances, can be together obtained in the isolated fraction by using the mentioned solvents (Fry 1986; Marry et al. 2006; Ponce et al. 2010; Raffo et al. 2011). As indicated by Renard et al. (2015), pectins show high affinity in the interaction with polyphenols liberated from the intracellular location during extraction of the cell wall biopolymers from tissues, after mechanical disruption of cells. The binding is due to a combination of hydrogen bonds and hydrophobic interactions, increasing the affinity with the degree of methyl-esterification of the homogalacturonans, and it is favored by increased ionic strength and decreased temperature. Also, phenolic compounds such as ferulic and coumaric acids (monomer, dimer and trimmer forms) can be found covalently bound to the arabinan chains of the rhamnogalacturonan I of pectins, as well as to the L-arabinose lateral substituents of the xylan backbone in hemicelluloses (Fry 1986; Marry et al. 2006; Scheller and Ulvskov 2010). In this cases, the polysaccharides can be chemically liberated after treatment with strong alkali (NaOH, KOH). Saura-Calixto (2011) established that dietary fibre and antioxidants are two recognized dietary factors in the prevention of chronic disease. The author indicated that dietary fibre has an essential role in intestinal health and appears to be significantly associated with a lower risk of developing coronary heart disease, stroke, hypertension, diabetes, and obesity. Regarding dietary antioxidants, they protect against oxidative damage to DNA, proteins, and lipids, and have a significant impact on the regulation of gene expression. Intake or plasma concentration of dietary antioxidants has been associated with the low risk of chronic disease in healthy diets. It has been suggested (Saura-Calixto 2011) suggested that even though an abundant scientific literature addresses dietary fibre and antioxidants separately as nonrelated compounds, probably because of the dif-

ference between their chemical structures and, hence, physicochemical and biological properties, as well as metabolic pathways, dietary fibre and a considerable amount of dietary antioxidants follow a common and synergistic physiological process within the gastrointestinal tract. Most reported dietary antioxidants are a wide variety of single molecules (vitamin C, tocopherols, carotenoids, low molecular weight polyphenols, and others) solubilized and totally or partially absorbed in the upper intestine. However, an appreciable amount of dietary antioxidants, mainly polyphenolics and some carotenoids, travel through the small intestinal lumen intact in tandem with the dietary fibre, reaching the colon, where they release the fibre matrix and produce metabolites and an antioxidant environment by the action of the enzymatic machinery of the bacterial microbiota. In this way, Broekaert et al. (2011) reported that arabinoxylan oligosaccharides (AOXs) are more powerful antioxidants than the free ferulic acid that they contain as esterified group. In addition, ferulate esterase of the gut microbiota liberates ferulic acid from hemicellulases and oligosaccharides into the caeco-colon lumen. Basanta et al. (2016) determined that the polyphenolic extract obtained from plum isolated fibres, mainly constituted by pentameric proanthocyanidins (170–200 mg/100 g plum fibre), showed a protective effect against the oxidative stress induced by tert-butylhydroperoxide on a Hek 293 kidney cell line, joined to a low cytotoxicity (50%-cytotoxic concentration > 100 μg/mL extract). Proanthocyanidins are catabolized in a relevant proportion by the colonic microbiota before they can be absorbed as the resulting products, which include free phenolic acids and phenyl-γ-valerolactones (Ou and Gu 2014).

4.1.4.2 Dietary Fibre and Hydration Properties

The hydration properties comprise the swelling, water-holding and water retention capacities and are tightly related to the thickening and gelling effects of dietary fibre.

Dietary fibre such as oligosaccharides, pectins, inulin of lower DP, soluble hemicelluloses, gums (alginates, carrageenans) and mucilages are water-soluble, viscous, and highly fermentable by the microorganisms of the intestinal tract. Because of its water-holding capacity, they delay gastric emptying (Huffman 2003). Therefore, many mucilages as well as pectins are also used for pharmaceutical purposes such as the mucilages obtained from *Plantago ovata* like the psyllium mucilage and mucilage of llanten, used as laxatives, as well as for protection of the intestinal epithelium. Wheat bran, cellulose and psyllium may help reduce constipation and the risk of colon disease because they absorb water, which increases bulking and promotes regularity. Soluble fibres include viscous fibres such as pectin, β-glucans, fructans (inulin, fructooligosaccharides), gum, mucilage (Soliman 2019). The physiological effects of soluble dietary fibres are attributed to their unique properties: viscosity and gel formation, and fermentability into the colon. Different dietary fibres might have different viscosities depending on their chemical composition (types of monomers), macromolecular structure and weight, concentration, pH, counter-ions, and ionic strength. Viscous soluble dietary fibres are believed to be more capable of inducing satiety compared to non-viscous soluble dietary fibres,

and hence delays gastric emptying, slowing digestion and the absorption of nutrients, including D-glucose, and reducing intestinal enzyme diffusion and the formation of an unstirred water layer. Viscous soluble dietary fibres are not being digested in the stomach. Instead, they are fermented in the colon and result in a rise in short chain fatty acids (SCFAs) (Lapasin and Pricl 1995; Salleh et al. 2019).

For healthy effects above described, a functional property like the hydration capacity (swelling and water holding capacity), inherent to dietary fibres, is involved, which is strongly associated to the chemical composition of dietary fibres. As a result of the hydration capacity, dietary fibres are able to immobilize water molecules by hydrogen bonding next to the hydroxyl groups of the polysaccharide macromolecules or low molecular weight carbohydrates and, consequently, to slow down water flow in the following layers of surrounding water. This behavior is manifested as viscosity or thickening effect, a very important property of dietary fibres and, especially, of higher molecular weight carbohydrates. The lower molecular weight saccharides also retain water molecules around, but the main effect of them is as osmotically active compounds (Schaller-Povolny et al. 2000). The dietary fibre has a varying capacity of producing viscous solutions upon dissolution and swelling in water. This capacity strongly depends on the molecular weight and concentration, and it is positively correlated to its solubility (Capuano 2017). Also, it can be favored by the presence of counterions such as potassium and calcium. Since the latter is a divalent ion, it produces the electrochemical crosslinking of pectin and alginate macromolecules, which leads to gelling of the aqueous system (Braccini and Pérez 2001). Hence, rheological properties of dietary fibres linked to their hydration capacity are not only related to their utility as additive or ingredient but also to their intestinal effects. Moreover, the gelation capacity of some soluble dietary fibres showed health benefits to the consumers and improved the commercial values of related functional food. As reported by Li et al. (2018), the soluble dietary fibre showed higher swelling and water holding capacities and viscosities than insoluble dietary fibre. These hydration properties are believed to be responsible for the delay in, for example, the glucose and cholesterol absorption in the small intestine and, hence, for the decrease in the blood glucose and cholesterol levels. The European Food Safety Authority (EFSA) has recognized in 2010 the scientific validity of nutrition and health claims regarding pectin as a nutritional supplement in the reduction of the post-prandial glycemic response, maintenance of normal blood cholesterol levels and the increases in satiety, leading to a reduction in the energy intake. Therefore, pectins' producers for food and pharmaceutical formulation were then suddenly confronted with an unexpected outcome, that is the use of pectin as a healthy additive or ingredient (Ciriminna et al. 2016). Pectin is a major fruit prebiotic that has been extensively studied and shown to promote a healthy, anti-inflammatory colonic microbiota ecosystem with greater microflora diversity than inulin (Dreher 2018).

On the other hand, the EFSA NDA Panel (2010) and the Federal Drug Administration in 2005, have recognized that the daily intake of 3 g of β-glucans from oat and barley contributes to maintain normal the cholesterol level in blood

(Othman et al. 2011). Therefore, the EFSA and FDA authorized the use of health claims for β-glucan from barley and oat (Kurek et al. 2018).

Swelling capacity is defined as the ratio of the volume occupied by the sample after immersion in excess of water and equilibration to the actual weight (Raghavendra et al. 2004). Hence, this parameter indicates how much the powder fibre matrix swells and its volume increases as water is absorbed. Water-holding capacity (WHC) is defined by the quantity of water retained by the fibres without the application of any external force, except for gravity and atmospheric pressure (Raghavendra et al. 2004). Thus, this parameter also includes the proportion of water loosely associated to the fibre matrix in addition to the strongly retained water. The water retention capacity (WRC) is defined as the quantity of water that remains into the hydrated fibre following the application of an external force (pressure or centrifugation). Therefore, it is indicating the fraction of water that it is strongly retained by the fibre polymers.

The maximum amount of water that the fibre can hold is a function of its chemical, physical and microstructural characteristics (Brett and Waldron 1996; Raghavendra et al. 2004). Beyond the chemical composition and macromolecular structure of the fibre (hydroxylation, methylesterification, charged groups, branching, molecular weight), particle size is hence a main characteristic that can decisively contribute to determine the hydration properties of the dietary fibre in the powder form (Cadden 1987). For the same chemical composition, the procedure by which a given particle size range is reached also contribute to determine the surface properties of the fibre material, that is, wettability or hydrophobicity. Consequently, the procedure used affect finally the swelling and hydration capacities of fibres as well as the final dissolution in the case of soluble dietary fibre. The rheological behavior is finally conditioned by the mentioned facts since it is a function of the capacity of the fibre biopolymers to interact with the water solvent, modifying its flow property. Reducing the particle size of wheat bran decreased the water-holding capacity, due, in part, to the collapse of its fibre matrix. Water absorption properties of cereal fibres are an important determinant of their reported stool bulking effects (Cadden 1987). Idrovo Encalada et al. (2019) obtained fibre powders from discarded carrots after elimination of the water soluble simple sugars and freeze-drying. For the same chemical composition (15% w/w uronic acids, 33% of neutral sugars, 23–25% of cellulose, 7–10% of lignin and ≈0.72% of total starch), the authors determined that swelling capacity increased significantly with the particle size of carrot fibre from 26.7 mL of water absorbed after18 h of equilibration per gram of 53 μm dried fibre, up to 36.3 mL/g for 210 μm of average particle size. Pectins present in the carrot fibres at (≈15% uronic acids' content) were mainly responsible for the water absorption and swelling capacity. The values of water holding and water retention capacities determined as the grams of water absorbed by the dried fibres after 18 h of equilibration per gram of dry fibre, were significantly lower for 53 μm carrot fibre than for 105 and 210 μm. On the other hand, Raghavendra et al. (2004) determined that the reduction in the particle size of coconut grating residue from 1127 to 550 μm, resulted in increased hydration properties, which was ascribed to the increase in the theoretical surface area and total pore

volume, as well as to an structural modification. However, below 550 μm, the hydration properties were found to decrease with decreasing particle size, which can be associated to the collapse of pores.

As early reported by Cadden (1987), the consumption of dietary fibre of cereals has been promoted for its prophylactic value in regulating colonic function. However, the addition of fibre to foods does not guarantee that the foods will become endowed with desirable physiological effectiveness. The addition of finely ground wheat bran or cellulose to a low-fibre diet has been reported to cause constipation in human subjects. Fibre supplements prepared by the food industry as food ingredients are often finely ground. Unfortunately, studies have shown that the processing of foods can alter the physical characteristics of the plant fibre and so affect the degree of microbial degradation and the ability of the fibre to absorb water and/or other compounds.

For a given chemical composition, the drying process used to obtained powders enriched in dietary fibre has a great effect on hydration properties of the product because it affects the microstructural characteristics of the powders obtained (Vetter and Kunzek 2003). In general, lyophilization generates powders with the highest active surface for interaction with water and, hence, with absolute re-hydration capacity. Spray-drying is also a high-quality drying process with respect to the wettability and re-hydration capacity of the powders obtained, and the particle size range can be managed through the nozzle used. On the other hand, drying in common chambers under limited convection combined with higher temperatures can produce powders with lower porosity and, hence, the lowest hydration capacity (Martinez-Las Heras et al. 2017). Fibres extracted as the ethanol (96% v/v) insoluble residues from persimmon peel and pulp showed that when freeze-dried, these fibres presented better hydration properties and oil holding capacity than those obtained after drying under 40 °C-air (≈7 h to constant weight). Freeze-dried persimmon peel and pulp fibres also demonstrated higher values of emulsion stability than commercial fibres such as those obtained from peach, lemon, orange and apple. Finally, the antioxidant activity of the smallest sized persimmon peel fibre obtained by freeze-drying was higher than that for lemon, orange and peach fibres (Martinez-Las Heras et al. 2017).

Beyond the drying processing used, the fibre powder obtained can be also modified by other physical methods which can imply the change in the particle size. In this sense, for a given chemical composition and particle size, the process used for reducing the particle size can also influence the hydration properties of the dietary fibre. Powder properties such as flowability and compressibility that pertain to bulk level of solid state are strongly influenced by changes in characteristics at the particle level, such as size, size distribution and morphology of particles (aspect ratio) (Sarrate et al. 2015). Liu et al. (2016) evaluated the effect of regular laboratory milling, ultra centrifugal rotor milling and ball milling on structural, physicochemical, and functional properties of the insoluble dietary fibre fraction that remained after heating the orange peel in water (1:5) for 2 h at 90 °C followed by centrifugation and freeze-drying. The matrix structure of the insoluble fibre fraction was destroyed but FTIR structure had no major change after grinding. Ultracentrifugal milling and ball

milling effectively decreased the average particle size of insoluble dietary fibre fraction (81.40 μm and 19.63 μm, respectively). As particle seize decreased, the bulk density and lightness of the insoluble dietary fibre fraction increased and a redistribution of fibre components from insoluble to soluble fractions was observed. Furthermore, the ball milled insoluble fibre exhibited significantly higher capacity to retard glucose diffusion. Ye et al. (2015) obtained insoluble fibre from orange pomace by elimination of the soluble fibre with 60 °C-water for 1 h of stirring. The insoluble fibre residue was dried under air at 60 °C for 48 h. The dried insoluble fibre was then ordinarily grinded (high-speed pulverizer), a sample of this procedure was then micronized for 8 min, while another sample was submitted to jet grinding. According to the $d_{0.90}$ diameter determined through light scattering, the particle sizes of the three milled products were respectively 750, 125, and 48.4 μm. As the particle size decreased, the fibre was enriched in the soluble component (the insoluble fibre was mostly lost upon intensive grinding), and a slight increase in crystallinity (52.84–62.20%) occurred. The latter was ascribed to the fact that lignin and hemicelluloses, existing in amorphous regions of the powders, were removed as the grinding was more intense. However, the swelling and water holding capacities were low and varied significantly but slightly as the particle size decreased, from 7.14 to 6.17 mL/g for the swelling capacity, and from 7.33 to 5.74 g water/g fibre for the water holding capacity.

Dubey et al. (2018) determined that milled cellulose showed significantly enhanced capacity for holding water (3.5–25 mL water/g), swelling (3–26.5 mL/g) and binding bile acids and sugars. The size reduction also resulted in increased fermentability of cellulose into SCFAs using three human fecal microflora samples. The increase in production of acetate (2880.60%), propionate (2738.52%), and butyrate (2865.89%) after fermentation of cellulose for 24 h was significantly enhanced by size reduction. Ang (1991) found that, depending on the fibre length, cellulose can retain 3.5–10 times its weight in water. A cellulose powder with at least 110 μm fibre length significantly increased the viscosity when dispersed in water at concentrations up to 3% w/v before sedimentation.

De Paepe et al. (2019) determined that modification of wheat bran particle size and tissue composition affects the colonization and metabolism by human faecal microbiota. Modification of wheat bran physicochemical properties largely affects the amount, but not the ratio of produced SCFAs, and that interindividual variability dictates the functional and composition response from the luminal microbiota to wheat bran supplementation. The wheat bran-attached microbiome composition was more affected by wheat bran structure. Micronization of unmodified bran from 1687 μm to 149 μm resulted in a higher SCFAs production after 24 h for all donors, except donor 7 and 9. This difference between micronized and unmodified bran disappeared again after 48 h and was not observed at 6 h. This result suggests that particle size only affects the rate of fermentation, confirming the finding from Stewart and Slavin (2009) that a reduction in average wheat bran particle size from 1239 μm to 551 μm increased SCFAs' levels starting from 8 h up till 24 h. The authors attributed the increased production of SCFAs to an increased surface area, providing a larger contact area for bacterial enzymes to access the substrate.

However, others claim that bran porosity more than surface area determines substrate accessibility to enzymes. Secreted extracellular enzymes are able to penetrate in nanometer size pores, whereas membrane-bound enzyme complexes, which are suggested to play a major role in the rate limiting primary degradation of wheat bran, are restricted to micrometer size pores. Changes in porosity may partly offset the effect of an increased surface area on enzyme accessibility, limiting the effect of micronization on fermentability.

As a consequence of all above described, swelling, water-holding and water retention capacities have to be determined after any extractive and modification procedures performed for extraction of dietary fibre enriched fractions. Intense shearing during grinding processes such as micronization, changes the insoluble fibre/soluble fibre weight ratio in the fibre product, with a general decrease. In spite of it, contrary to that expected, the hydration properties are decreased.

4.1.4.3 Dietary Fibre and Gut Microbiota

By considering the health benefits, Codex states that dietary fibre generally presents one or more of the following properties: (1) decreased intestinal transit time, increased stools bulk; (2) fermentation by colonic microbiota; (3) reduced blood total and/or LDL cholesterol levels; and (4) reduced post-prandial glycemia and/or insulin levels (Delzenne et al. 2019). The (1) and (2) functions are the essential ones for the nutritional effect of dietary fibre. These four properties were included in the EU Directive 2008/100/EC and applied, in recent years, for evaluating the benefits to health of a wide range of fibre ingredients by Health Canada's Food Directorate and the FDA. These two public organisms concluded that, for most current commercially available dietary fibre, sufficient scientific evidence is available for including them in the list of compounds that can be officially considered as dietary fibre.

In spite of the common characteristic of being non-digestible in the human small intestine, the dietary fibre is widely different in composition, structure and the way by which they feed the bacteria harboring the gut microbiota (Delzenne et al. 2019). The gastrointestinal microbiota has an important role in human health, and there is increasing interest in utilizing dietary approaches to modulate the composition and metabolic function of the microbial communities that colonize the gastrointestinal tract to improve health, and prevent or treat disease. One dietary strategy for modulating the microbiota is the consumption of dietary prebiotics (Holscher 2017). The International Scientific Association of Probiotics and Prebiotics defined "dietary prebiotics" as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (Davani-Davari et al. 2019). As indicated by Benítez-Paez et al. (2016), dietary fibres are major drivers of gut microbiota composition and function, stimulating the dominance of bacteria able to utilize these substrates as an energy source. Microbial species interact in vivo to form complicated food chains, and some of these relationships are centered on the glycan metabolism (Koropatkin et al. 2012). However, the effects vary depending on both the type of

fibre and the individual's microbiota. The primary and secondary metabolic pathways mediating specific fibre-induced effects on the metabolic phenotype remain unclear and, hence, it is not possible to personalize fibre-based interventions. Fibre is an instrumental dietary component that can be used to remodel gut microbiota composition and function to potentiate the beneficial effects of healthy diets on body weight management and metabolism. Experimental models revealed that diet-microbe interactions contribute to obesity, for example, by increasing lipid absorption or aggravating adipose tissue inflammation independently of adiposity, in the context of diets rich in saturated lipids (Benítez-Paez et al. 2016). According to actual evidence, it can be inferred that consumption of a varied diet with an important proportion of vegetables and fruits (cell wall carbohydrates and lignin, antioxidants) and, probably, also algae in some diets, gives rise to a typical gut microbiota that carries healthy benefits to the host. Conversely, a meat rich diet combined with low proportion of vegetables and fruits would promote the development of the microbiota responsible for anaerobic fermentation into the gut and deliver of sulphur compounds (Conlon and Bird 2015). The differences in bacterial community structures of native African populations reflected the diets of the hosts. Western diets, characterized by higher intakes of dietary animal proteins (as meat, milk and eggs), may deliver greater amounts of sulphur compounds to the colonic microbiota, thus favoring sulfidogenic hydrogen disposal. On the other hand, methane is the major hydrogen sink in Native Africans, who have lower intake of animal products and higher breath methane concentrations than the westernized populations.

The immune defenses along the intestine, including the mucus barrier, help prevent potentially harmful bacteria from causing tissue damage (Conlon and Bird 2015). The microbial metabolism contributes to the host immunity because microbial enzymes mediate the conversion of tryptophan into indole and indole derivatives that shape human host immune responses. The indole 3-aldehyde produced by the microbiome acts like an activating ligand for human host aryl hydrocarbon receptors, which are expressed by the immune cells. For example, the binding of indole induces the IL-22 secretion by innate lymphoid cells, promoting the secretion of antimicrobial peptides that protects the host from pathogenic infection by *Candida albicans*. Microbial production of SCFAs (acetate, propionate, butyrate, succinate and lactate) from dietary fibre also shapes host immunity, contributing to both innate and adaptive immune system functions (Guthrie et al. 2019).

The maintenance of a diverse and thriving population of beneficial gut bacteria helps keep harmful bacteria at bay by competing for nutrients and sites of colonization. Diet, particularly the use of a range of fibres, may be the best way of maintaining a healthy gut microbiota population (Conlon and Bird 2015).

4.1.4.3.1 Microbiota Enzymes' Machinery

Symbiotic microorganisms that reside in the human intestine are adept at foraging glycans, including those in dietary plants (starch, hemicellulose and pectin), animal-derived cartilage and tissue (glycosaminoglycans and N-linked glycans), and host

mucus (*O*-linked glycans). Most gut bacteria can possess multiple enzymes that have activity against isolated plant polysaccharides, but only a few gut bacteria, are directly engaged in the breakdown of recalcitrant insoluble substrates, such as those organized into the plant cell wall structure (Flint et al. 2008). Fluctuations in the abundance of dietary and endogenous glycans, combined with the immense chemical variation among these molecules, create a dynamic and heterogeneous environment in which gut microorganisms proliferate (Koropatkin et al. 2012). Descriptions of the microbial communities that live on and in the human body have progressed at a spectacular rate over the past 5 years, fuelled primarily by highly parallel DNA-sequencing technologies and associated advances in bioinformatics, and by the expectation that understanding how to manipulate the structure and functions of our microbiota will allow us to affect health and prevent or treat diseases. Among the myriad of genes that have been identified in the human gut microbiome, those that encode carbohydrate-active enzymes are of particular interest, as these enzymes are required to digest most of our complex repertoire of dietary polysaccharides (El Kaoutari et al. 2013).

The human gut microbiota is rich at the species level, but somewhat limited in terms of phylum-level bacterial diversity; furthermore, archaea are mainly represented by members of the genus Methanobrevibacter. The most commonly represented bacterial phyla in healthy adults are Firmicutes and Bacteroidetes, with significant numbers of Actinobacteria and Proteobacteria members also present. The relative proportions of these phyla sometimes diverge widely, reflecting not only interpersonal, geographical, lifestyle and temporal variations, and perturbations caused by disease, but also variations in the metagenomic protocols used to determine the composition of the microbiota. The ability to utilize complex dietary and host glycans is central to the survival of prominent members of the gut microbiota. Plants in the form of fruits, vegetables and cereals are major components of the human diet that provide dietary fibre (El Kaoutari et al. 2013). The biochemistry of the various host and dietary glycans that enter the gut is exceptionally diverse. Dietary fibre comprises many structurally diverse sugar moieties joined together by glycosidic bonds to form chains and branches. Generally, the more complex the polysaccharide, the more enzymes are required for its breakdown. Many different glycosidic linkages may be incorporated into a single polymer, so degradation of these polymers requires several linkage-specific degradative enzymes. Polysaccharide chain length or DP and branching of the fibre influence the ability of bacteria to utilize it as an energy source.

As reported by McKeen et al. (2019), dietary glycans are at the core of immunological interactions between host cells, microbes, and the mucosal matrix. Multiple pathways of immunomodulatory action have been identified, leading to the reclassification of functional polysaccharides as secondary metabolites and biological response modifiers. Flint et al. (2008) indicated that the human genome encodes, at most, only 17 enzymes for the digestion of food glycans, specifically starch, sucrose and lactose, as above mentioned. On the other hand, digestion of plant material occurs through fermentation, in which the chemical energy in a carbon source is converted into ATP that is used by cells in the anaerobic environment of the intestine

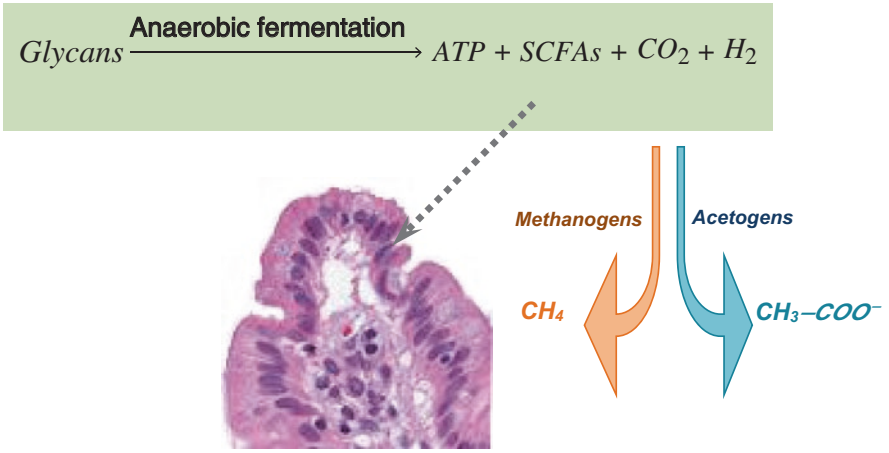

Fig. 4.1 Anaerobic fermentation reaction taking place in the colon lumen catalyzed by the enzymes of the microbiota, with production of energy as ATP, short chain fatty acids (SCFAs), carbon dioxide and hydrogen. These products are afterwards involved in the conversion to methane and acetate by the methanogen and acetogen microorganisms, respectively. SCFAs are absorbed by the colonocytes

(Fig. 4.1). The major end products of fermentation at the colon are the SCFAs butyrate, acetate and propionate, which provide approximately 10% of the calories that a human absorbs (the value varies depending on our diets), and are involved in numerous physiological processes. For example, SCFAs have been associated with a reduced risk of cardiovascular and inflammatory bowel diseases, and type 2 diabetes. Furthermore, butyrate is a main energy source for colonocytes and has been associated with a reduced risk of colorectal cancer.

Carbohydrate-active enzymes encoded by the human gut microbiome catalyse the breakdown of glycoconjugates, oligosaccharides and polysaccharides to fermentable monosaccharides. There are two types of enzyme that cleave glycosidic bonds between carbohydrates or between a carbohydrate and a non-carbohydrate moiety:

– Glycoside hydrolases (e.g. bacterial cellulases, mannanases, xyloglucanases, bacterial xylanases): cleave bonds by the insertion of a water molecule (hydrolysis), and they are classified into 130 families.
– Polysaccharide lyases: cleave complex carbohydrates using an elimination mechanism, and they are segregated into 22 families.

Bacterial pectinases are found in glycoside hydrolases and polysaccharide lyases families. An additional category of carbohydrate-active enzymes associated to the food digestion by microbiota is that of the carbohydrate esterases, which remove ester substituents from the glycan chains to facilitate the action of glycoside hydrolases and polysaccharide lyases (El Kaoutari et al. 2013).

Some microorganisms in the intestinal tract target dozens of glycans and possess the corresponding enzymatic tools for depolymerizing each of these molecules into their component sugars. Gut microorganisms vary widely in the number of different glycans that they are capable of targeting. As an example, the human gut symbiont *Bacteroides thetaiotaomicron* can degrade more than a dozen types of glycan, whereas some species are restricted to one or a few types (Koropatkin et al. 2012).

From an ecological perspective, species with broad glycan-degrading abilities can be thought as "generalists" that shift their metabolism from meal to meal, whereas species with narrower glycan-degrading potential can be considered "specialists" that focus on one or a few glycans. Specialists run the risk of becoming extinct in a host if their preferred nutrients wane for too long, so such microorganisms would most probably evolve to degrade ubiquitously abundant dietary glycans or host-derived mucins. Thus, the gut microbiota grown in hosts that consume vegetable, fruit and cereal reach diets has "specialists" species that can be absent in diets poor in these items. However, when a fully omnivorous diet is achieved after weaning, the composition of the microbiota stabilizes and experiences fewer temporal changes. Two bacterial phyla, Firmicutes and Bacteroidetes, are numerically dominant in the adult microbiota. Microbes of the first phylum are usually the most abundant, but the ratio of firmicutes to bacteroidetes can change over time and be influenced by different diets, especially those that promote changes in host adiposity. Actinobacteria is the third phylum that also contributes to the human microbiota, being in general underestimated due to the molecular approaches used. A predominantly vegetarian, high-fibre African diet is conducive to the growth of specific fibre-degrading species, which involve a higher prevalence of bacteroidetes and actinobacteria than of firmicutes and proteobacteria, while the opposite trend was observed in European individuals, who consumed a lower fibre diet, more typical of the Western societies (Koropatkin et al. 2012).

Beyond the influence of certain types of diet in shaping the composition of the microbiota, supplementing the diet with particular glycans can affect species abundance. Not all species that possess the potential to degrade a given glycan will do so successfully in vivo. As an example, inulin and shorter FOS selectively increase the abundance of *Bifidobacterium* spp., although many *Bacteroides* spp. are also able to use these glycans. The microbiota can change rapidly according to the composition of two following meals in the same day. A rapid shift from a high-fat diet to a high-carbohydrate diet results in community changes that are observable after just 1 day, but take several days to stabilize. *Bacteroides ovatus* has an enzyme machinery that targets arabinoxylans of maize. Also, enzymes to hydrolyze other hemicelluloses (equatorial β-1→4 link) such as β-glucans, galactomannan, glucomannan, xylans, and xyloglucan. *Bacteroides thetaiotaomicron* has two different groups of enzymes able to hydrolyze the equatorial-axial β-1→4 link of the galactan lateral chains of pectins (two enzymes), the arabinan side chains (two groups of six enzymes), the arabinogalactan side chains, the rhamnogalacturonan I and II, and the homogalacturonan backbone (seven enzymes) of pectins. Also, other groups hydrolyze the β-2→6 fructan link of levan, and the links of starch (Koropatkin et al. 2012).

Hemicelluloses such as the arabinoxylans of maize contain ferulate as pendant group and as crosslinker of these macromolecules (ferulate, di or triferulate esters. After fermentation in the gut by endo-β-1,4-xylanases (endoxylanases) that cleave β-1,4-glycosyl linkages within the poly-β-1,4-xylose backbone, readily soluble arabinoxylans of different DP, containing ferulates, can be produced, which are more powerful antioxidants than the free ferulic acid. In addition, ferulate esterase of the gut microbiota liberates ferulic acid from hemicellulases and oligosaccharides into the caeco-colon lumen (Broekaert et al. 2011).

The phylum Bacteroidetes possess the starch utilization system (Sus) as the efficient strategy for competing for this nutrient. In their outer membrane and the periplasm of these bacteria, the Sus works to sequentially bind starch to the cell surface, degrades it into oligosaccharides and transports them into the periplasmic space, where the oligosaccharides are degraded to even simpler sugars like D-glucose, and imported into the cell. Unique to Bacteroidetes are also the called Sus-like systems which function by a similar mechanism as Sus but harbor enzymes that are predicted to target glycans other than starch (Koropatkin et al. 2012).

Insoluble fibres such as cellulose, are generally poorly fermented by human gut microbes, but their presence in the diet increases gut transit rate and thus reduces the amount of time available for colonic bacterial fermentation of non-digested foodstuff (Holscher 2017). The ability to degrade cellulose seems to be essential for the disruption of most plant cell-wall structures of vegetable tissues, as non-cellulolytic bacteria have limited ability to solubilize this material. Cellulolytic bacteria are generally defined by their ability to degrade and grow on highly ordered forms of cellulose. In the human colon, the digestibility of cellulose from dietary fibre is reportedly far higher than that of the purified crystalline cellulose, and the cellulolytic bacteria that have been isolated from the human gastrointestinal tract have less activity than their rumen counterparts against more recalcitrant cellulosic substrates. Cellulolytic bacteria require the ability to degrade matrix polysaccharides, such as xylans, mannans and pectins, to access cellulose fibrils, although they do not necessarily use the solubilized products, which become available to other members of the community through cross-feeding. This task is performed by the cellulosome, which is a discrete, extracellular, multi-component, multi-enzyme complex that is found in anaerobic cellulolytic bacteria and provides enhanced synergistic activity among the different resident enzymes to efficiently deconstruct the intractable cellulosic and hemicellulosic substrates of the plant cell wall. Some of the components of the cellulosome are structural and some are enzymatic. Although the systems that have been described so far in the abundant Gram-negative Bacteroidetes seem to be most suitable to the sequestration of soluble polysaccharides, some Bacteroides species that have been reported in the human colon, particularly the *Bacteroides cellulosilyticus*, have activity against insoluble cellulose (Flint et al. 2008).

In addition to the degree of polymerization, the accessibility within the digesting food particles and solubility of complex carbohydrates impacts the location of their respective fermentation within the human gastrointestinal tract. Regional variations in microbial colonization of the colon exist, along its length, simultaneous to the decrease in transit velocity. Soluble fibres, such as FOS and pectin are metabolized

by bacteria more proximally in the gastrointestinal tract, ileum and ascending colon, while the least soluble fibres like cellulose, can be partially fermented in the distal colon where the slowest transit time and the highest bacterial density exist (Koropatkin et al. 2012; Holscher 2017). The most soluble easily digestible glycans are metabolized in the ileum, caecum and ascending colon at decreasing rates, as their solubility decreases. The brush-epithelium is covered by a thinner mucus, the transit is faster and lower bacteria density exists. Along the transverse and descending colon, the velocity of transit is also continuously decreasing while the concentration of bacteria increases simultaneously. Just in the sigmoid colon and rectum the mucus is thick, the transit is slow, and the highest bacteria density is found, with colonization of fibre particles and outer mucus layer. Therefore, the least soluble, indigestible glycans are fermented by bacteria located in the descending and sigmoid colon (Flint et al. 2008; Koropatkin et al. 2012; Holscher 2017).

4.1.5 Dietary Fibre and Bioactivity

Dietary fibre have gained attention over the past 20 years due to its bioactivity which means its potential health benefits in reducing the risks of many diseases, such as diabetes, cancer, cardiovascular diseases, and obesity. These benefits are related, in many cases, to its functional properties, in addition to their basic nutritional functions.

4.1.5.1 Dietary Fibre and the Glycemic Response

Diabetes mellitus (DM) is a metabolic disease that occurs when the body does not produce insulin (Type I diabetes) or the body does not use insulin properly (Type II diabetes), leading to high glucose concentration in blood (hyperglycemia) (ADA 2019). In 2017, approximately 425 million adults were living with diabetes and it is estimated that by 2045 this will rise to 629 million (IDF 2017). A healthy diet is key to manage type II diabetes, the most common type (ADA 2019).

Many prospective cohort studies have shown that a relatively high intake of dietary fibre (DF) is inversely associated with the risk of diabetes compared with a low intake (Nie et al. 2019).

DF can act in the small intestine as soluble polymer chains in solution, as insoluble macromolecular assemblies, and as swollen, hydrated networks (Eastwood and Morris 1992). Therefore, DF intake improves postprandial glucose and insulin response by slowing sugar absorption and causing a bulking effect in the stomach, and the added satiety results in the reduction of energy intake (Nie et al. 2019). DF may also be able to decrease gross energy of a food due to its lower energy density (Lattimer and Haub 2010). Goff et al. (2018) proposed four possible mechanisms for controlling glycemia:

1. Delay of gastric emptying (GE): high DF diets results in lower gastric emptying rates, slower rates of absorption of glucose into the blood and lower insulin responses, suggesting that GE is the predominant mechanism involved. This effect is attributed to the viscosity of soluble DF and to attenuation of enzyme action due to non-specific binding with insoluble DF.
2. Hormonal regulation: DF can affect the release of gastric and intestinal hormones regulating digestion and absorption. In addition, short-chain fatty acids resulting from DF colonic fermentation also stimulate the release of gut-derived hormones.
3. Reduced α-amylase activity in the small intestine: this effect can be attributed to various mechanisms such as the formation of DF-starch complexes where DF acts as a barrier between starch and enzyme, the adsorption of enzyme to DF leading to its inhibition, the reduction of water availability for starch hydrolysis and the slowing of enzyme and substrate diffusion due to increased viscosity, among others.
4. Delay of sugar absorption: DF might delay the diffusion of sugars in the small intestine.

These authors indicated that the rheological behavior of food not necessarily reflects its rheological behavior in the gut, so digesta viscosity is a more effective way of measuring glucose levels regulation than solution viscosity.

The chemical composition and structure as well as the molecular weight (MW) of polysaccharide chains influence the ability of DF to exert physiological functions. There are discrepancies about the effect of the solubility nature of DF and its beneficial effect in glucose levels regulation. Although it has been generally accepted a relationship between viscosity and reduction of blood glucose, the exact impact of viscosity is unclear (Goff et al. 2018). According to Gowd et al. (2019), several prospective cohort studies associate the intake of insoluble DF with a protective effect against insulin resistance and DM, while consumption of soluble DF gives little protection. The positive effects of insoluble DF are attributed to fermentation and short chain fatty acids production in gut microbiota. Short chain fatty acids promote the secretion of key hormones to prevent gluconeogenesis in the liver, activate intestinal gluconeogenesis and improve insulin sensitivity.

Nevertheless, most studies focus on the viscosity effect of soluble DF. In order to verify whether other fibre characteristics, beyond viscosity, can have an impact on glycemia and appetite sensations, Paquet et al. (2014) compared the effects of two juices of similar viscosity but enriched with guar gum/xanthan gum or konjac-mannan/xanthan gum mixture and a control non-enriched juice on the variation of glucose, insulin, C-peptide and appetite sensations in 20 healthy men with similar glucose, insulin and C-peptide concentrations before the consumption of the three juices. Juices enriched with fibres failed to significantly reduce postprandial glucose, insulin and C-peptide responses compared to the control beverage, but the beverage enriched with konjac-mannan/xanthan gum decreased significantly the appetite score, and increased fullness sensation suggesting that viscosity is not the unique factor influencing appetite responses.

Repin et al. (2018) studied the amylolysis of modified tapioca starch in simulated small intestinal conditions in the presence of each of four dietary fibre types (yellow mustard mucilage, soluble flaxseed gum, fenugreek gum, and oat gum) at concentrations to match for post-digestion viscosity. Studying the progress of amylolysis by measuring the decline of digesta apparent viscosity over time, they observed that supplementation of digesta with DF reduced the progress of both the digesta apparent viscosity decline and the changes in digesta reducing sugar content. Authors attributed these effects to the reduced diffusion of enzyme and/or substrate and concluded that to alter amylolysis to a similar extent, fibres have to be present at amounts resulting in similar post-digestion viscosity even though their concentrations may not match.

Fabek et al. (2014) investigated the effects that digestive processes in the stomach and small intestine have on the thickening capacity of six soluble DFs (guar gum, locust bean gum, fenugreek gum, xanthan gum, soluble flaxseed gum, and soy soluble polysaccharides). They performed a two-stage in vitro digestion, simulating gastric and small intestinal phases, in order to evaluate changes in viscosity. Gums were used at defined concentrations to create equi-viscous solutions. Their flow behavior was analyzed after exposure to simple dilutions, pH changes, and in vitro digestion. Authors observed minor effects of pH and digestive enzymes on fibre structure. Xanthan gum retained viscosity more than all other DF types. Later, using a dialysis system, protein and starch were mixed with gums to study glucose release in a food model, in vitro. Although all gums lowered glucose concentration, xanthan gum was the most effective. With these results, the authors concluded that digesta viscosity of soluble fibres does not depend on their initial viscosity or concentration but on their ability to resist changes during digestion.

In a later study, Fabek and Goff (2015) examined the effect of adding viscous soluble DFs on starch digestibility during simulated intestinal digestion. The model food consisted of tapioca starch (4% w/w), skimmed milk (8.65% w/w) and xanthan gum (4% w/w), guar gum (3% w/w), soluble flaxseed gum (7% w/w) or soy soluble polysaccharide (20% w/w). Gum concentrations were chosen to give matching viscosities. Solutions were submitted to a 3-stage in vitro digestion (salivary, gastric, and small intestinal phases). Light scattering results showed that the particle size of starch granules decreased through the digestion process. Microscopy showed granule surface degradation for the control, flax and soy solutions while this effect was attenuated for granules extracted from the guar gum and xanthan gum solutions, which had greater viscosities inside the digesta in comparison to the other treatments. The authors observed that including DFs that can retain viscosity during digestion, reduced starch hydrolysis and suggested that the increase in viscosity interferes with enzyme diffusion, leading to a reduced amylolysis. In addition, the authors considered the ability of some gums to allow granules agglomeration, thus reducing the area exposed to enzymes. Based on these results, authors suggested that the glucose-lowering ability of viscous DFs might be related to their ability to reduce the rate at which starch granules are hydrolyzed inside the lumen.

Using β-glucans, Kwong et al. (2013) studied the effect of varying solution viscosity on glycemic responses. For this, they changed solution volume, without

changing the β-glucan dose or MW. A total of 15 healthy subjects received six 50 g oral glucose beverages prepared with or without 4 g of high-MW (580,000 g/mol) or low-MW (145,000 g/mol) β-glucan, with a beverage volume of 250 or 600 ml. Postprandial plasma glucose concentration was measured over 2 h. The physico-chemical properties of the beverages were also measured. The high-MW β-glucan beverage, which was more viscous, achieved greater reductions in plasma glucose concentrations than the beverage with low-MW β-glucan. At the same MW, the 250 and 600 ml β-glucan beverages differed in viscosity but not in postprandial plasma glucose concentration. Authors concluded that β-glucan dose and MW are the most vital characteristics for improving the bioactivity of β-glucan solutions with respect to glycemic response.

Abirami et al. (2014) used the pulp and peel DF from *Citrus hystrix* and *Citrus maxima* to study their potential role in lowering postprandial serum glucose level through in vitro assays and observed that these DFs could effectively adsorb glucose, retard glucose diffusion and post-pone the release of glucose from starch to different extents.

Feinglos et al. (2013) performed a double-blind, placebo-controlled 20-week clinical study to evaluate the effects of psyllium (two different doses) on fasting blood glucose and glycosylated hemoglobin in 37 patients being treated for type-2 DM and on a restricted diet. Both doses of psyllium significantly lowered blood glucose and glycosylated hemoglobin compared to placebo treatment at week 12. The improvement in glycemic control observed was above that already conferred by a restricted diet.

To evaluate the effect of oat β-glucan on postprandial glycemia attenuation, Regand et al. (2009) prepared muffins, granola, porridge and pasta containing 4 g of β-glucan and control products with low β-glucan content prepared with wheat flour. They determined the viscosity and MW of β-glucan in vitro-digestion extracts and the fasting and postprandial blood glucose concentrations in 12 human subjects in a period of 4 weeks. Porridge and granola were the most effective in attenuating the glucose peak in blood glucose response and authors attributed this to the high MW of their components and to viscosity.

Steinert et al. (2016) assessed the effect of consuming a pre-load of a commercially available oat-bran at different concentrations before a test-meal of white bread on glycemic responses in 10 healthy humans. They observed a significant effect of dose on blood glucose reduction suggesting the use of oat bran as nutritional preload strategy in the management of postprandial glycemia.

Kubo et al. (2016) tested the combined effects of wheat albumin, which inhibits mammalian amylase, and DF, which retards sugars absorption, in a rat model of type 2 DM. The DF mixture (54.4% of total DF, 39.9% water soluble DF) consisted of oat, chicory root, guar bean, barley leaves, konjac potato, and seaweed. The bioactive ingredients were added to a soluble starch solution. Authors observed that the combined intake of both ingredients suppressed hyperglycemia more effectively than each separate intake. They also observed an improvement in liver and plasma lipids contents.

Regand et al. (2011) studied the effect of oat β-glucan in a granola model food on starch digestibility and glycemic responses. Blood glucose concentrations were measured before and after ingesting wheat and oat granolas, with 0.6 and 6.2 g of β-glucan, respectively, and two starch doses (40 and 60 g). The authors observed a reduction of in vitro starch digestibility and lower blood glucose levels when in vitro sample viscosity increased. Moreover, β-glucan was significantly more active in reducing blood glucose rise when the β-glucan/starch ratio was 0.16 rather than 0.11.

Rohajatien et al. (2018) studied the effect of feeding bitter melon fruit to rats with and without hyperglycemia in a 4 weeks experiment. At week 4 of experiment, they observed a decrease of 56% blood glucose level in hyperglycemia rats when compared to week 0, and ascribed these effects to the DF of melons, mainly pectin.

Huang et al. (2019) compared the in vitro hypoglycemic capacities of orange pomace and extruded orange pomace powders. The extruded pomace, which had a higher soluble DF content, was more effective to retard glucose diffusion and inhibit α-amylase activity than the non-extruded sample and authors suggested that a higher soluble DF content would lead to higher glucose adsorption and may contribute to the retarding of α-amylase hydrolysis of the starch molecules.

Cassidy et al. (2018) performed and extensive review on the effects of soluble DF (β-glucan, guar gum. psyllium, alginate) on postprandial blood glucose response. They concluded that overall, several soluble DFs have shown beneficial effects in lowering the postprandial blood glucose response however issues with palatability have limited their development in the functional food industry. Authors state that while research is scarce investigating the effect of processing on many of these soluble DFs, results from clinical studies show that some soluble DFs, mainly β-glucans that have undergone minimal processing can attenuate the postprandial blood glucose response when consumed with a high carbohydrate food or beverage.

Lu et al. (2013) studied the effect of replacing 25%, 15% or 10% wheat flour with okara powder (a byproduct of tofu or soy milk production process) to make noodles and bread enriched in DF, mainly insoluble DF, on glycemic response (GI) in vivo. The results showed that the GI of okara foods was markedly lower than that of control foods, with values for okara bread, okara steamed bread and okara noodle of 49, 54 and 52, respectively, referring to glucose (GI = 100). While the values obtained for control foods were 67 for bread, 86 for steamed bread and 77 for noodle.

It can be concluded that there are different mechanisms by which dietary fibres can help to control glycemia. The DFs that perform this control more efficiently, according to literature, are those that can exert the effects summarized in Fig. 4.2. Nevertheless, more systematic studies are necessary to clarify the effect of fibers from different sources on short-chain fatty acids production and of these compounds, on glycemia control.

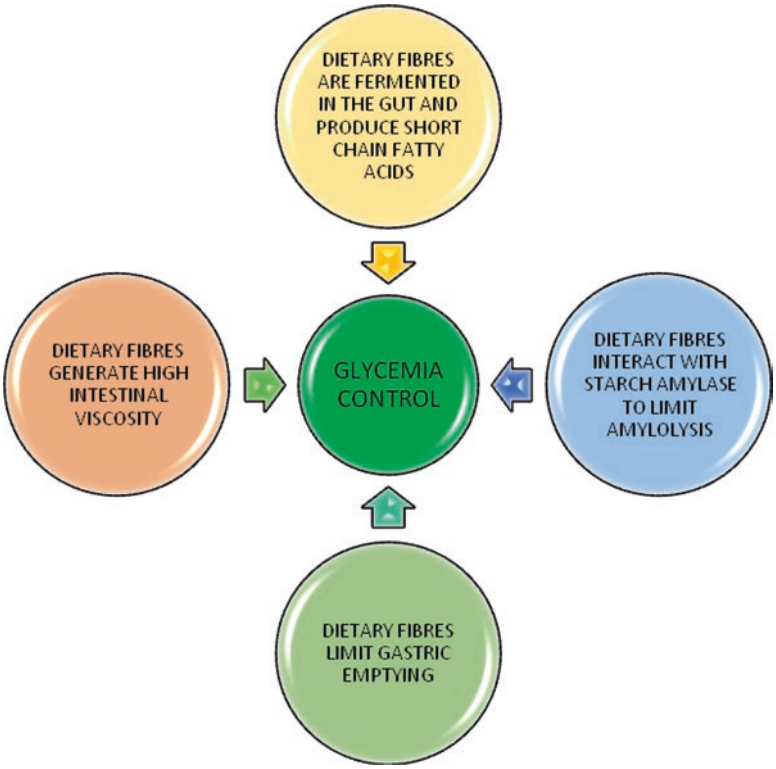

Fig. 4.2 Effects by which dietary fibres help to control glycemia

4.1.5.2 Dietary Fibre and Obesity

The accumulation of excessive fat in the body causes overweight and obesity, which lead to chronic health problems such as cardiovascular diseases and type-2 diabetes.

According to Maheshwari et al. (2019), β-glucans from oat and barley reduce appetite and weight providing satiation along with nutrition. Authors suggest this could be due to the high viscosity and water binding capacity of β-glucans, which prolongs the digestion in the gut. Huang et al. (2011) studied the effects of β-glucan from oats, on the activation of gut hormone, satiety, and weight loss in diet-induced obesity mice. Authors observed that the energy intake and body weight gain were lower with increasing β-glucan over 6 weeks. A gut-hypothalamic anorexigenic pathway was activated and the response was in a dose-dependent manner. The increased satiety appeared to be long-lasting without the development of a tolerance effect. In this study all diets had the same total fibre content having included insoluble DF from wheat in diets with lower β-glucan content and authors suggested that oat β-glucan may have some advantages over other sources of DF.

Hamden et al. (2018) studied the effect of pectin in high-fat/fructose diet induced obesity, hyperlipidemia and hyperglycemia. Administration of pectin to rats

decreased lipase activity improving body weight. Cholesterol and triglycerides also decreased. In addition, it was observed a decrease in α-amylase activity leading to lower blood glucose levels.

Drew et al. (2018) studied the effects of seven DFs (β-glucan, pectin, inulin, inulin acetate ester, inulin propionate ester, inulin butyrate ester or a combination of inulin propionate ester and inulin butyrate ester) in obesity prevention. During 8 weeks, mice were fed either high-fat, low-fat or high-fat/DF-supplemented diets. Results showed that all of the DFs prevented weight gain and produced similar responses in body composition and host gene expression in cecum and liver. While cecal bacterial profiles differed with each specific dietary fibre, authors observed collective outcomes in the expression of certain host genes and established common gene expression differences in the host. This implies that bacterial composition per se may not be causal in protecting against weight gain. In conclusion, diverse DFs prevented weight gain on a high-fat diet, despite giving rise to different cecal bacteria profiles.

Du et al. (2010) investigated the association of total DF, cereal DF, and fruit and vegetable DF with changes in weight and waist circumference in a 6.5-year follow-up study with 89,432 European participants. DF consumption was inversely associated with subsequent weight and waist circumference change. A 10-g/day total DF intake was associated with a reduction in body weight of 39 g/year and a reduction in waist circumference of 0.08 cm/year. When evaluating the effect of the fibre source, they observed that a 10-g/day cereal DF intake reduced body weight in 77 g/year and waist circumference in 0.1 cm/year, while fruit and vegetable DF was not associated with weight change but had a similar association with waist circumference. Authors concluded that there is a beneficial effect of DF intake, particularly cereal DF, in preventing body weight gain.

Bozzetto et al. (2018) reviewed epidemiological and observational studies concerning the effect of DFs on obesity-associated cardiovascular events. They found evidence from epidemiological studies that consuming more than 20 g DF/day is associated with body weight loss in the long term. From observational studies, authors also found an inverse association with DF intake and a percent body fat.

Samout et al. (2016) performed a study on rats evaluating the effect of apple pectin supplementation on obesity. Results showed that treatment with the aqueous extract of pectin decreased the weights of the rats. In addition, high-fat diet treatment induced severe liver and kidney damage as determined by several biomarkers in blood but when high-fat diet-treated rats were also fed pectin, all those biomarkers were restored to almost normal values. The apple pectin extract reduced lipid peroxidation and enhanced the expression of intracellular endogenous antioxidants.

Zhan et al. (2019) studied the effect of citrus pectin in mice that were first exposed to a typical environmental pollutant, p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE), in order to induce obesity. Pectin was supplied during and after interruption of p,p′-DDE exposure. They analyzed the body and fat weight gain, plasma lipid profile and insulin resistance of mice and analyzed gut microbiota composition and the levels of short-chain fatty acids. Results showed that pectin supplementation

reversed body and fat weight gain, dyslipidemia, hyperglycemia and insulin resistance and authors ascribed this to the regulating gut microbiota effect of pectin.

Bray et al. (2018) compared the effects of a high-fat cellulose diet (60% fat + 10% cellulose), a high-fat pectin diet (60% fat + 10% pectin), a low-fat cellulose diet (10% fat + 10% cellulose), and a low-fat pectin diet (10% fat + 10% pectin) on mice for 12 weeks. In high-fat diets pectin prevented additional weight gain while for low-fat diets, it was determined a weight loss of 22.2 and 25.4%, for cellulose and pectin, respectively. Both low-fat diets and high-fat pectin diet reduced fasting blood glucose, improved glucose tolerance and decreased fatty liver when compared to high-fat cellulose diet. Authors concluded that pectin could moderate some obesity-related morbidities in the presence of high fat.

Choi et al. (2016) isolated a pectic polysaccharide composed of rhamnogalacturonan I with arabinan and arabinogalactan chains from *Morus alba*. This polysaccharide was able to inhibit the proliferation of pre-adipocyte cells in a dose-dependent manner to 91, 75, 68 and 54% viabilities at sample concentrations of 50, 100, 200 and 500 μg/ml, respectively, compared to untreated control cells. Authors suggested that this polysaccharide is able to reduce the number of fat cells and the mass of adipose tissue and could be used for the treatment or prevention of obesity disorders.

It can be concluded that research supports a beneficial role of higher intake of DF in the prevention of obesity. According to Du et al. (2010) the mechanisms by which this role is developed are: (1) reduced digestion rate which stimulates the release of gut hormones promoting satiety, (2) increased viscosity in the case of soluble DF, (3) low energy density, (4) reduced postprandial blood glucose response, and (5) its acting as a mechanical barrier to the enzymatic digestion of other macronutrients such as fat and starch in the small intestine. More recent studies ascribed also the prevention of obesity to the regulation of gut microbiota by certain dietary fibres.

4.1.5.3 Dietary Fibre and Cancer

Almost 50 years ago, Burkitt (1971) observed lower rates of colorectal cancer among Africans who consumed a diet high in fibre. Ever since, most of the research on DF and cancer prevention has focused on colorectal cancer. Increased DF intake may lead to a dilution of fecal carcinogens, reduced transit time, and bacterial fermentation producing short-chain fatty acids with anti-carcinogenic properties (Kunzmann et al. 2015). Evidence from case-control studies also suggests that DF may be inversely related to breast cancer risk and this could be associated with the inhibition of intestinal reabsorption of estrogens by DF and the subsequent increased fecal excretion of estrogens (Aune et al. 2012).

Many investigations on DF and cancer have focused on pectin. Zhang et al. (2015) suggested that the antitumor capacity of pectin and its effect in colon cancer prevention is correlated with pectin probiotic activity. On the other hand, there is growing evidence that the arabinogalactan/galactan content of pectins provides a natural source of ligands to inhibit the biological functions of galectin-3 (Gal-3) (Morris et al. 2013). Elevated levels of Gal-3 in the serum have been linked to the

development of several different cancers as well as cancer metastasis (Zhang et al. 2015). It is important to remark that modification of pectin generates homogalacturonans and fragments containing rhamnogalacturonan I, which are pectin-derived products rather than pectins (Morris et al. 2013). Most researches performed on pectin and cancer prevention are based on pectin-derived fragments, which are more accessible to galectins. Moreover, pectin modification to degrade the polymer and to decrease its degree of esterification may produce antitumor activity by intervention in ligand recognition by Gal-3 (Zhang et al. 2015).

Bergman et al. (2010) compared the effects of citrus pectins with different degrees of esterification (DE: 30%, 60% and 90%) on the proliferative capacity of four malignant cell lines (2 human colon carcinoma cell lines, 1 human erythroleukemia cell line, and 1 Burkitt lymphoma cell line). Pectins with DE 30% or 60% at increasing doses caused a dose-dependent inhibition of colon carcinoma and leukemia cells but neither pectin affected Burkitt lymphoma cells. Authors concluded that as the cells that were affected by pectin express galectin receptors, while those cells that were not affected are deficient of this receptors, probably the antiproliferative effect of citrus pectin is due to its ability to inhibit galectin function.

Citrus pectin when modified by high-pH and temperature is rich in galactosyl, a ligand for Gal-3. Liu et al. (2008) studied the effect of modified citrus pectin in the inhibition of the expression of Gal-3 in liver metastasis of colon cancer. The study was performed with 75 mice injected with colon cancer cells. Liver metastasis of colon cancer was observed after 3 weeks. Mice were fed pectin through drinking water at concentrations of 0.0%, 1.0%, 2.5% and 5.0% (w/v) and the percentage of liver metastasis was 100%, 80%, 73.3% and 60%, respectively. The concentration of serum Gal-3 in pectin treated mice was significantly higher than that in the negative control group. Authors concluded that Gal-3 expression increases in liver metastasis and can be inhibited by modified citrus pectin.

Xue et al. (2019) studied the effects of ginseng pectin derivatives on Gal-3-mediated T cell activation and apoptosis. They isolated two fractions from ginseng roots, which were enriched in rhamnogalacturonan I: WGPA-UD was composed of GalA (24.6%), Rha (10.8%), Gal (30.8%), and Ara (20.6%), while RG-I-4 was composed of GalA (33.8%), Rha (21.8%), Gal (19.5%), and Ara (9.2%). Authors also prepared modified citrus pectin (85% GalA, 1.6% Rha, 9.3% Gal and 4% Ara) and purified potato galactan (11.3% GalA, 6.1% Rha, 70% Gal and 10.0% Ara). Both ginseng fractions inhibited apoptosis, but not activation, whereas potato galactan promoted activation, but not apoptosis, and citrus pectin affected both of these activities, indicating that these substances selectively act on different cell processes, even though they all bind Gal-3. Later, to investigate the anti-tumor activity of these samples they performed a study in mice where samples (10 mg/kg body weight) were administered daily following tumor cell inoculation. Authors observed that only ginseng samples WGPA-UD and RG-I-4 could inhibit tumor growth by 29% and 45%, respectively and demonstrated that ginseng pectins could selectively inhibit Gal-3-induced T-cell apoptosis, while not affecting T-cell activation.

Cobs-Rosas et al. (2015) studied the effect of pectins extracted from defatted rapeseed cake on cancer MCF-7 (human breast adenocarcinoma) and Caco-2

(human colorectal adenocarcinoma) lines. All the pectins extracted exhibited antiproliferative activity, being more effective on MCF-7 cells than Caco-2.

Cheng et al. (2011) studied the anticancer activity of structurally different ginseng polysaccharides: homogalacturonan- rich pectins, arabinogalactans with rhamnogalacturonan I domains, and one fraction containing glucan and arabinogalactan. The homogalacturonan rich fraction inhibited a human colorectal adenocarcinoma cell (HT-29) cell proliferation and induced apoptosis accompanied by the activation of caspase-3.

According to Wang et al. (2003), a pectic polysaccharide from *Centella asiatica* (L.) Urban could increase the immunological activity of T and B cells, being modulated by the carboxyl and acetyl groups of pectin.

Prado et al. (2019) extracted pectin fractions from papaya with ammonium oxalate and at different ripening-time points in order to relate changes in pectin structure with Gal-3 inhibition. Only one fraction, the less soluble one, was able to bind Gal-3 and diminished the proliferation of colon cancer cell lines. This fraction derived from an intermediate point of papaya ripening and had similar GalA content and degree of esterification from those of other ripening time points but it showed a lower MW peak and more exposed ramifications.

Fan et al. (2017) studied the effect of combining fish oil (containing polyunsaturated fatty acids) with fermentable DF in the prevention of colon cancer. Mice were fed diets containing 15% fat and 6% fibre by weight. The diets differed in the source of lipid (corn oil versus fish oil) and source of fibre (cellulose, which is poorly fermentable, versus highly fermentable pectin). The four dietary groups were corn oil/cellulose, corn oil/pectin, fish oil/cellulose, and fish oil/pectin. After 4 weeks of diet, authors observed that the combination of fish oil (containing ω-3 polyunsaturated fatty acids) and fermentable pectin (leading to butyrate production) acted coordinately to protect against colon cancer due, in part, to an enhancement of apoptosis across all stages (initiation, promotion, and progression) of colon tumorigenesis. Authors suggested that fish oil alters colonocyte mitochondrial membrane composition and function, creating a permissive environment for apoptosis induced by DF fermentation products

Triff et al. (2018) also investigated the effect of combining fish oil and fermentable DF in colon cancer. These authors suggested that the short-chain fatty acids produced by DF fermentation act as chemoprotectives and the polyunsaturated fatty acids in fish oil act as ligands for tumor suppressive nuclear receptors. They treated rats with a colon carcinogen and fed them diets containing fish oil, fermentable DF, a combination of fish oil and pectin, or control diet (with no fish oil or pectin). The fish oil/pectin diet generated unique epigenetic modifications and was the only one to induce the expression of chemoprotective genes.

Oh et al. (2019) performed a meta-analysis of prospective studies that included studies on fibre intake and outcomes including colorectal adenoma and colorectal cancer. Publications considered reported all DF sources (cereal/grain, vegetable, fruit, and legume) although for adenoma studies, there were no report on legume DF. From 4632 publications, 10 prospective studies (6 for colorectal cancer and 4 for adenoma) were included in the dose-response meta-analysis. They concluded

that although all DF sources may provide some benefits, the effect in colorectal cancer prevention is strongest for DF from cereals/grains.

It can be concluded that DF performs specific bioactive effects against certain cancers. According to literature, these effects are influenced by DF source and the high activity of pectin and its degradation products is remarkable.

4.1.5.4 Dietary Fibre and Cardiovascular Disease

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the United States and Europe.

The cardiovascular system is subject to life-style induced changes as well as natural deterioration due to the aging process. The vascular endothelium is a regulator of vascular homeostasis and endothelial disfunction contributes to the expression of CVD. A dysfunctional endothelium results in blood pressure desregulation and increased atherogenicity. Arterial disfunction, characterized by oxidative stress and inflammation-mediated endothelial disfunction and arterial stiffening, is the primary risk factor for cardiovascular diseases. Age, stress and dietary pattern have a significant role in modulating endothelial disfunction (Edirisingle and Burton-Freeman 2014).

Dietary fibre has been reported extensively as having a beneficial effect to prevent mortality due to CVD (Threapleton et al. 2013; Tang et al. 2018, Soliman 2019). Erkkila and Lichtestein (2006) informed that an increase in fibre intake reduces diet caloric density while soluble fibre exerts a beneficial effect on lipid and glucose metabolism but, according to the authors, data on its effect in arterial inflammation and coagulation are limited. Salas-Salvado et al. (2006) informed that dietary fibre decreases CVD risk independently of fibre type and concluded that, probably, this trend is associated not only to dietary fibre but also to numerous bioactive compounds (i.e. antioxidants) that are present in food rich in dietary fibre. Pita Lottenberg et al. (2010) stated that the immune and metabolic systems are closely related and act in an interdependent way. Inflammatory processes are associated with excessive fat tissue which has a pro-inflammatory activity which can help the development of other chronic diseases. Chronic diseases such as cardiovascular ones are associated with inflammatory processes due to the effect of low density lipoproteins that induce inflammation of the arteries endothelium. Inflammatory process markers are, for example, C-reactive protein, Interleukin-6 and leukocyte count. Casas et al. (2018) evaluated the effect of some constituents of the diet on CVD. They informed that there are different mediators of coronary artery diseases: C-reactive protein, interleukin IL-1, IL-18, IL-1β, IL-18, monocyte chemoattractant protein MCP-1 and tumor necrosis factor TNF-α, among others. These mediators are considered potential inflammation biomarkers and their expression may correlate with coronary artery diseases severity. And that these markers suffer a decrease when polyphenols are present in the diet. It must be remembered that polyphenolic compounds are present, in general, jointly with dietary fibre in fruit and vegetables.

The relationship between carbohydrate and dietary fibre intake and the risk of cardiovascular disease mortality in japanese was reported by Miyazawa et al. (2019). The study followed 8925 participants (3916 men and 5009 women) aged 30–79 years without CVD at baseline who participated in the National Nutrition Survey in Japan, concluding that higher intake of DF was associated significantly with a lower risk of CVD mortality in men and lower risk of stroke mortality in women. They also concluded that intake of carbohydrates, available carbohydrate and starch were not associated with the risk of CVD mortality in men or women.

Threapleton et al. (2013) reported that there are different mechanisms by which the DF can exert a protective effect on risk of CVD. Fibres with thickening effect can affect absorption of glucose and lipids in the small intestine, attenuating their postprandial rise and helping to maintain higher levels of satiety contributing to less weight gain. These authors also remarked that, additionally, soluble fibre is fermented in the large intestine giving origin to short chain fatty acids which reduce circulating levels of cholesterol. This constitutes a new point of view for the link between CVD risk and dietary fibre and it centers on the effect of dietary fibre on human gut microbiota.

McRae (2017) reported a review of meta-analyses concerning the dietary fibre beneficial effect for CVD prevention. The author concluded that dietary fibre produces a decrease in mortality and that this trend might be ascribed to: (a) reduced LDL cholesterol which originates in reduced cholesterol and fatty acid absorption, increased fecal bile acid excretion, bacterial fermentation that produces propionic acid that inhibits HMG- CoA reductase; (b) reduced blood pressure which originates in reduced glucose absorption and decreased insulin secretion; (c) reduced inflammation due to nuclear factor-κB inhibition by polyphenols leading to reduced C-reactive protein, tumor necroses factor α and interleukin-G.

Brunt et al. (2019) investigated the potential mediation of age-related changes in the gut microbiome on arterial dysfunction. For this purpose, they suppressed gut microbiota in young and old mice with a mixture of broad-spectrum, poorly absorbed antibiotics in drinking water for 3–4 weeks. They concluded that ageing alters the abundance of microbial taxa associated with gut dysbiosis and that, in old mice, the antibiotic treatment reverses arterial rigidity and attenuates vascular inflammation and oxidative stress.

Bartolomaeus et al. (2019) investigated the effect of short chain fatty acids, in particular, propionic acid, on cardiac damage mediated by hypertension and atherosclerosis. For this purpose, they developed a mice animal model. The hypertension was induced by means of infusion with Angiotensin II (1.44 mg/kg) for 14 days and, to accelerate the development of atherosclerosis, the mice were infused with 0.72 mg/kg of Angiotensin II for 28 days. To study the effect of propionate, mice received this compound (200 mM) in the drinking water *ad libitum* during the experiment. They studied the cardiac damage through histology, echocardiography, electrophysiology, immunofluorescence and flow citometry. As hypertensive stimuli like Angiotensin II, promotes the activation of T cells and macrophages, they also evaluated the mode of action of propionate through the study of the regulatory T cell depletion using antibodies. They concluded that propionate significantly

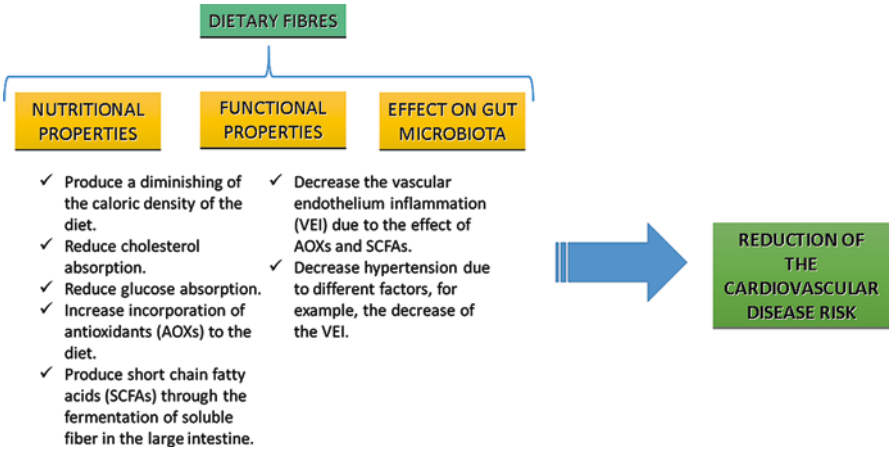

Fig. 4.3 Influence of dietary fibres on cardiovascular disease risk

attenuated cardiac hypertrophy, fibrosis, vascular dysfunction and hypertension showing the immune-modulatory effect of short chain fatty acids and their importance in cardiovascular health. These fatty acids are generated in the colon by means of the fermentation of dietary fibre present in the diet.

The different effects of dietary fibre on cardiovascular disease risk, according to bibliography, are summarized in Fig. 4.3.

It can be concluded that there is a close relationship between dietary fibre intake and the decrease in factors associated with cardiovascular disease, showing the positive effect of this nutrient in human health. Although, more information is needed in relation to the exact mechanism of action, it can be emphasized that the link between dietary fibre consumption and microbial flora of the gastrointestinal tract, immunity and cardiovascular disease, emerges as a promising working hypothesis that must be more deeply studied.

4.1.6 Conclusions

Research performed over the past 20 years showed that dietary fibre produces health benefits in reducing the risks of diabetes, obesity, cancer and cardiovascular disease. These benefits are related, in many cases, to their hydration, thickening, gelling, antioxidant properties and to their effect on gut microbiota.

Investigations in this area had been extensive but elucidation of the mechanisms involved in this bioactivity is not yet conclusive. However, the emergence of new hypotheses such as the linking of dietary fibre with gastrointestinal flora and immunity, illuminates the path of future studies to be carried out to clarify these mechanisms.

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Chapter 5
Lipids

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Abstract Chapter 6 provides information on substances of lipid origin that have had important effects for the treatment or prevention of diseases such as cancer, diabetes mellitus, cardiovascular disorders, obesity, among others. Information associated with metabolites of plant origin, as well as lipids of animal origin, food lipids, that have demonstrated hypoglycemic, anti-inflammatory, antiproliferative, hypocholesterolemic, antihyperlipidemic and antihypertensive effects is presented. The chapter also discusses topics dealing with the chemical structures of the reported lipids, their origin, synthesis, preclinical studies, in vitro, in situ, clinical studies, detailing dosage, method of administration, biochemical, molecular, genetic studies, and mechanisms of action.

Keywords Lipids · Diseases · Health · Food · Fatty acid

5.1 Introduction

Lipids are hydrophobic substances essential for living; currently, much is known about these molecules (Finkelstein et al. 2014). One of their classic functions is to form part of the plasma membrane of any type of cell, including agents such as viruses (Shepherd 2004). This chapter discusses the therapeutic properties of lipids as well as the type of food where they are found, whether they are of plant, animal, mineral origin. The lipid group includes fatty acids, phospholipids, waxes, sphingolipids, cerebrosides, gangliosides, terpenoids, and steroids, among others.

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5.2 Lipids and Cholesterol Diseases

The diseases associated with the high concentration of cholesterol are varied, for example, hypercholesterolemia, atherosclerosis, dyslipidemia, gallstones, among others (Platt et al. 2014). These diseases have been treated with drugs that lower the plasma cholesterol concentration, such as statins and ezetimibe (Taylor et al. 2013; Thongtang et al. 2012); however, some lipids can also inhibit intestinal cholesterol absorption, generating significant therapeutic effects and we can find them in different foods.

5.2.1 Hypercholesterolemia

Beta-sitosterol is a plant sterol that, biochemically, is classified within sterols or steroids, which are non-saponifiable lipids (Ulbricht 2016). It has been shown to have significant effects on the treatment of hypercholesterolemia for a long time. Some clinical trials date back to the 1990s when a group of patients was treated with beta-sitosterol at a dose of 12 g/day, demonstrating a significant decrease in the plasma concentration of total cholesterol and LDL cholesterol (Zák et al. 1990). β-sitosterol, as well as other plant sterols, have extensive reports as hypocholesterolemic agents. Still, its food formulation is complicated due to insolubility in water and, despite being lipids, it does not have a high solubility in oils. Its chemical properties prevent the existence of a variety of functional foods enriched with sterols. A widely used technique to formulate them is the use of emulsions (Yuan et al. 2019). This type of physicochemical system has been tested in experimental animals. Beta-sitosterol has been esterified with fatty acids (e.g, oleic and linoleic) and has been formulated in microemulsions to be administered in mice with hypercholesterolemia. The mice were fed a high-fat diet for 70 days. Once they presented hypercholesterolemia, they were treated with a b-sitosterol microemulsion esterified with linoleic acid at different concentrations. The dose with the highest cholesterol-lowering effects was 700 mg/kg/day (Yuan et al. 2019). The authors carried out the microemulsions using standardized methods from their laboratory and suggest that this type of formulations may be a guideline for making industrial products or functional foods.

5.2.2 Cholesterol in Metabolic Syndrome

Another of the beneficial effects of β-sitosterol has been reported in the metabolic syndrome, which is characterized by altering the concentration of lipids, carbohydrates, producing visceral obesity, and alterations in blood pressure (Desai et al. 2016). Associated with lipids, cholesterol accumulates excessively in cell

membranes in this syndrome. This effect disrupts the activity of cholecystokinin, an essential hormone in the gastrointestinal system (Desai et al. 2016). This hormone has its type 1 receptor, abbreviated as CCK1R. A group of American researchers analyzed the effect of β-sitosterol on cholecystokinin receptors in experimental animals, which expressed the human receptors, the study was carried out on the cells of the mice. β-sitosterol, at doses of 100 and 10 mM, was shown to improve CCK1R signaling in cells that had elevated cholesterol in their membrane, without affecting the binding between the receptor and its hormone (Desai et al. 2016). This suggests that this lipid could be used to treat one of the metabolic syndrome disorders, which would be visceral obesity since there is an accumulation of adipose tissue and excess cholesterol in cell membranes.

5.2.3 Cholesterol Gallstones

Other lipids that have shown effects for the treatment of cholesterol diseases are the so-called polyunsaturated fatty acids (PUFA) (Jang et al. 2019). These lipids have been evaluated concomitantly with ursodeoxycholic acid for the treatment of cholesterol gallstones. In this disease, there is an overproduction of mucin in the gallbladder, which generates bile sludge and, later, the gallstone. C57BL/6 mice fed a lithogenic diet and treated with ursodeoxycholic acid and PUFA at doses of 12.5 mg/kg/day and 51 mg/kg/day, demonstrating significant effects on gallstone dissolution; the most significant results were those that combined PUFAs and ursodeoxycholic acid because they decreased the expression of mucin genes, associated with the overproduction of bile sludge. Also, these fatty acids increased the concentration of phospholipids and bile salts in the bile, allowing mixed micelles to assemble that transport excess cholesterol in bile and gallstones (Jang et al. 2019).

In this way, it can be considered that functional foods rich in fatty acids of this type could serve as healthy foods in lithiasis people.

Cholesterol-associated diseases have statin-based therapy; however, these drugs produce critical adverse reactions. Statin hepatotoxicity and myotoxicity have been demonstrated in many studies, but remain the leading-edge drugs for the treatment of hypercholesterolemia (Adhyaru and Jacobson 2018). The development of functional foods rich in fatty acids, plant sterols, or other types of lipids, could replace statin therapy, supporting a healthy life in terms of eating, sleeping well, reducing stress, exercising. In the case of gallstones, we find a disease that has no pharmacological treatment, only surgical treatment (Portincasa et al. 2016). A diet rich in fatty acids, which have proven effective in dissolving gallstones, would be appropriate to manage this disease, which is one of the most frequent in the gastrointestinal system.

5.3 Biological Activity in Cancer

In recent years, the development of anticancer agents has shifted from non-specific drugs to cytotoxic drugs, which act towards dysregulated signaling pathways in cancer cells. Among these discoveries, tyrosine kinase inhibitor molecules have been found, which are overexpressed in tumors and have become a pharmacological target for these therapeutic agents (Heinrich et al. 2003).

These new drugs are well tolerated and have fewer adverse effects than the more widely used cytotoxic agents, there is a continuing need to develop new specific molecules that are well tolerated and provide more options in cancer chemotherapy, either as single agents or in combination with other drugs, and that can be used to develop new regimens cancer treatments. Cancer is a disease that weakens the body's immune system. Most of the people who fail due to this disease contracted other types of in-hospital infections that became opportunistic and evicted the body, due to their immunosuppressed state; For this reason, there is an emerging need to search for new therapies based on lipid compounds, which do not produce such severe immunosuppression effects.

There is reported evidence that many lipids and lipid analogs are critical regulators of oncogenesis. This information has arisen from investigations that have been carried out in tumor cells or experimental animals after dietary conditioning and the use of tumor cell xenografts. Exploration of such molecules in cancer therapy is at an early stage of research; however, many of them show considerable promise as future cancer therapies. When considering which lipid-based molecules could be developed, it is essential to solving particular problems that arise with lipid-based medications.

5.3.1 Prostaglandins and Ceramides

Although the biological properties of individual molecules seem promising, relatively few have managed to go through the drug development process due to chemical instability, rapid metabolism, and in some cases, the incidence of side effects. For example, several synthetic prostaglandin (PG) analogs have previously been developed as potential antiulcer, antihypertensive, and fertility control agents (Collins and Djuric 1993). Knowledge of the mechanisms of action through which lipids and their metabolites regulate tumorigenic processes requires background information on the growth and spread of cancer cells. Cancer has multiple stages, in which cells develop the capacity for unregulated proliferation, become resistant to proapoptotic stress that kills normal cells, and acquires the ability to migrate to other adjacent and distant tissues to establish secondary metastases (Murray et al. 2015).

Another example of functional lipids are ceramides, which can be found in many foods such as rice and wheat. Besides, they are metabolites of many medicinal

plants and are called phytoceramides (Canals et al. 2018). Ceramides are recognized for their signaling role in regulating cell proliferation, differentiation, and death. Hydrolysis of sphingomyelin produces a ceramide. This reaction is catalyzed by sphingomyelinases, whereas de novo synthesis is mediated by multiple ceramides synthases that produce endogenous ceramides, which have various types of fatty acids attached; the longest chain ceramides are proapoptotic. Accumulation of ceramide in cells occurs after treatment with anticancer agents or saturated fatty acids, such as palmitic acid (Merrill and Jones 1990). Direct addition of ceramide C2, at a concentration of 1 μM, has been shown to alter the mitochondrial transmembrane potential, forming channels, or targeting Bcl-2 proteins (B-cell lymphoma 2) (Garcia-Ruiz et al. 1997). These proapoptotic actions of ceramide are mediated by many molecules (Chen et al. 2008). Ceramide can be cleaved by ceramidase, which terminates the apoptotic actions of long-chain ceramides and is overexpressed in cancer cells (Seelan et al. 2000).

5.3.2 *Fatty Acids*

There are some lipids that inhibit the activity of enzymes dedicated to promoting tumorigenesis. Inhibition of COX-2 enzyme activity is an attractive strategy for preventing tumorigenesis and has been shown to be effective in colon, lung, and prostate cancer cells in in vitro assays (Kamijo et al. 2001; Nagatsuka et al. 2002) and models of xenografts in mice. Recently, it was discovered that a group of novel ins-3 monounsaturated fatty acids inhibited the proliferation and migration of breast cancer cells that overexpressed COX-2. In this study, monounsaturated fatty acid analogs with variations in the chain were synthesized (Fig. 5.1), which were evaluated in breast cancer cells, MDA-MB-468, which overexpressed COX-2. These fatty acids inhibited cell proliferation, activated the apoptotic pathway, decreased PGE2 production, as well as reduced cell invasion (Cui et al. 2012). These fatty acids, called MUFAs, managed to demonstrate high activity in this experimental model, and this study establishes a relationship between the activity of these fatty acids, depending on the length of their chain, therefore, converts them to longer chain MUFAs. In promising anti-inflammatory agents, as well as can be part of a new species of anticancer.

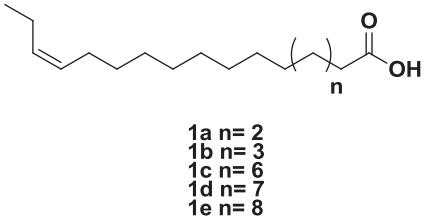

Fig. 5.1 Long-chain n-3 synthetic monounsaturated fatty acids active in breast cancer cells that overexpress the COX-2

5.3.3 *Alkylphospholipids*

On the other hand, there are the so-called Alkyl phospholipids (ALPs), which have shown antitumor activity (Berdel et al. 1981). Edelfosine has considered the first synthetic analog of ALPs evaluated as a possible anticancer agent, along with ilmophosine, which has a thioether residue instead of the methoxyl substituent (Fig. 5.2).

It is essential to mention that these phospholipids have been modified to improve their therapeutic activity against cancer. As seen in the figure below, structural modification to remove the glyceryl nucleus produced an alkylphosphocholine analog miltefosine and replacement of the choline moiety with a piperidine system produced periphosine (Fig. 5.3). The development of other molecules has been reported, for example, erucylphosphocholine and it's analog erufosine, which possess a 22-carbon fatty acid chain and a ω − 9-cis double bond. These structural developments have improved the selectivity of the agents for cancer cells over healthy cells and have enhanced their metabolic stability (Mollinedo et al. 1997; Ruiter et al. 1999; Gajate et al. 2004).

ALPs cause a number of antitumor actions in cells (Fig. 5.4), including interference with membrane lipid raft function, impaired PI3K/Akt survival signaling, inhibition of phosphatidylcholine synthesis, generation of ROS, and activation of endoplasmic reticulum stress (Gajate et al. 2012). That is why there is substantial evidence that these fatty acids have multiple potential pathways in their mechanism of action. ALPs decrease the viability of tumor cells in several ways. They promote cell cycle arrest in the G2/M phase by inducing the CDK inhibitor p21Cip1 and inhibit proliferative signaling of ERK and PI3K/Akt, possibly interfering with Raf-1 membrane association, leading to decreased Raf-1 kinase activity (Samadder and Arthur 1999; Elrod et al. 2007; Kumar et al. 2009).

In addition to what has already been mentioned, ALPs are well tolerated in preclinical studies. However, the clinical use of ALPs of synthetic origin has been

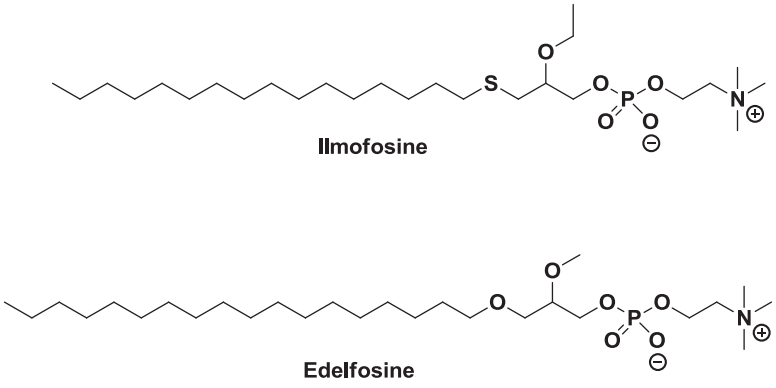

Fig. 5.2 Chemical structures of ilmofosine and edelfosine

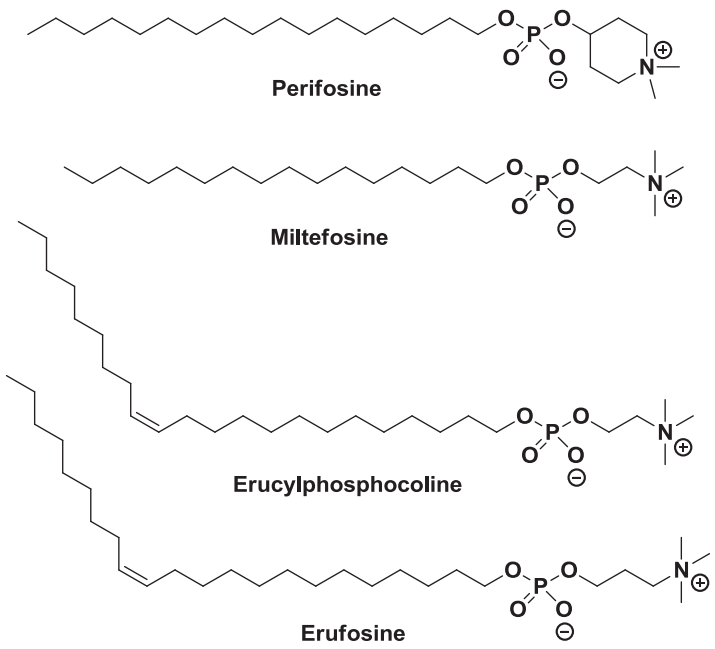

Fig. 5.3 Alkyl phospholipid structures that have been chemically modified to improve their anticancer properties

restricted due to their hemolytic potential and gastrointestinal toxicity, as well as other vital toxicities including fever, myalgia, arthritis, and pain (Berdel et al. 1987).

In addition to ALP treatment, concomitant therapies of these lipids with anticancer agents have been developed. The use of these combinations as forms of cancer treatments has been promising. In recent studies, periphosine increased the antineoplastic effect of lenalidomide and dexamethasone in multiple myelomas (Jakubowiak et al. 2012); also, studies with promising activity of the combination of capecitabine with peripheosine for metastatic colorectal cancer have been reported (Bendell et al. 2011). Other recent preclinical studies have identified more combinations of drugs containing peripheosine, for example, the mixture with the cyclin dependent kinase inhibitor SNS-032, which has shown a potential value in the treatment of human acute myeloid leukemia cells, within the effects cell death was increased if we compared the effect of the substances separately, probably due to a decrease in PI3K/Akt survival signaling by periphosine (Meng et al. 2013). The combination of periphosine with the mTOR inhibitor CCI-779 caused cell cycle arrest and inhibited growth in various human cancer cell lines (Pitter et al. 2011). These preclinical results suggest that inhibition of the PI3K/Akt/mTOR pathway at two points in the cascade may produce more optimal effects.

In addition to combinatorial therapies between ALPs and anticancer drugs, combinations between these molecules and radiation therapy have been developed. One of the first in vitro assays that showed radiosensitization potential was miltefosine

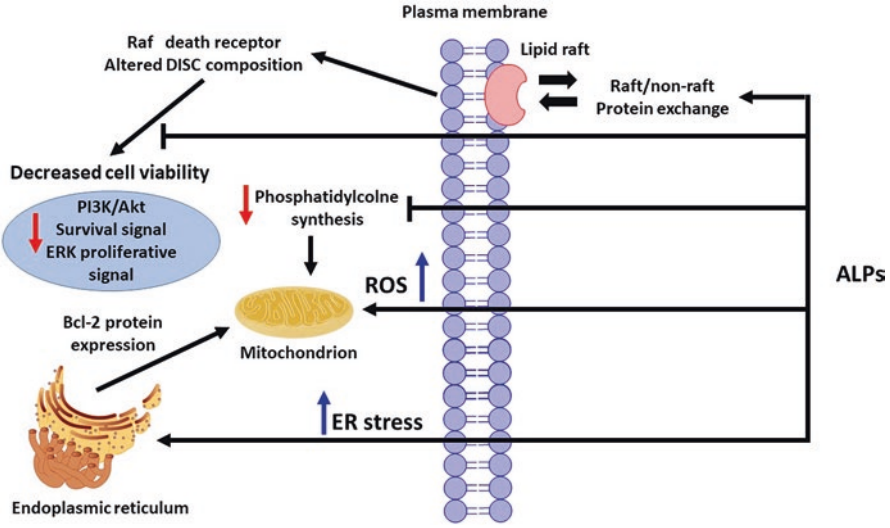

Fig. 5.4 ALPs decrease the viability of cancer cells by disrupting lipid rafts in the plasma and mitochondrial membrane, modulating the distribution of Raf death receptors, and affecting phosphatidylcholine synthesis. The production of reactive oxygen species and the stress of the endoplasmic reticulum promote apoptotic cell death. Disruption of the PI3K/Akt survival pathway and proliferative ERK signaling may also contribute to decreased cell viability produced by ALPs

on cell lines that excreted the activated Ras oncogene (Bruyneel et al. 1993). Subsequently, Berkovic et al., demonstrated that miltefosine and edelfosine affected clonogenic survival after radiation in squamous cell carcinoma KB (Berkovic 1998). Periphosine has been shown to improve cytotoxicity through radiation in both short and long-term trials. The most recent studies have demonstrated the increase in radiation-induced apoptosis and the elimination of clonogenic tumor cells by erucilphosphocholine (ErPC) in malignant glioma (Handrick et al. 2006). Although the cytotoxic mechanisms of action remain unclear, immunohistochemical analyzes of tumor tissue after treatment revealed a prominent apoptotic response, mediated by caspase 3 activity. Similar results were observed in a xenograft model of human prostate carcinoma, in which the combinatorial therapy of peripheosine and radiation, had a significantly more potent effect on tumor growth, unlike treatment with a single substance (Gao et al. 2011).

5.3.4 Omega Fatty Acids

Another class of lipids found in many foods and that have shown therapeutic effects are omega polyunsaturated fatty acids, particularly those in group 6 (ω-6 PUFAs). Although all ω-6 PUFAs can be consumed in the daily diet, the precursors of ω-6 are more abundant in seeds and vegetable oils; therefore, it is considered the primary

dietary source of all ω-6. Research on these fatty acids shows that PUFAs have some participation in the diet as inhibitors of cell proliferation, for example, in the Caco-2 colon cancer cell line (Dommels et al. 2003). At the same time, a high intake of these compounds also shows a protective effect against the development of cancer (Horrobin and Ziboh 1997). In addition to found activity found in these molecules, as well as in their derivatives, the family of polyunsaturated fatty acids has an important antitumor event.

Linoleic acid can be desaturated and converted to gamma-linolenic acid (GLA), which is associated with anticancer activities in vitro and in vivo models. For example, GLA inhibited cell growth of the human neuroblastoma lines GOTO, SK N-DZ, NKP, and NCG, a rat C6 glioma cell line, and the LLC-WRC256 rat carcinosarcoma cell line (Fujiwara et al. 1989; Colquhoun and Schumacher 2001). More interestingly, GLA-induced cytotoxicity was shown to exhibit high selectivity towards cancer cells without affecting the growth of non-cancer cells in ZR-75-1 human breast cancer lines, A549 lung cancer cells, and prostate PC-35 (Das 1992). Also, GLA has been shown to be cytotoxic to the 36B10 rat malignant astrocytoma cell line, without affecting normal astrocytes. And the radiation sensitivity of astrocytoma cells was improved, but not of normal astrocytes (Begin et al. 1986). In an experimental rat model for C6 glioma, the infusion of GLA was shown to increase the frequency of apoptosis and a decrease in tumor mass, without influencing neural tissue and normal vasculature (Vartak et al. 1998). Therefore, it is suggested that GLA is a possible anticancer therapeutic agent due to its high selectivity, as well as the ease of consuming it in daily food.

There is evidence that specific metabolites of ω-3 PUFAs exert antitumor actions on their own. An example of these is eicosanoid derivatives, which have decreased pro-inflammatory, proliferative, invasive, and pro-angiogenic responses compared to those formed from ω-6 PUFAs (Abou-el-Ela et al. 1989; Rose and Connolly 2000; Hardman 2002). The antiangiogenic activities of eicosapentaenoic acid (EPA) in human endothelial cells, including decreased invasion and endothelial tube formation, have been attributed to prostaglandin E3 (PGE3), derived from COX-2, and possibly to other metabolites; PGE3 directly suppressed the induction of the pro-angiogenic mediator angiopoietin-2 by vascular endothelial growth factor (VEGF). The mechanisms by which specific metabolites of PUFA-3 regulate angiogenesis and other associated processes have not been fully explained, but are related to the alteration in the signaling pathway for prostanoid receptors; therefore, eicosanoids derived from EPA ω-3 activate prostanoid receptors less efficiently than those derived from arachidonic acid ω-6 (Wada et al. 2007). Also, epoxides obtained through CYP-mediated metabolism of PUFA-3 have been shown to exert growth suppression and anticancer effects. These EPA epoxides decreased cell proliferation in endothelial tissues and activated apoptosis, leading to cell cycle arrest by activation of MAPK p38, which suppresses growth through down-regulation of cyclin D1 (Cui et al. 2011). Another study on epoxides demonstrated that they exert anticancer effects by suppressing VEGF-mediated angiogenesis, which resulted in decreased growth of the primary tumor and metastasis in vitro (Zhang et al. 2013). Figure 5.5 shows the structures of the epoxides evaluated in the said experiment.

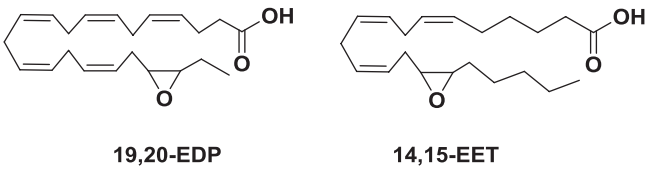

Fig. 5.5 Chemical structures of 19,20-epoxydocosapentaenoic acid and 14,15-epoxyeicosatrienoic acid with in vitro antiangiogenic activity

Some metabolites dependent on the 5-lipoxygenase (LOX) pathway have also presented antitumor activity, including 15-Hydroxyeicosatetraenoic acid and resolvins with antiproliferative capacity, and which are derived from arachidonic acid (Haeggström and Funk 2011). Among the resolvins that have anti-inflammatory activity, as well as inducing apoptosis when administered orally or intravenously, we find those derived from epoxidation reactions. These complex eicosanoids come from the biotransformation of DHA into 17S-hydroxy-DHA by the action of 15-LOX, then it is transformed into 7S hydroperoxy, 17S-hydroxy-DHA by 5-LOX and finally into resolvin D1, after epoxidation, which could involve CYP-mediated metabolism. Similarly, 4S-hydroperoxy, 17S-hydroxy-DHA is another product generated by LOX from 17S-hydroxy-DHA, which also undergoes epoxidation to produce resolvins D3 and D4. These resolvins exhibit anti-inflammatory properties in vivo when administered intravenously or orally (Dangi et al. 2009).

Some ω-3 PUFAs epoxides have become a different group of potential anticancer agents. A series of synthetic C20-C22 long-chain saturated fatty acid ep-3 epoxides (Fig. 5.6) have been evaluated for their antiproliferative and proapoptotic actions in human breast cancer cells. In these experiments, it was discovered that these epoxyeicosapentaenoic fatty acids are active on the MDA-MB-231 cell line, which increases caspase-3 activity and leads to downregulation of cyclin D1 and cell cycle arrest in the phase G1 (Dyari et al. 2014). These fatty acid epoxides were developed from naturally occurring 17,1-3 17,18-epoxy-EPA by removing additional olefinic bonds, due to the oxidation potential of isomeric epoxides, which stimulate proliferation and inhibit apoptosis. Synthetic ω-3 fatty acid epoxides impaired the viability of MDA-MB-231 cells and, to a lesser extent, MDA-MB-468, MCF-7, and T-47D cells; however, epoxides are unlikely to be suitable for in vivo application, due to their low stability, since epoxide hydrolase converts them to inactive diols (Inceoglu et al. 2008).

5.3.5 In Vitro and In Vivo Studies

Bhupender and coworkers synthesized acylamide derivatives from doxorubicin fatty acids (Fig. 5.7) and evaluated their anticancer activities in vitro. One of the synthesized molecules showed antileukemia activity, comparable to cytarabine.

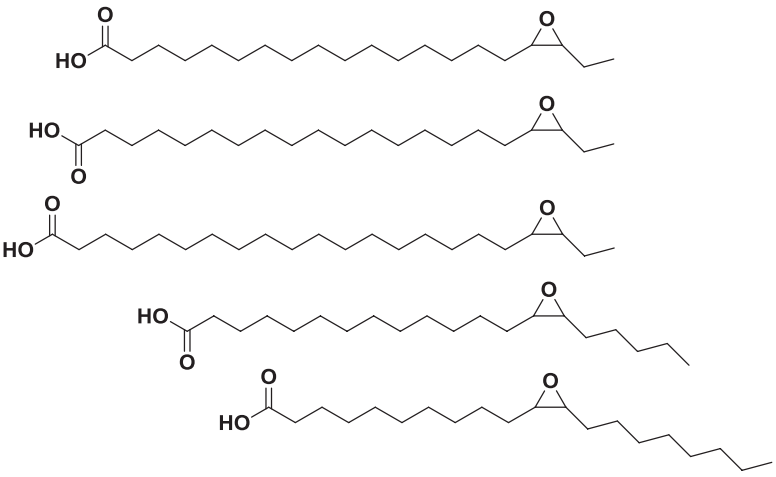

Fig. 5.6 Chemical structures of ω -3 Epoxyfatty acids

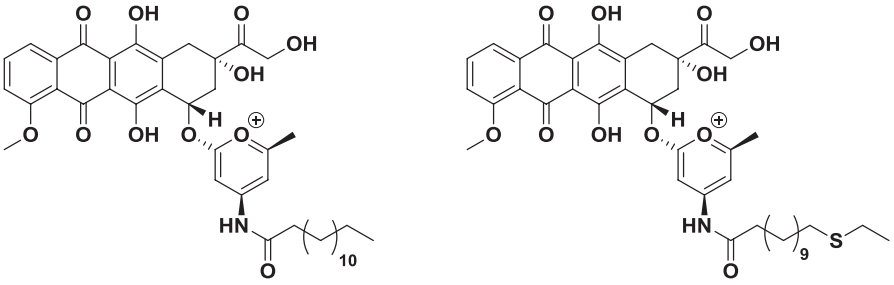

Fig. 5.7 Structures of fatty acyl amide derivatives of doxorubicin

These effects were associated with the chemical modification of the structure (Bhupender et al. 2011).

These researchers also synthesized fatty acyl ester derivatives (Fig. 5.8) of cytarabine and evaluated them as antileukemic agents, finding that some of them inhibited the growth of CCRF-CEM cells (Bhupender et al. 2010). On the other hand, Liu et al., reported the synthesis and antitumor evaluation of cytarabine N4 fatty acyl amino acid derivatives, to improve lipophilicity and bioavailability of cytarabine, where the antitumor activity determined in HL-600 cells and HeLa demonstrated that the derivatives were more active in HeLa cells than cytarabine, while most of them shown cytarabine-like activity in HL-60 cells. The length of the fatty acids in the derivatives seemed to have an impact on the observed business (Liu et al. 2009) (Fig. 5.9).

Zhang Chun-hong and his working group synthesized new panaxadiol fatty acid esters and evaluated them to determine their antitumor activity in Vero cells, finding a better antitumor effect compared to the 5-Fluorouracil control (Zhang et al. 2007).

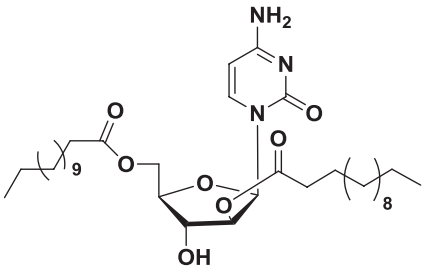

Fig. 5.8 Structure of 2',5'-dimyristoyl derivative of cytarabine

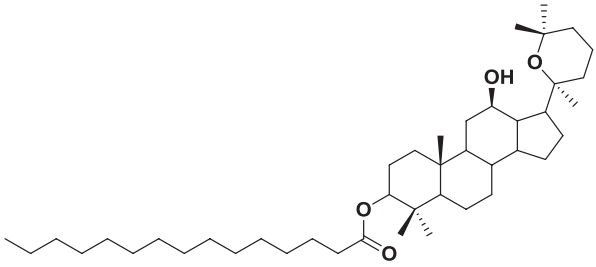

Fig. 5.9 Structure of new panaxadiol fatty acid ester

The antitumor activity of these panaxadiol derivatives is more reliable than the reference drug; Fig. 5.5 shows the chemical structures of the synthesized compounds, where the length of the fatty acid alkyl chain was modified, and the activities of each one were compared.

Jubie et al., have reported two works reporting on some new heterocyclic fatty acid conjugates and their anticancer evaluation in human lung carcinoma cell lines. These compounds demonstrated cytotoxicity on these cell lines (Jubie et al. 2013). The compounds of Fig. 5.10 possess a fatty acid chain substituted with 1,3,4-oxadiazole, which showed maximum cytotoxic activity. Furthermore, it was observed that the presence of toxophoric bonds -N = C-O- in the nucleus 1,3,4 oxadiazole might be responsible for the antitumor activity. This working group concluded that these compounds are good bioisterosters of amide and ester functionalities, with a substantial improvement in the biological activity of hydrogen bonding interactions with different objectives responsible for tumor development. The operation of these 1,2,4-triazole substituted fatty acid analogues depends on the length of the fatty acid chain and is therefore directly related to their antitumor activity (Jubie et al. 2013).

The chemical structures of the ceramides (Fig. 5.11) have allowed them to exert proapoptotic effects. A correlation has been found between the activation of apoptosis and its intracellular levels. The investigations have been able to continue because methods have been developed for the detection of ceramides, enzymatic inhibitors have been discovered to block the synthesis of ceramides and generators of ceramides have been identified that induce apoptosis (Lin et al. 2006). It has been investigated that ceramide can intervene, both in the intrinsic and extrinsic apoptotic pathway. Likewise, the concentration of this lipid is influenced by

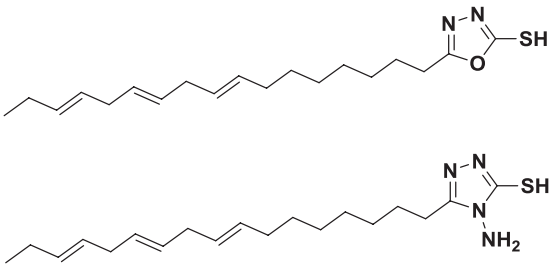

Fig. 5.10 Structures of novel 1,3,4-oxadiazole-2-thiol and 1,2,4-triazole-3-thiol fatty acid analogues

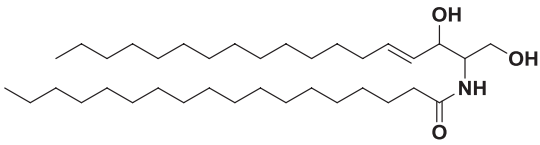

Fig. 5.11 Chemical structure of ceramide

stimuli, such as the deprivation of nutrients, cellular stress, the effect of drugs, heat, radiation or hypoxia, which is reflected in the cascade activation of caspases and dysfunction. In multiple organelles leading to apoptosis (Morales et al. 2007).

Due to the resistance of traditional cancer therapies, studies on ceramide metabolism show promising pharmacological treatments and alternatives.

The focus on the development of ceramide as an anticancer potential has led researchers to the design of analogs of this sphingolipid to give a new approach to cancer therapy; however, it is known that ceramide cannot cross cell membranes. Therefore its application as a therapeutic agent is limited (Kolesnick and Hannun 1999). From this point, it is where analogues of this sphingolipid are developed, which increase both its corrective action and the ability to cross cell membranes. The first indications of modifications to ceramide as proapoptotic agents occurred with the replacement of one of the fatty acid chains by a shorter acyl group, resulting in the derivatives called C2 and C6 (Fig. 5.12), which inhibited proliferation in tumor cells (Kolesnick and Hannun 1999). On the other hand, investigating the functionality of ceramide, other derivatives with anticancer activity have been obtained (Fig. 5.13), mainly the derivative that contains the phenyl ring together with those that have a sphingoid residue or an allyl fluoride and the derivative dihydroceramide Fluorides, which induced apoptosis in Molt-4 and K-422 leukemine cell lines (De Jonghe et al. 1999). Within this same exchange of functional groups to ceramide, the compounds derived from uracil, thiouracil, and 5,6-dimethylthiouracil (Fig. 5.14) were analyzed in the CCRF-CEM leukemia cell line, finding that the presence of pyrimidine rings is essential for apoptosis-inducing activity, these sphingolipid derivatives have also been shown to increase caspase-3 activity as well as cytochrome C release (Ghafourifar et al. 1999).

In conclusion, a large number of lipid-derived compounds with anticancer activity have been developed, constituting new lines of research for alternative cancer therapies. As reviewed, the influence of functional groups within a molecule of lipid origin can have various effects on human cancer lines. The development of these

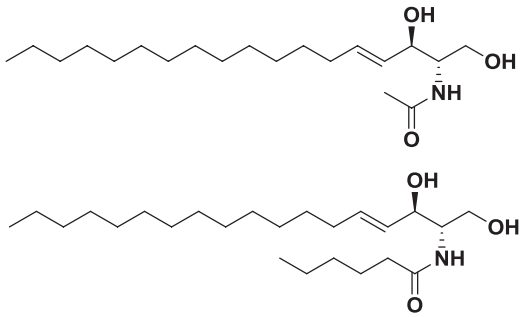

Fig. 5.12 Chemical structures of C2-ceramide and C6-ceramide

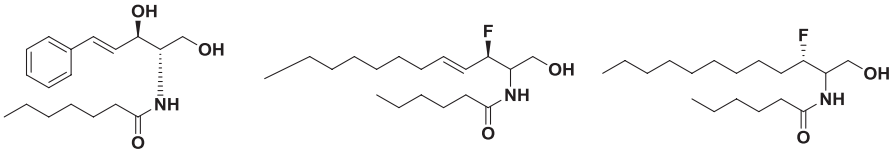

Fig. 5.13 Ceramide derivatives; with a phenyl ring, ceramide allyl fluoride and fluorinated dihydroceramide

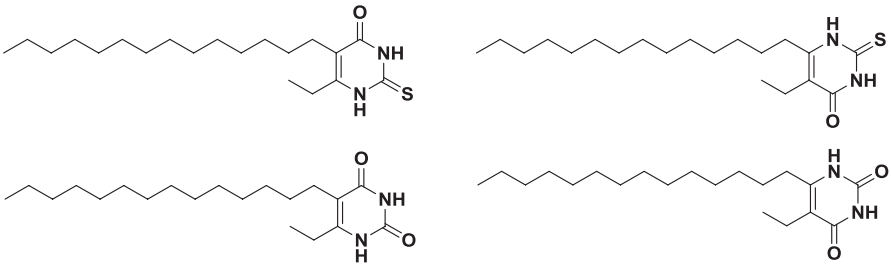

Fig. 5.14 Derivatives of ceramide of the uracil and thiouracil type

new molecules broadens the panorama in the search for more significant activity against cancer, but less toxic effects. Likewise, it is used that lipid molecules are endogenous and that the body can easily recognize them, reducing the reactions that may occur within a future therapy based on these compounds.

5.4 Effects on the Cardiovascular System

One of the most prevalent conditions that produce cardiovascular damage is atherosclerosis; this disease has a strong relationship with lipid metabolism (Torres et al. 2015); however, the events it triggers are associated with heart disease and blood pressure problems, due to the formation of atheroma plaque. Among the lipids that

have shown essential effects against atherosclerosis, we find short-chain fatty acids, for example, butyric acid (Ohira et al. 2017).

5.4.1 Atherosclerosis and Cardiovascular Risk

This acid is also found referred to as butyrate, and it has reported significant anti-inflammatory, apoptotic and antioxidant properties in different experimental models; These properties are related to the development of atherosclerosis, since, within its pathophysiology, there are numerous inflammatory and oxidative processes (Aguilar et al. 2014). In a study carried out in ApoE konckout mice, the effect of butyrate was evaluated, which was added to 1% in the rodent diet for 10 weeks; the study was complemented using endothelial cell lines, which were treated with 0.5 mM butyrate, after being stimulated with oxidized LDL. The effects of butyrate were surprising since the appearance of atheroma plaque was reduced in 50% of the treated mice, macrophage migration was inhibited by decreasing the production of monocyte chemoattractant protein 1, cell adhesion protein 1 vascular and 72 kDa type IV collagenase (Aguilar et al. 2016); thanks to the fact that the output of this last protein was reduced, the collagen deposits in the atheroma plaques increased, forming a kind of protection factor. The authors concluded that butyrate could delay the formation of atherosclerosis, stabilizing atheroma plaque, and lowering platelet glycoprotein 4 in macrophages, leaving important points to investigate a future therapeutic target (Aguilar et al. 2016). It is imperative to recognize the role of this acid in some functional foods that may be part of the atherosclerotic patient's diet.

5.4.2 Omega-3 and Coronary Disease

Omega-3 fatty acids also have essential reports in coronary diseases, for example, eicosapentaenoic acid (Brinton and Mason 2017). This lipid has been administered in hypercholesterolemic patients, who also receive statin therapy. The effects found are translated to the decrease of coronary events in the patients (Alfaddagh et al. 2017). A clinical study evaluated the effect of eicosapentaenoic and docosahexaenoic acids on coronary heart disease caused by atherosclerosis. These acids were administered at doses of 2 and 4 g per day. The results were significant since many parameters associated with the formation of atheroma plaque were reduced, and antioxidant and anti-inflammatory mechanisms of action were revealed (Nakao et al. 2018).

Different reviews have shown that omega fatty acids are effective in preventing coronary events caused by atherosclerosis (Abdelhamid et al. 2018); however, there is little evidence on the effect of lipids on other diseases of the cardiovascular system. Fatty acids can be obtained from foods rich in unsaturated fats; other studies have evaluated supplements enriched with fatty acids; that is, they are already pre-

sented under some pharmaceutical form or special presentation. More specific studies estimate fatty acids reactively in different models in vivo, in vitro, even in clinical trials.

Some authors have recommended functional foods fortified with phytosterols as a primary source to prevent cardiovascular diseases, mainly those associated with high cholesterol levels, including prestigious health institutions that have supported this initiative (Köhler et al. 2017). Foods rich in phytosterols have been shown to be effective in lowering the plasma concentration of total cholesterol and LDL cholesterol, being critical factors in avoiding cardiovascular risk (Patch et al. 2006).

One of the disadvantages of phytosterols is their poor solubility in water, so they must use different systems to be administered or used as ingredients in functional foods. Some tests that have been done with these lipids consisting of lecithin emulsifications, others include the use of margarine to be administered. These lipids have had essential effects in preventing acute myocardial infarctions (Ortega et al. 2006).

5.5 Effects on Inflammation

Inflammation can be conceptualized as a primary way in which the body reacts to harmful stimuli such as irritation, toxic compounds, infection, or irradiation; the vital signs are warmth, redness, pain, and swelling. The aim of this process is removing injurious stimuli and favors the healing process (Chen et al. 2018). However, some specialist suggests that it shifts the metabolic balance towards catabolism; being a pathological process, not a defensive reaction (Stankov 2012). The inflammatory process underlies primary hyperalgesia (a painful response to a stimulus that is usually not painful), contributing to peripheral sensitization in nerve damage, especially if accompanied by tissue damage (American Chronic Pain Association 2018). Acute inflammation is generally self-limited allows that cellular and molecular events efficiently minimize impending injury or infection. However, if it fails to resolve, chronic inflammation can appear, contributing to a variety of diseases (Chen et al. 2018). During inflammation are promoted leukocyte migration from blood to the damaged tissues and the generation of pro-inflammatory chemokines, cytokines, and lipids mediators, which are fundamental to start and maintain the phenomenon (Shapiro and Fazio 2016).

5.5.1 Lipids as an Inflammation Mediator

Lipids are the second energy fuel and the main component of cell membranes. There are also recognized as a protagonic role as regulators of intracellular and intercellular processes in maintaining tissue homeostasis and inflammation so have been named "bioactive lipids" (Chiurchiù and Maccarrone 2018). These lipids originate from host essential fatty acids, which could be regulated by diet and by

synthetic optimized mimetics of these molecules as nutritional supplements (Serhan et al. 2014). These molecules generated from omega-6 or omega-3 essential polyunsaturated fatty acids precursors, are esterified into membrane lipids and act activating specific G protein-coupled receptors (GPRs). A bioactive lipid classification by their biosynthesis and function is classical eicosanoids, specialized pro-resolving mediators (SPMs), sphingolipids/lysoglycerophospholipids, and endocannabinoids (eCBs) (Chiurchiù and Maccarrone 2018).

Classic eicosanoid includes leukotrienes (LTs) and prostaglandins (PGs) that arise from the oxidation of arachidonic acid (AA) and related (PUFA) by cyclooxygenase (COX), lipoxygenase (LOX), cytochrome P450 (CYP) enzymes and via non-enzymatic free radical mechanisms (Rogerio et al. 2015). Cells are specialized in produce certain kinds of eicosanoid, but concentration changes accord with physiological conditions of the tissues in which they in (Dennis and Norris 2015). In situations of tissue damage or injury, innate immune cells, like granulocytes, monocytes, and macrophages, are conducted, and it is produced by classical eicosanoids. The result is an acute inflammation, characterized by when so-called "cardinal signs": heat, swelling, redness, pain, and loss of function (Nathan 2002). Therefore, classical eicosanoids are involved in the initiating steps that permit leukocytes and specifically neutrophils to leave, via diapedesis, postcapillary venules (Serhan et al. 2014), are enhancers of innate and adaptive immune activation and thus involved in many inflammatory diseases; despite (PGD2) and (PGE2) possess anti-inflammatory effects (Dennis and Norris 2015).

Specialized pro-resolving mediators (SPMs) participate in reducing inflammation and facilitate the restoration of tissue contributing to homeostasis through removal, relief, recovery, regeneration, and remission, a process called to as "resolution of inflammation". They are produced by the very same immune cells recruited in the inflammatory zone to selflimiting and minimized the noxious stimulus (Chiurchiù and Maccarrone 2018). The SPMs are originated from omega-6 AA and omega-3 PUFAs docosahexaenoic acid (DHA), docosapentaenoicacid (DPA) and eicosapentaenoic acid (EPA), through the same enzymes that produces classical eicosanoids: COXs, LOXs, and P450 (CYP). At the same time, SPMs have been subdivided into six kind: AA-derived lipoxins LXs (LXA4 and LXB4); EPA-derived E-series resolvins (RvE1–3); DHA-derived D-series resolvins (RvD1–6); protectins and neuroprotectins (PD1/NPD1 and PDX) and their sulfido-conjugates (PCTRs); maresins and their conjugates (MaR1, MaR2 and MCTR1–3) and, the DPA-associated 13-series resolvins (RvT1–4) (Serhan et al. 2014). The receptors that mediate SPMs activity are five: formyl peptide receptor 2 or ALX (FPR2), GPR32 or DRV1, chemerin receptor 23 or ChemR23 (ERV), leukotriene B4 receptor 1 (BLT1) and GPR18 (DRV2), differentially expressed in tissues and with a broad affinity for each lipid mediator (Chiurchiù and Maccarrone 2018). Recent evidence shows that impaired metabolism and SPMs function are associated with persistent inflammation reaching chronicity, such as rheumatoid arthritis, cystic fibrosis, neurological diseases, and atopic dermatitis (Rincón et al. 2015).

Lysoglycerophospholipids and sphingolipids are other classes of bioactive lipids distributed in the plasma membranes that show a tremendous molecular diversity

due to their linkage with molecules such as serine, choline, ethanolamine, inositol or and other fatty acids (e.g., phosphoinositides and ceramides) responsible for the outcome of inflammation (Serhan 2014). These modulate a great variety of cellular processes that are relevant for tissue adaption to inflammatory events. Some of them included lysophosphaditylcholine (LPC), lysophosphatidilinositol (LPI), and their byproduct lysophosphatidic acid (LPA) involved in relevant aspects of tissue biology, such as plasma membrane shaping, cell growth and death, and inflammatory cascades (Chiurchiù and Maccarrone 2018). It is speculated that its sustained effects are linked with a variety of chronic inflammatory diseases, for instance, obesity and diabetes, chronic obstructive pulmonary disease, cancer, atherosclerosis, inflammatory bowel disease, neuroinflammatory disorders, and rheumatic artritis (Serhan et al. 2014; Rogerio et al. 2015). For example, sphingolipids as ceramide and its byproducts ceramide 1-phosphate (C1P) and sphingosine 1-phosphate (S1P) participate in numerous inflammatory processes and are responsible for controlling intracellular traffic and signaling, cell growth, adhesion, vascularization, survival, and apoptosis. Excessive ceramide signaling conditions adipose tissue inflammation and insulin resistance, which occurs in metabolic syndrome and type 2 diabetes by inducing hyperactive immune cells such as macrophages and B cells (Chiurchiù and Maccarrone 2018).

Endocannabinoids (eCBs) are endogenously bioactive lipids produced by mammals capable of binding to and activate the same receptors as the main psychoactive component of marijuana Δ9-tetrahydrocannabinol, named type CB1 and CB2. Two of them are anandamide (*N*-arachidonoyl ethanolamine or AEA) and 2-arachidonoylglycerol (2-AG), which also comprise -AG-ether, O-arachidonoylethanolamine, and palmitoylethanolamide (PEA) (Bruni et al. 2018). According to the inflammatory state from tissue, eCBs also interact with peroxisome proliferator-activated receptors (PPARs) and members of the transient receptor potential (TRP) channels, GPR55 (Chiurchiù and Maccarrone 2018). Consequently, modulation of the eCB system through various therapeutic and nutritional strategies allows reducing the inflammatory processes in which cytokines are released, infiltrate leukocytes and reactive species are produced, as neurodegenerative diseases (Witkamp and Meijerink 2014; Balvers et al. 2013)

5.5.2 Bioactive Lipids in Preclinical Trial

Rheumatoid arthritis is an autoimmune affection of onset around the age of forty, characterized by severe joint inflammation, deformation, pain, and movement limitation. In vitro tests, omega-3 polyunsaturated fatty acid eicosapentaenoic acid (20,5, EPA) reduces gene expression, particularly cyclooxygenase (COX-2), which participates in inflammatory processes leading to the production of leukotriene B4 (LTBA4) and prostaglandins E2 (PGE2). Linolenic acid (18,3, ALA) was also tested on these models, although it was found to have less potency than EPA (Hurst et al. 2010). Moreover, PUFAs have been evaluated by their anti-inflammatory properties

linking the neuro-immune modulating features to its biological effects. It has been explored the supplementation with 20 g/kg of fish oil (FO) finding than this treatment attenuated the stress-induced neuroinflammation and promoted dysregulation the of neurotransmission system, with NLRP3 and NF- κB decrement in certain rat brain areas (Tang et al. 2018). On the other hand, omega-3 PUFA treatment ameliorated DOX-induced oxidative stress in the prefrontal cortex and hippocampus, showing than this supplementation attenuated neuroinflammation. Some research suggests than apoptosis induced by stress, oxidative, and neurotransmitter system abnormalities and pro-inflammatory cytokines, may contribute to the physiopathology of depression (Wu et al. 2016). Furthermore, PUFAs also have been demonstrated as a potential treatment against neurobiological side-effects associated with depression. The omega-3 PUFAs can effectively protect against chemotherapeutic agents like Doxorubicin (DOX) in a dose of 1.5 g/kg over three weeks, and the results shows than the PUFAs supplementation significantly mitigated the behavioral changes induced by the neurotoxicity of DOX, and also alleviated the induced neural apoptosis and the induced depressive-like behaviors in rats. The fish oil (FO) has a rich content of PUFAs (EPA 34%, DHA 24%), and it has been proved than the treatment with 1.5 g/kg ameliorated depressive-like behaviors induced by lipopolysaccharide (LPS) repeated administration through modulation of reactive oxygen species (ROS). The improvement of serotonin, dopamine, and glutamate neurotransmission system was observed, conferring neuro-immune modulating features to PUFA (Dang et al. 2018).

Many preclinical studies are showing that cannabinoids can be beneficial in treating pain and inflammation, among other clinical conditions (Bruni et al. 2018). For example, through a triple trial, the anti-inflammatory and antinociceptive efficacy of cannabidiol (CBD) was measured by inhibiting zymosan-induced swelling of the mouse leg and to relieve zymosan-induced pain. In the same study, CBD also sharply reduced in vivo TNF production evaluated by an ELISA kit. Hence the author concluded that cannabinoids are involved in the inhibition of chronic inflammation symptoms (Gallily et al. 2018).

5.5.3 Bioactive Lipids in Clinical Trials

Few clinical trials have explored the beneficial effects of PUFAs on illnesses. Fish oil-derived PUFA supplementation is recommended for the relief of symptoms in many inflammatory diseases. The main reason for this is that omega-3 EPA and DHA promote the inhibition of the enzyme COX-1 (more than the COX2), reducing the products of arachidonic acid metabolism, as does the lowest dose of acetylsalicylic acid, an NSAID (Dennis and Norris 2015). In the case of rheumatoid arthritis, stearidonic acid (18, 4 or SDA) and its EPA and DHA derivatives, present in seed oils such as chia, can play an essential role in human metabolism in its prevention or treatment, because it has been demonstrated at a clinical level that reduces inflammatory symptoms (Miles and Calder 2012). Patients with hepatic diseases like non-

alcoholic steatohepatitis (NASH) treated with a diet richer in omega-3 PUFAs (64% alpha-linolenic ALA, 16% eicosapentaenoic EPA, and 21% docohexanoic DHA acids) show positive changes evaluated by the NASH activity score (NAS) in plasma biochemical markers of inflammation, lipid metabolism and liver function (Nogueira et al. 2016). Atopic dermatitis is a skin disease that is mainly characterized by its dryness, which leads to its scaling and irritation and causes annoying symptoms such as itching. In these patients, there is a reduction in the activity of the A6-desaturase, necessary to convert the ALA of the diet into SDA and EPA, so including an SDA supplement would be required for the treatment of the disease. On the other hand, echium oil, rich in SDA, has shown for years, local utility in some types of dermatitis, inhibiting up to 60% the release of pro-inflammatory prostaglandins (PGE2) with respect to untreated control tissues (Coupland et al. 1996; Guil-Guerrero 2007). Added to this, pro-inflammatory mediators LTB4 and PGE2 are present in the sebaceous glands of the skin being associated with acne. Blocking them with PUFAs (SDA or EPA) can, therefore, reduce acne lesions, constituting a therapeutic alternative (Alestas et al. 2006).

Although major depression is not an inflammatory disorder, it is well known that chronic inflammation (infection, for example), increased the rate of major depressive disorder and reduced the responsiveness of antidepressants and to psychotherapy. In this sense, the fish oil (FO) biological effects have been evaluated against the major depressive disorder (MDD) in humans being by proton magnetic resonance spectroscopy in the bilateral dorsolateral prefrontal cortex (DLPFC) an anterior cingulate cortex and of teenagers. It is found that 16.2 g/day correlating positively with a low score depressive symptom, with a trend in the small dose group, although further studies are needed to evaluate these changes in a larger controlled trial (McNamara et al. 2016) and if it can also be reduced in patients suffering from joint pain or inflammation.

5.5.4 Pharmacodynamic and Pharmacokinetic of Bioactive Lipids.

The Wageningen University & Research, a partnership between Wageningen University and Wageningen Research Foundation is interested a novel mechanisms underlying the anti-inflammatory activity of omega-3 fatty acids, that involve the formation, biological activity and kinetics of fatty acid amides as DHEA (N-docosahexaenoylethanolamine). This research focuses on immune-modulating properties of these PUFAs using peripherical blood mononuclear cells (PBMCs), macrophages, microglial cells, and mice model of colitis. To elucidate the mechanism of action and kinetics properties of these compounds in gastrointestinal- and neurological disorders in mice and human tissue after being submitted to inflammatory conditions or by diet modifications are used spectroscopy techniques (LC-MS/MS). Consequently, is possible to develop novel nutritional and/or phar-

macological intervention strategies (Witkamp and Meijerink 2014; Balvers et al. 2013).

In the case of cannabinoids, they are metabolized by liver and gut enzymes, suffering a first-pass hepatic metabolism; likewise, they have specific pharmacokinetic requirements, demonstrate reduced gastrointestinal permeability, and cause irritation. Also, cannabinoids show low oral bioavailability to treat inflammation, so other routes of administration such as transdermal, intranasal, and transmucosal must be used. Due to its hydrophobic nature, they may be susceptible to choice for nanoparticulate pharmaceutical systems, with the advantage of being administered by multiple routes (Bruni et al. 2018).

5.5.5 Functional Food Based on Bioactive Lipids

The functional foods are a beneficial effect on health more than necessary nutrients, promoting a reduction of risk of disease. The role of dietary lipids in wellbeing as protectors or potential therapeutic targets has been explored in last year (Rey et al. 2019). Humans can synthesize many fatty acids but are unable to desaturate long-chain fatty acids at either C3 or C6 from the methyl end, making them essential, as the PUFAs (Ballabio and Restani 2012). The importance of the dietary lipids has few considered in nutritional researches, even less in the technology of functional food (Meyner and Genot 2017) Besides this limiting aspect, lipid oxidation (rancidity) is the primary process involved in reducing shelf-life food. It modifies the nutritional value, texture, color, taste, and aroma leading to taste and flavors unacceptable (Lima et al. 2013). The oxidation of PUFAs and other bioactive lipids is associated with several mechanisms; one of them is the phenomenon of unsaturated lipid peroxidation that runs in parallel with oxidative stress (Nowak 2013). Therefore, essential unsaturated lipids contained in fishmeal and meat show severe trouble in food stability. In the case of chicken meat, has been tested the dietary supplement with conjugated linoleic acid, reducing the concentrations of malondialdehyde (MDA), a final product of oxidative degradation of fats (Narciso-Gaytán et al. 2011).

The oral drug fingolimod was developed as a first-line treatment for multiple sclerosis (an inflammatory disease), due to its ability to down-regulate S1PR1 and to sequester highly pathogenic T cells within the lymph nodes, avoiding brain myelin injuries. Fingolimod is responsible for reducing blood-brain barrier dysfunction, diminishing the production of sphingolipids from reactive astrocytes, as ceramides (Van Doorn et al. 2010).

Finally, in relation to cannabinoids, regulation is stringent, particularly for phytoremediation and other herbal products such as marijuana, because it is required prior to its commercialization, the performance of strict and well-controlled preclinical and clinical trials, which clearly demonstrate therapeutic efficacy against pain and inflammation, the therapeutic interval and low risk for patients (Nathan 2002; Rincón et al. 2015).

The inflammatory process is related to four main lipids: classical eicosanoids, specialized pro-resolving mediators (SPMs), lysoglycerophospholipids /sphingolipids, and endocannabinoids that play significant roles in inflammation, and dysregulation of one or more of them may lead to inflammation-associated disorder. Most of these bioactive lipids and several elements of their intricate metabolism and signaling are differentially dysregulated in many chronic inflammatory diseases, so the study of the role they play as part of the diet will allow the design of new therapeutic strategies based on robust and safe functional food. In the case of cannabinoids, alternatives to systemic oral delivery as nanoparticle techniques should be considered once the therapeutic doses have been correctly established to treat inflammatory disorders without risk to the patient and in accordance with the legislation of each country.

5.6 Therapeutic Activity in Obesity

The World Health Organization (WHO) defines obesity as abnormal or excessive fat accumulation that presents significant risk factors for several chronic diseases, like diabetes, cardiovascular diseases and cancer (World Health Organization (WHO) 2018) Obesity has been increasing worldwide in the last 40 years; in 2016 there were about 650 million adults, about 41 million children under 5 years old and more than 340 million children and adolescents from 5 to 19 years old with this condition. Currently, there are some strategies to reduce the incidence of obesity, but the traditional treatment and public health interventions are proving inadequate control of the global epidemic in this condition (Afzal 2017). There are multiple approaches and strategies used to treat obesity, including lifestyle modifications (healthy dietary, increasing exercise, behavioral therapy), pharmacotherapy, and surgery (mainly bariatric), the latter being the most risking of the interventions (Wyatt 2013).

Obesity, due to its metabolic complexity, acts as a stressful agent, both adipocyte metabolism and the organs responsible for the metabolism process, including liver, muscle, and pancreas, resulting in insulin resistance and type II diabetes mellitus (DM II). The obesity and the progressive expansion of adipocytes lead to the decreased blood supply to these lipid cells ending in hypoxia. These events have been related to the necrosis of macrophages and their infiltration into the fat tissue, allowing an overproduction of active metabolites called adipocytokines, such as glycerol, plasminogen activator inhibitor-1 (PAI-1), C-reactive protein (CRP), and pro-inflammatory mediators, including tumor necrosis factor-alpha and interleukin-6 (TNF-α and IL-6), and free fatty acids. These changes initially lead to an inflammatory process in adipose tissue; then, it expands to a systemic inflammation associated with the development of various obesity-related diseases (Figueiredo et al. 2017a).

In this context, some substances play an essential role in mediating inflammation and related disorders. Some studies have shown that omega-3 polyunsaturated fatty

acids (PUFAω3) have significant biological effects, which can contribute to the treatment of obesity and metabolic disorders related (de Mello et al. 2018).

This has led to the search for more treatments to reduce this epidemic, such as the use of substances with a high content of polyunsaturated fatty acids, or the consumption of these acids directly.

5.6.1 Polyunsaturated Fatty Acids (PUFAs)

Fatty acids are the main components of membrane lipids, typically contain 12 to 24 carbon atoms forming hydrocarbon chains. Based on the presence and number of double bonds, maybe of the type: saturated fatty acid (no double bonds), monounsaturated (one double bond), and polyunsaturated (with two or more double bonds). Polyunsaturated fatty acids (PUFA) include two series: omega-6 (ω6) and omega-3 (ω3), depending on which is the first carbon double bond (Fig. 5.15). The exogenous conversion of these fatty acids form compounds which are precursors of biologically relevant mediators, such as arachidonic acid (ARA), docosahexaenoic acid (DHA) and eicosapentaenoic (EPA) (Wiktorowska-Owczarek et al. 2015).

The increased consumption of omega-6 contributes to inflammation, oxidative stress, endothelial dysfunction, and atherosclerosis since arachidonic acid is metabolized in pro-inflammatory eicosanoids. Furthermore, EPA and DHA have an anti-inflammatory ability due to the reduction of the adhesion molecules VCAM-1 and ICAM-1 as well as MCP-1 chemokines, metalloproteinases matrix, and pro-inflammatory cytokines. Therefore, by decreasing the omega-6/3 ratio, the inflammatory response can be reduced (DiNicolantonio and O'Keefe 2018).

PUFAω6 intake does not inhibit the antiinflamtoria ability of omega-3; even this combination (at low ratio omega-6/3) is associated with lower levels of inflammation. This was demonstrated in the study Health Professionals Follow-Up Study

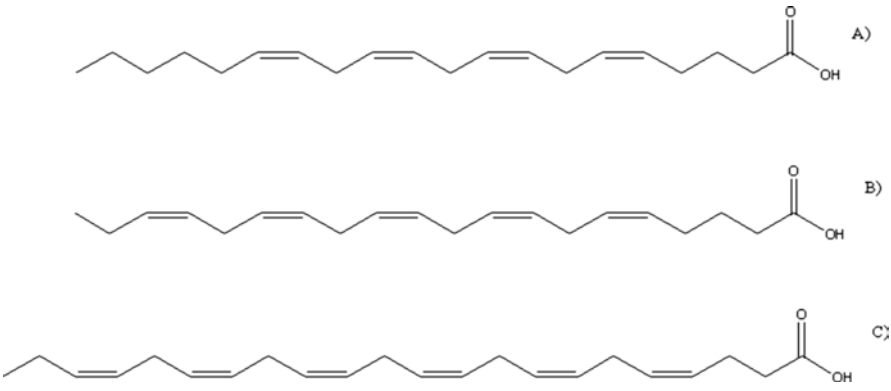

Fig. 5.15 Examples of structures of different polyunsaturated fatty acids. (**a**) Arachidonic acid, omega-6; (**b**) eicosapentaenoic acid, omega-3; (**c**) docosahexaenoic acid, omega 3

(HPFS), which was a prospective cohort investigation of 51,529 professional men health USA, between 40 and 75 years, with the baseline in 1986. Also, we conducted the Nurses' Health Study II, a prospective cohort of 116,671 nurses between 25 and 42 years, with the baseline in 1989. After applying a number of exclusion criteria, the sample for such research was 859 subjects (405 men and 454 women). The participants were determined in serum sTNF-R1, sTNF-R2, IL-6, CRP, all markers of pro-inflammatory cytokines. With multiple regression analysis, it was observed that there is a statistically significant inverse association between dietary PUFAω3 and plasma levels of soluble TNF-receptor 1 and 2. These relationships depend on the intake of PUFAω6, suggesting that at low levels of PUFAω3 intake, the PUFAω6 are associated with high levels of inflammatory markers; however, at higher levels of omega-3 together with the consumption of omega-6, the combination of both types of fatty acids is associated with lower levels of inflammation (Pischon et al. 2003).

On the other hand, Mantzioris et al. in 2000 developed a study with healthy male volunteers, who were provided with enriched α-linolenic acid (ALA) food (cooking oil, margarine, salad dressing, and mayonnaise), eicosapentaenoic and docosahexaenoic acid (sausage and salt sauce), and food rich in naturally PUFAω3 as linseed meal and fish. Subjects added to these foods diet for four weeks, whereby the fatty acid intake, plasma cell fatty acids and eicosanoid production, and monocyte-derived cytokines were measured. On average, volunteers consumed 1.8 g/day of EPA + DHA, while the daily intake of ALA was 9 g/day. With this, EPA was increased on average three times in plasma, platelets and mononuclear cell phospholipids. There was also a significant decrease in PGE2, IL-1b, and TXB2 synthesis, pro-inflammatory cytokines related to the development of obesity, and another metabolic syndrome (Izaola et al. 2015).

5.6.2 Fish Oil

Currently, there are dietary supplements in the market based on fish oil (FO), which contain PUFAω3, EPA, and DHA (Mantzioris et al. 2000).

A study in two groups of male C57BL/6 administered with fish oil (low dose = 1.2%, high dose = 2.4%), showed that subjects delivered with this oil gain less weight compared to those without, and intake of this substance reduces fat accumulation and induces the expression of uncoupling protein 1 (UCP1) in mitochondrial brown adipose tissue (BAT). Also, it increases oxygen consumption and rectal temperature, as well as upregulation of β3 adrenergic receptors (β3AR) and UCP1, in white adipose tissue (WAT) and in interscapular brown adipose tissue; added to this, the urinary excretion of catecholamines and norepinephrine is enhanced. Everything described indicates that it promotes thermogenesis (Mason and Sherratt 2017). BAT is an essential factor in the regulation of energy homeostasis. It's controlled by the sympathetic nervous system and mitochondrial uncoupling protein 1 (UCP1). That

is why it can provide novel strategies for the treatment of obesity in humans (Kim et al. 2015).

In another study, male C57BL/6 mice were administered with a low-fat diet (LFD) and high-fat diet (HFD), as well as being supplemented with fish oil (0%, 3% or 9%), all treatments were for 6 months. Mice were measured bone structure, body composition, and serum cytokines bone-related. The animals fed with HFD increased serum TNF-α, leptin, and tartrate-resistant acid phosphatase (TRAP). Similarly, serum osteocalcin fell and bone-specific alkaline phosphatase. Moreover, the intake of fish oil decreased fat mass, serum TRAP, and expression of TNF-α in adipose tissue. The bone content of long-chain PUFAω3 increased, and the PUFAω6 decreased, with the elevation of FO content in the diet. Therefore, the increased FO in the diet may decrease adiposity and thus mitigate bone deterioration induced by HFD, possibly by reducing inflammation and bone resorption (Contreras et al. 2016).

Furthermore, in a double-blind, placebo-control trial, supplements derived PUFAω3 fish oil at a dose of 2.4 g/day for 6 months was administered. This treatment decreased the levels of triglycerides (TG) and increased HDL-C levels in patients with type 2 diabetes with abdominal obesity. However, there were no changes in total cholesterol, LDL-C, LDL-C index /HDL-C, body composition, and glucose compared to subjects administered placebo (Cao et al. 2020).

5.6.3 Linseed Oil

Flaxseed is one of the oldest cultivated grains in all civilizations; It is used today primarily as a nutritional supplement, especially its oil. Linseed oil is an essential source of PUFAω3, in which the α-linolenic acid (ALA) represents approximately 50% of these (Wang et al. 2017).

A study with rats Wistar (Rattus norvegicus) fed diets based on linseed oil and sesame oil (independent groups and a third with both oils) for 60 days was performed. Bodyweight (throughout the experiment, twice a week), adiposity index, triglycerides, total cholesterol, LDL, HDL, non-HDL, and glucose in serum were evaluated at the end of the experiment. The diets enriched with flaxseed and sesame oils were rich in PUFAω3, being higher in linseed. The adiposity index was lower in animals with diets supplemented with linseed oil. Also, this group showed lower levels of total cholesterol, triglycerides, and showed less weight gain. This demonstrated that diets supplemented with flaxseed oil improve the biochemical and morphometric parameters of experimental animals, explaining that the presence of sources of PUFAω3 benefits the quality of food (Goyal et al. 2014).

Another study was carried out in C57BL/6 male mice, where they were divided into different groups: fed with a low-fat diet (LFD), high-fat diet (HFD), and two groups with the same diets supplemented with flaxseed oil, plus a control group. All diets lasted 16 weeks. The animals were weighed twice weekly, as well as the reg-

istration of food consumption. With these data, the caloric intake of each group was calculated, and adipose tissue biopsies were taken for histological analysis. HFD mice develop obesity with insulin resistance, a fact that was attenuated by supplementation with linseed oil; even with medium doses of this, the metabolic activation of macrophages in adipose tissue (ATM) is blocked, so insulin signaling in adipose tissue was improved (Figueiredo et al. 2017b).

5.6.4 Bile Acid and Derivatives

Bile acids come from cholesterol metabolism. In their chemical structure, they preserve the core nucleus of cholesterol; therefore, they are considered substances with lipid properties (Thakare et al. 2018; Marin et al. 2015). Bile acids are synthesized in the liver and are responsible for forming bile salts, which are the body's natural emulsifiers (Macierzanka et al. 2019). The body synthesizes cholic acid and chenodeoxycholic acid abundantly; however, there are other bile acids such as deoxycholic and ursodeoxycholic (Chiang 2009). The latter has been used for decades for the treatment of cholestasis and cholesterol gallstones (Guarino et al. 2013).

Ursodeoxycholic acid (UDCA) is a secondary bile acid derivative metabolism (Fig. 5.16) that has been proposed as a potent treatment for inflammatory bowel disease (Yu et al. 2017).

A study conducted in male C57BL/6, were divided into three groups fed with a regular diet, high-fat diet (HFD), and HFD supplemented with UDCA 0.5% w/w, for 8 weeks. It showed that mice fed with HFD + UDCA had less body weight gain compared to other animals. Similarly, the glucose level was decreased in this group

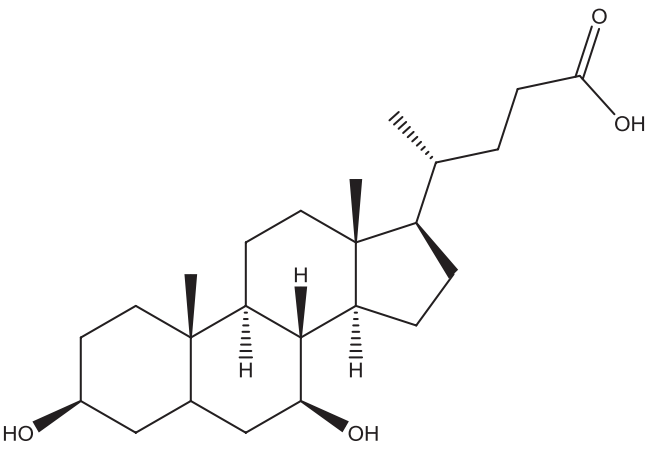

Fig. 5.16 Chemical structure of ursodeoxycholic acid

compared to only HFD were fed (He et al. 2018; Zhang et al. 2019). This research is opening new search strategies for potential obesity treatments, with this molecule.

Taking supplements and enriched with polyunsaturated fatty acids, with a higher proportion of omega-3 to omega-6 food, can induce obesity reduction due to the decrease of pro-inflammatory cytokines as well as preventing other metabolic disorders. However, it is necessary to emphasize that adequate dietary management regarding these PUFAs should be considered, as well as the consumption of fiber, unprocessed sugar, and exercise since their inadequate use can lead to other problems such as hypertension.

Bile acids have also been shown to be protective factors against obesity and lipid accumulation. In a study of transgenic mice, which overexpressed the limiting enzyme in the synthesis of bile acids, cholesterol 7a-hydroxylase, it was shown that taurokenodeoxycholic, taurodeoxycholic, taurocolic and tauro-β-murolic bile acids decreased plasma lipid concentration, such as lysophosphatidylcholines, phosphatidylcholines, sphingomyelins, and ceramides. These effects occurred in mice that were fed a high-fat diet and suggest anti-obesity results (Qi et al. 2015).

Another bile acid that has shown significant effects against obesity is chenodeoxycholic acid. This acid was evaluated in an in vitro model, using 3 T3-L1 adipocytes, which were exposed to high concentrations of glucose and different doses of the acid. Adipocytes demonstrated oxidative capacities, probably of fatty acids, a significant effect in the treatment of obesity (Teodoro et al. 2016).

The use of bile acids also represents a significant challenge for the pharmaceutical and food industries, since they are substances derived from cholesterol and cannot be solubilized in water, they also tend to form emulsions, which are very complicated systems to use in some food. Furthermore, the high concentration of bile acids can be toxic to cells, so its use and consumption should be moderate. Some bile acids produced by other animals have been used in capsule form to treat problems of obesity, cholesterol, diabetes, bile, but their biological impact on health must be considered.

The information presented in the chapter highlights the effects of some lipids against cancer, which is a chronic degenerative disease, to a lesser extent we report effects on the cardiovascular system, obesity, inflammation and cholesterol diseases. Whatever the disease, what is sought in the future is to find adequate means to be able to ingest or administer lipids. If we consider the core part of the theme of this book, we are faced with many disadvantages, because lipids are difficult to manipulate for the pharmaceutical and food industries. Its null or poor solubility in water prevents them from making formulations that can be administered orally, without presenting problems. Associated with the latter, emulsions can be formulated to be ingested, but these types of preparations are unpleasant to the eye, since they coexist two phases that are immiscible with each other, but that can coexist thanks to a surfactant agent. The emulsions can be administered intramuscularly, which would be an alternative for lipid treatment. The food industry also faces many difficulties when formulating products for human consumption,

which use lipids as active ingredients. These substances can be dissolved with similar ones that could harm the body, especially if you have a disease associated with cholesterol, triglycerides, dyslipidemias. Therefore, they must also invest a lot of inputs in creating the right vehicles to formulate food with healthy lipids. All these factors constitute constant research, which has left different products on the market, such as emulsions, capsules, ointments. The development of a functional food becomes more complex because every food needs to have a pleasing presentation for the client, in terms of smell, color, flavor, texture, and appearance. The information collected will allow taking different bibliographic sources in order to amplify a particular topic that readers choose.

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Chapter 6
Marine Bioactives

Reza Tahergorabi and Mehdi Abdollahi

Abstract Marine organisms are a rich source of bioactive compounds. Bioactive compounds are compounds with health-promoting effects. Consumption of these compounds may lower the risk of diseases such as heart diseases, cancer, diabetes, osteoporosis, and other complications. Recently, marine bioactives have attracted much attention due to their enormous health benefits. This book chapter provides a succinct review of the recent studies about marine bioactives including proteins, peptides, amino acids, fatty acids, sterols, polysaccharides, oligosaccharides, phenolic compounds, photosynthetic pigments, vitamins, and minerals. It also discusses the bioactives derived from marine bacteria as well as different techniques used for marine bioactives recovery.

Keywords Marine organisms · Bioactives · Health · Seafood

6.1 Introduction

More than 70% of the earth is covered by the seas, oceans and aquatic environments. Many living creatures including aquatic plants and animals exist in these environments with potential health benefits that have not been discovered yet. Many studies have been conducted so far to explore the world under the water and to find a cure for many diseases that the world population is dealing with. However, we are yet far from exploring these valuable resources of the aquatic world. Earlier studies with Greenlandic Inuit or Eskimos indicated that having a great number of seafoods in the diet increases well-being and health (Bang et al. 1986; Rangel-Huerta and Gil 2018). This was probably the milestone of a series of studies on the effect of seafood consumption on human body. Since that time, scientists found that marine organisms including plant and animals contain bioactive compounds which may

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promote health in human being. According to these studies, marine organisms may provide bioactive compounds with different activities including anticoagulant, calcium-binding, anti-obesity, and anti-diabetic, antioxidant, anti-hypertensive, anti-HIV and anti-proliferative activities (Bleakley and Hayes 2017; De Jesus Raposo et al. 2013; Abdul et al. 2016). Thus, this chapter discusses the bioactive compounds of different types of marine organism. It also reviews their applications in health, cosmetic and food industries.

6.2 Marine Proteins

Proteins have a fundamental, physiological and nutritional role in the human body as major structural components of all cells. They also act as hormones, enzymes, and antibodies and have a critical role as carries in both cell walls and blood. On top of that, proteins e.g. collagen provide structural support in connective tissues, cells, and skin. As a food component, proteins have essential nutritional roles by providing energy and amino acids which are vital for growth and maintenance in our body. Foods from marine resources are generally recognized as a great source of proteins containing all the essential amino acids close to the proportion suitable for human beings (Hamed et al. 2015). Marine animal-based foods contain relatively higher proportion of protein on a wet weight basis (average 17.3%) than meats from terrestrial animals (13.8%), despite having a higher moisture content than most terrestrial meats (Tacon and Metian 2013). Marine animals muscle usually contains lower amount of stroma proteins (e.g. collagen and elastin) than red meat which ranges from 1 to 3% in finfish up to 10% in shark and ray fish. Myofibrillar protein content in marine animals ranges between 65 and 75% and it ranges between 20 and 35% for sarcoplasmic proteins (Venugopal 2008). Marine invertebrates e.g. oyster, mussel, clam, and squid exceptionally have another type of protein in their strained muscles called paramyosin which ranges between 3 and 19%. Proteins from marine animals have high digestibility and biological value as well as having essential amino acids especially lysine much higher than proteins from plant foods (Wang et al. 2018). These proteins are also rich in amino acids e.g. methionine and lysine which are limited in terrestrial meat proteins (Tacon and Metian 2013; Khalili Tilami and Sampels 2018).

Beyond their nutritional value, recent studies have shown that proteins from marine foods and their hydrolysates can also exert health effects on the human body. For many years, health effects of seafoods consumption such as dyslipidemia and heart diseases have been attributed to high content of mega-3 fatty acids found in their oil. However, most recent studies have shown that marine proteins may also play a key role in beneficial health effects of marine foods. Various physiological health effects and bioactivities such as mitigating effects on obesity, metabolic syndrome, inflammation, type II diabetes (insulin sensitivity or glucose tolerance), cardiac risk factors (high blood pressure and triacylglycerol levels), osteoporosis, and reduced circulating concentrations of lipids have been reported for marine proteins

in either animal models or human trials which are summarized in Table 6.1 and briefly reviewed in the following.

6.2.1 Antiobesity Properties of Marine Proteins

Obesity which is morphologically seen as overweight and extra body fat accumulation is as worldwide health issue. This excessive body weight has shown strong association with heart disease risk factors e.g. insulin resistance, type-2 diabetes, dyslipidemia, metabolic syndrome, and high blood pressure. Several studied have shown that sole inclusion of fish protein in diet can effectively protect against obesity-related disorders especially formation of adipose tissue mass in animal models as summarized in Table 6.1. For example, a diet with a mixture of several marine protein sources (ling, rosefish, cod, wolfish and muscle from a scallop) could reduce fat mass in rats compared with the diets containing a mixture of chicken, pork, and beef as main protein source (Holm et al. 2016). However, the effects of preventing obesity were more evident in cod protein containing diets. Proteins from other fish including salmon, herring, bonito, and mackerel were also added to high fat diet and their effects on rats were compared with diets with casein. Despite equal energy intake among all groups, it was an only salmon protein-containing diet that significantly reduced weight gain (Pilon et al. 2011). These two studies suggest that beneficial physiological effects of marine proteins are highly governed by their sources. The latter study also found that consumption of salmon diet also increased circulating calcitonin levels in the rats which might have also played role in reduction of weight gain in the studied rats. Salmon calcitonin is a widely studied bioactive peptide in fish protein with 32 amino acids with blood calcium lowering activity 40–50 times more potent than human calcitonin (Aadland et al. 2015). It has been clinically used for more than 30 years for treatment of metabolic bone disease e.g. osteoporosis, paget disease, and bone metastases by inhibiting osteoclast activity (Pilon et al. 2011).

6.2.2 Hypolipidemic Properties of Marine Proteins

Another reported health benefit for marine food proteins is related to their effects on lipid metabolism which is also related to coronary artery disease. Animal studies have shown that defatted protein from Alaska Pollak could decrease serum cholesterol in rats through the inhibition of cholesterol and bile acid absorption and the enhancement of cholesterol catabolism in the liver (Hosomi et al. 2009). Also, similar beneficial effects have been observed in both rabbits fed with cod protein compared to casein and milk proteins and in rat fed with herring and salmon protein hydrolysates (Bergeron and Jacques 1989; Drotningsvik et al. 2016). When protein from crab, scallop, cod, and chicken was tested on obesity-prone mice, a significant

Table 6.1 Bioactive properties of fish proteins studied in animal models

Studied bioactivity	Protein source	Study condition	Main results	Reference
Anti-obesity	Bonito, herring, mackerel, or salmon	Male Wistar rats 4 weeks	Lower weight gain and reduced fat accumulation in salmon protein fed mice	Pilon et al. (2011)
	Ling, rosefish, cod, wolffish, and scallop	Healthy male mice 12 weeks	Less fat mass accumulation, decreased feed intake and diminished weight gain	Holm et al. (2016)
	Cod, crab, and scallop	Obesity-prone male mice, high sucrose and high-fat diet	Scallop-fed mice gained less body and fat mass	Tastesen et al. (2014)
Hypolipidemic	Shrimp, squid and octopus defatted protein	Male rats, 19 days	Decreased hepatic cholesterol	Tanaka et al. (1998)
	Alaska pollock	Male Wistar rats 4 weeks	Decreased cholesterol in serum and liver	Hosomi et al. (2009)
	Cod + scallop	Female mice, 13 weeks	Lower serum levels of leptin and LDL cholesterol	Jensen et al. (2016)
	Blue whiting water-soluble protein	Male obese rats 5 weeks	Lower serum and liver cholesterol	Drotningsvik et al. (2018)
	Herring and salmon by-products protein hydrolysate	Obese rats 4 weeks	Reduced serum HDL and LDL-cholesterol, and higher serum TAG, MUFA and n-3: n-6 PUFA ratio	Drotningsvik et al. (2016)
Antidiabetic	Cod	High fat diet fed rats 4 weeks	Fully prevented the development of insulin resistance in rats	Lavigne et al. (2001)
	Bonito, herring, mackerel, or salmon	Male Wistar rats 4 weeks	Improved insulin sensitivity	Pilon et al. (2011)
	Bonito	Type-2 diabetes mellitus rats 6 weeks	Improved T2DM-induced bone frailty	Ochiai et al. (2015)

(continued)

Table 6.1 (continued)

Studied bioactivity	Protein source	Study condition	Main results	Reference
Anti-hypertensive	Fish	Spontaneously hypertensive rats (SHR) for 8 weeks	significant reduction of blood pressure	Ait-Yahia et al. (2003, 2005)
	sardine	Male Wistar rats 3 weeks	decrease of diastolic blood pressure and heart rates	Khelladi et al. (2018)
	sardine by-products	Obese rats 4 weeks	Lowered blood pressure	Affane et al. (2018)
Anti-inflammatory	Cod protein	Bupivacaine-injured skeletal muscle rats 4 weeks	Promoting growth and regeneration of skeletal muscle after trauma	Dort et al. (2012)
	Shrimp protein hydrolysate	Bupivacaine-injured skeletal muscle rats 4 weeks	Facilitated resolution of inflammation after muscle injury	Dort et al. (2016)
	Bonito, herring, mackerel, or salmon	Male Wistar rats 4 weeks	Reduced expression of both tumor necrosis factor–α and interleukin-6	Pilon et al. (2011)

reduction in lipid metabolization was found in scallop fed mice (Tastesen et al. 2014). Scallop protein could significantly reduce plasma triacylglyceride, non-esterified fatty acids, glycerol and hydroxybutyrate in mice. Most of the mentioned studies have used fillet or muscle of marine animals as a source of protein in their studies. However, a more recent study by Drotningsvik et al. (2018) have evaluated anti-obesity effects of water soluble proteins from a pelagic fish called blue whiting. Obese rats fed with a diet containing the water soluble proteins (1/3 of protein in their diet) from blue whiting had lower levels of serum and liver cholesterol compared to rats fed with 100% of casein in their diet. This was most likely related to lower hepatic cholesterol synthesis in the rats fed with the water soluble proteins.

In line with the above mentioned animal studies, a randomized control trial comparing the effects of consuming protein from cod, pollock, saithe, and scallops with lean meat: chicken, beef, turkey, pork, egg, and low-fat milk in a Norwegian group found a reduction in both fasting and postprandial circulating triglycerides concentrations in the participants (Aadland et al. 2015). Also, cod protein supplementation to thirty-four overweight adults for 8 weeks could help lipid metabolism in the participants and reduce LDL cholesterol (Vikoren et al. 2013).

6.2.3 Antidiabetic Properties of Marine Proteins

Type 2 diabetes is another health issue associated with obesity and related to sugar metabolism in the body. In this disorder, the human body becomes resistant to the effect of insulin or loses the capacity to produce insulin. Some studies have shown that seafood and even fish protein can reduce insulin resistance and thereby increase capacity to store glucose as glycogen and minimize the risk of type 2 diabetes (Nkondjock and Receveur 2003). For instance, feeding rats with a high-fat, high-sucrose diet containing cod protein (having 91% protein and 0.19% lipid) as protein source completely hindered the development of insulin resistance and glucose intolerance in the animals (Lavigne et al. 2001). Control rats fed with the same diet but containing soy protein isolate and casein as protein sources showed improvement in fasting glucose tolerance and peripheral insulin sensitivity (Lavigne et al. 2000). Nevertheless, insulin resistance was detected in the rats fed with soy protein and casein. The author showed that the ability of cod protein in preventing insulin resistance caused by obesity in those rats could be partly related to the direct effect of amino acids in the cod protein on insulin-stimulated glucose uptake in skeletal muscle cells (Lavigne et al. 2001). In line with the previous studies, feeding rats with diet containing salmon protein also promoted their insulin sensitivity (Pilon et al. 2011). Ochiai et al. (2015) showed that defatted protein produced from dried bonito fish (*Katsuwonus pelamis*) could effectively diminish the bone frailty caused by insulin resistance and type 2 diabetes mellitus in young rats (Ochiai et al. 2015). This study could confirm that fish protein can also be a marine bioactive that can potentially help in mitigating bone frailty independent from the effects found for poly unsaturated fatty acids.

A more recent randomized double-blind study on 93 overweight adults evaluated the effect of protein from herring and salmon protein hydrolysate as well as cod protein on glucose regulation and markers of insulin sensitivity in the participants (Hovland et al. 2019). The participants received the fish proteins (2.5 g/day) as well as a mixture of casein and whey (as control) as tablet. They did not report fat content in the proteins. The study showed that consumption of the low dosage of cod protein or herring protein hydrolysates could promote glucose regulation in overweight adults. However, they did not find any significant effect for salmon protein hydrolsyate (Hovland et al. 2019).

6.2.4 Antihypertensive Properties of Marine Proteins

Blood pressure or hypertension is another important risk factor for cardiovascular disease which is the largest cause of death globally (Vasdev and Stuckless 2010). Normal blood pressure should be 120/80 mmHg and elevation of one or both parameters causes heart workload increase and results in a condition called hypertension (Jensen and Mæhre 2016). The beneficial effects of marine proteins on hypertension

have been studied in both animal models and less frequently in clinical trials. For example, a 20% replacement of intact fish protein in the diet of spontaneously hypertensive rats (SHR) for 8 weeks significantly reduced blood pressure in the animals compared to those eating the casein protein (Ait-Yahia et al. 2003; Ait Yahia et al. 2005). A more recent study showed that a diet containing 20% of sardine protein and 2% of lemon zest induced a significant decrease of diastolic blood pressure and heart rate values in rendered diabetic and hypertensive rats compared with casein containing diet (Khelladi et al. 2018). Also, purified protein from sardine by-products could induce lowered blood pressure in obese rats compared with casein (Affane et al. 2018). Although studies on the effects of intact marine proteins are rare, a large number of studies have shown that total protein hydrolysates from different marine sources such as salmon (Enari et al. 2008), cod (Jensen et al. 2014), cobia (Yang et al. 2013) and jellyfish (Liu et al. 2012) have significant blood pressure reducing effect on SHR. Also, evaluations on chronic effect of total protein hydrolysates from some marine sources such as seabream (Fahmi et al. 2004) and jellyfish (Liu et al. 2012) on SHR have shown a significant reduction of blood pressure even comparable to that of captopril. When it comes to human studies the results are not easily judged. For example, a randomized trial with 33 medicated patients with coronary heart disease showed that cod protein as main protein source in diet could reduce both systolic and diastolic blood pressure in the patients (Erkkilä et al. 2008). However, supplementation of salmon protein hydrolysate capsules to overweight adults for 2 months had no effect on blood pressure of the patients (Enari et al. 2008).

6.2.5 Anti-Inflammation Properties of Marine Proteins

Inflammation is normally considered as a regular reaction of our immune system to harmful stimuli which has a critical role in our life. However, inflammation disorder can cause a vast variety of diseases such as cancer, atherosclerosis, and ischemic heart disease, colitis, Crohn's disease and so on. Anti-inflammatory effects of omega-3 containing fish oil are widely agreed but recent studies have shown that fish proteins and most probably their hydrolysate may have anti-inflammatory effects.

For example, defatted cod protein added to the diet of rats with artificially injured muscle promoted resolution of inflammation in their muscles compared to casein and defatted peanut protein. The cod protein could significantly reduce density of neutrophils and ED1+ macrophages at day 14 and 24 post injury in the injured muscles of the rats (Dort et al. 2012). Addition of defatted peanut protein to the diet of the rats with injured muscles had no anti-inflammatory effect and even reduced their muscle mass recovery (Dort et al. 2012). The authors later showed that the anti-inflammatory effect observed for cod protein is related to its high levels of arginine, glycine, lysine and taurine by supplementing casein with a mixture of those amino acid in similar amount to their levels in cod protein (Dort et al. 2016).

In a later study, Dort et al. (2016) reported similar anti-inflammatory effects for shrimp protein hydrolysate in rats with artificially injured muscle. Anti-inflammatory activity was also reported for proteins from four different fish species including bonito, salmon and herring and mackerel. Proteins from the named fish could mitigate expression of both tumor necrosis factor–α and interleukin-6 in visceral adipose tissue of rat compared with casein (Pilon et al. 2011).

6.2.6 Brain Health Effects of Marine Proteins

Age-related diseases such as dementia and Alzheimer's disease that are progressive disorders causing brain cell death and loss of memory are also growing in the aging population around the world. Beneficial effects of fish consumption against the cognitive related disease have been widely studied but it has been mainly related to the function of omega-3 fatty acids (Kühn 2014). However, a recent study has shown that parvalbumin which is recognized as most common allergen in fish can cause cross-reactions with human amyloidogenic proteins and inhibits amyloid formation of α-synuclein which is mostly associated with neurodegenerative disorders such as Alzheimer's and Parkinson's (Werner et al. 2018). The authors suggested that beneficial effects of fish on brain health might be also partly explained by its protein function. However, further studies are needed to make a concrete conclusion in this regard.

6.2.7 Marine Algae Proteins and Their Bioactivity

Proteins from marine plants i.e. seaweed and microalgae are also an emerging type of marine proteins that have gained massive attention recently as more sustainable and marine origin vegetarian protein alternatives. Proteins in seaweed are a structural component of their cell wall and have physiological roles as enzymes and pigments (Pimentel et al. 2019). Protein contents in seaweeds can reach up to 47% dry weight in Rhodophyceae (red seaweeds) and 9–26% dry weight in Chlorophytes (green seaweeds), followed by the lowest at about 3–15% in Phaeophytes (brown seaweeds). However, protein content of seaweeds varies substantially by change in season and geographical locations and environmental conditions (Okolie et al. 2018).

Two typical proteins found in seaweeds with bioactive properties are lectin and phycobiliproteins. As glycoproteins with high specificity binding with carbohydrate, lectins have found a wider range of application e.g. in blood grouping, antiviral (including human immunodeficiency virus type 1(HIV-1)), cancer biomarkers, and targets for drug delivery (Bleakley and Hayes 2017). Lectins from algal sources have also shown other bioactive properties such as antinociceptive, antibacterial, antiviral, antiadhesion, cytotoxic, and mitogenic properties (Okolie et al. 2018).

Phycobiliproteins are photosynthetic proteins that have critical role in light capturing in red seaweeds. They are water-soluble and inherently fluorescent which makes them a useful biomaterial for application in some immunological methods (Pal and Suresh 2016). Phycobiliproteins are also used as natural colorants in the food and cosmetic industry. In addition, these proteins have shown a wide range of bioactive properties such as hepatoprotective anti-inflammatory activities, antitumor, antioxidant, antiviral and neuroprotective properties (Bleakley and Hayes 2017). These multifunctional bioactivities of phycobiliproteins have led to their application in treatment of some disease e.g. arteriosclerosis, serum lipid reduction, and lipase inhibition (Okolie et al. 2018).

Protein hydrolysates and peptides generated by enzymatic hydrolysis of proteins from a wide range of seaweeds have also shown several bioactive properties such as antioxidant (Heo and Jeon 2008; Wang et al. 2010), antihypertensive (Athukorala and Jeon 2005; Cian et al. 2012), antiproliferative (Athukorala et al. 2006) and antidiabetic (Harnedy et al. 2015) properties. However, results are mainly limited to in vitro studies which call for more research on animal models and human trials for a better understanding of their application potentials. This has also made seaweeds as one of the fastest-growing research fields for recovery of marine origin bioactive compounds.

Altogether, recent studies have shown that health benefit effects of marine foods go beyond their omega-3 PUFAs and their protein can play a significant role in their bioactivity. However, more human studies in clinical and intervention trials on pure and especially defatted marine proteins are needed to support bioactivities found in vitro models and animal models. Also, effects of processing, storage and cooking methods on the bioactivity of marine proteins need to be considered in future studies and recommendations.

6.3 Marine Peptides

Peptides are short chains of amino acids connected with peptide bonds with usually between 3 to 20 amino acids (Jo et al. 2017). Bioactive peptides may naturally exist in marine organisms to perform some physiological roles in their body or be generated artificially by enzymatic hydrolysis of marine proteins. The enzymatic hydrolysis method has gained great attention in the food industry and it has been used for extraction of bioactive peptides from a wide range of marine resources such as fish, crustaceans, mollusks, algae, and microorganisms, especially during the last two decades. Different types of marine animals such as fish, shrimp, lobster, crab, mussel, clam, jellyfish, sea cucumber, sea urchin, squid, oyster, sponges, rotifers and etc. have been used for production of bioactive peptides using enzymatic hydrolysis (Proksch et al. 2010; Bordbar et al. 2011; Ngo et al. 2012; Harnedy and FitzGerald 2012; Jo et al. 2017). In addition, seafood industry has already lost more than 50% of its biomass as by-product e.g. fish head, frame, tail, bone, skin, viscera, blood and

shells which have been targeted as a great substrate for production of marine bioactive peptides (Atef and Mahdi Ojagh 2017; Ishak and Sarbon 2018).

Bioactive peptides are inactive within the parent protein structure but as soon as they are released using the hydrolysis, they show various bioactive properties depending on their amino acid composition and sequence (Ngo et al. 2012). Thanks to the almost endless number of variations that can happen in amino acid composition and sequence, marine bioactive peptides have shown several types of bioactivity including antihypertensive, antiproliferative, anticancer, antioxidant, antimicrobial, anti-inflammation, anticoagulant and opioid agonists or antagonists properties (Proksch et al. 2010; Bordbar et al. 2011; Ngo et al. 2012; Harnedy and FitzGerald 2012; Samarakoon and Jeon 2012; Jo et al. 2017). In the light of these explanations, bioactive peptides may be able to potentially improve human health and reduce disease risk as nutraceuticals and pharmaceuticals. In parallel, promotion in consumers' awareness about the association between food and health has led increase in demand for functional foods (Jo et al. 2017). Thus, bioactive peptides produced from marine organisms, representing more than 50% of our global biodiversity, can be a great source of bioactive compounds to be used as nutraceuticals and functional foods (Kim and Wijesekara 2010; Suleria et al. 2015). Thus, in the following, an overview of most recent bioactive peptides produced from different marine resources as well as seafood processing by-products and their bioactive properties is presented.

6.3.1 Marine Peptides with Antioxidant Activity

Antioxidants play an important role in our body by reducing negative effects from the excessive generation of reactive oxygen species (ROS) such as superoxide anion (O^{2-}) and hydroxyl (OH^{1-}) radicals. However, imbalance between generation of ROS and ability of endogenous antioxidants in human body in their detoxification can cause oxidative stress. This imbalance has been associated with several chronic health issues such as heart disease, stroke, high blood pressure, cancer, inflammatory disease and aging (Valko et al. 2007). Bioactive peptides with ability to scavenge free radicals and ROS or stopping lipid peroxidation by interrupting the radical chain reaction have been extracted from protein hydrolysate of different marine animals and plants. These peptides are normally called antioxidant peptides and have been isolated from fish and shrimp muscle and their processing by-products e.g. head (Yang et al. 2011; Chi et al. 2015a), frame (Je et al. 2005, 2007), skin (Zhang et al. 2012), bone (Baehaki et al. 2015), swim bladder (Zhao et al. 2018), viscera (Villamil et al. 2017), and shrimp peeling by-products (Ambigaipalan and Shahidi 2017). For example Chi et al. (2015b) extracted three antioxidant peptides from tuna head by-products with sequence of Trp-Glu-Gly-Pro- Lys (WEGPK), Gly-Pro-Pro (GPP), and Gly-Val-Pro-Leu-Thr (GVPLT), with molecular weights of 615.69, 269.33, and 485.59 Da, respectively. The antioxidant activity of the isolated peptide was most likely related to high concentration of hydrophobic and/or aromatic amino

acid residues in their sequence. However, the mechanism of their antioxidant activity was different where GPP indicated highest in vitro radical scavenging activity (IC_{50} = 1.9-2.4) but WEGPK inhibited the peroxidation of linoleic acid. Also, a peptide (Lys-Thr-Phe-Cys-Gly-Arg-His) with molecular weight of 86.1 kDa produced from croaker (*Otolithes ruber*) muscle with enzymatic hydrolysis could promote the endogenous cellular antioxidant enzymes in Wistar rats (Nazeer et al. 2012). The peptide elevated the activities of catalase (CAT), glutathione-S-transferase (GST) and superoxide dismutase (SOD) in the animals.

Other marine animals including crab (Yoon et al. 2013), squid (Sudhakar and Nazeer 2015), oyster (Umayaparvathi et al. 2014; Zhang et al. 2019a), mussel (Wang et al. 2013), clam (Chi et al. 2015a), jellyfish (Zhuang et al. 2009a), and sea cucumber (Zhou et al. 2012) have been used for production of antioxidant peptides. For example, Sudhakar and Nazeer (2015) could separate a 679.5 Da peptide from cuttlefish (*Sepia brevimana*) by enzymatic hydrolysis with the sequence of Ile/Leu-Asn-Ile/Leu-Cys-Cys-Asn with a remarkable inhibition of linoleic acid autoxidation in a model system.

Marine algae are also considered as a rich source for isolation of antioxidant peptides due to their highly unstable living conditions in ocean experiencing extraordinary low light intensities and high oxygen concentrations (Samarakoon and Jeon 2012). For example, a peptide with sequence of Glu-Leu-Trp-Lys-Thr-Phe recovered from enzymatic hydrolysis of *Gracilariopsis lemaneiformis* proteins with α-chymotrypsin showed a significant free radical scavenging activity with an EC_{50} value of 1.514 mg/ mL (Zhang et al. 2019b). The authors suggested low molecular weight and hydrophobic and/or aromatic amino acids in the sequence of the purified peptides as main reason for its relatively good antioxidant activity.

6.3.2 Marine Peptides with Antihypertensive Properties

Peptides produced form marine organisms have been widely investingated as bioactives with antihypertensive properties. Antihypertensive peptides can modulate physiological regulation of blood pressure by inhibiting the activity of angiotensin-I converting enzyme (ACE) (Abdelhedi and Nasri 2019). ACE can regulate blood pressure by converting angiotensin-I to angiotensin-II. The later is a potent vasoconstrictor and also inactivates the vasodilator bradykinin (Li et al. 2004). Side effects created by treatment of blood pressure with synthetic ACE inhibitors such as captopril, enalapril, alcacepril have made interest in finding natural alternatives including bioactive peptides (Kim and Wijesekara 2010). From a mechanistic point of view, synthetic drugs inhibit ACE by blocking its action while ACE inhibitory peptides react with ACE and prevent its attachment to Angiotensin I (Ngo et al. 2012). However, the mechanism of action has not been well understood for some bioactive peptides. Numerous studies have shown antihypertensive activity of marine-derived bioactive peptides in both in vitro and in vivo. Bioactive fractions obtained by enzymatic hydrolysis of cobia head with papain showed an ACE

inhibitory IC_{50} of 0.24 mg/ml which was intensified after incubation with gastrointestinal enzymes (Yang et al. 2013). Oral administration of the bioactive peptides to SHR in a dosage of 150–1200 mg/kg body weight could reduce systolic blood pressure in a dose-dependent manner in the rats. Similar blood pressure-lowering effect was found in SHR fed with bioactive peptides from jellyfish *Rhopilema esculentum* (IC_{50} = 1.28 mg/ml) (Liu et al. 2012), oyster (IC_{50} = 66 μmol/L) (Wang et al. 2008), sea bream scale collagen (IC_{50} = 0.57 mg/ml) (Fahmi et al. 2004), yellowfin sole (*Limanda aspera*) frame (IC_{50} = 28.7 μg/ml) (Jung et al. 2006), bigeye tuna dark muscle (*Thunnus obesus*) (IC_{50} = 26.6 μM), chum salmon (*Oncorhynchus keta*) skin (IC_{50} = 18.7 μM) (Wang et al. 2008).

The antihypertensive effect of marine bioactive peptides has been also reported in some human studies. For example, daily administration of 3 g of a 3 kDa permeate of protein hydrolysate from dried bonito could significantly reduce systolic blood pressure in borderline and mildly hypertensive human subjects (Fujita et al. 2001). Also, 300 and 500 mg daily uptake of protein hydrolysate from a seaweed (*Undaria pinnatifida*) showed the same effect in mildly hypertensive subject groups consuming its jelly after 8 weeks (Kajimoto et al. 2002). Similarly, a daily intake of 1.6 g oligopeptide from Nori (*Porphyra yezoensis*) resulted in a significant reduction of systolic blood pressure in participants with high-normal blood pressure after 12 weeks (Kajimoto 2004). In addition, consumption of a beverage (100 ml) containing 2 g of salmon muscle protein hydrolysate for 12 weeks significantly reduced systolic and diastolic blood pressure in 60 mildly and high-normal hypertensive participants (Enari et al. 2007; Norris et al. 2013).

6.3.3 Marine Peptides with Antiproliferative and Anticancer Properties

Cancer is one of the top leading causes of death among the global population and is continuously increasing which has made it a big threat for the global population (Ezzati et al. 2002). Cancer is the abnormal growth and uncontrolled proliferation of cells caused by certain mutations in cellular DNA which destabilize cell division and death process (Le Gouic et al. 2019). This uncontrolled cell division can finally lead the formation of tumor which may limit its location or invade and spread to other parts of body (Ezzati et al. 2002). Production of antiproliferative peptides that can induce cell death by apoptosis has gained interest as a way for treatment of cancer. Different peptides from marine organisms have shown antiproliferative and anticancer properties. Among the studied organisms that can produce toxins; sponges, mollusk and tunicates have been the most effective and studied aquatic organisms (Suarez-Jimenez et al. 2012). However, peptides with antiproliferative effect have been also isolated from other marine organisms such as marine snails (Kim et al. 2013), oyster (Umayaparvathi et al. 2014) and fish (Song et al. 2014) and snow crab by-products (Doyen et al. 2011). Two peptides with molecular weight

ranging from 390 to 1400 Da separated from enzymatic hydrolysate of tuna dark muscle showed antiproliferative activity against human breast cancer cell line MCF-7 (Hsu et al. 2011). The purified peptides had an amino acid sequence of Leu-Pro-His-Val-Leu-Thr-Pro-Glu-Ala-Gly-Ala-Thr and Pro-Thr-Ala-Glu-Gly-Gly-Val-Tyr-Met-Val-Thr. The two peptides exhibited a dose-dependent inhibition effect of the cancer cells with IC_{50} values of 8.1 and 8.8 μM. Also, a peptide with amino acid sequence of YALPAH from hydrolysate of half-fin anchovy (*Setipinna taty*) induced PC-3 cell apoptosis at the concentration of 4.47 μM (Song et al. 2014). The peptide showed an IC_{50} of 8.1 mg/ml and its antiproliferative activity was correlated to its positive charge intensity in a way peptide with the highest positive charge intensity showed the strongest antiproliferation. Anticancer peptides found in the studied hydrolysates from marine organisms have all had very low molecular weight and all contained active amino acids including Pro, Gly, Lys, Arg, and Tyr. This might be because low molecular weight peptides have higher mobility and diffusivity than larger peptides which facilitates their interaction with cancer cells and promote their anticancer activity (Ishak and Sarbon 2018).

6.3.4 *Marine Peptides with Skin, Bone, and Joint Health Effects*

Several factors including chronological aging, dermatological disorders, and environmental conditions can cause skin properties loss. This can be even intensified undesirable lifestyle and photo-aging (Fu et al. 2018). Collagen peptides from marine foods have gained great interest as a sustainable ingredient with antiaging and skin health promotion properties. A large number of studies have shown that collagen peptides from different marine sources such as fish scale (Wang et al. 2017) fish skin (Pyun et al. 2012) and jellyfish (Zhuang et al. 2009b; Fan et al. 2013) could increase collagen production in rats and significantly decrease matrix metalloproteinases (MMP) expression. For example, Song et al. (2017) showed that ingestion of collagen peptide from silver carp skin at 50, 100 and 200 mg/kg body weight increased moisture contents of the skin of mice subjected to UV-induced photoaging. It also significantly increased the skin components and improved the antioxidative enzyme activities in both serum and skin of the animals. In addition, they found that low molecular peptides were more effective than high molecular weight collagen peptides. In contrast, ingestion of gelatin (>120 kDa) from silver carp did not lead to any significant change compared to control mice. Later Liu et al. (2019) showed that collagen peptides form silver carp skin promotes the photoaging skin cell repair by activating the TGF-β/Smad pathway to promote procollagen synthesis and suppressing AP-1, MMP-1 and MMP-3 protein expression to prevent collagen degradation. Similarly, oral ingestion of collagen hydrolysate from Nile tilapia scale increased the collagen content and antioxidant enzyme activities and

improved the appearance and structure of skin after 6 months in mice (Wang et al. 2017).

A clinical study on 64 individuals for 12 weeks evaluated the effect of collagen peptides from catfish skin on human skin hydration and elasticity, and wrinkling when it is orally consumed. This randomized controlled trial showed that daily intake (1000 mg/day) of low-molecular-weight collagen peptide from the fish skin significantly promoted hydration, elasticity, and wrinkling in human skin (Kim et al. 2018). It has been also shown that gelatin hydrolysate from fish skin resulted in significantly higher content of hydroxyproline-containing peptides in human blood compared with gelatin hydrolysate from porcine in 5 h after ingestion (Ohara et al. 2007; Ichikawa et al. 2010). This means collagen source can affect quantity and structure of hydroxyproline-containing peptides in human blood after their oral administration which would govern their health benefit. This may suggest marine collagens as a more promising source for functional food development. However, further clinical studies are needed to fully support this.

Bone related disorders such as osteoporosis and osteoarthritis are also considered as a common disease in the global aging population (Daneault et al. 2017). Marine collagen peptides have also shown a positive effect in treatment of osteoporosis, joint disorders, and osteoarthritis (Aleman and Martinez-Alvarez 2013). For example, collagen hydrolysate from silver carp skin improved mineral density, increase bone hydroxyproline content, enhance alkaline phosphatase level and reduce tartrate-resistant acid phosphatase 5b (TRAP-5b) activity in serum of chronologically aged mice (Zhang et al. 2018). Also, a significant reduction of bone loss was observed in mice supplemented with collagen hydrolysate from fish compared to a control protein suggesting benefits of hydrolyzed collagen for osteoporosis prevention go beyond the effect of simple protein supplementation (Wauquier et al. 2019).

Altogether, bioactive peptides from marine resources have shown a wide range of bioactive properties which have made them a promising source for the development of functional foods as a route to benefit from these biologically active ingredients in human health promotion. However, further studies on the efficacy of marine bioactive peptides when added to food products is needed.

6.4 Marine Amino Acids

Seafood products such as fish, crustaceans, and mollusks are very good sources of essential amino acids (EAA) and contain proteins with a very high biological value. Proteins from marine animals are a rich source of methionine (5.9 to 6.4% of total EAA) and lysine (18.2–19.6% of total EEA) (Tacon and Metian 2013). This makes marine products a good substitute for these amino acids which are normally considered as limiting amino acids in plant-based proteins. Marine plants especially brown seaweeds are also a reach source of aspartic acid and glutamic acid. Other abundant amino acids in edible seaweeds e.g. *Palmaria palmata* and *Enteromorpha* include

histidine, leucine, isoleucine, methionine, and valine (Pal and Suresh 2016). Also, content of valine, threonine, isoleucine, leucine, methionine, and phenylalanine in *Sacharine latissima* and proteins from this brown seaweed met the WHO/FAO's adult and infant recommended dietary intake level set by WHO/FAO/UNU (Abdollahi et al. 2019).

Marine foods are also considered as an important source of taurine which is a biologically active amino acid. Taurine is naturally occurring Sulphur-containing amino acid (2-aminoethanesulphonic acid) in the human body which does not include in protein sequence or structure, but it plays very important biological role in our body. A wide range of biological actions including beneficial effect on cardiovascular health, protection against ischemia-reperfusion injury, modulation of intracellular calcium concentration, and antioxidant, antiatherogenic and blood pressure-lowering effects have been reported for taurine (Xu et al. 2008). It can be partially synthesized in the body, but diet is the main source of taurine in healthy people. Seafoods especially mollusks are rich source of taurine and a large part of seafood health benefits has been associated with their high levels of taurine. For example, Dragnes et al. (2009) reported a range of 57 mg/100 g in haddock to 510 mg/100 g in blue mussel when studying different seafood including cod fillet, salmon fillet, saith fillet, haddock fillet, cod roe, peeled shrimp and deshelled mussel. Among the studied fish fillets, saithe had the highest content with 162 mg/100 g. They also found substantially higher content of taurine in cod roe, shrimps and blue mussel than all the studied fish fillets. A level of 70 and 240 mg/100 g wet weight has been also reported for oyster and clam (Lourenço and Camilo 2002; Harnedy and FitzGerald 2012). However, taurine content of seafood products can be strongly affected by processing conditions, cooking, and storage. Since taurine is a water-soluble compound, products subjected to soaking, brining or washing experience a great loss of taurine compared to freshly caught products (Dragnes et al. 2009).

6.5 Marine Oils and Fatty Acids

Marine food products are considered as the major food source of long-chain omega-3 fatty acids especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). A great scientific and public interest has been created toward consumption of marine omega-3 polyunsaturated fatty acids since studies found a significantly lower incidence of cardiovascular disease (CVD) in Greenlandic Inuit or Eskimos having a great number of seafoods in their diet compared to Western populations (Bang et al. 1986; Rangel-Huerta and Gil 2018). Long-chain omega-3 fatty acids including EPA and DHA are very insufficiently produced from their plant origin precursor alpha-linolenic acid in human body (Keefe et al. 2019). Thus, they must be necessarily provided by our diet and/or supplementation with marine fatty acids. Marine foods are in general considered as a rich source of long-chain omega-3 fatty acids but there is large variation in the content of these fatty acids among different types of seafood.

6.5.1 Marine Sources of Omega-3 Fatty Acids

The muscle of fatty fish such as salmon, trout, herring, mackerel, sardine, anchovy, albacore and tuna contains high amounts of EPA and DHA. For example, 100 g of cooked salmon and herring or 200 g of sardine can provide 2 g of EPA +DHA. This will cover the recommendations for daily intake of omega-3 fatty acids (0.25–2 g) by World Health Organization (Itsiopoulos et al. 2018). Demersal fish such as cod and halibut store oil mainly in their liver thus have low content of EPA and DHA in their muscle.

Fish oil is also a very important source of long-chain omega-3 and is the richest available source of EPA and DHA. Global production of fish oil is around 0.8–1 million tons which are mainly produced from whole pelagic fish including anchoveta, sardine, capelin, blue whiting, menhaden, and herring especially in southwest America (Auchterlonie 2018). Also, almost a quarter of global fish oil is produced from fish processing by-products which its share is increasing as a more sustainable alternative. More than 75% of global fish oil production is used for animal feeding, especially in aquaculture. Around 21% of its global production is directly used for human consumption as omega-3 capsules, infant formulas and pharmaceuticals and functional food supplements which are expected to have major growth in demand for fish oil (Seafish 2018). Although the major part of fish oil is used for feed, still marine oils are one of the most popular supplements in the world. For example, marine origin omega-3 products are used by 6.5% of the population in the USA which represents 37% of supplement users in the country (Albert et al. 2016).

Other emerging marine sources of omega-3 fatty acids are krill and algae and copepods oil as shown in Fig. 6.1. Krill oil contains high levels of phospholipids and represents a good source of EPA and DHA up to 12–50 g of long-chain omega-3

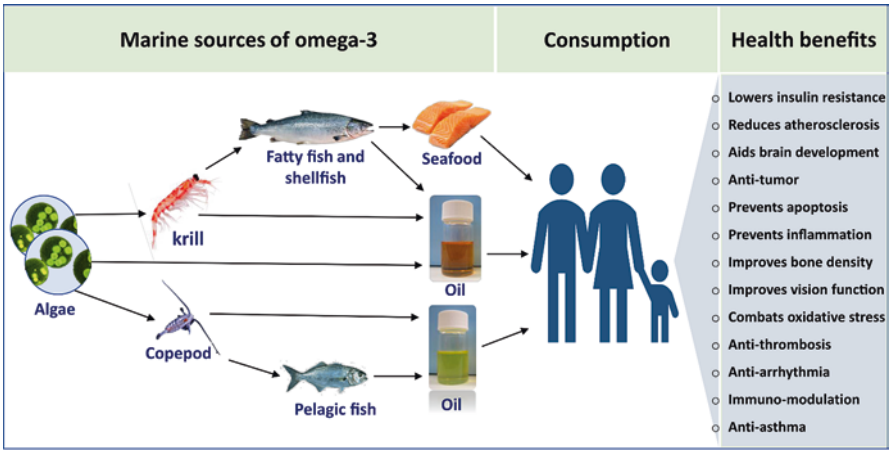

Fig. 6.1 Marine sources of omega-3 fatty acids can be directly consumed as seafood products or used for the production of fish oil and omega-3 concentrates

fatty acids per 100 g oil depending on species (Adarme-Vega et al. 2014). Krill oil is mainly produced by harvesting a krill species known as *Euphasia superba* and compared to fish oil stores 30–65% of long-chain omega-3 fatty acids as phospholipids while it is mainly stored as triglycerides in fish oil (Burri and Johnsen 2015). Several studies have shown that since cell membrane is made of phospholipids, this similarity may increase physiological fatty acid absorption of krill oil compared to fish oil (Andraka et al. 2019). Some review papers have recently gathered researches on bioavailability and health benefits of krill oil (Burri and Johnsen 2015; Andraka et al. 2019). Recently a Norwegian company called Calanus has started marketing oil extracted from a small copepod called *Calanus finmarchicus* as a new source of marine long-chain omega-3 fatty acids. Omega-3 fatty acids are mainly stored as wax esters in this copepod and are sold as the only commercially available marine source of wax esters.

Marine microalgae are another emerging source of marine omega-3 fatty acids which is considered as a vegetarian and sustainable marine alternative. Microalgae are primary producers of long-chain omega-3 fatty acids which are later accumulated in other marine organisms including krill and fish. They can have oil content of 10-50% of their body weight which can store omega-3 as 30–70% of their fatty acids (Martins et al. 2013).

6.5.2 Health Benefits of Marine Omega-3 Fatty Acids

Marine omega-3 polyunsaturated fatty acids are among the most studied and documented food bioactives with health benefits during the last four decades and some of their health benefits are summarized in Fig. 6.1. Beneficial health effects of marine omega-3 polyunsaturated fatty acids on CVD by preventing sudden cardiac death, congestive heart failure, and ischemic stroke have been reported in many clinical studies and reviewed by Bowen et al. (2016) and Elagizi et al. (2018). Recently 3 large randomized control trials on the potential benefits of marine omega-3 fatty acids on the occurrence of CVD have been conducted (Keefe et al. 2019). First study was done on 8179 patients suffering from coronary heart disease and showed that daily intake of highly purified omega-3 product (4 g/day) containing EPA reduced the risk for major adverse CVD by 25% (Bhatt et al. 2019). The two other large trials were conducted in primary prevention populations (Bowman et al. 2018; Bhatt et al. 2019). They also indicated that daily intake of purified fish oil (1 g/day) providing 840 mg/day of EPA and DHA significantly diminished risks of death due to coronary heart disease. It was especially effective in those who did not consume fish and seafood frequently (Bowman et al. 2018; Bhatt et al. 2019). The authors concluded that high doses of marine omega-3 fatty acids should be consumed for patients with coronary heart disease on statins having elevated triglycerides and in primary prevention for people who do not consume at least 1.5 meals of seafood/week (Keefe et al. 2019).

Omega-3 fatty acids especially DHA are primary structural fatty acids in the brain membrane phospholipids thus their beneficial neuroprotective effects against dementia have been also reported (Karr et al. 2011). A large number of studies have also evaluated the effects of omega-3 fatty acids on cognitive decline or Alzheimer's disease (Sinn et al. 2010). Long-chain omega-3 fatty acids have a vital role for normal development of brain and their levels decrease in the brains of people with Alzheimer's disease (Karr et al. 2011; Shahidi et al. 2018). Studies with biological and animal models have shown that omega-3 fatty acids can improve blood flow, reduce inflammation and/or amyloid-β pathology which giving them ability of primary prevention of cognitive decline (Fotuhi et al. 2009; Jicha and Markesbery 2010). This is in line with observational studies on human which also suggests consumption of omega 3 fatty acids can reduce cognitive decline with aging (Canhada et al. 2018). However, Fotuhi et al. (2009) concluded in their review that the existing data may support the role of these fatty acids in slowing cognitive decline in elderly people without dementia, but not for the prevention or treatment of dementia, including Alzheimer disease.

Other important health benefits reported for marine long-chain omega-3 fatty acids includes preventing or slowing the progression of age-related macular degeneration (Ghasemi Fard et al. 2019; Punia et al. 2019), anticancer properties (Manson et al. 2019). It has been also reported that they can reduce oxidative stress (Heshmati et al. 2019), and have immuno-modulatory activity. This makes them a prominent supplement recommended for prevention or treatment of inflammatory disorders e.g. rheumatoid arthritis (RA), Crohn's disease, ulcerative colitis, psoriasis, asthma, lupus and cystic fibrosis (CF) (Ruxton et al. 2004).

6.6 Marine Sterols

Sterols are a group of lipids that are also found in marine organisms with different biological roles as hormones and signaling molecules (Pal and Suresh 2016). They are also a structural component of cell membrane providing membrane fluidity and permeability. Sterols have been isolated from different marine sources such as diatoms (Belt et al. 2018) and sponges (Heidary Jamebozorgi et al. 2019) but marine algae are considered among the most important marine sources of bioactive sterols (Abdul et al. 2016). The main type of sterol found in brown algae is fecosterol while red algae contain mainly cholesterol and green algae (Chlorophyceae) contain mainly Taergosterol and 24-ethylcholesterol (Sánchez-Machado et al. 2004). A wide range of biological activities have been also reported for sterols from marine organisms including antioxidant, antidiabetic, anti-inflammatory and anti-HIV properties, anticancer activity, hepatoprotective, antiobesity, anti-osteoarthritic and anti-osteoporotic effects as well as anti-hyperlipidemic and anti-arteriosclerosis effects (De Jesus Raposo et al. 2013; Abdul et al. 2016).

6.7 Marine Polysaccharides

Marine animals that are used as muscle food contains normally low contents of polysaccharides but shells of crustaceans such as shrimp and crab as well as squid pen are a rich source of chitin which is one of the most important marine polysaccharides (Fig. 6.2). Chitin or poly (β-(1-4)-N-acetyl-D-glucosamine) is the second most abundant polysaccharide on the earth which is industrially produced from marine shell waste stream (Ngo et al. 2015). However, chitin has poor solubility due to its crystalline structure which limits its application. Thus, chitin is converted to chitosan which is generated by deacetylation of chitin through enzymatic or chemical processes. Chitosan is soluble in weakly acidic solutions and has antioxidant and antimicrobial properties. It is widely used for biomedical applications such as drug delivery, wound healing, tissue regeneration, as well as food protection, agriculture, textile, cosmetics, paper making and wastewater treatment (Muxika et al. 2017). Also a recent systematic review of randomized controlled trials by Huang et al. (2019) concluded that chitosan consumption might be a useful adjunctive pharmacological therapeutic tool for bodyweight management, particularly in overweight/obese participants.

Another bioactive polysaccharide extracted from marine animals is chondroitin sulfate which is a sulfated glycosaminoglycan. Cartilage of some marine animals such as shark and ray for many years have been considered a good source of this polysaccharide. More recently other marine sources such as sea cucumber (Myron et al. 2014), fish (Vázquez et al. 2016) and shrimp by-products (Palhares et al. 2019) have been introduced as alternative marine sources for extraction of chondroitin sulfate. It is an essential component of the extracellular matrix of connective tissues. This glycosaminoglycan has various biological and vital roles in human body. This ranges from help in function and elasticity of the articular cartilage and hemostasis

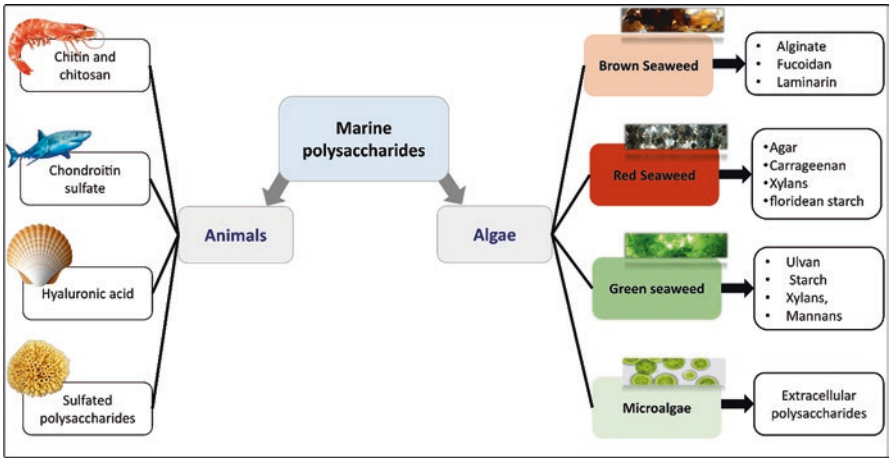

Fig. 6.2 Marine polysaccharides and their potential animal and algae sources

up to regulation of cell development, cell adhesion, proliferation and differentiation (Vázquez et al. 2013). A wide range of commercial products of chondroitin sulfate is marketed as nutraceuticals with cartilage regeneration, anti-inflammatory activity and osteoarthritis properties (Volpi 2009). The products mainly contain low/medium-molecular weight chondroitin sulfate (inferior to 20 kDa) and are orally consumed to treat and prevent osteoarthritis (Michel et al. 2005; Vázquez et al. 2013).

Hyaluronic acid, also called hyaluronan, is another polysaccharide or more exactly a mucopolysaccharide which is naturally found in organisms (Vázquez et al. 2013). It has a huge number of medical applications e.g. ophthalmic surgery, orthopedic surgery and rheumatology, drug delivery systems, pulmonary pathology, joint pathologies, and tissue engineering (Giji and Arumugam 2014). It has been traditionally extracted from terrestrial sources, but more sustainable sources especially marine organisms have recently attracted great attention. It has been isolated from some marine animals such as bivalve mollusk *Amussium pleuronectus* (Kanchana et al. 2013), fish eyeball (Amagai et al. 2009; Murado et al. 2012), liver of marine stingray *Aetobatus narinari* (Sadhasivam et al. 2013). Sulfated polysaccharides have been also isolated from some marine animals such as sponges (Jridi et al. 2018), clam (Souissi et al. 2019) and tuna processing by-products (Jridi et al. 2018).

Algae, especially seaweeds, are the most important sources of marine bioactive polysaccharides. Brown seaweed is a source of alginate, fucoidans, and laminarin (Fig. 6.2) (Fedorov et al. 2013). Fucoidans are a group of sulfated polysaccharides that have structural role in cell wall of brown seaweeds and are one of the most studied marine polysaccharides during the last decade (Sanjeewa et al. 2017). Fucoidans have shown a wide range bioactive properties including antiviral, anticoagulant, antitumor, anti-inflammation, anti-allergy, antiobesity and antioxidant properties (Vo and Kim 2013). Laminarin is also a polysaccharide with a small molecular weight (~5 kDa) found in brown seaweeds which has shown different bioactive properties such as anticancer, anti-inflammatory, anticoagulant, and antioxidant effects (Kadam et al. 2015). Both fucoidans and laminarins are considered as interesting marine bioactive compounds for application in functional foods.

Red seaweeds are the source of sulfated galactan (agars and carrageenans), xylans, and floridean starch (Pal and Suresh 2016). Carrageenans are also a group of sulfated polysaccharides with great interest in food industry due to their excellent physical properties, such as thickening, gelling, and stabilizing abilities (Jiao et al. 2011). At low molecular weight they have also shown different bioactive properties e.g. as promising anticancer and antitumor activities possibly due to their antiviral and antioxidant properties, and stimulation of antitumor immunity (Raman and Doble 2015).

Green algae contain ulvan, starch, xylans, mannans, and ionic polysaccharides which contain sulfate groups. Uronic acids, rhamnose, xylose, galactose, and arabinose are also found in this type of algae (Pal and Suresh 2016). Ulvan is a water-soluble sulfated polysaccharide found in green seaweed of the order *Ulvales* and it has the gel-forming capacity and several bioactive properties and health benefits which have been reviewed in may papers (Kim and Li 2011; Ngo and Kim 2013).

6.8 Oligosaccharides

Sugar molecules consisting of 2–10 monosaccharide units are called compound sugar or oligosaccharides. Many functions have been reported for oligosaccharides extracted from marine resources including immunostimulant, antioxidant, anticarcinogenic and antitumor effects (Mussatto and Mancilha 2007). Some of the oligosaccharides may be used as prebiotics to promote probiotic bacterial growth. Examples include xylooligosaccharides and fructooligosaccharides which cannot be digested in the gastrointestinal tract and act as prebiotics. Some of the most important marine oligosaccharides are chitin, carrageenan, agar, and alginate oligosaccharides which are produced by chemical or enzymatic hydrolysis of their primary polysaccharides. Food applications of marine oligosaccharides have been reported as low-sweetness humectants and bulking agents. They are also used as stabilizers in cosmetic industry (Lordan et al. 2011).

6.9 Phenolic compounds

Macro and microalgae contain-antioxidant compounds called polyphenolic compounds. Phenolic acids, hydroxycinnamic acids, simple phenols, coumarins, xanthones, naphthoquinones, flavonoids, stilbenes, anthraquinones and lignins are 10 classes of polyphenolic compounds that can be recovered or isolated from marine organisms (Ibañez et al. 2012). For instance, extract of marine brown algae such as *Eisenia bicyclis, Ecklonia kurome, H. fusiformis, and Ecklonia cava* polyphenolic is called phlorotannins. This bioactive compound imparts many functions including antioxidant, antibacterial, chemo-preventive, UV-protective, and antiproliferative effects… Eckol, phlorofucofuroeckol A, dieckol, and 8,8-bieckol which are few examples of phlorotannins have been effective against phospholipid peroxidation Shibata et al. (2007) experimented these phlorotannins and found out that they resemble ascorbic acid and tocopherol in terms of antioxidant activity.

6.10 Photosynthetic Pigments

These are pigments that are able to absorb solar energy for photosynthesis. Mainly, carotenoids and chlorophyll in macroalgae are the photosynthetic pigments. Carotenoids act as antioxidants, and provitamin A. They have anticancer, and cardioprotective effects. They are also effective against macular degeneration. β-carotene and astaxanthin are generated by microalgae and have been employed in food industry. Examples of these microalgae include *Dunaliella salina*, *Haematoccous pluvialis*, *Nanochloropsis oculat*, *Chlorerlla sorokiniana* (Pizarro and Stange 2009).

Dunaliella salina is used for mass production of the β-carotene and it can produce β-carotene up to 14% of its dry weight (Miyashita 2009). Cultivation of the *Dunaliella salina* is easier than the other plants and produces both cis and trans isomers of carotene with high bioavailability. In addition, under irradiance stress, *Dunaliella salina* accumulates a large amount of zeaxanthin which contributes to disease preventions (Yeum and Russell 2002).

Haematoccous pluvialis, is cultivated in both open and closed culture systems and produces chlorophylls and carotenoids. *Haematoccous pluvialis*, is able to produce astaxanthin as 1.5–3% of its dry weight under stress conditions. Several European countries and USFDA approved *Haematoccous pluvialis*, as a dietary supplement for human consumption. Astaxanthin has 10 times stronger activity than carotenoids which promotes anticancer, anti-inflammatory effects. That is why astaxanthin has been utilized by nutraceutical, cosmetics and food and feed industry (Rasmussen and Morrissey 2007).

Some of the reported bioactivities of the β-carotene include free-radical scavenging which alleviates the issues with coronary heart disease, cancer, premature aging, and arthritis. Carotenoid extract of *Chlorella ellipsoidea* exerted strong antiproliferative effect on human colon cancer cells, including induction of apoptosis (Klassen 2010).

Chlorophylls which are mainly produced by all classes of algae and cyanobacteria have been used as a coloring agent in food and drinks. They also impart anticancer effects. Marquez and Sinnecker (2007) found that dietary chlorophyll exhibits antimutagenic effects and reduces tumor cell growth. Diet high in chlorophyll may also reduce the risk of colon cancer.

Astaxanthin is a type of carotenoid which is found in yeast, salmon, trout, krill, shrimp, and crayfish. Astaxanthin supplementation of obese mice diet showed a decrease in body weight, skeletal muscle and adipose tissue (Yuan et al. 2011). Studies also have shown that insulin resistance could be alleviated using astaxanthin. This could be related to activation of post-receptor insulin signaling (Arunkumar et al. 2012). It appears that the greatest amount of astaxanthin can be found in *Haematococcus pluvialis* which is a chlorophyte algae. Astaxanthin has been effective to reduce cardiovascular risk markers of oxidative stress and inflammation according to clinical studies. It has been also effective for improving blood status (Riccioni et al. 2011; Yuan et al. 2011).

Chlorophylls extracted from brown algae have antioxidant activities in methyl linolenate systems. Normally chlorophyll b shows stronger antioxidant effect than chlorophyll a due to the presence of an aldehyde group in chlorophyll group b. However, the mechanism of action is unknown (Lanfer-Marquez et al. 2005).

Neither carotenoids nor chlorophyll can be synthesized by animal tissues. Thus, these molecules must be obtained from food, particularly seafood organisms are the major sources of these compounds.

Phycobiliproteins are a class of pigments (composed by a protein and chromophore called phycobilin) in marine red algae such as *Porphyridium cruentum* and cyanobacteria which are used as fluorescent markers when linked to antibodies, A-protein, biotin, lectins, and hormones (Aneiros and Garateix 2004). Phycocyanin

and phycoerythrin are two of the most known phycobiliproteins. They act in the immune system and anti-inflammatory agents. Phycocyanin is also used in perfumes and eye makeup powders as well as food colorants due to its stability (Kadam and Prabhasankar 2010).

Fucoxanthin extracted from *Hijikia fusiformis* is also one of the main antioxidant molecules with free radical scavenging activity. This activity might be due to double allenic bonds at the C-70 position (Sachindra et al. 2007). Fucoxanthinol has been extracted from *Undaria pinnatifida*. *Undaria pinnatifida* also contains another metabolite called halocynthiaxanthin. Both metabolites have antioxidant activity. Studies have shown that fucoxanthin has higher antioxidant activity than fucoxanthinol and halocynthiaxanthin due to the presence of an allenic bond.

6.11 Vitamins

B vitamins particularly vitamins B_1, B_2 and B_{12} are found in large quantities in seaweeds. According to Kim and Taylor (2011), two-third of the human requirement of vitamin C and adequate amount of vitamins A, B_2 and B_{12} can be obtained through consumption of 100 g of seaweed. Vitamin B_{12} is mainly found in some of the red macroalgae such as *Palmaria longat* and *Porphyra tenera* and green seaweeds. However, the highest concentration of vitamin B_{12} is 0.768 mg/kg for *Porphyra*. Vitamin B_{12} is also found in microalgae (*Spirulina platensis*) at 7 mg/kg. Vitamin B_{12} is a co-factor enzyme and cobalt-containing tetrapyrrole related to chlorophyll and heme. Megaloblastic anemia, chronic fatigue syndrome, and neuropsychiatric disorders are few serious conditions due to vitamin B_{12} deficiency. Red and brown algae are the excellent sources of folic acid and folate derivatives.. For instance, 100 g of dry *Undaria pinnatifida* provides 150 μg folic acid (Misurcova 2011). *Dunaliella salina* is a halophile green micro-algae which is a great source of β-carotene (provitamin A), as well as thiamine, pyridoxine, riboflavin, nicotinic acid, biotin and tocopherol (Drokova and Popova 1974).

Vitamin C or ascorbic acid acts as an antioxidant as well as immune system support. This vitamin is found in *Spirulina platensis* at high concentration (80 mg/kg). It is also found in *Porphyra umbilicalis* which traditionally consumed to prevent scurvy (Karleskint et al. 2012). While *Undaria pinnatifida* and *Laminaria digitate* are significant sources of vitamin E and C, diatom *Haslea (Navicula) ostrearia* is particularly rich in vitamin E. *P. cruentum* is another microalga rich in vitamins C, E (tocopherols) (Lordan et al. 2011).

The best sources of vitamin D are fatty fish. *Nannochloropsis oculate* is one of the algae that contain vitamin D as well. Rickets in infants and children and osteomalacia in adults are among the diseases due to vitamin D deficiency (Luten 2009).

Vitamin E is a mixture of tocopherols including α-, β-, and γ-tocopherols. Red, green and brown seaweeds are the main sources of α-tocopherol. β- and γ-tocopherols are mainly found in Phaeophycean. Vitamin E is useful in cardiovascular disease prevention and it has antioxidant activities. type of seaweed processing as well as

seasonal, environmental and physiological changes all may influence the vitamin E content. For instance, α-tocopherol in dehydrated *Himanthalia longate* and canned *Himanthalia longate* was 33.3 and 12 μg/dry weight, respectively (Ravishankar et al. 2005).

6.12 Minerals

Macroalgae are great sources of minerals. Geography, season and environmental condition of the harvested seafood all affect the mineral contents of the macroalgae. *U. pinnatifida*, sargassum and *Chondrus crispus*, *Gracilariopsis* can be considered as a dietary supplement to the daily intake of minerals such as Na, K, Ca and Mg, as well as trace minerals like Fe, Zn, Mn and Cu (Taboada et al. 2010).

Osteoporosis and hypocalcia are two of the conditions caused by Ca deficiency in the diet. Ca is also needed during lactation and pregnancy. The high amount of Ca is found in seaweeds. Fishbone which is considered a fish processing by-product is also a good source of Ca. Almost 30% of the fishbone is collagen however, 60–70% of the fishbone is composed of Ca, phosphate and hydroxyapatite. Fishbone can be incorporated into food products. However, they should become soft enough to be edible. In order to make them edible, different techniques and methods such as hot water treatment and acetic acid solutions are used (Nguyen et al. 2011).

Hydroxyapatite is another compound from fishbone which can be used for rapid bone repair after major trauma or surgery because it is stable at physiological pH and functions actively in bone bonding.

The most promising characteristic of seaweed is high I content which is an important factor in growth patterns and metabolic regulations. Kelp is one of the seaweeds which contains high amount of I. Production of thyroid hormones such as thyroxine and triiodothyronine depend upon I in the diet. Stillbirth, abortion, cretinism, goiter and mental disorders are few ailments due to lack of enough I in the diet (MacArtain et al. 2007).

Some of the minerals in seafoods are more abundant than land animals or plants. For instance, *Palmaria palmata* is a seaweed and an excellent source of iron which contains 8 g/serving of dry algae. This amount of iron is even higher what is found in 100 g of raw sirloin steak. However, high content of arsenic in some seaweeds is a place of concern for their direct consumption as food (MacArtain et al. 2007).

6.13 Bioactive Compounds Derived from Marine Bacteria

Several biologically important bioactive compounds can be extracted from bacteria that live in marine environment. Most of these bacteria live under harsh conditions including high pressure, cold and dark situations. However, regardless of these conditions, they produce valuable bioactive compounds that are necessary to study.

6.13.1 Antibacterial Effects

Marinispora (strain NPS008920) is a marine actinomycete that has been isolated from Cocos Lagoon, Guam. This strain was found in the sediment samples collected from this area. The compositional analysis of this strain revealed a series of novel 2-alkylidene-5-alkyl-4-oxazolidinones, lipoxazolidinone A, B, and C. These compounds have shown potent antibacterial activities similar to linezolid (Zyvox) which is a commercial antibiotic. Minimum inhibitory concentration (MIC) tests showing that this antibiotic has potent antibacterial activity with 1.56–15.57 mM against gram-positive bacteria and 37.38 mM against two strains of *Haemophilus influenzae (*Barbachyn and Ford 2003).

Marinispora is a marine actinomycete. A new strain of this genus called NPS12745 was found in the sediments off the coast of San Diego, California. Two important marine antibiotics i.e. chlorinated bisindole pyrroles, and lynamicins A-E were discovered in this strain. These two antibiotics have shown strong antibacterial activity against *S. aureus* (MSSA, MRSA: methicillin-resistant), *Staphylococcus epidermidis* and *Enterococcus faecalis*. Therefore, this strain has the potential to be used in combat against those infections that have been caught in a hospital and are potentially caused by organisms that are resistant to antibiotics (McArthur et al. 2008).

Pseudomonas stutzeri (CMG 1030) is one of the 100 species of bacteria that was found in the intestinal tract of fish collected from the Baluchistan coast in which borders the Gulf of Karachi, Pakistan, *Pseudomonas stutzeri* (CMG 1030) showed potent antibacterial effect against different types of pathogens including MRSA strains. zafrin (4b-methyl-5,6,7,8-tetrahydro-1(4b-H)-phenanthrenone) is an ethyl acetate extract of *Pseudomonas stutzeri* (CMG 1030) which was able to kill *Bacillus subtilis* faster than ampicillin, vancomycin or tetracycline. The mechanism of action for zafrin is similar to nisin and it does not disintegrate the bacterial cell wall

and Triton X-100, which disrupts the cell membrane. It was suggested that the mode of action of zafrin is via the disruption of the cytoplasmic extract collected from red alga *Laurenica spectabilis* in Ras-Gharib coast of the Red Sea, Egypt is active against pathogenic microorganisms with MIC of 0.1–10 mg ml^{-1}. This extract was effective against most of the Gram-positive and Gram-negative bacteria as well as against pathogenic fungi such as *Candida albicans, Aspergillus niger* and *Botrytis fabae* (Isnansetyo et al. 2003).

6.13.2 Anticancer Effects

Marine bioactive compounds have been also explored for anticancer effects. *Micromonospora marina* is a bacterium which was found in In 1997 in soft corals of Indian oceans. the mycelial extract of this bacterium contains a novel depsipeptide named thiocoraline Clinical studies revealed that Thiocoraline is able to inhibit

DNA polymerase-a. PharmaMar is a pharmaceutical company that currently studies this compound for commercialization (Romero, et al. 1997; Newman and Cragg 2004).

Marine fungus *Curvularia* sp. (strain no. 768) was found on a red alga called *Acanthophora spicifera*. The macrolide apralactone A, a 14-membered phenyl acetic acid macrolactone, as well as six further curvularin macrolides that were extracted from this fungus, have shown anticancer activity against 36 human tumor cell lines (Greve et al. 2008).

6.13.3 Antidiabetic Effects

Diabetes mellitus is a condition that the body does not produce enough insulin and as a result, the blood glucose level is high. The number of patients is increasing annually throughout the world (World Health Organization 1985). Aquastatin A is a compound that was isolated from a marine fungus *Cosmospora* sp. SF-5060 which was found at Gejae Island, Korea. Studies have shown that this compound has strong inhibitory effect against protein tyrosine phosphatase 1B (PTP1B).

Further analysis revealed that the EC50 value of this compound is 0.19 mM. PTP1B is able to regulate the insulin and leptin receptor-mediated signaling pathways. Therefore, it could be future solution to diabetes and its complications (Seo et al. 2009).

6.14 Extraction Techniques for Marine Bioactives

6.14.1 Super Critical Fluid Extraction (SFE)

This method was proposed by Hannay and Hogarth in 1879. SFE is a method that uses solvents at temperature and pressure above their critical points. The major advantage of this technique is minimum use of toxic organic solvents. The most commonly used solvent is carbon dioxide (CO_2) to extract natural resources such as marine bioactives. Although CO_2 is an environmentally friendly solvent which is considered as GRAS for use in food industry, however, low polarity of the CO_2 is one of the major drawbacks that should be solved by using cosolvents or polar modifiers to change the polarity of the CO_2 (Björklund et al. 2005). Methanol at 1–10% may be used to expand the CO_2 range of polarity. Propane, butane, and dimethyl ether have also been proposed to use to increase the polarity of the CO_2. However, none of these solvents fulfill the principles of Green Chemistry. As for marine bioactives extraction, CO_2 has the benefit of high diffusivity, and ease of tuning the temperature and pressures that have been applied. Also, utilization of CO_2 provides a solvent-free extraction method. CO_2 can be easily converted from liquid form to gas after completion of the extraction for ease of recovery (Ibañez et al. 2012).

6.14.1.1 Application of SFE to Macroalgae, Microalgae, and Cyanobacteria

As we discussed earlier, due to the low polarity of CO_2, this method is beneficial for compounds with low polarity. However, if CO_2 used at mild pressure and temperature conditions, it allows obtaining volatile compounds without affecting its properties. The volatile compounds produced by aquatic organisms play a critical role in chemical defense mechanisms and food gathering of the organisms. Microalgae share their ecological niche with bacteria and other microorganisms. As a result, microalgae secrete compounds with antibacterial, antifungal, and often antiprotozoal activities (El Hattab et al. 2007). For instance, the extract obtained from *Dunaliela salina* which is a green microalga using the SFE method with CO_2 at 314 bar and 9.8 °C showed strong antimicrobial activity against the pathogens *Escherichia coli*, *Staphylococcus aureus*, *Candida albicus*, and *Aspergillus niger*. This activity is probably due to the presence of indolic compounds, polyunsaturated fatty acids, and compounds related to the metabolism of carotenes such as β-ion-one and neophytadiene in microalgae extract (Mendiola et al. 2005).

Bioactive lipids such as essential fatty acids also are extracted using the SFE. For instance, *Spirulina platensis* was studied for this purpose. The maximum extraction yield was obtained at 350 bar and 40 °C and a flow rate of 24 kg/CO_2/h. Similarly, vitamin E extraction was studied in *Spirulina* and a tocopherol enrichment of more than 12 times the initial concentration of the tocopherol in raw material by extraction with neat CO_2 at 361 bar and 83.3 °C was achieved. Carotenoids were also extracted from *Chlorella vulgaris* and *Spirulina*. The addition of polar modifiers such as ethanol in the supercritical CO_2 allowed the extraction of more polar carotenoids but also chlorophylls, thus decreasing the selectivity of the extraction process. Other bioactive compounds such as diolefins have been extracted from *Botrycoccus braunii* using SFE. *Botrycoccus braunii* is able to store large number of hydrocarbons with long-chain (25–31 carbon atoms) which can be used as a substitute for paraffinic and natural waxes (Mendiola et al. 2005).

Phenolic compounds from marine resources have been also extracted using the SFE method. A hyphenated technique was used to isolate isoflavones from sea macroalgae. In this technique, samples are pretreated using sonication, followed by extraction using SFE with modified CO_2 and 3% of MeOH/H_2O mixture at 350 bar and 40 °C for 60 min (Klejdus et al. 2010).

6.14.1.2 Application to Invertebrates

Bioactive compounds from invertebrates such as crustacean including krill, crawfish, crab or shrimp as well as squid, urchin, and starfish have also been extracted using the supercritical CO_2 method (Félix-Valenzuela et al. 2001).

Astaxanthin, the pigment responsible for the orange-pink coloration of the crustacean is abundant in their shell waste. They are also able to modify some carotenoids such as β-carotene and transform them into astaxanthin. For the first time,

Yamaguchi and his colleagues in 1986 were able to apply SFE to crustacean waste. They extracted nonpolar lipids, mainly triglycerides and astaxanthin from krill using one-step extraction utilizing SC-CO_2 at 60 °C and 245 bar.

Sea urchin gonads and squid viscera are rich in PUFA which are normally discarded. However, these are nutritious from a human nutritional standpoint (Zhu et al. 2010). Palmitic, oleic, eicosapentaenoic acid and docosahexaenoic acid were extracted from squid viscera using SC-CO_2 with 1.5% ethanol and temperatures between 25 °C and 50 °C and pressure range from 80 to 170 bar (Chun et al. 2010).

6.14.2 Pressurized Liquid Extraction (PLE)

There are different names for pressurized liquid extraction including pressurized fluid extraction (PFE), enhanced solvent extraction (ESE), high-pressure solvent extraction (HPSE) or accelerated solvent extraction (ASE). The main advantage of this method is the simultaneous application of pressure and a liquid with a temperature higher than its boiling point. Therefore, it reduces the amount of solvent that is needed for extraction, so it is considered as a green extraction technique. It also allows for faster extraction of materials. (Turner and Ibañez 2011).

6.14.2.1 Applications to Macroalgae, Microalgae, and Cyanobacteria

Reduced extraction time and the possibility of automation are some reasons for popularity of the PLE method for recovery of bioactive compounds from marine resources. Carotenoids from *Dunaliella salina* were extracted using PLE and the results showed that the temperature is the main factor that influences the recovery. The best yield was with ethanol at 160 °C and 17.5 min (Breithaupt 2004).

Carotenoids such as fucoxanthin and other oxygenated carotenoids from brown macroalgae such as *Eisenia bicyclis*, *Cytoseira abies-marina*, and *Himanthalia elongate* have been isolated using pressurized liquid extraction. It has been reported that this technique could be used to extract bioactive compounds from cyanobacteria or algae as well (Shang et al. 2011).

6.14.3 Pressurized Hot Water Extraction (PHWE)

This method is also known as subcritical water extraction, pressurized low water (PLPW) extraction, or superheated water extraction (SHWE) is a particular use of PLE with water as extracting solvent. This method uses water at temperatures above the atmospheric boiling point. However, it keeps it in the liquid form by using the pressure. Water is the greenest solvent can be used (Teo et al. 2010).

6.14.3.1 Application to Macroalgae, Microalgae, and Cyanobacteria

PHWE at high temperatures may generate new antioxidant compounds. Plaza et al. (2010) used this technique to study the antioxidant properties of *Chlorella vulgaris* and *Sargassum vulgare.* The application of this technique at high temperatures may produce new compounds with antioxidant activities.

6.14.4 Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE)

In an ultrasound-assisted extraction system, acoustic cavitation is used disrupt the cell walls and reduce the particle size of the target compounds as well as enhancement of the contact between the solvent and the target compounds. However, in microwave-assisted extraction, the microwave radiation is used to induce movement of polar molecules and rotation of dipoles to heat solvents and to promote transfer of target compounds from the sample's matrix into solvent (Ying et al. 2011).

6.14.4.1 Application to Macroalgae, Microalgae, and Cyanobacteria

Mainly carotenoids were extracted using this technique from microalgal genus *Dunaliella*. The prosses performed on *Dunaliella tertiolecta* led to rapid pigment extraction mainly because of the absence of frustule in microalgae cells thus allowing immediate solvent penetration (Pasquet et al. 2011).

6.14.4.2 Application to Marine By-Products

The bioactive compounds from fish processing by-products have not been studied using MAE method. fatty acid profile composition of the lipids recovered from cod liver and mackerel fillet using this technique were studied by Batista et al. (2001). Mackerel fillet and cod liver contained lipid content of 5.6% ± 0.4% and 62.6% ± 3.1%, respectively. These results indicated that application of microwave-assisted extraction could be a replacement for the conventional method due to its efficiency.

6.14.5 Isoelectric Solubilization and Precipitation

Isoelectric solubilization and precipitation (ISP) is a method of recovery of proteins and lipids from seafood and seafood processing by-products. Generally, processing fish into fillets generates large quantitates of by-products including trimming, heads,

fish frames, skin and scale which are normally discarded. However, these by-products are valuable and nutritious resources of highly functional proteins and omega-3 fatty acids that if recovered properly cane be added to food products. Tahergorabi et al. (2015); Tahergorabi et al. (2012) and Tahergorabi et al. (2011) have applied this method to isolate the protein fish whole fish as a model for fish processing by-products as well as poultry products.

The ISP process is carried out in five steps. In the initial step, the fish or fish processing by-products are ground and homogenized with a ratio of 1:6 (w: w) of water. In the second step, the pH of the solution is adjusted to 11.50 ± 0.05 with 10N NaOH. In the third step, the homogenate is transferred to centrifuge tubes and centrifuged at 10,000 × g. This step separates the solution into three layers including the fat on the top, protein solution in the middle and the insoluble and impurities at the bottom. In the fourth step, the protein solution is transferred to a beaker and the pH is adjusted to isoelectric point (5.5 ± 0.05) with 10N HCl. In the last step, the solution is centrifuged, and the protein is recovered from the solution.

6.15 Conclusions

Extracts of marine organisms have demonstrated bioactive properties that impart health benefits. The bioactive compounds not only are extracted from the marine organisms but also are extracted from their processing by-products. Hence, they have attracted much attention from food, cosmetic and drug industries in the past few years. As a result, many methods have been designed to extract these valuable compounds from marine resources. Incorporation of these compounds in food may also offer functional food products that could target specific health issues. However, this may emerge the issue of overexploitation of the marine resources. Therefore, responsible and sustainable strategies must be devised to use these limited and valuable resources.

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Chapter 7
Food and Plant Bioactives for Reducing Cardiovascular Disease Risk

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Abstract Cardiovascular diseases (CVDs) are the leading cause of mortality and disability worldwide, taking an estimated 17.9 million lives each year. The economic burden for CVDs is estimated to have been 906 billion dollars in 2015 and is expected to rise to 22% by 2030. In this context, the scientific community is highlighting the need to support a concept of "preventive medicine", based first of all to the lifestyle change, and if necessary, the use of nutraceutical substances as well. The evidence-based prescription of these molecules seems a viable option, especially in people in primary prevention from chronic diseases and, in the specific, in patients with suboptimal values of blood pressure, cholesterolemia and triglyceridemia. Within the world of nutraceuticals, in the last years, a growing interest has been directed to food and plant bioactives, which may have a potential disease preventing and therapeutic use. In particular, bioactive peptides derived from both animal and plant derivatives demonstrated a significant anti-hypertensive and lipid-lowering effect in randomized clinical trials (RCTs). Furthermore, some polyphenols isolated from foods or plants, exert anti-inflammatory and anti-oxidant activity, which could strengthen the prevention of chronic diseases. Other bioactive compounds extracted from food or plant derivates and used to support cardiovascular risk patients include polyunsaturated fatty acids (PUFAs), lycopene, alliin, plant sterols, monacolin k and berberine. Nevertheless, although bioactive molecules showed their effectiveness in the studies conducted up to today, further long-term RCTs are necessary to confirm these effects to allow their preventive use.

Keywords Cardiovascular disease · Prevention · Nutraceuticals · Cholesterol · Blood pressure · Clinical evidence

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7.1 Introduction

Cardiovascular diseases (CVDs) are the leading cause of mortality and disability worldwide, reaching 31% of deaths and taking an estimated 17.9 million lives each year (World Health Organization 2015). The leading causes of premature death in Europe are atherosclerosis-related diseases, being responsible for 38% of deaths in men and 42% of deaths in women under 75 years old (Perk et al. 2012). The economic impact worldwide of CVDs is estimated to have been 906 billion (US) dollars in 2015 and will tend to increase by 22% by 2030 (Bloom et al. 2011).

Among the modifiable cardiovascular risk (CVR) factors, the most common in the general population with a prevalence about 25–45% in Western countries is represented by essential hypertension (≥140 mmHg the systolic blood pressure (SBP), and ≥90 mmHg the diastolic (DBP)). Despite the availability of adequate and well tolerated antihypertensive drugs, the significant prevalence of hypertension in the general population and especially in elderly individuals, it is responsible for the majority of CVDs in people at different CVR profile (Cicero and Colletti 2015). Other important CVR factors include elevated total cholesterol (TC) (>5 mmol/L) and low density lipoprotein cholesterol (LDL-C) (>3 mmol/L for patients at low and moderate risk for coronary heart disease (CHD), >2.6 mmol/L for patients at high risk and >1.8 mmol/L for patients at very high risk) while high concentrations of high density lipoprotein cholesterol (HDL-C) are considered protective in certain conditions (Mach et al. 2020). Therefore, both hypertension and LDL-C are considered the fundamental CVR factors and the main targets of both nutraceutical and drug therapies (Colantonio et al. 2016).

Several non-pharmacological and pharmacological interventions have been proposed for ameliorating the abovementioned CVR factors (Cicero et al. 2017a, 2019). In particular, the use of some nutrients and nutraceuticals has demonstrated to have favourable anti-hypertensive and lipid-lowering effects (Cicero and Colletti 2016; Cicero et al. 2018). Among these, plant and food bioactives represent a heterogeneous group of compounds potentially useful in the prevention of chronic diseases. An important class of bioactives include the **bioactive peptides** (BPs), a large number of peptides contained in a wide range of food sources of both animal and plant origin, and generated by enzymatic processes, chemical hydrolysis, fermentation or gastrointestinal digestion processes from food proteins (Aluko 2015). In the last years, an increased number of publications has highlighted regarding their potential effect on lipid metabolism, blood pressure, anticancer and immunomodulatory activities, but they seem to have antimicrobial, analgesic, antioxidant and anti-inflammatory effects, as well (Cicero et al. 2017b).

Another example of "bioactive molecules" includes the broad category of **polyphenols** (PPs). PPs are various secondary plant metabolites, structurally characterized by at least one aromatic ring linked to phenolic-, carbon-, hydroxyl- or other groups. They exist with different structures (Table 7.1) in fruits, vegetables, nuts, herbs, cocoa, tea and other plants and plant products. In particular, flavonoids, lignans, phenolic acids, and stilbenes represent the main four classes of PPs present in food (Fig. 7.1) (García-Villalba et al. 2010). Among flavonoids, the flavonols, flava-

Table 7.1 Polyphenol classes, compounds and content in common foods (adapted from Tangney et al. 2013)

Polyphenol class	Polyphenol subclass	Compounds	Dietary source (mg/serving)			
			<25	25–50	50–100	>100
Flavonoids	Flavonols	Kaempferol, Myricetin, Quercetin	Black tea, walnuts, black beans, dark chocolate, red wine, almonds	Spinach, plum	Capers	
	Flavanones	Naringenin, Hesperitin	Red wine		Grapefruit, orange	
	Flavan-3-ols	Catechin, Epigallocatechin, Gallate, Procyanidin	Grape juice, plum, white wine, almonds, blueberry	Red wine, cocoa	Dark chocolate	Black and green tea
	Flavones	Apigenin, Luteolin	Oregano			
	Anthocyanins	Anthocyanidin, Anthocyanin	Red wine	Black beans, plum	Blueberry	
	Isoflavones	Daidzein, Genistein		Tofu		
Lignans	Lignans	Lariciresinol, Secoisolariciresinol			Flaxseed	Sesame oil
Phenolic acids	Hydroxy-benzoic acid, Hydroxy-cinnamic acid	Egallic acid, Vanillic acid, Caffeic acid, Ferulic acid	Grape juice, red wine, rosemary, grapefruit, dark chocolate, white wine, cocoa, oregano, rolled oats,	Flaxseed, black tea, green olives, black beans	Plum	Coffee, walnut, blueberries
Stilbenes	Stilbenes	Resveratrol	Red wine			
Other polyphenols	Tyrosol, Curcuminoids	Hydroxytyrosol, Curcumin	Olive oil, coffee, red wine		Green olives, turmeric	

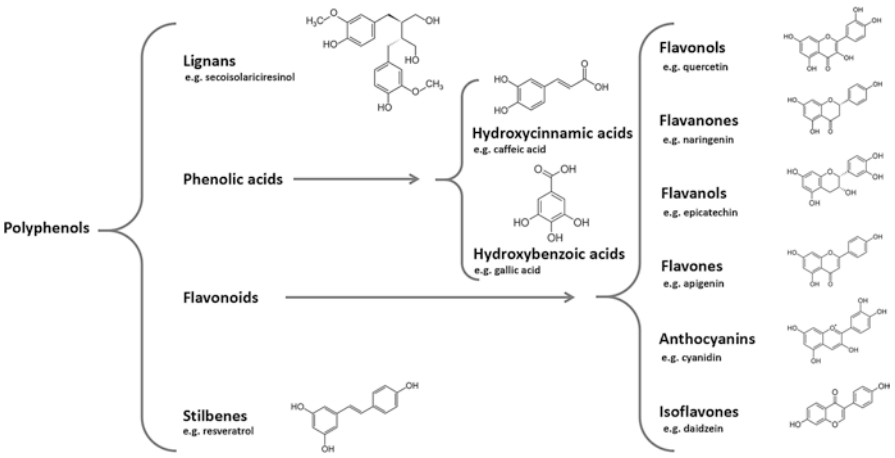

Fig. 7.1 The four classes of PPs and their chemical structures. PPs that are categorized as "other polyphenols" with particular chemical structures such as tyrosol and curcuminoids, are not included. Adapted from Spencer et al. (2008)

nols, flavones, flavanones, isoflavones, and anthocyanins are of great interest in clinical and pre-clinical researches, while among non-flavonoids class appertain the lignans, hydrolysable phenolic acids and stilbenes (Tomé and Visioli 2016). Literature data on PPs suggest that they could potentially exert an effect on lipid profile, blood pressure and insulin resistance, especially by reducing the oxidation of LDL-C and improving the endothelial function. PPs from green tea, grape, berries, cocoa, and soy are the most studied and the most effective ones in clinical practice (Cicero and Colletti 2018a).

Finally, bioactive compounds can also include molecules not typically classified as "polyphenols" or "peptides" but with multiple engaging activities in CV prevention. For example, molecules like berberine or monacolin k are well known to act as lipid-lowering agents, while alliin and pycnogenol have anti-hypertensive activity, representing a potential management option for people in primary prevention.

This chapter aims to analyse the role of bioactive substances derived from food and plants in prevention and treatment of CVDs, reporting the results of RCTs and meta-analyses associated.

7.2 Methods

A systematic search strategy was conducted to identify trials in both the Cochrane Register of Controlled Trials and MEDLINE (January 1970 to May 2020). The terms 'food bioactives', 'plant bioactives', 'cardiovascular diseases', 'hypertension' and 'dyslipidemia' were incorporated into an electronic search strategy. Then the selected references were screened for application on CVDs or CVR factors. All of

the citations included in the electronic strategy have been reviewed in order to identify potentially relevant articles for this chapter and the eligibility of the potential trials as well. Finally, the authors selected papers reporting recent comprehensive reviews or meta-analyses, or original studies *in vitro* and *in vivo* and clinical trials on BPs with an action on CVDs.

7.3 Blood Pressure Lowering Effect

According to the European guidelines for hypertension management (Unger et al. 2020), the nutraceutical approach might represent a good strategy for people with borderline values of blood pressure (BP) and an adjuvant in combination with anti-hypertensive drugs in patients with moderate hypertension (Borghi and Cicero 2016; Sirtori et al. 2012). In addition, the conventional treatment could be associated with some typical side effects, including loss of taste, hyperkaliemia, skin rashes, cough, sleep apnoea, angioedema and erectile dysfunction. In this regard, the anti-hypertensive nutraceuticals (Table 7.2) might be used to reduce conventional side effects, especially in subjects in primary prevention with pre- or mild-hypertension, also to improve the economic burden on health due to potential reduction of CVDs (Houston 2013).

7.3.1 Bioactive Peptides

Different types of **bioactive peptides** (BPs) derived from both animals and plant sources, with antihypertensive activity, have been investigated in the last years from a large number of research studies (Fig. 7.2) (Bhat et al. 2015; Hartmann and Meisel 2007). The anti-hypertensive action of some BPs seems to be related to the inhibition of angiotensin converting enzyme (ACE), responsible for conversion of angiotensin I in angiotensin II. However, BPs are able also to increase the activity of certain vasodilating agents including eNOS (endothelial-Nitric oxide synthases that increased production of endothelial NO), reduce the activity of the sympathetic system and inhibit the production and release of renin (Pripp 2008).

Two important factors can interfere with the anti-hypertensive efficacy of BPs: the degradation by gastro-intestinal peptidases and its poor absorption into the blood stream. In particular, BPs are probably absorbed by a saturable transporter peptide (PEPT1) as well as by the paracellular or transcellular route (Rotimi 2015). Concerning these two factors, the anti-hypertensive activity of BPs will be significantly different.

An important source of proteins is milk, that contains a good number of BPs including the tripeptides Valine-Proline-Proline (VPP), Isoleucine-Proline-Proline (IPP) and FFVAPFPEVFGK, YLGYLEQLLR peptides (Cicero et al. 2013). Several RCTs have underlined the effects of milk bioactives in CV prevention: in particular,

Table 7.2 Food and plant bioactives with possible blood pressure lowering effect

Plant/food bioactive	Active daily doses	Expected effects on BP	Direct vascular effects
Beetroot (inorganic nitrates)	321–2790 mg of nitrates equivalent to 70–500 mL of juice	−3.5 mmHg SBP −1.3 mmHg DBP	↑ FMD, ↓ PWV
Bioactive peptides (IPP, VPP)	5–60 mg	−2 mmHg SBP - 1 mmHg DBP	↑ FMD, ↓ PWV
Cocoa flavonoids	200 mg	−2 mmHg SBP	↑ FMD, ↓ PWV
Garlic (S-allylcysteine and derived polysulfides)	1200–2400 mg of aged garlic extract	−9 mmHg SBP −4 mmHg DBP	Not investigated
Karkadé	2–6 cups	−7.5 mmHg SBP −3.5 mmHg DBP	Not investigated
Non roasted green coffee (chlorogenic acid)	40 mg	−2.6 mmHg SBP −3.1 mmHg DBP	Not clear
Omega-3 PUFAs	3–4 g (EPA + DHA)	−4 mmHg SBP −3 mmHg DBP	↑ FMD, ↓ PWV
Lycopene	15–50 mg	−5 mmHg SBP	↑ FMD, ↓ PWV
Pomegranate (gallic acid, ellagic acid, punicalagin A and B and punicalin A and B)	240 ml	−5 mmHg SBP −2 mmHg DBP	Not investigated
Pycnogenol	100–200 mg	−4 mmHg SBP −3 mmHg DBP	↑ FMD, ↓ PWV
Resveratrol	>300 mg	−9 mmHg SBP −6 mmHg DBP	↑ FMD, ↓ PWV
Tea (flavan-3-ols)	2–6 cups	−2 mmHg SBP −1.2 mmHg DBP	↑ FMD, ↓ PWV

DHA docosahexaenoic acid, *EPA* eicosapentaenoic acid, *FMD* flow mediated dilation, *IPP* isoleucine–proline–proline, *PWV* pulse wave velocity, *VPP* valine–proline–proline

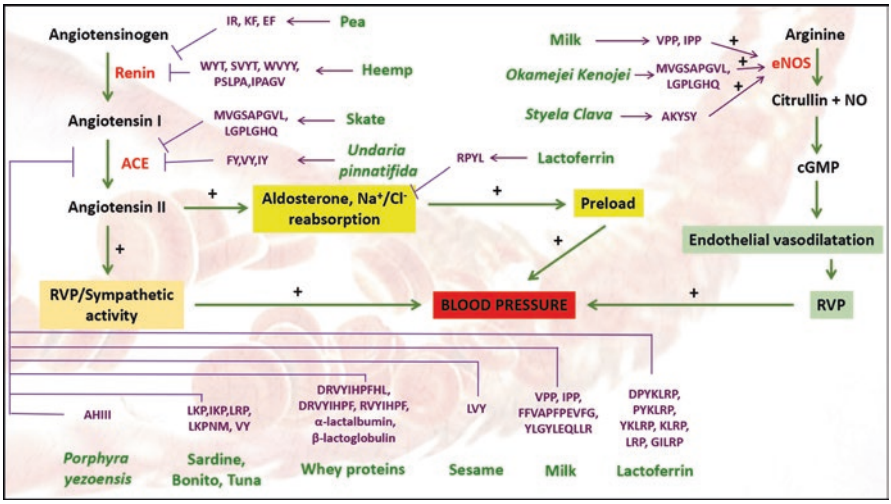

Fig. 7.2 BPs with evidence on the reduction of blood pressure: proposed mechanisms of action. *ACE* angiotensin converting enzyme, *cGMP* cyclic guanosin monophosphate, *EF* glutamate–phenylalanine, *eNOS* endothelial nitric oxide synthase, *FY* phenylalanine–tyrosine, *IKP* isoleucine–lysine–proline, *IPP* isoleucine–proline–proline, *IR* isoleucine-arginine, *IY* isoleucine-tyrosine, *KF* lysine–phenylalanine, *LKP* leucine–lysine–proline, *LRP* leucine–arginine–proline, *LVY* leucine–valine–tyrosine, *NO* nitric oxide, *RVP* renal venous pressure, *VPP* valine–proline–proline, *VY* valine–tyrosine, *WYT* tryptophan–tyrosine–threonine

the tripeptides VPP/IPP have shown a variable anti-hypertensive efficacy at dosages between 5 and 100 mg/day, although it has been more evident in Asian subjects. In a meta-analysis of 18 RCTs, BPs showed to reduce both Systolic Blood Pressure (SBP) (−3.73 mmHg, 95%CI: −6.70, −1.76) and Diastolic Blood Pressure (DBP) (−1.97 mmHg, 95%CI: −3.85, −0.64) (Cicero et al. 2011a). In addition, these peptides could modulate pulse wave velocity (PWV) in mildly hypertensive subjects, with an excellent safety profile (Cicero et al. 2011b, 2016).

A rich source of BPs are also whey proteins, which are converted in BPs through different treatments such as the enzymatic hydrolysis by trypsin, alcalase or pepsin. In particular the DRVYIHPFHL, DRVYIHPF, and RVYIHPF peptides, have shown anti-hypertensive activity with an inhibitory action on the renin angiotensin system (RAS) system, both in normotensive/pre-hypertensive and in obese subjects (Yadav et al. 2015; Nongonierma and FitzGerald 2015).

Several studies report also a potential anti-hypertensive action of BPs isolated from cow's milk. Studies on animals and humans have shown that lactorphins lower both SBP and DBP by normalizing endothelial function while α-lactalbumin and β-lactoglobulin, that are obtained from enzymatically hydrolysed whey, are able to reduce blood pressure inhibiting the ACE (Dong et al. 2013). Several marine peptides with anti-hypertensive activity have been detected in some fish such as tuna, bonito and sardine (LKP, IKP, LRP), but also in *Okamejei kenojei* (MVGSAPGVL, LGPLGHQ) and *Styela clava* (AHIII). The presence of these BPs has led to an

increase of endothelial NO levels and aorta vasodilation in rats even if data on humans are still lacking (Cheung et al. 2015).

Finally, the intake of plant proteins such as those derived from barley, soy, oak and pea proteins seems to be associated to mild but significant lower blood pressure levels (Altorf-van der Kuil et al. 2010; Malaguti et al. 2014). In particular, some extract peptides from cereals such as isoleucine–valine–tyrosine (from wheat germ), isoleucine–aspartate–proline (from hydrolysis of gliadin) or those oats and barley extracts showed strong ACE inhibitory action (Motoi and Kodama 2003; Nirupama et al. 2015). However, it is not easy to discriminate between the effect of plant proteins and other associated dietary components on blood pressure level. In this regard, isoflavones consumed with soy might be the real compound responsible for anti-hypertensive action of this functional food. In fact, a recent meta-analysis, including hypertensive patients, showed that soy isoflavones intake is associated to a decrease of both SBP (−5.9 mmHg, 95%CI: −10.5, −1.3, $p = 0.01$) and DBP (−3.3 mmHg, 95%CI: −6.5, −0.2, $p = 0.04$) (Liu et al. 2012).

7.3.2 Polyphenols

Polyphenols (PPs) are secondary plant metabolites naturally present in plants and plant products such as fruits, vegetables, nuts, herbs, cocoa, and tea. After ingestion, PPs undergo the first structural variations by the acid environment of the stomach with the exception of acid-resistant structures. In the small intestine, about 5–10% of PPs undergo the action of both glucosidase and hydrolase enzymes which facilitate their absorption in the blood.

The other 90% of PPs (in the conjugated form) is metabolized by gut microbiota that is responsible for the absorption of low molecular weight metabolites as simple phenols. Depending on the type of bacteria, PPs can undergo different enzymatic reactions of hydrolysis, dehydroxylation, demethylation, and decarboxylation.

Finally, PPs absorption is severely limited for a phase II metabolism both locally and in the liver. The excretion of PPs is mainly urinary (Zanotti et al. 2015).

One of the most important sources of polyphenols and in particular **flavan-3-ol** compounds are both **black tea** (BT) and **green tea** (GT). In a dose-response meta-analysis of 18 prospective cohort studies (including 11,306 and 55,528 deaths from CVDs and all causes), GT consumption was significantly inversely associated with CVDs (one cup/day increment, −5%) and all-cause mortality (one cup/day increment, −4%), whereas BT consumption was significantly inversely associated with all cancer and all-cause mortality. In particular, for CVDs mortality, the summary RR for the highest vs. lowest category of GT and BT consumption were 0.67 (95% CI 0.46, 0.96) and 0.88 (95%CI 0.77, 1.01), respectively. For all-cause mortality, the summary RR for the highest vs. lowest category of GT and BT consumption were 0.80 (95% CI 0.68, 0.93) and 0.90 (95% CI 0.83, 0.98), respectively (Tang et al. 2015). These data might be explained in part by the anti-hypertensive properties of both GT and BT

probably associated with the action of tea flavonoids on endothelial function and thus, the improvement of arterial compliance (Grassi et al. 2008a). In particular, the meta-analysis of Liu et al. showed a blood pressure (BP)-lowering effect of tea if consumed as 2–6 cups per day, for at least 4 weeks. Moreover, GT appears to have an antihypertensive action superior to that of BT (mean reduction of SBP after GT consumption: 2.1 mmHg, 95%CI –2.9 to −1.2; mean reduction of SBP after BT consumption: 1.4 mmHg, 95%CI –2.4 to −0.4; mean reduction of DBP after GT consumption: 1.7 mmHg, 95%CI –2.9 to −0.5; mean reduction of DBP after BT consumption: 1.1 mmHg, 95%CI –1.9 to −0.2, compared to baseline values) (Liu et al. 2014). The greater BP-lowering effect of GT compared to BT might be due to the higher content of phytochemicals that contribute to improve vascular function and reduce the numbers of reactive oxygen species (ROS) in the vascular system (Ihm et al. 2012). However, even BT consumption seems able to improve arterial compliance measured by byrachial artery flow mediated dilation (FMD) (Grassi et al. 2009).

Another type of tea (*Hibiscus sabdarifa* L., English: roselle, red sorrel) also known as **karkadé** is well known to contain high amounts of vitamin C and polyphenols including **flavonoids** (such as quercetin and luteolin), **organic** and **phenolic acids** (such as citric, hibiscus, or protocatechuic acids), and **anthocyanins** (such as cyanidin-3-o-sambubioside, cyanidin-3-o-glucoside, or delphinidin-3-o-sambubioside). The phytocomplex of polyphenols seems capable of exercising strong antioxidant activity and to inhibit the tone of smooth muscle (Sarr et al. 2009). A meta-analysis of 5 RCTs and 390 participants showed a significant effect of karkadé consumption in lowering both SBP (− 7.6 mmHg, 95% CI − 9.7 to −5.5, $p < 0.00001$) and DBP (−3.5 mmHg, 95% CI −5.2 to −1.9, $p < 0.0001$).

Several dietary flavonoids are able to exert positive effects on vascular stiffness, reducing ROS and inflammatory markers and improving NO metabolism (Habauzit and Morand 2012). In particular, **cocoa** powder, obtained by pulverizing the bean, contains PPs from 12 to 18% of dry weight depending on variety, growing region and processing operations of the bean (Fernández-Murga et al. 2011). Cocoa is rich in **flavanols**, and in particular **(−)-epicatechin** which represents about 35% of the total cocoa PPs. In addition, in this "functional food" there are also (+)-catechin, (+)-gallocatechin, (−)-epigallocatechin and (−)-epicatechin-3-o-gallate, even if in smaller quantities. Finally, cocoa contains dimeric or trimeric forms of flavanols such as procyanidin B1, B2 and C1 and other polyphenols like quercetin, apigenin, luteolin and naringenin (Oracz et al. 2015). Nevertheless, a particular attention was placed on flavanols, which appear to protect the vascular function, increasing NO bioavailability. In this regard, several studies including both healthy and hypertensive patients have shown a correlation between the dark chocolate consumption and the improvement of arterial stiffness (FMD) (Grassi et al. 2008b, 2012). In a meta-analysis of 20 RCTs and 856 healthy people, the administration of flavanol-rich cocoa products (30–1080 mg of flavanols, mean = 545.5 mg in 3.6–105 g of cocoa products) for 2–18 weeks revealed a statistically significant reduction of both SBP (−2.8 mmHg, 95%CI − 4.7 to −0.8, $p = 0.005$) and DBP (− 2.2 mmHg, 95%CI −3.5 to −0.9, $p = 0.006$) compared with control (Ried et al. 2012). A more recent meta-analysis

from the Cochrane collaboration including 40 RCTs and 1804 subjects has confirmed data obtained by the abovementioned meta-analysis (Ried et al. 2017).

Finally, future important evidence on the benefit of cocoa PPs in CV health will be provided by the ongoing Cocoa Supplement and Multivitamin Outcomes Study (COSMOS), coordinated by the Department of Epidemiology of the Brigham and Women University (Boston, USA). This study will investigate the effect of cocoa flavonoids in reducing the risk of major CV events, in a sample of 18,000 subjects (aged ≥60 years) randomized to receive for 4 years either to placebo capsules or to the isolated cocoa extract (Brigham and Women Unibersity 2016).

Another important source of polyphenols is *Punica granatum* L. (**Pomegranate**), well known to provide several health benefits. Pomegranate juice is fruit juice particularly rich in antioxidant bioactives such as **gallic and ellagic acids, punicalagin A and B and punicalin A and B**. These molecules have been studied in different conditions including hypertension (Zarfeshany et al. 2014). A recent meta-analysis of 574 individuals and 8 RCTs (Sahebkar et al. 2017) demonstrated the antihypertensive effect of pomegranate juice with daily doses >240 cc (SBP −4.9 mmHg, 95%CI −7.7 to −2.2, $p < 0.001$ and DBP -2.0 mmHg, 95%CI –3.7 to −0.3, $p = 0.021$) compared to control.

Coffee is a frequently consumed beverage, even if there has been a long-standing controversy regarding its safety on BP and CVD. However, recent, well-controlled studies have demonstrated that coffee may reduce BP in people especially with borderline values, probably due to the presence of **chlorogenic acid**. Nevertheless, hypertensive subjects with uncontrolled BP should avoid consuming large doses of caffeine (Loader et al. 2017).

Despite several authors attribute the BP-lowering effect of coffee to the presence of chlorogenic acid (Watanabe et al. 2006), other studies have shown that this molecule is in part inhibited by hydroxyhydroquinone (HHQ), which is formed through the coffee roasting processes (Yamaguchi et al. 2008).

For this reason, coffee could reduce BP inversely to the HHQ content as demonstrated with the supplementation of decaffeinated green coffee bean extract (significant reduction of SBP and DBP and improvement of PWV compared to the control ($p = 0.01$ for all)) (Revuelta-Iniesta and Al-Dujaili 2014).

Resveratrol is a tri-hydroxy-stilbene polyphenol particularly concentrated in grape. Many studies *in vitro* and *in vivo* have shown the anti-hypertensive effects of this molecule through a multiplicity of mechanisms that can be summarized in: anti-ROS activity, stimulation of endothelial production of NO and protection of vascular stiffness, and prevention of platelet aggregation (Li et al. 2012). A recent meta-analysis of 17 RCTs (36 treatment arms and 681 people) concluded that resveratrol does not exert a BP-lowering effect. Nevertheless, considering only type 2 diabetic patients, SBP was significantly reduced by resveratrol treatment (−8.8 mmHg, 95% CI −12.5, −5; $p < 0.001$), probably related to the positive effects of this molecule on insulin sensitivity (daily dosages >300 mg/day). Similar results were obtained in patients with non-alcoholic fatty liver disease (NAFLD) (Fogacci et al. 2018).

The main important aspects of resveratrol which potentially strongly influence the effectiveness of the treatment regard its very-low bioavailability, the pharmaceutical formulation tested and the dosage and the length of the treatments. In this regard, new drug delivery systems (DDS) intended to enhance resveratrol bioavailability have been developed in the last years (Amri et al. 2012).

7.3.3 Other Bioactive Compounds

Garlic is a functional food particularly rich in **polysulfides**. Among these, **S-allylcysteine** might play a pivotal role as BP-lowering agent because stimulating the vascular gasotransmitter hydrogen sulfide (H_2S) and the production of vascular NO, reducing the peripheral vascular resistances (Ried and Fakler 2014). Garlic organosulfur compounds act also as ACE inhibitory and calcium channel blockers (Butt et al. 2009). In particular, the study of *Williams* et al. suggests that S-allylcysteine improves the endothelial function in patients with CAD (coronary artery disease) (Williams et al. 2005). A recent meta-analysis (9 RCTs and 482 people) showed a positive anti-hypertensive action of aged garlic extract administered for 8 to 26 weeks (SBP −9.1 mmHg; 95%CI −12.7 to −5.4; DBP −3.8 mmHg; 95%CI −6.7 to −1.0 compared to the placebo) (Rohner et al. 2015). The BP-lowering effects of garlic seems to be additive to the one of the conventional treatments (Reid et al. 2010). However, its use is partially limited because gastrointestinal side effects are not uncommon.

Lycopene is a carotenoid, particularly concentrated in tomatoes. This molecule has antioxidant, anti-ROS and anti-inflammatory activities even if the antihypertensive mechanism of action of lycopene is still unclear. Several studies have demonstrated that lycopene reduces the degree of oxidation of LDL and improves the FMD in humans (Müller et al. 2016). A meta-analysis of six RCTs suggested a significant BP-lowering effect (SBP mmHg −4.9, 95%CI −8.8, −1.1, $p = 0.012$) of lycopene with dosages of 10–50 mg/day for 4–12 weeks. Nevertheless, lycopene intervention had no statistical effect on DBP (Li and Xu 2013).

Although lycopene supplementation might be considered to reduce SBP, the tomato intake provided more favourable results on CV outcomes than did lycopene supplementation (Burton-Freeman and Sesso 2014).

Pycnogenol, the bark extract of *Pinus pinaster*, is considered another antioxidant molecule that protects cell membranes from oxidative stress and might exert an anti-hypertensive action through the inhibition of ACE and the increase of vascular NO. Accordingly to this, a small RCT including pre-hypertensive subjects showed that the administration of 150 mg/day of pycnogenol, for a duration of 12 weeks, improved the vascular function (FMD) (Hu et al. 2015).

In addition, pycnogenol decreases myelo-peroxidase activity and high sensitivity C reactive protein (hs-CRP), improves renal cortical blood flow and reduces urinary albumin excretion all properties that support its effect on human BP (Maimoona et al. 2011).

Beetroot is a natural source of **inorganic nitrates** with BP-lowering activity both in pre-hypertensive and hypertensive patients, especially if consumed as juice (250 mL/day) (Kapil et al. 2015). A meta-analysis of RCTs showed that beet juice administration (321–2790 mg of nitrates) is associated with dose-dependent changes in SBP (−4.4 mmHg, 95%CI −5.9, −2.8, $p < 0.01$) (Siervo et al. 2013). Similar results were obtained by a more recent meta-analysis (Bahadoran et al. 2017).

Once ingested, inorganic NO_3^- metabolizes *in vivo* to nitrite (NO_2^-) and subsequently it is introduced into the bloodstream. NO_2^- exerts its effects through its conversion to functional nitrogen oxides (NO_x), including NO (Clements et al. 2014). In addition, beet juice is rich in betalains (responsible for the red colour of beetroot) and PPs. Betalains are antioxidants molecules that act as donators of electrons, suggesting a role in protection against oxidative stress and hypertension as well (Gandía-Herrero et al. 2016).

Finally, omega 3 **Polyunsaturated Fatty Acids** (PUFAs), in particular eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) extracted from fish and algae, have demonstrated in several RCTs to possess anti-hypertensive effects. The possible BP-lowering mechanisms of PUFAs could be summarized in: (1) the enhancement of the bioavailability of NO via activation of eNOS (endothelial NO synthase), (2) the regulation of prostaglandins synthesis balance and the enhacement of the vasodilating ones, (3) the reduction of insulin-resistance, (4) the regulation of vascular tone modulating the parasympathetic nervous system and (5) the suppression of the RAS system (Cicero et al. 2009). In a meta-analysis of 70 RCTs, the administration of PUFAs (0.3–15 g/day) assumed for 4–26 weeks has been demonstrated to reduce SBP (−1.5 mmHg, 95%CI −2.2 to −0.8) and DBP (−1.0 mmHg, 95% CI −1.5 to −0.4) compared to the placebo. The subgroups analysis showed the strongest BP-lowering effect among untreated hypertensive subjects (SBP = −4.5 mmHg, 95%CI −6.1 to −2.8 and DBP = −3.0 mmHg, 95%CI −4.3 to −1.7) (Miller et al. 2014). Another meta-analysis of RCTs also shows that PUFA supplementation (900–300 mg/day) is associated to improvement in both pulse wave velocity ($p < 0.01$) and arterial compliance ($p < 0.001$) (Pase et al. 2011).

7.4 Cholesterol Lowering Effect

Another important CVR factor is represented by dyslipidemia. Many available RCTs and meta-analyses of RCTs have shown a correlation between the reduction of the levels of low-density lipoprotein cholesterol (LDL-C) and the reduction of relative risk of CVDs (Hobbs et al. 2016). In particular, a meta-analysis of the Cholesterol Treatment Trialists' (CTT) Collaboration, including 14 RCTs and 90,056 individuals, demonstrated a greater reduction in coronary and vascular events, which was related to a greater decrease in absolute levels of LDL-C (Baigent et al. 2005). In addition, in a report from the CTT Collaboration on more than 170,000 people, it was stated that with the lipid-lowering drug therapy, each further

reduction of LDL-C by 40 mg/dl (~1 mmol/l) decreased by 1/5 the risk of revascularization, CAD and ischemic stroke, underlining that a reduction of LDL-C of 125 mg/dl (3.2 mmol/l) could lead to a decrease in risk of about 40–50%, in the absence of an increased risk of cancer or non-CV-related death (Gay et al. 2016). 1 mmol/l is a reduction that is achievable through lifestyle improvements associated with lipid-lowering nutraceuticals (Table 7.3) (Tang et al. 2015).

7.4.1 Bioactive Peptides

The bioactive peptides (BPs) with major clinical evidence on the reduction of cholesterolaemia are those derived from soy, lupine and milk proteins (Fig. 7.3) (Butteiger et al. 2016). Peptides from cowpea and from Mucuna pruriens have also shown a lipid-lowering activity (Marques et al. 2015).

In a recent meta-analysis of 35 studies, soy proteins and in particular B-conglycinin globulin have shown a lipid-lowering effect with a reduction in LDL-C of −4.83 mg/dl (95% CI: −7.34, −2.31), triacylglycerols (TAG) of −4.92 mg/dl (95%CI: −7.79, −2.04) and a significant improvement in HDL-C of 1.40 mg/dl (95%CI: 0.58, 2.23). In particular, hypercholesterolemic patients have benefited greatly from the reduction of LDL-C (−7.47 mg/dl, 95%CI: −11.79, −3.16) compared with healthy subjects (−2.96 mg/dl, 95%CI -5.28, −0.65) (Tokede et al. 2015a).

The lipid-lowering mechanisms of action regarding soy and lupine proteins could be attributed to the inhibition of the hydroxymethylglutaryl-coenzymeA (HMG-CoA) reductase enzyme, up-regulation of LDL receptors, regulation of the Sterol regulatory element-binding protein 2 (SREBP2) pathway and the increase of the faecal excretion of bile salts (Lammi et al. 2014).

The proteins derived from lupine (50 mg/day) demonstrated clinical efficacy in the reduction of very low density lipoprotein (VLDL) and LDL level in a rat model: in fact, an increased number of LDL receptors in HepG2 hepatoma cell line has been observed with the conglutin gamma (extracted from lupine) (Sirtori et al. 2012).

Another peptide with lipid-lowering action that interacts with micelle formation and absorption of exogenous cholesterol is derived from the hydrolyzate extracted of *Mucuna pruriens* (Herrera Chalé et al. 2016). Peptides from cowpea have also demonstrated to inhibit cholesterol synthesis and its solubilisation into micelles (Marques et al. 2015).

A bioactive peptide derived from milk is β-lactotensin; at a dose of 100 mg/kg per os it showed a significant lipid-lowering activity in mice, with an increased excretion of bile acids in the faces (Yamauchi et al. 2003). It acts probably via the action on neurotensin receptor 2 (NTS2) and D1 receptors, which results in higher levels of synthesis of bile acids from cholesterol, enhanced further by the direct action of β-lactotensin on mRNA (Yoshikawa 2015).

Table 7.3 Food and plant bioactives with possible lipid lowering effect

Plant/food bioactive	Active daily doses	Expected effects on lipid profile	Direct vascular effects
Apple	100–800 mg of polyphenols	0/−15% LDL, 0/+15% HDL (only in hypercholesterolemic subjects)	Unclear
Berberine	500–1000 mg	−15/−20% LDL, ↓ ApoB, TG, hs-CRP, IL-6, MCP-1, ICAM-1, VCAM-1, MMP-9	↑ FMD, ↓ PWV
Bergamot	500–1000 mg of bergamot polyphenols fraction	−5/−10% LDL, ↓ sdLDL, hs-CRP, TNF-α	↑ FMD, ↓ PWV
Berries	320 mg/1 g of dry extract	−5/−30% LDL, 0/−20% TG, +10/+30% HDL	Unclear
Bioactive peptides	10–100 mg/day (IPP, VPP)	−0/−5% LDL	Not investigated
Cocoa and dark chocolate	400–1000 mg of polyphenols	0/−5% LDL	↑ FMD, ↓ PWV
Coffee	150 ml (300 mg of polyphenols)	0/−5% LDL, 0/+5% HDL, 0/−13% TG	Unclear
Curcumin	300–1000 mg	0/−5% LDL, ↓ hs-CRP, IL-6, MCP-1, ICAM-1, VCAM-1, MMP-9	↑ FMD, ↓ PWV
Grape	200–800 mg of total polyphenols	–0/5% ↓ ApoB, ApoE ↑ ApoAI, ApoAII	↑ FMD, ↓ PWV
Monacolin K	3–10 mg	−15/25% LDL, ↓ LDL, ApoB, hs-CRP, MMP-2, MMP-9	↑ FMD, ↓ PWV
Nuts	30 g/day	−5/−10% LDL	↑ FMD, ↓ PWV
Olive oil	25 ml/day (polyphenols: 366 mg/kg)	0/−5% LDL	Unclear
Plant sterols and stanols	3 g/day	−5/−15% LDL	Unclear
PUFAs	1–4 g (EPA + DHA)	−5-20% TG, ↓ sdLDL, hs-CRP, TNF-α, ↓ adhesion molecules	↑ FMD, ↓ PWV
Soy	40–80 mg/day of soy-derived isoflavones	0/−5% LDL, +0/5% HDL, 0/−13% TG	Unclear
Tea	170–850 mg/day of tea catechins	0/−5% LDL, ↓ oxyLDL	↑ FMD, ↓ PWV

APO apolipoprotein, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *FMD* flow mediated dilation, *HDL* high density lipoprotein, *hs-CRP* high sensitivity C reactive protein, *ICAM* intercellular adhesion molecule 1, *IPP* isoleucine–proline–proline, *LDL* low density lipoprotein, *oxyLDL* LDL oxidated, *MCP* monocyte chemoattractant protein, *sdLDL* small dense LDL, *TG* triglycerides, *PWV* pulse wave velocity, *TNF-alpha* tumor necrosis factor alpha, *VCAM* vascular cell adhesion protein, *VPP* valine–proline–proline

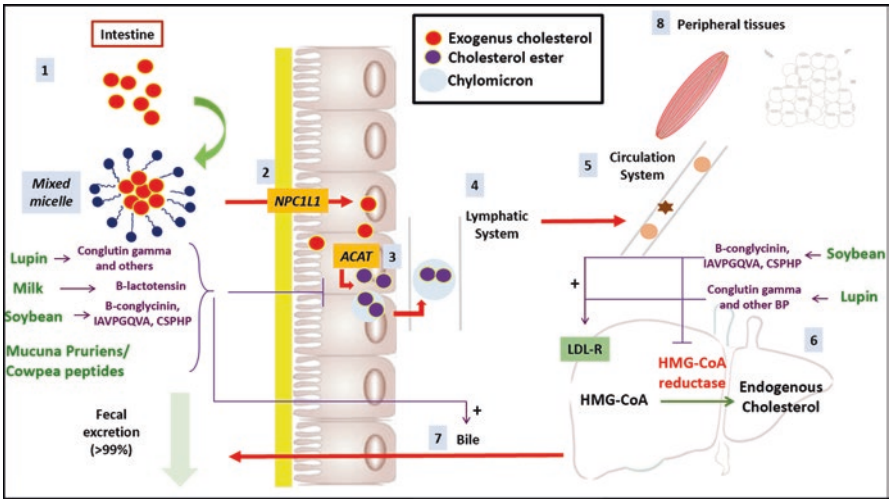

Fig. 7.3 Main bioactive peptides with evidence on cholesterol metabolism: proposed mechanisms of action. (1) Bioactive peptides arrive into intestinal lumen with exogenus cholesterol after a meal (2). The meal fats form the mixed micelles with pancreatic and bile secretions, that facilitate the entrance into enterocytes via the NPC1L1 transporter presents on the brush border membrane of the enterocyte. Into enterocytes, the cholesterol is a substrate for intestinal ACAT (3), and after it is incorporated into chylomicrons to reach the bloodstream (5) through the lymphatic system (4). The cholesterol reaches the liver (6), but a percentage is re-excreted into the intestinal lumen and used for the bile synthesis and thus eliminated through the faeces (7). The cholesterol (trough the lipoproteins) is taken up by several peripheral tissues such as muscle and adipose tissue (8). *ACAT* Acyl-CoA cholesterol acyltransferase, *HMG-CoA* hydroxymethylglutaril-CoA, *LDL-R* low-density lipoprotein-receptor, *NPC1L1* NiemannPick C1 like 1

7.4.2 Polyphenols

The putative lipid-lowering mechanisms of action of polyphenols include the reduction of oxidative stress of the lipoproteins, the inhibition of hepatic synthesis of LDL, the enhancement of the number of hepatic LDL-receptors and the reverse cholesterol transport by the stimulation of transporters such as ABCG1, ABCA1 and SR-BI, the activation of AMPK (AMP-activated protein kinase) and PPAR-gamma (peroxisome proliferator-activated receptor gamma) (Tomé and Visioli 2016).

Bergamot is the common name of the fruit *Citrus bergamia* and it contains high levels of **flavonoids** such as neohesperidin, neodesmin, naringin, neoeriocitrin, rutin, rhoifolin and poncirin. Specifically, the 3-hydroxy-3-methyl-glutaryl flavanone–enriched fraction (HMGF: brutieridin, melitidin, and HMG neoeriocitrin) acts as a statin by inhibiting HMG-CoA reductase and ACAT, lowering the formation of cholesterol esters. Bergamot contains also naringin, a bioactive molecule that acts with several anti-atherosclerotic mechanisms, including the inhibition of LDL oxidation and ROS activity and the activation of AMPK. Nevertheless, the final effect might be due also to other components like neoeriocitrin, melitidin, and rutin (Di Donna et al. 2009).

The evidence indicates both quantitative and qualitative lipid-lowering effects of bergamot, especially through the reduction of both small dense (sd)-LDL and triglycerydes (TG) levels and the improvement of HDL-C levels. For this reason, people with MetS and NAFLD who are intolerant to statins may benefit from bergamot supplementation. However, the clinical literature is still poor because data regarding bergamot comes from a single research unit and aren't confirmed by other groups yet. In addition, data on vascular stiffness is still lacking.

Gliozzi et al. has conducted a study including 77 patients with mixed dyslipidemia divided into five groups: placebo (n = 15), 10 mg of rosuvastatin (n = 16), 20 mg of rosuvastatin (n = 16), 1000 mg of bergamot (bergamot-derived polyphenolic fraction (BPF); n = 15), and 1000 mg bergamot plus 10 mg of rosuvastatin (n = 15). After the treatment the study showed a reduction of LDL-C from a baseline value of 4.94 mmol/l to a value of 2.97 mmol/l after 10 mg of rosuvastatin; to 2.26 mmol/l after 20 mg of rosuvastatin; to 2.92 mmol/l after 1000 mg of BPF; and to 2.33 mmol/l after 1000 mg bergamot plus 10 mg of rosuvastatin (Gliozzi et al. 2013).

The same author underlined a significative effect of bergamot on TC, LDL-C and TG in people with Mets and NAFLD (Gliozzi et al. 2014).

The consumption of **apple polyphenols** (0.21–1.43 g/day) both as a juice and as a fruit might protect and reduce the ROS and oxidation of lipoprotein as well. However, data regarding LDL-C and TC reduction are still contrasting. In a RCT, the treatment with 600 mg/day of apple polyphenols in 71 subjects with BMI between 23 and 30 resulted in a significant decrease of LDL-C levels (Nagasako-Akazome et al. 2007). Similar results were obtained in mildly hypercholesterolemic patients and with the consumption of two apples/day (*Annurca* apple) for 4 months (LDL-C –14.5% and HDL-C + 15.2% ($p < 0.001$ for all)) (Tenore et al. 2016). However other studies showed no benefits on the lipid profile and vascular function, despite an improvement of the oxLDL (Ravn-Haren et al. 2013; Vafa et al. 2011).

A rich source of PPs and in particular **anthocyanins** are **berries**. Several RCTs have evaluated the effects of blueberries, strawberries, chokeberries (*Aronia melanocarpa L.*) and cranberries supplemented as fresh fruit, juice or freeze-dried extract as well in CV prevention (Basu et al. 2010).

The study of Qin et al. showed that the supplementation with 320 mg/day of berry-derived anthocyanin in dyslipidemic subjects improved LDL-C, TG and HDL-C compared to the placebo group ($p < 0.001$ for all) (Qin et al. 2009). Similar results were obtained after the consumption of 200 ml of chokeberry juice (386 ± 9.7 mg of total phenolics) (Skoczynska et al. 2007) and in hypertensive patients (Oszmianski and Wojdylo 2005) as well.

Even the consumption of **cranberry** for 12 weeks**,** has demonstrated to decrease significantly LDL-C (from 3.3 ± 0.2 to 2.9 ± 0.2 mmol/l, $p = 0.005$), TC ($p = 0.020$) and TC/HDL-C ratio ($p = 0.044$) compared with placebo, in a RCT of 30 diabetic subjects (Lee et al. 2008). Cranberry juice (480 mL/day) also demonstrated good antioxidant efficacy in patients with metabolic syndrome (Basu et al. 2011).

Finally, even the *Vaccinium arctostaphylos L* (better known as Caucasian whortleberry) and PPs from strawberries seem able to improve the lipid profile in people

with mild dyslipidaemia or MetS, as demonstrated by several RCTs, probably because of the high presence of PPs flavonoids (Kianbakht et al. 2014; Basu et al. 2014).

The **flavonoids** present in **dark chocolate** (DC) could possess lipid-lowering activity as demonstrated in a pilot study of 28 healthy people treated with 700 mg/day of flavonoids for 1 week. The results showed a significant reduction of LDL-C by 6% ($p < 0.018$), hs-CRP levels ($p < 0.04$) and platelet aggregation ($p < 0.006$) and an improvement of HDL-C by 9% ($p < 0.0019$) (Hamed et al. 2008). Even the RCT of Mursu et al. (2004), including healthy volunteers, showed similar conclusions. DC (27–100 g/day) or cocoa flavanols (850–993 mg/day) were administered also in grade I hypertensive patients (Grassi et al. 2005), in obese people (Di Renzo et al. 2013), in menopausal women with type 2 diabetes (Curtis et al. 2013) and in the elderly (Mastroiacovo et al. 2015), with satisfactory results in the reduction of cholesterolemia and inflammation markers as well.

DC seems to be a lipid-lowering agent also in people at high CVR, in addition to increase the levels of HDL-C and decrease the oxidation of LDL ($p < 0.05$ for both) (Khan et al. 2012). Moreover, Baba et al. underlined a greater reduction in patients with serum cholesterol ≥3.23 mmol/l at baseline (Baba et al. 2007).

The meta-analyses of Hooper et al. (2012) (42 RCTs and 1297 participants) and Shrime et al. (2011) (24 RCTs and 1106 participants) shows an improvement of HDL-C and a slight but significant reduction of LDL-C after the assumption of DC or flavan-3-ol-rich cocoa derived products.

However, some studies are still conflicting (Desideri et al. 2012; West et al. 2014; Nogueira Lde et al. 2012; Neufingerl et al. 2013) and contrasting with the above-mentioned results even if some of them reported a great statistical heterogeneity. For this reason, larger and longer RCTs with a specific population sample are needed to have more consistent and clear results. Finally, the consumption of DC or cocoa PPs is associated with an improvement of arterial stiffness (FMD) and insulin resistance (HOMA-IR) (Hooper et al. 2012; Shrime et al. 2011). In general, DC compliance is excellent and side effects are negligible.

In the last years, an increased number of publications has pointed the attention on the potential protective effects of **coffee and its bioactives** (including caffeine, chlorogenic acid, caffeic acid and hydroxyhydroquinone) against oxidative stress and related chronic disease risk. Concentration of total polyphenols in coffee are about 200 mg/100 mL (Fukushima et al. 2009). However, despite that the moderate consumption of coffee (2–4 cups/day) seems to be associated with reduced CVR (Crippa et al. 2014), data regarding the lipid-lowering activity are still contrasting (Cai et al. 2012; Grioni et al. 2015).

Grape is particularly concentrated in **anthocyanins**, **flavanols**, **flavonols**, **proanthocyanidins** and **stilbenes**. Nevertheless, although the correlation between the consumption of grape (as fruit, juice or nutritional supplement as well) and the anti-inflammatory, anti-hypertensive, anti-platelet, anti-oxidant and ameliorative of endothelial function is now known, data on lipid profile are still unclear (Castilla et al. 2006; Vaisman and Niv 2015). In the study of Sano et al. the administration of 400 mg/day of proanthocyanidins (extracted from grape seed), for 4–12 weeks, in

healthy people, has shown no significant changes in LDL-C and TC as well compared to baseline, even if the supplement significantly decreased the LDL oxidation ($p < 0.001$) (Sano et al. 2007). Similar results were obtained from Diaz-Rubio et al. in 28 healthy subjects treated with 200 ml/day of pomegranate and grape juice (Díaz-Rubio et al. 2015), Siasos et al. in 26 healthy smoker subjects (965 mg/day of total PPs for 2 weeks) (Siasos et al. 2014) and in subjects with metabolic syndrome (Sivaprakasapillai et al. 2009). Finally, even the meta-analyses by Feringa et al. (9 RCTs and 390 participants included) and Sahebkar et al. (10 RCTs and 11 treatment arms) showed no quantitative effects of grape seed or resveratrol on lipid profile (Feringa et al. 2011; Sahebkar et al. 2015). In contrast to these results, some RCTs have found benefits on lipid profile. In this regard, the intake of 800 mg/day of grape PPs, in healthy males, for 2 weeks, resulted in lower TC and TG after the consumption of high fat meal (van Mierlo et al. 2010). Similar results were obtained in a further study involving 60 healthy volunteers assuming 700 mg/day of polyphenol-rich grape extract supplement or placebo (Yubero et al. 2013).

The consumption of at least 30 g/day of **nuts** or > 4 times/day is known to reduce the CVR by 37% (mean reduction of 8.3% for each weekly serving of nuts) (Kelly and Sabaté 2006). The reason of CV protective activity of nuts might also be explained due to their lipid-lowering action. In fact, nuts are particularly rich in PPs (phenolic acids, proanthocyanidins, flavan-3-ols and ellagitanninis) and other **bioactive substances** such as plant sterols and stanols, linoleic acid, alpha-linolenic acid, gamma-tocotrienols, the L-damming and other micronutrients. The effects of nuts consumption were evaluated in a metaanalysis of 25 trials (583 men and women with or without dyslipidemia and not in treatment with conventional therapies) conducted in seven countries. The analysis showed a relationship between the consumption of nuts (mean assumption: 67 g/day) and the reduction of the levels of TC (0.28 mmol/l; −5.1%), LDL-C (0.26 mmol/l; −7.4%), LDL-C/HDL-C ratio (0.22; −8.3%), and TC/HDL-C ratio (0.24; −5.6%) ($p < 0.001$ for all). In addition, in people with hypertriglyceridemia (TG > 1.70 mmol/l), nuts reduced also TG levels by 0.23 mmol/l (−10.2%) ($p < 0.05$). In general, the effects of nut consumption were dosage-dependent and the greatest efficacy was obtained in patients with elevated levels of cholesterol at baseline (Sabaté et al. 2010).

Similar results and conclusions were obtained by a sub-group of the "Prevention with Mediterranean diet" (PREDIMED) study, where the Mediterranean diet, enriched in the consumption of nuts, has demonstrated to improve the lipid profile and to reduce the CVR in patients at high CVR (Medina-Remón et al. 2016).

Another component of the Mediterranean diet are **olives** with a high content of **polyphenols** such as oleuropein and hydroxytyrosl. Nevertheless, data from RCTs regarding their lipid-lowering activity are still conflicting (Cicero and Colletti 2018b). For example, despite a further meta-analysis of eight cross-over RCTs and 355 participants showed a slight reduction in SBP ($p < 0.001$) and LDL oxidation ($p = 0.05$) following the consumption of olive PPs, no significant effect was observed on TC, LDL-C, HDL-C and TG (Hohmann et al. 2015). In contrast to this study, other RCTs are in countertendency. In a multicentre, crossover study, including 200 volunteers and six research centers from five European countries, the lipid-lowering

activity of three types of olive oil was tested. In particular, for a single dose of olive oil (25 ml/day), the content of PPs was 366 mg/kg (Oil A), 164 mg/kg (Oil B) and 2.7 mg/kg (Oil C). The treatment was characterized by 3 weeks for each type of oil, alternated by 2-week washout periods. At the end of the study the improvement of HDL-C was proportional to the intake of PPs (Oil A: +0.045 mmol/l (95% CI 0.02, 0.06 mmol/l) as well as the reduction of oxidized LDL-C (Oil A: −3.21 U/l (95% CI −5.1, −0.8 U/l), while the reduction of TG was on average 0.05 mmol/l for each treatment (Covas et al. 2006).

Similar results were obtained in 60 pre-hypertensive patients (Lockyer et al. 2016). Even the assumption of yogurt enriched with olive PPs (50 mg/day) has demonstrated to improve the LDL-C ($p = 0.06$) and lipid peroxidation ($p < 0.05$) in 16 healthy subjects (Georgakouli et al. 2016). Finally, the PREDIMED study has demonstrated that the consumption of extra virgin olive oil rich in PPs is associated to reduced risk of CVDs and mortality in individuals at high CVR, if associated with a healthy lifestyle (Guasch-Ferré et al. 2014).

Other bioactive compounds which are particularly interesting for their lipid-lowering action are **soy isoflavones**. A meta-analysis of 11 RCTs showed that **daidzein** and **genistein**, the main soy isoflavones, significantly reduced serum TC by 0.10 mmol/l (3.9 mg/dl or 1.77%; $p = 0.02$) and LDL-C by 0.13 mmol/l (5.0 mg/dl or 3.58%; $p < 0.0001$). The cholesterol-lowering activity was larger in people with higher values of TC and LDL-C at baseline (Taku et al. 2007), and dosages >80 mg of isoflavones led to better results as well. The improvements in HDL-C appeared only in trials of >12 weeks duration (Zhan and Ho 2005; Tokede et al. 2015b).

Tea extract from the leaves of *Camellia sinensis* is the second most consumed beverage in the world after water. It is well known to possess health properties because of the high presence of bioactive substances. As mentioned before, there are many kinds of tea, according to the manufacturing processes: green tea (GT), black tea (BT) and oolong tea (OT, produced by partial fermentation) (Khan and Mukhtar 2007). The most important catechin present in tea is a flavan-3-ol, the **(−)-epigallocatechin gallate** (EGCG), that represent around the 50–80% of total catechins in tea, even if (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epicatechin (EC) are present in small quantities as well.

Tea catechins are able to reduce the lipid peroxidation and might improve the lipid profile, probably interfering with the micellar solubilization and absorption of intestinal cholesterol, acting as activator of the AMPK that stimulate lipogenesis, enhancing the hepatic LDL-receptors expression and the biliary excretion of cholesterol, and reducing the endogenous synthesis of cholesterol through the inhibition of HMG-CoA reductase (Shishikura et al. 2006). In the meta-analysis by Onakpoya et al. (20 RCTs and 1536 subjects), the consumption of 250–1200 mg/day of GT extract or of 170–850 mg/day of EGCG has demonstrated to reduce TC of 0.13 mmol/l (95%CI: 0.2, 0.07, $p < 0.0001$) and LDL-C of 0.19 mmol/l (95%CI: 0.3 to 0.09, $p = 0.0004$) (Onakpoya et al. 2014). However, GT seems not to influence the plasma HDL level (Zheng et al. 2011). Similar results were obtained in patients with chronic stable angina (Lee et al. 2016). The cholesterol-lowering effectiveness of GT was found to be greater in RCTs with longer durations of intervention. In

addition, GT extract has shown a mild but significant antihypertensive effect. Regarding the type of tea, the consumption of BT compared to GT has shown analogue results on LDL-C reduction (Wang et al. 2014). Moreover, GT is associated with an improvement in FMD (Lin et al. 2016) and PWV (Park et al. 2010). Regarding the safety profile, the supplementation of tea or catechins is considered safe and well tolerated. However, elevated dosages of GT could be responsible of iron and folate deficiency, reducing their intestinal absorption. Therefore, particular attention should be given to its intake in pregnant women and in women at risk for pregnancy (Onakpoya et al. 2014).

7.4.3 Other Bioactive Compounds

The rhizome of *Curcuma longa* contains a large amount of curcuminoids and in particular **curcumin**, which represents the major phenolic compound present in the spice turmeric. Several RCTs and meta-analyses have shown the anti-inflammatory and antioxidant activities of curcumin. Moreover, this nutraceutical acts as a lipid-lowering agent trough the inhibition of the expression of the NPC1L1 transporter and the increase of the cholesterol efflux via ABCA1 expression (Kumar et al. 2011). However, the results regarding the effects of curcumin on lipid profile are still unclear (Sahebkar 2014).

Plant sterols and stanols (PS), are molecules structurally similar to cholesterol, differing in the side chain at C24 that presents a methyl or ethyl group (campesterol and B-sitosterol, respectively) or an extra double bond at C22 (stigmasterol). Stanols are instead the saturated derivatives of sterols. PS are present in different plant sources such as vegetable oils, seeds, legumes, nuts, and fat spreads and if administrated in fed state, they decrease the intestinal absorption of exogenous cholesterol through the competition with it in the formation of solubilized micelles and in the NPC1L1 transporter.

However, the ATP-binding cassette protein family (ABCG5 and ABCG8) shuttles and blows out the majority of sterols and stanols in the intestinal lumen (PS bioavailability <1–2%).

The cholesterol-lowering activities of PS have been highlighted in different meta-analyses of RCTs. One of the most recent meta-analyses, including 41 RCTs and 2084 subjects, demonstrated the effectiveness of PS (mean dose: 1.6 g/day, range: 0.3–3.2 g/day) on cholesterolemia, with a significant reduction of LDL-C of 0.33 mmol/l (12.8 mg/dl; −8.5%) compared to the placebo (Ras et al. 2013). PS might also have an impact on TG but only in people with high TG levels at baseline. No differences in efficacy have been underlined between sterols and stanols at dosages up to 3 g/day (Cicero et al. 2017a).

Monacolin K is a secondary fermentative component of red yeast rice (RYR) obtained by the fermentation of a particular yeast (in general *Monascus purpureus*) in rice (*Oryza sativ*a), that is well known to possess a lipid-lowering activity. The main lipid-lowering mechanism of action of RYR concerns the inhibition of

HMG-CoA reductase. A recent meta-analysis of 20 RCTs showed that 2–24 months of supplementation with RYR, reduced LDL-C by 39.4 mg/dl (1.02 mmol/l, −1.20 to −0.83) compared to the placebo, not different from low-intensity statin treatments (40 mg of pravastatin, 10 mg of simvastatin, 20 mg of lovastatin). In addition, the supplementation with RYR significantly reduced TG (−23 mg/dl, −0.26 mmol/l; range: −0.35 to −0.17) and increased HDL-C (0.3 mg/dl, 0.007 mmol/l; range: 0.03–0.11) compared to the placebo.

Concerning the safety profile, RYR administration is in general well tolerated as highlighted by a meta-analysis where the incidence of cases of liver abnormalities and kidney injury was similar in both RYR and control groups and the incidence of developing muscular symptoms was lower in RYR groups (0–23.8%) compared with control groups (0–36%) (Gerards et al. 2015). Similar data was obtained in a previous Chinese meta-analysis that included 93 RCTs and 9625 volunteers (Liu et al. 2006). Pleiotropic activities of RYR include the improvement of FMD and arterial stiffness and the reduction of inflammatory markers (hs-CRP). Finally, this nutraceutical has been studied to evaluate its effects on CV outcomes. In this regard, a large trial including 66 hospitals in China and 445 patients of 65–75 years old, with a history of myocardial infarction, has evaluated the effects of RYR for a mean of 4 years. People were randomized in two groups (placebo vs RYR). At the end of the study, only patients in the active group showed a reduction in the risk of CHD (−31.0%; $p = 0.04$), all-cause mortality (−31.9%; $p = 0.01$), stroke (−44.1%; $p = 0.04$), and the need for a coronary revascularization (−48.6%; $p = 0.07$) (Zhao et al. 2004).

Berberine (BBR) is a quaternary benzylisoquinoline alkaloid particularly concentrated in different parts of various plants (e.g. *Coptis chinensis*, *Hydrastis canadensis*, *Berberis aristata*).

The lipid-lowering mechanisms of BBR are essentially two: first, it is an inhibitor of proprotein convertase subtilisin/kexin type 9 (PCSK9), limiting the degradation of the hepatic LDL-receptor, and second, it acts directly on the expression of LDL-receptor, causing an upregulation of the receptors through a post-transcriptional mechanism that stabilizes their mRNA.

The lipid-lowering efficacy of BBR (500–1000 mg/day) has been confirmed by a recent meta-analysis of 27 RCTs and 2569 participants. The results showed a reduction of LDL-C of −25.14 mg/d (−0.65 mmol/l, 95%CI, −0.75 to −0.56; $p = 0.00001$), TG of 34.5 mg/dl (−0.39 mmol/l, 95%CI, −0.59 to −0.19; $p = 0.0001$) and an improvement of HDL-C of 2.71 mg/dl (0.07 mmol/L, 95%CI, 0.04–0.10; $p = 0.00001$). These effects might be additive to statin treatments, and could improve glucose metabolism and blood pressure as well (Meng et al. 2012).

In 61 patients undergoing percutaneous coronary intervention, the supplementation with BBR (300 mg, t.i.d., for 30 days) in addition to standard therapy, has demonstrated to reduce matrix metalloproteinase (MMP)-9, intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, C-reactive protein, interleukin-6 and monocyte chemoattractant protein-1 ($p < 0.001$ for all) compared to baseline values (Lan et al. 2015). BBR assumption is usually safe. Mild

diarrhea, constipation and abdominal distension can appear with the use of high dosages (>1 g/day).

Finally, **PUFAs** (in particular **EPA** and **DHA**) from both fish and vegetal origins, represent a valid nutraceutical to reduce TG in the blood (by 18–25%), even if their effects on LDL-C and HDL-C are clinically insignificant. Nevertheless, data on CVD outcomes have produced conflicting results, and their clinical efficacy appears to be related to non-lipid effects. In addition, low dosages of PUFAs (e.g. 400 mg/day of EPA plus DHA) do not significantly reduce TG levels, as confirmed in an RCT of 4837 post-myocardial infarction patients (Kromhout and Giltay 2010). A meta-analysis involving 20 RCTs and 63,030 patients showed that the treatment with PUFAs did not have an impact on a composite CVD endpoint or total mortality but was associated with a significantly decreased rate of vascular death (Kotwal et al. 2012).

7.5 Discussion and Future Perspectives

Current progress in bioactive compounds is an exciting and growing research field, even if this potential should not be surprising. In fact, bioactive peptides are able to control and modulate the cellular communications and functions as well, while the polyphenols are able to regulate the inflammation and oxidative stress at the base of chronic diseases.

This renewal of interest in therapeutic "bioactive molecules" derived from food and plants, might be due to some limitations of conventional treatments, including frequent development of drug resistance, poor delivery, non-specificity, side effects and economic costs (Craik et al. 2013).

Studies conducted *in vitro* and *in vivo* demonstrated the effectiveness of bioactive compounds in prevention of CVDs and, since now, the excellent tolerability profile. The blood pressure and cholesterol lowering molecules tested in humans confirm their optimal tolerability and safety. However, despite that the safety profile seems to be good, the presence of proteins and hydrolysates bioactives might exacerbate or induce allergic reactions. In this regard, longer and larger RCTs are needed to verify the safety of these substances (Franck et al. 2002). In addition, there is the need of solid pharmacokinetic studies to determine the active dosages and the frequency of administration, and to analyze the variability in biological effects. In fact, bioactive compounds include a large class of different substances with different pharmacodynamic and pharmacokinetic profiles. In addition to the different chemical structures, other aspects which may influence the bioavailability and the effectiveness of the bioactive compounds are the pharmaceutical forms and the presence or not of other substances including excipients and other molecules.

Other limits regarding the prescription of bioactive substances in clinical practice concern the limitations of the studies, including the short duration (almost never more than 8 weeks) and the restricted sample of enrolled subjects. All these factors might contribute to explain the great heterogeneity of the results obtained from the

studies (Fanali et al. 2018). It is also necessary to standardize extractive processes for bioactive compounds.

In conclusion, the results obtained since now in studies *in vitro* and *in vivo* and in clinical trials are encouraging and have shown the great potential of bioactive molecules in CV prevention. However, several aspects need to further confirmations, such as the influence of gut microbiota on bioactives bioavailability, the little knowledge of the active metabolites, the dosages of administration and the standardization of products, and number and characteristic of people enrolled in the studies.

Moreover, longer and larger RCTs are needed to confirm the effects of bioactive compounds in CVDs as well as in prevention of CVR factors, and to promote activities and potential prescriptions in clinical practice. Finally, a cost-benefit analysis should be done to understand the utility of these compounds in relation to the economic burden of chronic diseases with negligible side effects.

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Chapter 8
Bioactives for Neuronal and Immune Functions

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Abstract The positive effects of certain dietary nutrients and phytochemicals on human health include the prevention of a non-communicable diseases (NCD) as well as the enhancement of the healing processes by decreasing the time needed for healing and improving the outcomes. A diet that is low in saturated fats and carbohydrates and that is high in fiber, antioxidants such as polyphenols and monounsaturated and omega-3 fatty acids, phytosterols and probiotics are known as a healthy diet. It has been shown that polyphenols are interfering with immune cells regulation, gene expression and pro-inflammatory cytokines' synthesis. As such, these molecules are associated with extended health benefits, playing an important role in the prevention and treatment of various chronic conditions, such as neurological disorders. Omega-3 fatty acids are known for their positive health effects through their anti-inflammatory properties as well as their impact on gut microbiota. DHA and EPA are known for being essential in neuronal/brain functioning and its immunomodulatory properties. Intestinal immune stress associated with low omega-3 availability might be also involved in the development of neuro-inflammation and progression of related diseases. Further studies are needed in order to understand the real impact and benefits of omega-3 fatty acids on the development of non-communicable diseases (NCD) including neurological conditions that are developed as a consequence of neuro-inflammation.

Keywords Immune · Neuro · Omega-3 · Polyphenols · DHA · EPA

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8.1 Introduction

8.1.1 Nutrients/Bio-Actives Components from Food

"Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (World Health Organization (WHO) 2019). It is well known that the consumption of certain food has an impact on our health due to its health-promoting properties. Many studies have shown the positive effects of certain dietary nutrients and phytochemicals on human health. These positive effects include the prevention of a non-communicable diseases (NCD) such as some specific chronic diseases (e.g., obesity, diabetes, cardiovascular diseases, cancer, and neurodegenerative conditions) as well as the enhancement of the healing processes by decreasing the time needed for healing and improving the outcomes (Davinelli et al. 2016; Yasmeen et al. 2017; Urquiaga et al. 2017). These effects that nutrition has on human health are especially important in an aging population that is in increase worldwide (Bruins et al. 2019).

The primary food nutrients are macronutrients and micronutrients. These are called essential nutrients as the human body cannot make them or at least not in sufficient quantity. Macronutrients are carbohydrates, proteins, and fats, while micronutrients are vitamins and minerals. Macronutrients provide energy to the body, while the deficit in micronutrients is related to many non-communicable diseases, including cognitive and neuromuscular function impairments (Bruins et al. 2019).

Different food contains different nutrients with a variety of the quality of carbohydrates and fats (Haase et al. 2018). High-energy food that has low nutrient value and low essential nutrient intake seems to have a tremendous influence on overall health, leading to various health problems, including mental health problems (Parletta et al. 2013). However, the mechanisms responsible for the effects of food components on health remain elusive (Yasmeen et al. 2017). Increased interest in the diet-related disease risks and potential beneficial effects that food bioactive nutrients can have on human health will most probably lead to improved therapeutic approaches in the future (Rescigno et al. 2017).

What is now considered as a healthy diet is an intake of vegetables and fruits, nuts, seafood, whole grains, and olive oil. In other words, a diet that is low in saturated fats and carbohydrates and that is high in fiber, antioxidants such as polyphenols and monounsaturated and omega-3 fatty acids, phytosterols and probiotics are known as a healthy diet (Urquiaga et al. 2017).

On the other hand, the importance of essential fatty acids and their effects on the development and progression of mental diseases has been more investigated recently, and the results show that omega-3 fatty acids are crucial for our wellbeing and that can be used in preventing and/or treating various diseases (Wysoczanski et al. 2016).

8.1.2 Nutrition and Immune System

It is well known that the role of the immune system is to protect the host homeostasis and general health. In order to achieve that, it must constantly monitor for harmful non-self molecules/ invading pathogens and adapt over time so that one may detect and neutralize evolving pathogens that try to avoid neutralization by the immune system (Childs et al. 2019; Gutierrez et al. 2019). A resilient immune system has the capacity to adapt quickly, and that ability to establish and maintain the appropriate immune response in challenging circumstances is called immune fitness. The healthy immune system is programmed to resolve and to return the tissue to the pre-inflamed state (restore tissue homeostasis). When that resolution of inflammation is contained in time and space, our body is in a state of immune fitness. However, when our body over-reacts and has poor and/or the inappropriate resolution of inflammation, it extends the time for pro-inflammatory mechanisms leading to tissue damage and pathology. This has a significant impact on the pathogenesis of chronic inflammatory diseases, including mental diseases (Barnig et al. 2019).

There are two major groups of the immune cells:

- cells of the innate immune system that represents the first line of defense and cells of the adaptive immune system that is specialized and more effective but its action is delayed; these cells are phagocytes such as monocytes, macrophages, neutrophils, tissue dendritic cells and mast cells;
- cells of the adaptive immune system; these are T cells that are involved in cell-mediated immunity and B cells that are responsible for humoral immunity.

The immune system disorders lead to various diseases, from autoimmune diseases that are the result of the hyperactive immune system to inflammatory diseases and life-threatening infections that are the result of the immune system that is less active than normal. For the optimal function of the immune system cells, a healthy diet that contains adequate nutrients is essential. Nutrition that supports the immune cell functions is important not only to help initiation of the effective immune response but also to help to avoid chronic inflammation by resolving rapidly the response (Childs et al. 2019). Low-grade inflammation that can be triggered by certain food components can lead to fatigue, depression as well as the development of a variety of other immune diseases. Actually, it was shown that chronic low-grade inflammation is present in virtually all non-communicable diseases (Prescott 2013).

Some micronutrients (e.g. vitamin D), as well as macronutrients (e.g. polyunsaturated fatty acids), have specific immune regulatory characteristics. It seems that dietary omega-3 fatty acids promote specific immune functions, but clarifying the mechanisms of nutrient supply effects on the different immune cells and their metabolism and signaling is a challenge that future research may resolve and thus help optimizing omega-3 supplementation, lately extremely popular, in busting the immune response and treatment of different diseases (Bjelica et al. 2020; Gutierrez et al. 2019; Kedia-Mehta and Finlay 2019).

Polyphenols, due to their specific structures, act as enzyme inhibitors (activators), activators or suppressors of particular signaling pathways, scavengers of reactive free radicals, etc. In that way polyphenolic component, specifical resveratrol may reduce inflammation and this was shown in ischemic heart disease patients by reducing inflammatory and fibrinolytic biomarkers (Bruins et al. 2019).

8.1.3 Nutrition and Mental Health

Worldwide, mental disorders are increasing with more than 450 million people suffering from depression, intellectual disabilities, schizophrenia, and drug abuse disorders. Moreover, an increase in the dementia rate is alarming with the incidence of 4–6 million new cases per year. Despite the significant burden that these mental disorders carry, the importance of healthy dietary patterns in the prevention of these disorders and the improvement of mental health has been poorly investigated so far (Silva and Sobarzo-Sanchez 2019; Parletta et al. 2013).

Currently available psychopharmacological therapy and complementary psychotherapeutic procedures have moderate efficacy, unwanted side effects and high risk of relapse. Thus, there is a need for new approaches in prevention and managing the progression of mental disorders (Mörkl et al. 2018; Opie et al. 2017). The knowledge we have about the nutrition impact on mental health is mainly based on experiments in vitro, animal research and epidemiological studies and only on some clinical trials (Davison et al. 2012). Even though more clinical research is required for proving that dietary patterns and food components can improve neuronal and cognitive impairments, existing evidence suggests that specific dietary factors may influence the lower risk of depression and other mental disorders (Silva and Sobarzo-Sanchez 2019; Opie et al. 2017). These factors include omega-3 fatty acids, B vitamin complex (vitamin B1, pantothenic acid and folate), vitamin E, vitamin D, magnesium, zinc and amino acids that are precursors to neurotransmitters (Mörkl et al. 2018; Morris 2016; Lakhan and Vieira 2008).

As known, apart from genetics, the potential cause of mental disorders is inflammationand neurotransmitter imbalance with growing evidence that nutrition and gut microbiota play an important role in mental health through anti-inflammatory and anti-apoptotic as well as neurogenesis supporting effects (Mörkl et al. 2018). Recently conducted studies show that traditional whole-food diets such as Mediterranean style diet that includes high consumption of fish, olive oil, vegetables, fruits, legumes, nuts and seeds, can help patients with mental disorders and might reduce the risk of developing depression in healthy population (Mörkl et al. 2018; Bersani et al. 2017). Increased consumption of nutrient-dense food, high in fiber and omega-3 polyunsaturated fatty acids and low in sugar and saturated *trans* fats provide the foundation for optimal brain function (Opie et al. 2017; Morris 2016) through the modified synthesis of neurotrophins and neurotransmitters as well as reduced neuroinflammation.

Deficiency in neurotransmitter precursors such as amino acid tryptophan, tyrosine, and its precursor phenylalanine as well as S-adenosylmethionine that facilitates the production of neurotransmitters in the brain lead to the mental disorders (Lakhan and Vieira 2008). Neurotransmitters are also generated in the gut by the gut microbiota. Namely, gut bacteria could synthesize 5-hydroxytryptamine (5-HT), gamma-amino acid, butyric acid, dopamine and short-chain fatty acids (Wang and Wang 2016).

In addition, a reduced level of plasma brain-derived neurotrophic factor (BDNF) that is essential for axonal growth, neuronal survival and plasticity is observed in patients with depression (Opie et al. 2017). Proteins provide amino acids that are neurotransmitter precursors and vitamin B play a significant role in the synthesis of these neurotransmitters. On the other hand, lipids and essential fatty acids from phospholipids in brain cell membranes ensure the membrane integrity and maintain the release of neurotransmitters, cytokines and hormones (Davison et al. 2012).

Oxidative stress and inflammatory processes contribute to neurodegeneration and psychiatric disorders (Scapagnini et al. 2012; Ng et al. 2008; Pandya et al. 2013). Antioxidants such as vitamins and minerals and polyphenols reduce the negative effects of oxidative stress, but other mechanisms also can reduce inflammation markers through a specific impact on intestinal microbiota. The enteric nervous system is connected to the central nervous system with a bidirectional communication pathway. Therefore, our dietary habits that modulate gut bacteria and its metabolites have an impact on the overall inflammation including neuroinflammation that is known to be involved in the mental disorder pathogenesis (Mörkl et al. 2018; Opie et al. 2017).

8.2 Polyphenolics as Healthy Food Ingredients

A great number of recent studies deal with polyphenols as one of the most promising healthy food ingredients. Many scientists support the attitude that the increase in and regular consumption of this kind of food is linked to numerous health benefits. The main source of of dietary polyphenols are vegetable, fruit and some legumes. It was estimated that the average daily intake of dietary polyphenols is nearly 1 g/person (Scalbert and Williamson 2000). Many scientists support the attitude that the increase in and regular consumption of this kind of food reduce the risk of various chronic diseases such are obesity, cardiovascular diseases, diabetes, and even certain types of cancer. The health benefit of polyphenolics is due to the wide spectrum of their biological activities: antioxidant, antimicrobial, anti-inflammatory, anti-aging, imunomodulatory, hemopreventive, anticarcinogen, anti-atherosclerosis, anti-angiogenic etc. Han et al. (2007), Cvejic and Gojkovic-Bukarica (2016), Raškovic et al. (2019). These activities are due to their specific structures, which enable them to interfere in many biochemical reactions, acting as enzyme inhibitors (activators), scavengers of reactive free radicals, activators or suppressors of particular signaling pathways, interrupting or inducing gene expressions etc.

8.2.1 Chemical Diversity and Natural Sources of Polyphenolics

Polyphenolic compounds are widely distributed in plants, where they are incorporated in many physiological, mainly defense mechanisms. They are one of the largest groups of secondary plant metabolites, with diverse structures, from simple phenols to large polymers such are tannins and procyanidols. Till now several thousand polyphenols in plants have been identified and classified in several ways (Belščak-Cvtanovic et al. 2018). Based on their chemical structure and carbon chain, they are divided into 16 major classes: simple phenols, benzoquinones, phenolic acids, phenylacetic acids, acetophenones, phenylpropanoids (hydroxycinnamic acids, coumarins, isocoumarins, chromones), naphtoquinones, xanthones, stilbenes, antraquinones, flavonoids, lignins (Harborne 1989).

With regard to their role in nutrition, a group of dietary polyphenols can be distinguished. They are defined as a large group of molecules contained in plant-derived foods commonly consumed as fruits, vegetables, herbs, and beverages. Among them, five major classes are recognized: phenolic acids (hydroxybenzoic and cinnamic acid derivatives), flavonoids (flavones, flavonols, flavanones, flavanols, omegaavones, catechins, anthocyanidins etc.), stilbenes and lignans (Han et al. 2007). It is important to note that many kinds of cereal, like wheat, barley, corn, millets, sorghum, rice and rye which are widely used in the everyday diet also contain various polyphenolics that enhance their healthy properties (Shahidi and Ambigaipalan 2015).

The most abundant polyphenols in the diet are phenolic acids and flavonoids. Phenolic acids are highly distributed in vegetables, fruit and beverages, especially coffee, tea, and beer. They are found in cereals such are wheat (caffeic, vanillic, ferulic gentisic, p-coumaric acids), barley (salicylic, p-hydroxybenzoic, p-coumaric, syringic acids, also flavonoids anthocyanins, proanthocyanidins etc.), sorghum (protocatechuic acid, caffeic acid, cinnamic and vanillic acids). Chlorogenic acid (3-caffeoyilquinic acid) is the main phenolic in potato extract and is identified as a major antioxidant and critical anti-proliferative compound in many cancer cells (Roleira et al. 2015).

The most common flavonoids in the diet are flavones and flavonols. Flavanones are highly presented in fruit, chocolate, tea and coffee; flavonols (quercetin and its derivatives) in various foods: vegetables (celery, broccoli spinach, onions), cereals (beans, sorghum), fruit (apples, cranberry, blueberries), spices, red wines etc., flavones (mainly luteolin) in celery seeds, parsley, broccoli, millets, legumes and many others, anthocyanins, in red fruit, cherries, plums, strawberries and oranges, proanthocyanidins in berries, also in nuts, beans and some cereals; stilbenes in grapes and red wine (Atanackovic et al. 2012; Cvejić Hogervorst et al. 2018, 2019; Han et al. 2007; Cvejić et al. 2017).

8.2.2 Antioxidant Activity of Dietary Polyphenolic

One of the most important features of phenolic compounds is their antioxidant potential. Numerous papers deal with the antioxidant activity of various phenolics, especially those present in food. Their presence in food is very important in attributing food as functional or healthy.

The antioxidant potential of particular phenolic compounds mostly depends on the number and arrangement of hydroxyl groups and their ability to donate hydrogen or electron, and thus inactivate reactive radical species such are hydroxyl radicals, alkyl peroxyl radicals, superoxide and many others. Besides, plant phenolics with two adjacent –OH groups or other chelating structures can bind transition metal ions (TMI) and prevent TMI-driven generation of harmful reactive oxygen species (Rice-Evans et al. 1997).

Among all phenolics, flavonoids and phenolic acids have been distinguished as the major antioxidants in food. Their activity is related to their structural features but also varies in dependence on the environmental systems, e.g. lipophilic or hydrophilic.

Flavonoids According to Rice-Evans et al. (1996), the main relevant criteria for radical scavenger effectiveness of flavonoids are:

1. the *o*-dihydroxy structure in the B-ring which is important for the higher stability of radical form as a consequence of electron delocalization. It was found that 3′,4′-hydroxyl groups in the B-ring contribute about 25% to luteolin antioxidant activity.
2. The importance of the 2,3-double bond in flavonoid rings may be seen by comparing the antioxidant activity of catechin (with three hydroxyl groups in the B-ring) and epigallocathehin-gallate with additional gallic acid moiety (three phenolic groups) with quercetin, which has fewer hydroxyl groups but the presence of the 2,3-double bond. It was found that this structural feature doubles the antioxidant activity of quercetin (Salah et al. 1995). However, in contrast to the aqueous phase, the significance of the 2,3-double bond decreases in lipophilic interactions.
3. Free hydroxyl groups (3-OH group in A- and 5-OH C-ring) together with the 4-oxo group contribute to overall antioxidant capacity. It is evident that glycosylation of free hydroxyl groups diminishes the antioxidant potential of all flavonoids.
4. Metal-chelating- potential: one more structural feature of flavonoids is their ability to chelate metal ions, especially iron and copper, thus preventing the formation of ROS. For this property, the most important are the *o*-diphenolic groups in the 3′,4′-position in the ring B and the ketol structure, 4-oxo, 3-OH or 4-oxo, 5-OH in the C ring of the flavonols (Fig. 8.1)

Phenolic Acids Concerning phenolic acids as dietary antioxidants two classes are distinguished: hydroxybenzoic acids and hydroxycinnamic acids. Their antioxidant activity is correlated with the numbers of free hydroxyl groups whereas the carboxyl

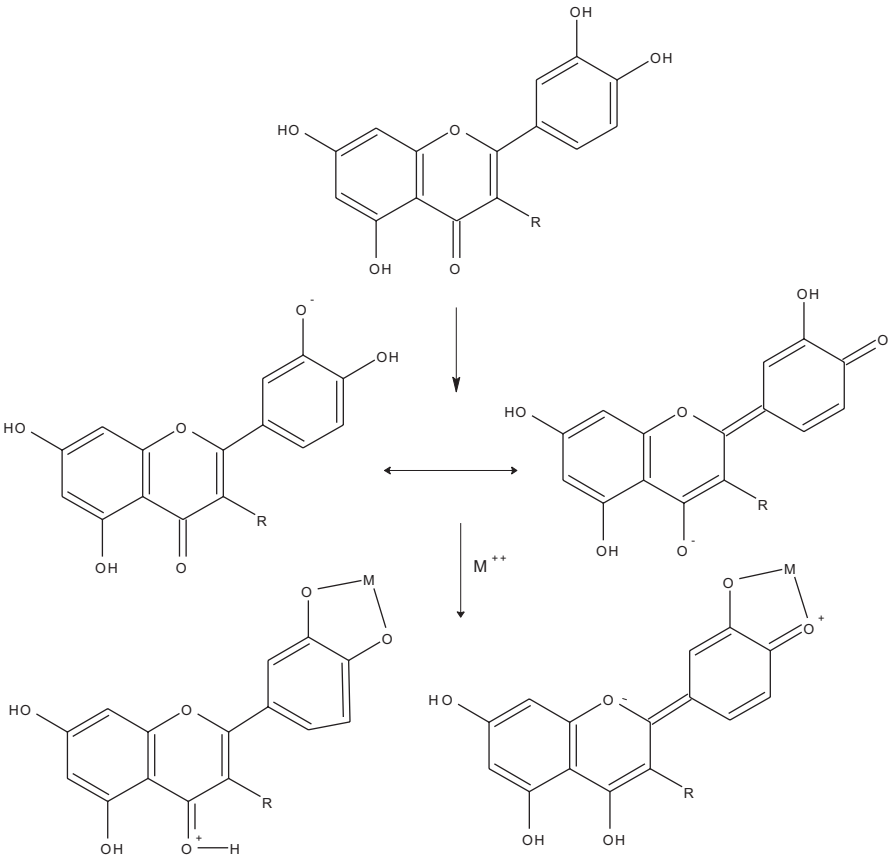


Fig. 8.1 Mechanism of metal-chelating reactions of flavonoids

group, directly attached to the benzene ring, has a negative influence on the H-donating abilities of phenolic groups, thus diminishing their potential. In the case of hydroxybenzoic acids, the number and position of hydroxyl groups, as well as the proximity of the carboxylic group, determine their antioxidant potential. It was found that derivatives with the *o*-diphenolic group in *m*-position towards a single carboxylic group (such as resorcylic acid) have the highest antioxidant potential. Incorporation of an additional $–CH_2$ group in hydroxyphenyl acetic acid enhances antioxidant potential, decreasing the influence of the carboxylate group and its electron-withdrawing effect. Gallic acid, with three hydroxyl groups, exhibits the highest antioxidant potential in comparison to others (Chen et al. 2015). In phenylpropanoids, the additional ethylenic group significantly increases antioxidant abilities. The presence of the Ph-CH=CH-COOH group ensures greater H-donating ability and radical stabilization than in benzoic acid derivatives (Leopoldini et al. 2011). Therefore, widely distributed hydroxycinnamic acids such as caffeic, ferulic or chlorogenic, are considered to be powerful dietary antioxidants. Hydroxylation

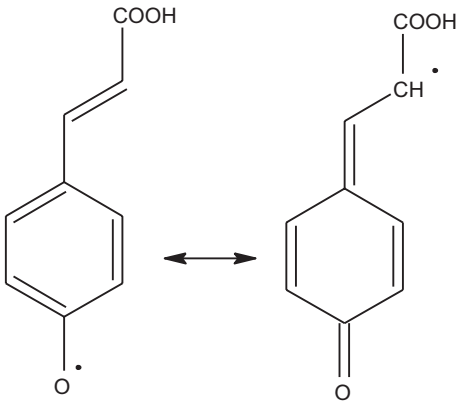
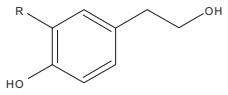


Fig. 8.2 Resonance structure of *p*-coumaric acid

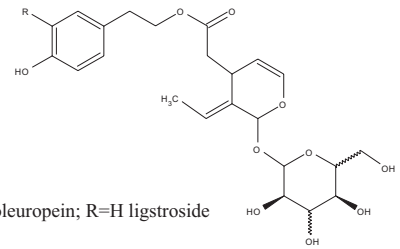
in the 3,4-position enhances antioxidant activity. This could be explained by the delocalization of unpaired electrons derived from 3,4-hydroxyl groups with the –CH=CH-COOH group, which contributes to the energetic stability of formed phenoxyl radicals (Fig. 8.2).

Beside flavonoids and phenolic acids, great attention is devoted to stilbenes (1,2-diphenylethylene), in particular *trans*-resveratrol (*trans-R*, 3′,4′,5′-trihydroxystilbene) and its glucoside. These phenolics are synthesized in plants in response to pathogen attack and are classified as phytoalexins. Hundreds of studies report the beneficial effect of resorcinol on the neurological and cardiovascular systems, also in the prevention and reduction of cancer diseases (Almagro et al. 2013). The antioxidant activity of resveratrol is documented by numerous studies. It was reported that the ability of resveratrol to neutralize different radical species as well as its metal chelating activity is significantly higher than synthetic antioxidant and α-tocopherol (Gǔlcin 2010). Another phenolic compound currently receiving worldwide attention is a diarylheptanoid, curcumin (diferuloylmethane), whose main compound in turmeric (*Curcuma longa*). Most of its health benefits are explained through its antioxidant and anti-inflammatory potential (Hewlings and Kalman 2017). A meta-analysis of randomized control data shows that supplementation with curcuminoids significantly improves antioxidant status in experimental groups, by enhancing plasma antioxidant enzymes (SOD and CAT) and glutathione GSH (Sahebkar et al. 2015). The mechanism of radical scavenging ability of curcumin is presented in Fig. 8.3.

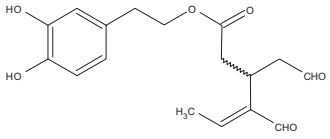
Impact on Lipid Peroxidation the ability to inhibit lipid peroxidation is one of the most important features of flavonoids, especially in regard to their health benefits in preventing cardiovascular diseases and arthritis (Mimica-Dukić et al. 2012). Free radical-mediated peroxidation of unsaturated fatty acids leads to their decomposition and the formation of lipid peroxyl radicals and lipid peroxides. It is well-known that polyphenols can intercept these chain reactions by reducing generated lipid radicals and hydroperoxides. It was found that quercetin was more effective than



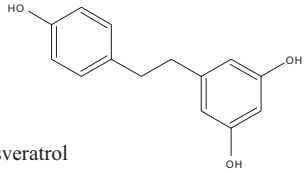
R=OH hydroxy tyrosil; R = H tyrosyl



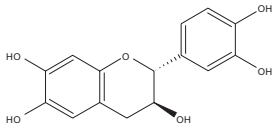
R= OH oleuropein; R=H ligstroside

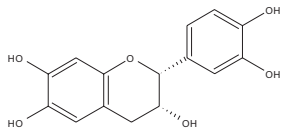


oleacin



resveratrol





(+)-catechin(-)-epicatechin

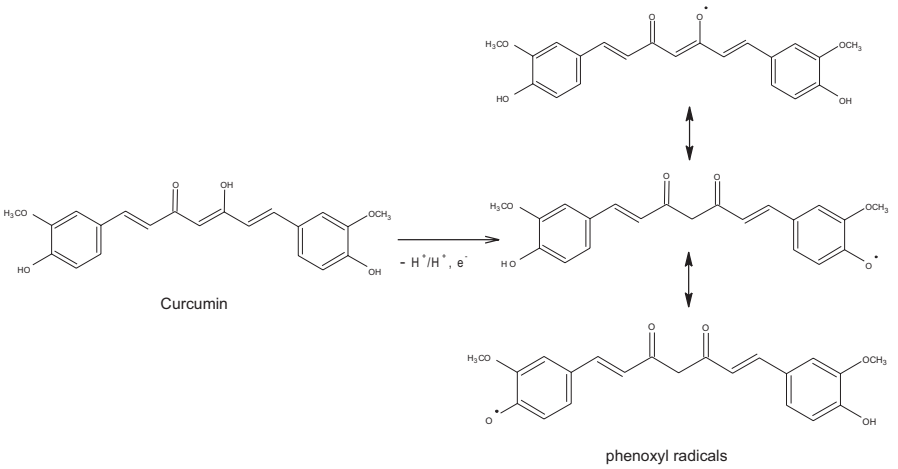


Fig. 8.3 Mechanism of radical scavenger activity of curcumin

catechin in the protection of LDL from oxidation (Rice-Evans et al. 1996; Shahidi and Ambigaipalan 2015).

However, some studies indicate that plant polyphenolic can exert prooxidant activity, especially in the presence of a higher concentration of metal ions (TMI, Fe and Cu). The direct prooxidant activity is the result of the generation of phenoxyl radicals or complex with TMI, which can induce lipid peroxidation, DNA damage and mutagenesis. Besides, it was reported that high concentrations (100 mM) of flavonols, myricetin and gossypetin, can affect LDL by covalent modification of the apoB100 protein. Even so, it is unlikely that these polyphenols will achieve so high concentrations in vivo (Rice-Evans et al. 1996).

Yang et al. (2012) explored the structure-related antioxidant/prooxidant activates of quercetin and p-coumaric acids and their derivatives by molecular modeling, using NADPH/peroxidase/H_2O_2 and DNA cleavage systems. They discovered that prooxidant activity in the NADPH/peroxidase/H_2O_2 system was in decreasing order, quercetin 3-O-glucoside > p-coumaric acid > rutin > quercetin > ferulic acid. Similar results were obtained for DNA cleaving activity. Thus, glycosidation, number and position of hydroxyl groups, also hydrophilicity and concentration predominantly affect the prooxidant ability of certain phenolic compounds (Yang et al. 2012).

Impact on Endogenous Antioxidant Enzymes A very important role of dietary phenolics is their ability to affect antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) glutathione peroxidase (GPx), glutathione transferase (GT), peroxidase (Px) and the level of endogenic antioxidant glutathione (GSH). Many previously published results show that polyphenolic compounds improve antioxidant defense mechanisms, both in vitro and in vivo. Phenolic extracts from parsley, celery, marigold, and elderberry significantly increased antioxidant enzymes SOD, CAT, GPx and GR, and decreased lipid peroxidase (LP) in animals exposed to oxidative stress induced by carbon tetrachloride (Mimica-Dukić and Popović 2007; Jakovljević et al. 2001; Popović et al. 2001, 2005). Fernandez-Pachon et al. (2009) reported that human consumption of red wine, 300 mL/day, for 1 week, significantly increase the activities of antioxidant enzymes: SOD, CAT, GR, GPx, and overcame oxidative stress. Furthermore, they found that wine consumption increases both SOD activity and SOD gene expression. Catechin, proanthocyanidin B4, curcumin, quercetin, resveratrol significantly increases activities of SOD, GST, CAT, GPx and GR in different in vitro studies (Han et al. 2007).

Impact on Enzymes Involved in Oxidation Besides the ability to increase the activity of antioxidant enzymes, polyphenols are able to modulate enzymes involved in the oxidation process, such are cyclooxigenase (COX), lipoxygenase (LOX), inducible NO-synthase (*i*NOS). Not least is their inhibitory effect on Xanthine oxidase (XOD) and NADH-oxidase, key enzymes in a respiratory burst, which leads to the uncontrolled release of reactive oxygen species, particularly superoxide radical and hydrogenperoxide (Hussain et al. 2016). Curcumin expressed high in vivo antioxidant and protective activity in rats exposed to liver injury by increasing the

activities of antioxidant enzymes CAT and SOD while decreasing the activities of iNOS and myeloperoxidase (MPO) (Shen et al. 2007).

In addition, dietary phenolics exert their influence on human health by acting as a modulator on other enzymes closely connected with particular metabolic failure and pathological conditions. It was found that particular phenolics increase the activities of enzymes such as angiotensin I-converting enzyme (ACE), α-amylase and α-glucosidase, lipase, cholinesterases, and tyrosinase, which are related to hypertension, type II diabetes, obesity, Alzheimer's diseases, inflammation and skin hyper-pigmentation (Goncalves and Romano 2017).

8.2.3 Oxidative Stress, Immune System and Polyphenolics

Oxidative stress is an imbalance between the excessive generation of reactive oxygen (ROS) or nitrogen molecular species (RNS), and their elimination by the antioxidant systems in cells and tissues. If prolonged, overproduction of ROS/RNS can cause damage to the main cellular molecules, proteins, lipids, and DNA, resulting in the development of many chronic diseases. There is increasing evidence that oxidative stress plays an important role in pathogenesis and the development of neurodegenerative, cardiovascular and kidney diseases, diabetes, diabetic nephropathy, lung diseases, eye diseases, autoimmune diseases, liver diseases etc. (Rahman et al. 2012).

The effect of polyphenolics on immune systems has been documented by various studies. They can affect immune cells, modulate cytokine production and pro-inflammatory gene expression (Yahfoufi et al. 2018). They may also modulate immune responses by affecting? epigenetic mechanisms and selectively activate and inactivate gene expression. Curcumin from turmeric (*Curcuma longa*) and epigallocatechin gallate (EGCG) from green tea can induce epigenetic change by inhibiting DNA-methyltransferase-1 (DNMT1), the enzyme responsible for the methylation of C5 sites of cytosine in DNA molecules leading to the development of various diseases. Besides, it was found that polyphenols regulate the intestinal mucosal immune response. In vivo experiments have shown that polyphenols enhance intestinal mucosal immunity by increasing populations of intraepithelial T cells and mucosal eosinophils (Ding et al. 2018). Polyphenols participate in immune systems responses by modulating different signaling pathways. They can bind to receptors on immune cells and thus trigger intracellular signaling pathways:

a. the nuclear factor NFkB signaling pathway which plays a key role in DNA transcription, cytokine production, and cell survival. Its activation is control-inhibited by IkB proteins (IkBs). Phosphorylation of IkB leads to ubiquitination and degradation of IkBs, leading to activation of NFkB and expression of proinflammatory cytokines, chemokines, immunoreceptors, growth factors, NOS, COX-2 etc. (Yahfoufi et al. 2018). It was reported that several phenolics compounds modulate NFkB activation and reduce inflammation. The most potent are quer-

cetin luteolin, isoflain, catechin and epicatechin, hydroxytyrosol etc. (Rahman et al. 2006). This is important considering that the disorder of NFkB has already been confirmed as being associated with cancer, inflammation, asthma, neurodegenerative diseases and heart diseases (Baldwin 2012).

b. The mitogen-activated protein kinases (MAPKs) signaling pathway is highly involved in cell growth, proliferation, death and differentiation. MAPKs regulate gene transcription and transcription factors involved in inflammation. The ability of polyphenols to block MAPK pathways indicates their therapeutic potential against the inflammation process. Among them, luteolin, chrysin, kaempferol and quercetin exhibited the highest potential to moderate MAPKs (Chen et al. 2004).
c. Polyphenolic compounds also participate in NO signaling pathways, improving endothelial NO-synthase (eNOS) expression and activity. Resveratrol, curcumin, quercetin and catechin in green tea were found to increase vasodilatation in coronary arteries through NO generation. Polyphenols-mediated NO signaling is of great therapeutic significance, especially with cardiovascular diseases (Forte et al. 2016);
d. Martínez-Huélamo et al. (2017) have recently summarized the results of numerous studies focused on the interaction of phenolic compounds with the nuclear transcription factor (erythroid-derived 2)-Like 2 (Nrf2) signaling pathway. Nrf2 is of crucial importance in regulating the expression of antioxidant enzymes and protein, in cells and tissues exposed to oxidative stress. According to the results presented, it seems that modulation of Nrf2 by phenolic compounds in olive oil (oleuropein, tyrosol, oleacein, ligstroside, etc.) and wine polyphenolics (quercetin, epicatechin, catechin, tyrosol, gallic acid, resveratrol, and caffeic acid) may be associated with the extent of their health benefits, with special focus on cognitive abilities and neurodegenerative disorders (Fig. 8.4)
e. However, most of these activities arise from the ability of polyphenolics to scavenge and diminish the generation of ROS and maintain redox equilibrium balance. Excessive ROS production disturbs the redox equilibriums affecting many cellular signaling pathways, which in turn leads to cellular dysfunction and the development of various diseases (Table 8.1). It was reported that ROS affects cell-signaling proteins (NF-κB, MAPKs, Keap1-Nrf2-ARE, and PI3K-Akt), ion channels and transporters (Ca^{2+} and mPTP), and modifyingprotein kinase and Ubiquitination/Proteasome System (Zhang et al. 2016). Evidently, by scavenging ROS, polyphenolic compounds significantly diminish the harmful effects of ROS.

8.2.4 Polyphenols-Oxidative Stress: Inflammation

One of the explanations of how oxidative stress influences a wide range of chronic diseases is a tight connection between oxidative stress and inflammation. The initiation of the inflammatory process is the most important physiological response of the immune system, triggered by various exogenous and endogenous inducers. The

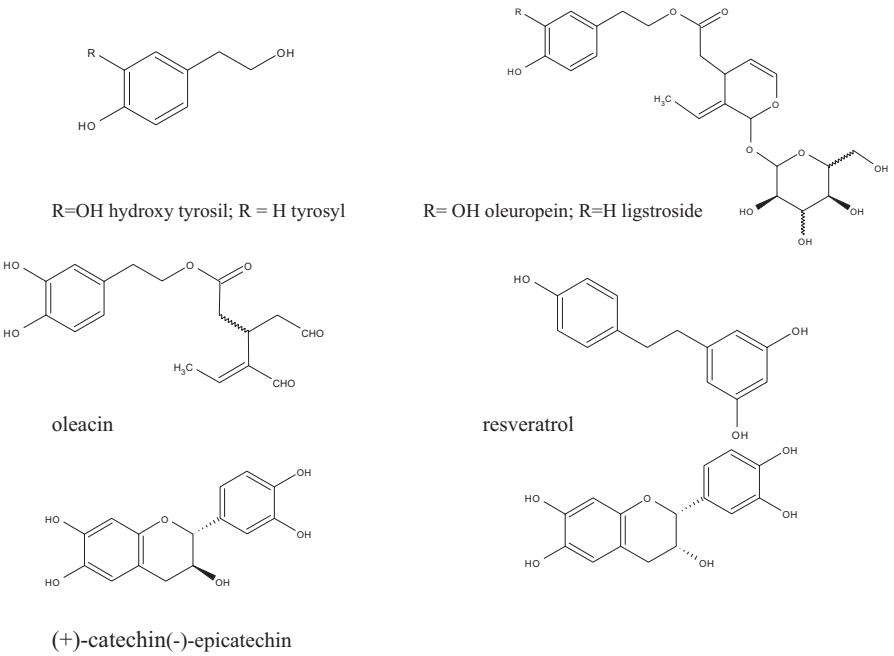


Fig. 8.4 Most active phenolic compounds in olive oil and grape wine (Martínez-Huélamo et al. 2017)

Table 8.1 ROS homeostasis in the cell

ROS homeostasis in cells									
Source of ROS	MiTRC	NOX	TNF-α	EGF	IL-1β	TNFR	cPLA2	TLR	MyD88
ROS neutralization		SOD	GPx	GST	MT3	FHC	DDH1		

MiTRC mitohondrial respiratory chain, *NOX* NADPH oxidases, *TNF-α* tumor necrosis factor-*α*, *EGF* epidermal growth factor, *IL-1β* Interleukin-1*β*, *TNFR* tumor necrosis factor receptor, *cPLA2* cytosolic phospholipases; domain, *TLR* toll-like receptor, *MyD88* myeloid differentiation factor 88, *SOD* superoxide dismutase, *GPx* glutathione peroxidase, *GS* glutathione S-transferase, *MT3* metallothionein-3, *FHC* ferritin heavy chain, *DDH1* dihydrodiol dehydrogenase

most important endogenous inducer associated with oxidative stress is the accumulation of advanced glycation end products (AGEs) and oxidized cellular lipoproteins. AGEs can attach to other proteins and inactivate them, or interact with their receptors (RAGE), stimulating several signaling pathways which in turn activate transcriptions of pro-inflammatory genes (Fishman et al. 2018). In addition, ROS produces oxidized lipids (LDL), recognized by macrophages, which will then generate various inflammatory mediators (cytokines, chemokines, vasoactive amines etc.) and promote pro-inflammatory signals. On the other hand, one of the main consequences of inflammation is the induction of oxidative stress, the production of ROS, RNS, AGEs, and several other compounds that lead to tissue damage (Colitti et al. 2019). Recently, Valacchi et al. (2018) introduced the term "*OxInflammation*"

to describe the *vicious circle* linking oxidative stress to mild chronic inflammation. Thus, oxidative stress and inflammation or immune response are in the reciprocal cause, intensifying or reducing each other. Therefore, it can be assumed that most of the harmful effects of ROS or RNS on human health are associated with the tight interaction between oxidative stress and immune response, especially inflammation. This particularly applies to neuroinflammation, neurodegeneration, arthritis, cancer and diabetes (Popa-Wagner et al. 2013). Due to high lipid content and high oxygen consumption, the brain is extremely exposed to oxidative stress. High oxygen concentration promotes the excessive generation of ROS. Apart from this, the neuronal membranes are rich in polyunsaturated fatty acids (PUFA) which are highly susceptible to ROS. The brain is also enriched with redox-metals like iron and copper that increase ROS production. All these lead to the development of various neurodegenerative diseases, especially Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), andamyotrophic lateral sclerosis (ALS) (Singh et al. 2019). Unfortunately, the current treatment of most neurodegenerative disorders, especially AD, is unsatisfactory, with poor long-term efficacy and many undesirable side effects. Therefore, many studies are focused on seeking drugs that will eliminate and reduce the main risk factors that lead to these disorders. As oxidative stress and inflammation have already been shown to enhance and initiate many neurodegenerative processes, it is reasonable to expect that polyphenolics, as proven antioxidants and anti-inflammatory agents, could be used in preventive and adjunct therapy. This is supported by in vitro and in ex vivo studies of the effect of grape seed polyphenol extract containing proanthocyanidins, catechin, epicatechin and gallic acid, on several neurodegenerative diseases. It was found that grape extract inhibits in vitro aggregation of neurotoxic amyloid-beta isoform protein (Aβ) which is one of the main factors for developing AD. Besides, grape extract attenuates the formation of tauopathies, another clinical manifestation in AD and dementia (Herman et al. 2018). Treatment with grape-seed polyphenols also improved multiple neuropathological conditions in PD model systems. Experiments on animals showed that chrysin, catechin, genstrain, quercetin and naringenin significantly reduced oxidative stress in primary rat midbrain cell cultures (Mercer et al. 2005). Many other in vitro and in vivo studies support the protective role of polyphenolics in the progression of neurodegenerative failure through its anti-inflammatory and antioxidant activities (Herman et al. 2018).

8.2.4.1 Dietary Polyphenols as Modulators of Cyclooxygenase Pathway of Arachidonic Acid Metabolism: Impact on Prostaglandin E_2 and Thromboxane A_2 Production

Eicosanoids are products of methabolic pathway of arachidonic acid (AA), a fatty acid found in in the cell membrane phospholipids. These lipids evince various biological activities in normal physiology, including vasoconstriction/dilatation, ovulation, platelet and renal function. Also, they have an important role in mediation of inflammatory response (Smith 1989; Morita 2002). In this sense, regulation of their

production has been determined as importan target for anti-inflammatory therapy. Common NSAIDs (non-steroidal anti-inflammatory drugs), which are derivatives of propionic (ibuprofen and naproxen), enolic (meloxicam and piroxicam), acetic (diclofenac, sulindac, indomethacin) or salicylic acid (aspirin), inhibit enzymes involved in AA metabolism and therefore alter eicosanoid production. These drugs are the first choice in the treatment of aches, pains or fever, regardless of certain risks and side effects, which may occur during repeatedly intake of NSAIDS over a long period (Cairns 2007). Consequently, there is a certain need to use alternative inhibitors, which can provide adequate therapeutic potential with minor side effects. For a better understanding of the anti-inflammatory activity of existing and new, potential therapeutics, such as natural products are, key targets in AA metabolism should be recognized.

AA metabolism is triggered by different inflammatory stimuli: tumor necrosis factor α, β (TNFα, β), interleukin-1 (IL-1), lipopolysaccharide (LPS), different cytokines, etc. (Tanabe and Tohnai 2002). Phospholipase A_2 (PLA_2), initially activated enzyme, releases AA from membrane phospholipids. Free AA can be converted to structurally diverse eicosanoids by three pathways determined by three classes of enzymes: cyclooxygenases (COX), lipoxygenases (LOX) and epoxygenases.

The most important step of the COX pathway is dependent on COX-1 and COX-2 (prostaglandin H synthase-1 and -2 (PGHS-1/2)), since the expression and activity of these enzymes direct amount of common intermedier prostaglandin (PG) H_2. The reaction catalyzed by COX has two phases: it begins with the formation of the cyclic endoperoxide PGG_2 from AA, which is then reduced to PGH_2. PGH_2 is transformed by different, terminal synthases (prostaglandin and thromboxane) to PGE_2, PGI_2 (prostacyclin), PGD_2, $PGF_{2\alpha}$, and thromboxane A_2 (TXA_2) (Chandrasekharan and Simmons 2004; Smith 1989). These products exert wide range of activities: PGE_2 and PGI_2 have gastroprotective role in the gastric mucosa, and participate in the regulation of salt and water excretion in the kidney; overexpressed PGE_2 can cause inflammation and pain in joints, as well as pain and fever in the central nervous system; PGI_2 produced in endothelial cells effects platelet inhibition and vasodilatation; TXA_2 in is a regulator of platelet activation and vasoconstriction (Cairns 2007). So, modulation of PGs and TXs production can be considered as tool for modulation of pain, fever, thrombosis and overall inflammation processes.

It is obvious that dietary polyphenols have been extensively investigated in numerous in vitro model systems which are related to inflammation, in vivo studies on animals were also done, but data on human studies are quite limited. Also, although dietary polyphenols are ubiquitously found in vegetables, fruits, and plant-based beverages, the most studies consider isolated compounds, some metabolites, usually plant extracts and eventually whole foods, and therefore enabling to determine anti-inflammatory potential, rather than claiming on real activity of particular food (García-Lafuente et al. 2009; Mitjavila and Moreno 2012; Roleira et al. 2015).

In terms of AA metabolism, most of the researches was focused on COX-2 inhibition, followed by issues on COX-1/2 selectivity. Although COX-1 and COX-2 have almost identical structures, affinities to same supstrates and catalyze

same reactions, their functions are different. Constitutively expressed COX-1, present in almost all cells, is involved in gastric protection, platelet aggregation and renal water balance. During inflammation, COX-2 is highly up-regulated particularly in the macrophages, monocytes, fibroblasts and endothelial cells (Smith 1989; Morita 2002). Overexpression of COX-2 leads to overexpression of PGs and TXAs, thus contributing to numerous pathological processes. So, COX-2 became a significant therapeutic target for inflammation, and even selective COX-2 inhibitors were developed (coxibs). But, coxibs, as well as NSAIDs have certain side effects, and, alternative inhibitors (natural products) with minor side effects are particularly needed.

Therefore, this chapter will provide a general overview of the most apparent findings on the common polyphenols which can modulate AA metabolism at certain points—enzymes involved in cyclooxygenase pathway and consequent reduction of PGE_2 and TXA_2 production.

Dietary Polyphenols as PLA_2 Inhibitors

PLA_2 family includes at least 10 members, which are classified into three groups: secretory ($sPLA_2$), cytosolic ($cPLA_2$) and calcium-independent ($iPLA_2$). Increase of PLA_2 leads to increased release of AA and consequently increased production of PGs and TXs. This can contribute to development of cardiovascular diseases, arthritis, inflammatory gastrointestinal disorders and disturbed neuronal homeostasis. PLA_2, which is normally expressed in the pancreatic, gall bladder and gastrointestinal epithelial cells, is upregulated in ulcerative colitis and Crohn's disease. Upregulation of gastrointestinal PLA_2 effects gut permeability, thus contributing to infectivity (Haapamäki et al. 1999). Also, it was found that cPLA2 is involved in the pathogenesis of multiple sclerosis-like diseases (Kalyvas and David 2004). It was proven, in mouse models of Alzheimer's disease, that released AA alter neuronal and synaptic activity (Sanchez-Mejia and Mucke 2010).

Several polyphenolics have been tested and they differently modulate PLA_2 activity: quercetin and rutin selectively inhibit groups of PLA_2; curcumin inhibits phosphorylation of cPLA2, and therefore inhibit activation; catechin and anthocyanidins cyanidin, malvidin, peonidin, petunidin, delphinidin, and pelargonidin act also as PLA_2 inhibitors, and stilbene resveratrol suppressed PLA_2 expression by reducing oxidative stress (Lindahl and Tagesson 1993; Lindahl and Tagesson 1997; Hong et al. 2004; Dreiseitel et al. 2009; Sun et al. 2017). Although these results can suggest that, for example, foods rich in quercetin can be potential ant-inflammatory agents or that a modulatory role for berry polyphenols in phospholipid metabolism can be suggested according to content and activity of anthocyanins, only several papers consider whole plants (extracts). Among several medicinal plants used for skincare and beauty, water extracts of *Cassipourea flanaganii* (Schinz) Alston. and ethanolic extracts of well-known medicinal food *Andrographis paniculata* Nees expressed notably in vitro inhibitory activity against $sPLA_2$ (Kishore et al. 2016; Thibane et al. 2019). According to experts opinion, until 2016 none of the synthetic inhibitors studied in clinical trials have reached the market (Kokotou et al. 2017).

Dietary Polyphenols as COX-1 and COX-2 Inhibitors

Since the COX activity is the crucial step in PGs and TXA2 production, this enzyme has been in the focus of eicosanoid-related inflammation processes. Unmitigated production of PGs and TXA2, mainly caused by rapid induction of COX-2 in inflammation processes, impaired renal function, GI tract integrity, nerve and brain, ovarian and uterine function, and thrombosis in individual tissues and organs. In addition to different physiologic and pathophysiologic roles of COX-1 and COX-2, side effects of common NSAIDs (gastric ulceration, complications of gastrointestinal bleeding, perforation, obstruction, renal dysfunction), as a result of non-selective COX-1 inhibition, and increased risk of cardiovascular issues after long-term treatment with COX-2 selective inhibitors, directed search for novel, natural inhibitors.

The anti-inflammatory activity of polyphenols can be, at least partially, attributed to modulation of COX activities on both transcriptional and enzyme levels. One of the first evidence was that quercetin can inhibit PLA_2, and that luteolin, galangin and morin inhibit COX (Bauman et al. 1980; Lee et al. 1982). Considering cyclooxygenase inhibitory activity, some conclusions on the structural characteristics of flavonoids and activity relationship were reached.

COX-1 inhibitory activity is typical for molecules with the C2-C3 double bond, which is important for the planarity of the molecule (Fig. 8.5). Oppposite findings are stated for 3-OH group in C ring: according to Wang and Wang (2016), it can diminish activity or, according to Roleira et al. (2015), it has no effect. The example of a good COX-1 inhibitor (Roleira et al. 2015), flavone **1** is shown in Fig. 8.5.

Structure of the most active COX-2 inhibitors is characterized by 4-oxo group, C2-C3 double bond, as well as OH groups in C5 and C7 positions (C ring). 3′,4′-Dihydroxyl moiety in B ring lowers the potency of COX-2 inhibitors, and additional B-ring hydroxyl group leads to the loss of inhibitory activity (Takano-Ishikawa et al. 2006). But, these rules were not in total agreement with the results of Ribeiro et al. (2015a, b). Nevertheless, docking study showed that catechol moiety forms hydrogen bonds with Tyr385 and Ser530 in hydrophobic pocket of enzyme and strengthen binding of flavone. The other explanation would be that overall activity of these compounds can be rather consequence of scavenging activity, which results in reduced amount of pro-oxidant reactive species implicated in overexpression of COX-2 (Takano-Ishikawa et al. 2006; Mello et al. 2011). The examples of good COX-2 inhibitors (Takano-Ishikawa et al. 2006; Ribeiro et al. 2015a, b), flavone 2 and baicalein (3) are shown in Fig. 8.5.

The activity of glycosides of various flavonoids was also tested, and Takano-Ishikawa et al. (2006) found that they exhibit lower activity than their aglycones. It can be, to some extent, accounted to their lower permeability through the cell membrane.

Some structural features of flavonoids were correlated with COX-1/2 selectivity: likely, less substituted flavonoids were more potent inhibitors of COX-1 than COX-2, since COX-1 active site has a smaller volume (Ribeiro et al. 2015a, b). According to Ribeiro et al. (2015), the flavonoids **4** and **5** are the examples of potent selective COX-2 inhibitors. Interestingly, some plant extracts could be selective also, as it was demonstrated for chamomile extract (Srivastava et al. 2010).

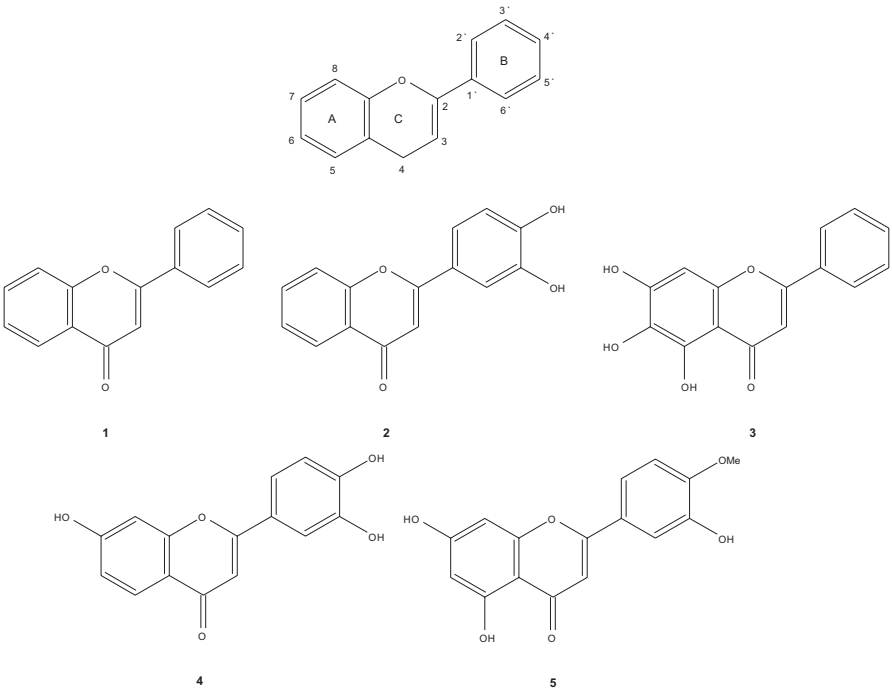


Fig. 8.5 The general structure of flavones, potent COX-1 inhibitors (**1**), COX-2 inhibitors (**2**, **3**), and selective COX-1/2 inhibitors (**4**, **5**)

To some extent, potency of COX-inhibition and COX-selectivity can be predicted according to structural properties of flavonoids. But, plant extracts are mixtures of numerous compounds, which are combined in different ratios and can exert synergistic (or antagonistic) activities. To determine their activity, in vitro and in vivo studies are undoubtedly needed and present certain challenge. An impressive number of in vitro researches have been done in order to prove COX-2 (COX-1) inhibitory activity of either isolated natural compounds or different plant extracts (Attiq et al. 2018; Kim and Park 2019; Bakar et al. 2018; Beara et al. 2010, 2012a, 2014, 2015; Lesjak et al. 2011, 2014; Beara et al. b; Nađpal et al. 2016, 2018; Šavikin et al. 2017).

Dietary Polyphenols as TXAS Inhibitors

TXAS catalyzes the final step in TXA_2 synthesis, and its inhibition can disturb TXA2 production and activity leading to modulation of platelet function and reduced risk of cardiovascular diseases. Also, increased TXAS expression occurs in active inflammatory bowel disease, that contribute to mucosal inflammation and intramucosal thrombogenesis (Lipsky et al. 2000). Since direct inhibition of TXAS can lead to the accumulation of PGH_2, a precursor of TXA_2, alternative dual inhibitors of both enzyme and corresponding receptors were found to be promising anti-angiogenic agents (Leval et al. 2006). But, there are evidence that effect on platelet

activity, which can be at least partially caused by TXA_2 modulation, could be achieved by consumption of different foods rich in polyphenols, such as garlic and onion (Moon et al. 2000; Ro et al. 2015; Simin et al. 2013), ginko (Kudolo et al. 2002), ginseng extracts (Jin et al. 2007; Lee et al. 2012), and even red wine (Renaud and de Lorgeril 1992; Majkić et al. 2019). Regarding isolated compounds, TXAS can be inhibited by fisetin, kaempferol, morin and quercetin (Tzeng et al. 1991; Lesjak et al. 2018), as well as green tea catechins (Son et al. 2004), while genistein, apigenin, quercetin and luteolin are able to bind and block TXA2 receptors (Guerrero et al. 2007).

Dietary Polyphenols as PGES Inhibitors

PGES include three groups: constitutive cytosolic PGES (cPGES), coupled with COX-1, constitutive membrane PGES-2 (mPGES-2) and inducible mPGES-1, which is coupled with COX-2. Ever since its role was discovered, mPGES-1 was targeted as a point for regulation of inflammation and its inhibitors were found to be a possible alternative to NSAID-s (Koeberle et al. 2016). Also, mPGES-1 is up-regulated in the dopaminergic neurons of patients with diagnosed Parkinson's disease, as well as in intestinal-type gastric adenocarcinomas and gastric cancer cell lines (Ikeda-Matsuo et al. 2019; van Rees et al. 2003).

Studies have been shown that curcumin and epigallocatechin gallate from green tea inhibit mPGES-1 dependent production of PGE_2 at sub-molar concentrations, exerting significantly stronger activity than activity against COX-2 (Koeberle et al. 2009a, b), while ellagic acid (Karlsson et al. 2010), kaempferol and isorhamnetin (Hämäläinen et al. 2011) inhibit LSP-induced expression of mPGES. Some other natural products, such as hyperforin from St. John's Wort (Koeberle et al. 2011), boswellic acids and some other triterpenoic acids from frankincense (Verhoff et al. 2014), embelin from fruits of Embelia ribes (Schaible et al. 2013) etc. It is interesting that most of these compounds also inhibited 5-lipooxygenase, thus presenting a new class of dual 5-LO/mPGES-1 inhibitors.

8.3 Omega-3 Fatty Acids

8.3.1 Main Compounds and Sources

Essential fatty acids are linoleic acid or LA (18:2n-6) and α-linolenic acid or ALA (18:3n-3), polyunsaturated fatty acids (PUFAs) with 18 carbon atoms, belonging to n-6 PUFAs (omega-6) and n-3 PUFAs (omega-3)families, respectively. These are called essential fatty acids as cannot be produced in the human body and must be taken from food (Wysoczanski et al. 2016). Fish and seafood are the main sources of omega-3 fatty acids, but vegetables and seed oils (e.g. flax, soy, canola, olive and walnut), as well as algae, also provide these fatty acids (Wysoczanski et al. 2016; Grosso et al. 2016; Cvejić Hogervorst et al. 2019).

After consumption, linoleic acid is transformed into arachidonic acid (AA), a precursor of cytokines that facilitate inflammation. On the other hand, α-Linolenic

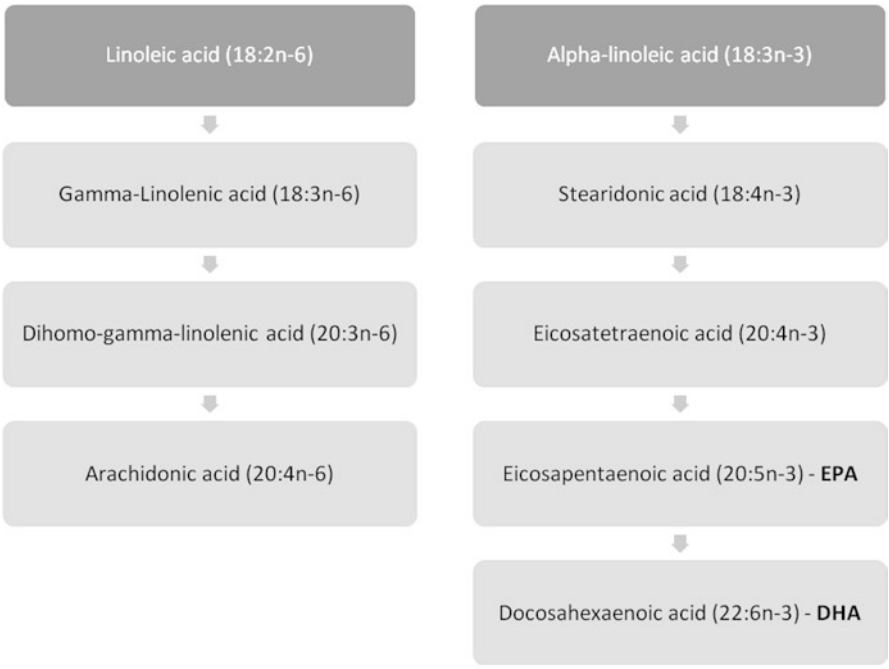
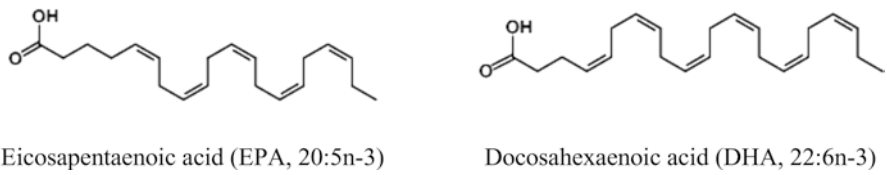


Fig. 8.6 Essential fatty acids derivatives—transformations that occur in the organism



Eicosapentaenoic acid (EPA, 20:5n-3) Docosahexaenoic acid (DHA, 22:6n-3)

Fig. 8.7 Structure of EPA and DHA

acid is transformed into eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) that have anti-inflammatory effects (Figs. 8.6 and 8.7).

Furthermore, omega-3 PUFAs that increase EPA in the cell membrane compete with the enzymes that convert AA into pro-inflammatory omega-6 eicosanoids. Increased omega-3 dietary intake with an omega-6/omega-3 ratio not above 5 should help in maintaining the non-inflammatory eicosanoid balance that consequently influences the cytokine balance (Wysoczanski et al. 2016; Grosso et al. 2014). DHA is essential for the proper function of the brain and retina. It builds the neuronal phospholipids membranes and positively modifies the immune and inflammatory response (Wysoczanski et al. 2016; Grosso et al. 2016). There is also evidence that omega-3 facilitates serotonin release by membrane fluidity increase and inhibition of prostaglandin formation (Patrick and Ames 2015). Furthermore, these essential

fatty acids are involved in neurogenesis and neuroplasticity, and through that can have positive effects on mental disorders, especially depression (Bourre 2004).

Deficiency in omega-3 fatty acids reduces vision and cause a decrease in cognitive and behavioral functioning as per the research on rodents (Wysoczanski et al. 2016; Fedorova et al. 2009). Results of the first meta-analysis of all observational studies concerning the influence of omega-3 intake on the decreased depression risk conducted by Grosso et al. support the initial hypothesis that consumption of dietary omega-3 fatty acids decreases the potential of depression development. It is also shown that EPA has better therapeutic effects on the depressive symptoms than DHA (Politi et al. 2013; Rizzo et al. 2012; Rondanelli et al. 2010) and the possible reason might be higher EPA's anti-inflammatory action, but further research is required in order to better understand the specific EPA and DHA roles (Grosso et al. 2016). On the other hand, DHA might have a more significant effect on the cortical and hippocampal atrophy due to its neuroprotective properties. It has experimentally shown that DHA has a regulatory role in apoptotic processes and consequently improve neuron survival (Reimers and Ljung 2019).

8.3.2 Biological Activity

Anti-Inflammatory Effects of Omega-3 Fatty Acids

Established connection between immune and nervous systems enables the direct influence of one system to another. Even though the mechanisms of immune system influence on the proper brain function are still to be clarified, it is evident that immune dysregulation promotes neurodevelopmental disorders. The immune system provides defense against pathogens in the first line by phagocytes (macrophages)and granulocytes (neutrophils). Microglia are myeloid glial cells located throughout the brain, and spinal cord that are brain resident macrophage cells and they act as the first and main form of active immune defense in the central nervous system (CNS). These cells are responsible for the production of both pro-inflammatory and anti-inflammatory cytokines. When there is an imbalance in the immune molecules, microglial response is triggered including the increased production of pro-inflammatory molecules such as tumor necrosis factor (TNF), interlukin-1β (IL-1β) and interleukin 6 (IL-6) that promotes neuronal damage leading to the brain pathologies (Laye et al. 2018; Filiano et al. 1617; Hsiao and Patterson 2012). Therefore, any substance that can limit the inflammation should be a new research target when it comes to the prevention and treatment of mental disorders. The influence of bioactive dietary components and omega-3 on the neuroinflammation is now becoming evident (Davinelli et al. 2016; Hoppenbrouwers et al. 2019).

Omega-3 fatty acids decrease inflammation through the following mechanisms:

1. *Modulation of signaling pathways*

Incorporation of DHA into membrane phospholipids alters receptor-signaling interactions. Also, the DHA level influences the membrane fluidity and the localization of several pro-inflammatory receptors leading to decreases pro-inflammatory activity (Laye et al. 2018).

2. *Control of gene expression*

Omega-3 fatty acids alter the signaling pathways controlling the expression of genes that encode the proteins involved in inflammation (many cytokines, adhesion molecules, and COX-2). These effects can be explained by DHA's and possibly EPA's ability to disrupt membrane lipid raft formation of inflammatory cells. In that way, nuclear factor kappa B (NF-κB) and TLR4 activation are reduced and inflammatory signaling initiated. Another mechanism of DHA's and EPA's action is an activation of peroxisome proliferator-activated receptor γ (PPAR-γ) that is an anti-inflammatory transcription factor that also inhibits NF-κB activation and therefore reduces the production of cytokines, TNF-α and IL-6 (Calder 2017, 2015).

3. *Reduction of pro-inflammatory eicosanoids*

Oxidation of EPA and DHA leads to the synthesis of eicosanoids (prostaglandins, thromboxane, and leukotrienes) that are anti-inflammatory signaling molecules. These eicosanoids antagonize the pro-inflammatory eicosanoids produced from AA and by competing for the same enzymes involved in the synthesis and consequently reduce the production of AA derived eicosanoids (Laye et al. 2018; Calder 2017).

4. *Effects on microglia*

In vitro studies showed that omega-3 fatty acids have anti-inflammatory effects on microglia. More in vivo studies are need in order to confirm omega-3 PUFAs modulation effects on microglia. In vivo studies conducted so far demonstrated that low dietary intake of omega-3 fatty acids during the perinatal period causes the enhanced phagocytic activity of microglia in the offspring (Laye et al. 2018).

The recommended daily intake of omega-3 polyunsaturated fatty acids for an anti-inflammatory effect is up to 1.8 g (Grosso et al. 2016). The aim of increased dietary intake of omega-3 fatty acids is to maintain the high level of blood EPA and DHA and consequently their increase in the brain. Brain PUFAs control microglia activity and its role in neuro-inflammatory processes. Besides, changes in the composition of cell membranes caused by increased EPA and DHA content lead to changes in lipid raft formation and signaling pathways as well as alterations of gene expression and production of eicosanoids and other anti-inflammatory signaling molecules (Laye et al. 2018; Calder 2017).

Omega-3 Fatty Acids and Gut Microbiome

The gut microbiome is a community of trillions of bacteria and fungi that inhabit the gastrointestinal tract and have an essential influence on the host's susceptibility to disease. There are thousands of different species, but approximately 60% are from phyla *Bacteroidetes* and *Firmicutes*. Among them, the most common genera are *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *Clostridium*, *Escherichia*, *Streptococcus*, and *Ruminococcus* (Costantini et al. 2017). The other species that

are most abundant belong to phyla *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia* and *Cyanobacteria* (Li et al. 2018).

These bacteria could improve the food fermentation and enhances the host's uptake of nutrients by processing indigestible food components. However, this is not the only function that gut bacteria have, it also has a direct impact on the host's immune system. Therefore, dysbiotic states such as an imbalance in the microbiome composition can results in the immune system activation, potentially causing neurodegenerative diseases, as mentioned above (Hirschberg et al. 2019; Ohlsson et al. 2019; Li et al. 2018).

The bidirectional connection between brain centers and the gastrointestinal tract is known as the gut-brain axis. Through it, gut microbiome influences the brain function through at least three pathways (Li et al. 2018; Feng et al. 2018; Raybould 2010; Powley et al. 2008) (Table 8.2).

The acquisition of microbiome starts *in utero*, as confirmed by the presence of a microbiota community in amniotic fluid and placenta (Costantini et al. 2017). The way of birth and subsequent breastfeeding have an influence on the composition of the microbiome that is gradually developed during the first 3 years of life (Yatsunenko et al. 2012). This means that a mother's diet can also influence the proper development of microbiota (Costantini et al. 2017). The factors that may affect adult gut microbial communities apart from the host's genetics are numerous, such as the geographical belonging and environmental factors, lifestyle (including the hygienic habits and stress exposure), some medications such as antibiotics or probiotics and different diets (Hirschberg et al. 2019). More specifically, it was shown that dietary habits are crucial in the creation of differences in the composition of microbiota between human individuals (Costantini et al. 2017).

Diet rich in saturated fatty acids is connected to the reduction of microbiota richness with the increased production of lipopolysaccharides (LPS)-producing bacteria such as *Enterobacteriaceae* and decreased production of LPS-suppressing bacteria such as *Bifidobacterium*) (Costantini et al. 2017; Moreira et al. 2012). On the contrary, intake of polyunsaturated fatty acids, specifically omega-3 PUFAs, results with the increased number of *Bifidobacteria* that seems to be responsible for the decrease in gut permeability that is important in maintaining the integrity of intestinal epithelia. Moreover, omega-3 supports the production of butyrate-producing bacteria (e.g., *Eubacterium rectale*, *Eubacterium ramulus*, and *Roseburia cecicola*). Butyrate is together with acetate and propionate the most abundant short-chain fatty

Table 8.2 Brain function regulation pathways

Pathway	Interaction/influence	Result
Immuno-regulatory	On immune cells	Changed levels of cytokines and prostaglandins
Neuroendocrine	On neurotransmitter secretion	Affected hypothalamic-pituitary-adrenal (HPA) axis
Vagus nerve	On the enteric nervous system	Affected brain functions, stress responses, mood and behavior

acid (SCFA). Short-chain fatty acids (SAFAs) are the predominant gut bacteria metabolites formed from otherwise indigestible fiber, whose reduced amounts are connected to inflammatory processes in the human body (Costantini et al. 2017; Haase et al. 2018; Barcenilla et al. 2000). As such, they influence the neurodevelopmental and behavioral disorders that are in correlation with the inflammation, as mentioned above.

Even though it was found that an association between essential omega-3 PUFAs and gut microbiome diversity in healthy adult people exist, the evidence from randomized clinical trials assessing the effect of omega-3 polyunsaturated fatty acids (PUFA) on human gut microbiota is scarce (Watson et al. 2018). Therefore, further research is needed in order to better explain the interaction of the gut microbiome, the diet, and the CNS immunopathology.

8.3.3 Effects on Depression

There are 13 clinical trials of omega-3 fatty acids in the treatment of depression that have been registered and have results according to clinicaltrials.gov. Out of these 13, only 7 have the published results that are presented in the table below (Table 8.3).

These studies examining the efficacy of omega-3 fatty acids on depression as well as meta-analysis have no consistent results related to the significance of omega-3 fatty acids efficacy. Omega-3 fatty acids as a therapy for depression are not significantly different from placebo based on the results of 4 out of 7 studies, as mentioned above. However, 3 out of 7 studies have shown a benefit for the omega-3 treatment of depression symptoms. Possible reasons for conflicting results could be unreliable outcome measurements, non-standardized diagnostic procedures, and other methodological flaws. In order to understand the real impact and benefits of omega-3 fatty acids on depression and other mental health disorders, further studies with a larger sample size are needed. These studies should be designed in the way that the therapeutic levels of omega-3 fatty acids needed for the improvement of depression symptoms are determined.

8.4 Overall

It has been shown that diet can influence the development of inflammation and various metabolic alterations through the effects of specific nutrients on different lines of actions, such as immune signaling, reactive species, microbiome composition, etc. In case when the immune system does not have appropriate resilience and ability to adapt, pro-inflammatory mechanisms could provoke tissue damage as well as various pathologies, consequently leading to the development of chronic inflammatory diseases, including neurological conditions.

Table 8.3 ClinicalTrials.gov search results (accessed November 3, 2019)

Title	Condition	Interventions	Omega-3 dose	Number of patients	Treatment duration	Results	Publication
Omega-3 for depression and other cardiac risk factors 2	Depression	Drug: EPA as an omega-3 supplement Comparator: placebo	2 g/day	144	10 weeks	No differences between drug and placebo	Carney et al. (2019)
The role of omega-3 fatty acids in adolescent depression	Depressive disorder, major	Drug: 2:1 EPA/DHA as omega 3 dietary supplement Comparator: placebo	1.2 g/day increased gradually by 0.6/2 weeks to a possible maximum daily dose of 3.6 g/day	16	10 weeks	No differences between drug and placebo	Gabbay et al. (2018)
Omega 3 for treatment of depression in patients with heart failure	Depression	Drug: 2:1 EPA/DHA fish oil Drug: almost pure EPA Comparator: placebo	2 g/day	108	12 weeks	Changes in cognitive depressive symptoms and social function were in favor of the omega-3 supplementation	Jiang et al. (2018)
Omega-3 supplementation and depression clinical trial	Depressive symptoms	Drug: fish oil omega-3 EPA-rich soft gels Comparator: placebo	3.17 g/day (EPA = 2.15 g; DHA = 1.02 g)	216	8 weeks	No added benefit in the reduction of the symptoms of depression in HIV-infected pregnant women	Opiyo et al. (2018)

Omega-3 fatty acids to improve depression and reduce cardiovascular risk factors	Cardiovascular diseases|depression|heart diseases|myocardial infarction|angina, unstable	Drug: sertraline plus omega-3 Comparator: sertraline plus placebo	2 g/day	122	10 weeks	Maybe an effective treatment for depression, but the required dosage and duration of treatment may depend on the patient's baseline level of omega-3 fatty acids	Carney et al. (2016)
Fish oil for the treatment of depression in patients with multiple sclerosis	Multiple sclerosis|depression	Drug: fish oil concentrate Comparator: placebo	6 g/day (2.1 g EPA and 1.5 g DHA)	39	3 months	No differences between drug and placebo for treatment-resistant depression in MS	Shinto et al. (2016)
Does fish oil prevent depression in pregnancy and postpartum?	Depression	Drug: EPA-rich fish oil supplement Drug: DHA-rich fish oil supplement Comparator: placebo	1060 mg EPA plus 274 mg DHA 900 mg DHA plus 180 mg EPA	126	Information not available	May provide a safe and well-tolerated means for pregnant women to reduce their risk for depression	Mozurkewich et al. (2011)

The inflammation triggered by oxidative stress is the cause of many chronic diseases. Antioxidant activity of polyphenols target different inflammatory components consequently exhibiting anti-inflammatory effect. It has been shown that polyphenols are interfering with immune cell regulation, gene expression and pro-inflammatory cytokines' synthesis. As such, these molecules are associated with extended health benefits, playing an important role in the prevention and treatment of various chronic conditions, such as neurological disorders.

Omega-3 fatty acids are known for their positive health effects, regarding their anti-inflammatory properties as well as their impact on gut microbiota. DHA and EPA are known for being essential in neuronal/brain functioning in close connection to its immunomodulatory properties, thus strongly influencing the development of non-communicable diseases (NCD), also including neurological conditions developing as a consequence of neuroinflammation. Intestinal immune stress associated with low omega-3 availability might be also involved in the development of neuroinflammation and progression of related diseases.

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Chapter 9
Bioactives Functionalization and Interactions

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Abstract In the battle with nutrient deficiency, the production of food enriched with bioactive compounds is becoming a modern trend. There are numerous types and sources of bioactive compounds used for this purpose, ranging from compounds isolated from medicinal plants to those extracted from food waste. Although many foods are marketed as functional foods, the problem with bioactive compounds, in and from food sources, is that the health claims and their bioavailability are still not fully explored. There are many examples of bioactive's functionalization health claims connected to their functional properties and their interactions in foods. This chapter leads the reader from the basic steps of acquiring bioactive compounds to their bioavailability analysis, protection and further improvement of their functional properties. The chapter also takes into account the fortification of foods with bioactive compounds as a strategy to reduce the occurrence of chronic illness as well as challenges that lie ahead for scientists dealing with all the aspects of bioactives, from processing to health claims.

Keywords Bioactives · Bioavailability · Extraction · Food fortification · Microencapsulation · Delivery systems

9.1 Introduction

The food enriched with bioactives has become not only a modern trend but also a discussion among scientists what steps are necessary to ensure the quality and stability of such foods. Although many foods are marketed as functional foods, meaning that they include vitamins, minerals, and other supplements, the problem with bioactives in and from food sources is that the health claims and their bioavailability

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are still not fully explored. Scientists are not only working on ways to improve the extraction processes for higher yield of the desired bioactive compound but are also trying to preserve their bioactivity and bioavailability. There are many examples of bioactives functionalization and interactions in foods; therefore, this chapter aims to further familiarize the reader with the process of bioactives extraction, its health effects, and bioavailability, as well as with the methods of further preserving and improving their functional properties. Furthermore, a short overview of the current strategies of bioactives application to battle chronic illness is presented, as well as the future challenges which lie among scientists, all the way from the extraction and solubilization process to full characterization of their bioavailability and interactions, and further functionalization of their properties.

9.2 Extraction and Solubilization of Bioactives

9.2.1 Extraction

Bioactive compounds or bioactives, are present in various biological sources and are important for the development of food additives and compounds utilized in health treatment (Jin et al. 2016; Sosa-Hernández et al. 2018). Bioactives can be found in small amounts in plants such as fruits, vegetables, whole grains, medicinal and aromatic plants (Gökmen 2016; Xu et al. 2017). Because of various positive effects on human health, the interest of bioactives also increased in different industries such as biomedical, pharmaceutical, cosmetic, food and chemical (Smith 2003; Azmir et al. 2013; Delattre et al. 2016). However, usage of bioactives in the above mentioned industries suggests the requisite for using appropriate and standardized extraction methods of these components from plants (Azmir et al. 2013; Mutalib 2015; Sosa-Hernández et al. 2018). Qualitative and quantitative characteristics of bioactives depend on the chosen extraction procedure (Smith 2003; Azmir et al. 2013; Delattre et al. 2016; Sosa-Hernández et al. 2018). Well-known classical extraction processes are often used for the extraction of bioactive compounds from different plant sources. These processes largely support the utilization of organic solvents, heat, and mixing. The existing classical (conventional) extraction processes are solid-liquid extraction, Soxhlet extraction, maceration and hydrodistillation (Azmir et al. 2013; Hosseini et al. 2018; Al Rashid et al. 2019). Solid-liquid extraction (SLE) is generally used for extracting bioactives from various plant sources. The solid-liquid extraction process includes extraction of bioactives with organic solvents, such as methanol, ethanol, acetone, or the aqueous phase of solvent mixtures (Taamalli et al. 2013; Gadkari et al. 2014; Xu et al. 2017). The choice of the solvent is relying on the character (polar or nonpolar) of the compound to be extracted. The extraction yield of bioactives is influenced by various working parameters such as the time needed for the extraction, temperature, polarity and solvent type, solvent to plant material ratio, and extraction cycles. Although per-

forming the solid-liquid extraction is simple, some disadvantages occur such as the requirement for the exploitation of high amounts of toxic organic solvents, the longer time required for the extraction, low extraction efficiency, additional step such as solid-phase extraction to eliminate unwanted compounds (Xu et al. 2017). Soxhlet extraction is a technique in which the bioactive is extracted from the plant or detached from interfering compounds (Garcia-Ayuso et al. 2000). Soxhlet extraction is applied when the bioactive has limited solubility in a solvent. The advantage of this method is that instead of moving several portions of the warm solvent through the sample, only one batch of solvent is recycled. Thermolabile compounds cannot be extracted by this method due to their degradability at prolonged heating (Nafiu et al. 2017). Disadvantages of Soxhlet extraction include the longer time required for the extraction, high amount of solvent use and a mandatory evaporation step after the sample has been extracted (Lopez-Avila 2000). Maceration represents the extraction of essential oils and bioactive compounds (Bromberger Soquetta et al. 2018). When applying maceration as an extraction procedure, the sample has to be ground into smaller particles. Grinding ensures an increase of the sample surface area to obtain a good mixture with the solvent. Occasional shaking increases the diffusion phenomenon and removes the concentrated solution from the sample surface (Azmir et al. 2013; Bromberger Soquetta et al. 2018). Hydro distillation can be defined as an extraction procedure of bioactives and essential oils from plant materials. The advantage of this method includes the fact that organic solvents are excluded from the process. Hydro distillation can be executed before the drying of plants (Azmir et al. 2013). Hydro diffusion, hydrolysis, and heat decomposition are included in this extraction technique. Disadvantages of this method include degradation of compounds at high temperatures, significant consumption of water, energy and time (Petigny et al. 2014).

Disadvantages of classical extraction methods are the longer time required to obtain a suitable amount of bioactives, utilization of expensive organic solvents, solvent evaporation, low extraction selectivity and degradability of bioactives at higher temperatures (Azmir et al. 2013; Bromberger Soquetta et al. 2018; Sosa-Hernández et al. 2018). To overcome the above-mentioned limitations, new extraction techniques such as supercritical-fluid extraction, microwave-assisted extraction, ultrasound-assisted extraction, pressurized-liquid extraction, enzyme-assisted extraction, high-voltage electrical discharges, and high hydrostatic pressure, have been developed. These new extraction techniques comply with the standards brought by the U.S. Environmental Protection Agency (EPA) and are considered "green". These techniques include environmentally friendly working conditions, "green" solvents, water use, higher extraction efficiency, energy savings (low environmental and economic influence), safe product design (Lenardão et al. 2003; Azmir et al. 2013; Bromberger Soquetta et al. 2018; Sosa-Hernández et al. 2018). Supercritical-Fluid Extraction (SFE) is often used for the extraction of bioactives with high-added values, i.e. pigments and fatty acids (García-Pérez et al. 2017). The often-used supercritical fluids are CO_2, ethane, butane, pentane, nitrous oxide, ammonia, trifluoromethane and water (Silva et al. 2016; Xu et al. 2017). SFE is performed with minimal solvent utilization as compared to other extraction techniques, less extrac-

tion time, increased safety and selectivity. Major disadvantages include the use of non-polar CO_2 which is inappropriate for the extraction of bioactives that are polar and high capital costs (Xu et al. 2017). Microwave-Assisted Extraction (MAE) can be used for the extraction of bioactive phenolics with high-added values, phytonutrients, functional foods and pharmaceutical ingredients from biomaterials (Li et al. 2013; Sosa-Hernández et al. 2018). MAE utilizes the effect of microwave energy to separate the desired compound from the plant matrix in the solvent. Methanol, ethanol, and water are commonly used as solvents (Xu et al. 2017). The advantages of MAE include low energy consumption and temperature, minimal solvent use, short extraction period and inhibition of thermolabile compounds degradation (Wang et al. 2011; Ma et al. 2012). Ultrasound-Assisted Extraction (UAE) can be used for the extraction of proteins, essential oils, polysaccharides, dyes, peptides, pigments, and bioactives (Briones-Labarca et al. 2015; Tiwari 2015). Ultrasounds are used for the disruption of plant cell walls, thus releasing the desired components from bioresources (Roselló-Soto et al. 2015). Factors that regulate the ultrasound effect include pressure, temperature, sonication time and frequency (Rajha et al. 2015). The use of UAE enables a shorter time of extraction, energy and solvent reduction. Ultrasound waves ensure efficient mixing, lower temperature, faster energy transfer, and increases the final yield (Chemat et al. 2008). Pressurized-Liquid Extraction (PLE), or accelerated solvent extraction (ASE), pressurized fluid extraction (PFE), enhanced solvent extraction (ESE) and/or high-pressure solvent extraction (HPSE) (Nieto et al. 2010), separates solutes from a plant matrix. PLE technique uses high pressure, allowing solvents to stay in the liquid phase beyond their normal boiling point (Azmir et al. 2013). The use of PLE encloses the low consummation of organic solvents, the shorter time required for the extraction and polar compounds extraction (Sosa-Hernández et al. 2018). PLE method can be applied for the extraction of different types of compounds from different matrices (Kaufmann and Christen 2002; Smith 2003; Tang et al. 2008). Enzyme-Assisted Extraction (EAE) is a technique in which enzymatic pretreatment is included, to enhance the extraction efficiency. The addition of enzymes such as pectinase, α-amylase, and cellulase during EAE leads to breakage of the cell wall and hydrolysis of the polysaccharides and lipids, which are included into the structure of cell wall, to release intracellular bioactives (Rosenthal et al. 1996; Sosa-Hernández et al. 2018). The EAE method depends on the type of used enzyme and its concentration, particle size of plants, plant to solvent ratio and time needed for the hydrolysis (Niranjan and Hanmoungjai 2004). Compared to conventional techniques, EAE offers advantages such as high selectivity, overall efficiency, the fast extraction process, low energy consumption, low consumption of toxic solvents and process recyclability (Shen et al. 2008; Alam et al. 2017). High-Voltage Electrical Discharges (HVED) is a technology that can be used for the extraction of products with the high-added-value from different food sources (Barba et al. 2015). The electrical breakdown of water is a phenomenon included in HVED. The electrical breakdown of water is followed by bubbles cavitation, high-amplitude pressure shock waves, the formation of active species, turbulence, etc. The advantage of HVED includes an increase in an extraction efficiency because damaging the cell wall leads to the release of intracellular molecules from

the cell cytoplasm (Boussetta et al. 2013; Rajha et al. 2015). The disadvantage of the HVED extraction process includes the occurrence of free radicals that can react with biomolecules and antioxidants (Bromberger Soquetta et al. 2018). High Hydrostatic Pressure (HHP) is a technique developed to become a replacement for processes that include the transfer of thermal energy and include extraction under high pressures (100–1000 MPa) (Briones-Labarca et al. 2015). This technology can be considered green since electric power is required (Andrés et al. 2016). It can be used for food that is safe for use regarding a microbiological point of view, without changes in physical, chemical, nutritional and sensory characteristics of foods (Escobedo-Avellaneda et al. 2011). The high pressure causes protein denaturation. The yield of HVED extraction can be increased using solvents that can pass through the cell wall and reach the bioactives present in cells (Briones-Labarca et al. 2015).

9.2.2 Solubilization of Bioactives

Most bioactives are hydrophobic, and, therefore, have low solubility in water as a green solvent (Clardy and Walsh 2004). To overcome the above-mentioned concerns, the use of safer alternative solvents such as ionic liquids (ILs) has been suggested (Ventura et al. 2017). ILs are liquid molten salts at temperatures below 100 °C (Seddon 1997) and usually consist of large and unsymmetric organic cations (e.g. tetraalkyl phosphonium, pyridinium tetraalkyl ammonium, pyrrolidinium, imidazolium) and organic or inorganic anions (e.g., bromide, hexafluorophosphate, tetrafluoroborate) (Xiao et al. 2018). ILs are known as alternatives to organic solvents because of their physicochemical properties such as negligible vapor pressure, non-flammability, high thermal and chemical stability (Arce et al. 2007; Garcia et al. 2012). ILs can also be considered as designable solvents since their properties and structure can be tuned using different combinations of cations and/or anions (Rogers and Seddon 2003) and can, therefore be used to extract bioactive substances which are not extractable using water or organic solvents. For this purpose, IL-water mixtures can be used (Brandt et al. 2011). The viscosity of the IL phase can be significantly lowered by adding the water into hydrophilic ILs (Blahušiak and Schlosser 2014). The IL–water mixtures show adequate extraction efficiency for polar bioactives, such as polyphenols, carbohydrates, saponins, alkaloids, etc. (Liu et al. 2011; Ribeiro et al. 2013; Zhu et al. 2015).

Along with ILs, deep eutectic solvents (DES) have been recognized as important solvents for several applications (Dai et al. 2013; Gonzales et al. 2020). DES is a liquid that is formed when at least two solid compounds are mixed in conditions that lower their melting points to form a eutectic mixture (Zhang et al. 2012; Smith et al. 2014). According to Choi et al. (2011), there might be DES-like media in nature playing many biological roles and hypothesized that this new kind of DES, named natural deep eutectic solvents (NADES), might be present in living organisms. Since NADES are primarily composed of natural compounds, they are a very promising option for green chemistry and are candidates to replace the toxic organic

solvents (Choi et al. 2011; Oomen et al. 2020). To date, over 200 natural product combinations have been identified as NADESs, all of which have very different physical and chemical characteristics and selectivity (Gonzales et al. 2020), which is why they cannot be regarded as general solvents (Dai et al. 2013). Factors, such as solvent type, pH, temperature, water content, and hydrogen bonding locations should be optimized to use NADES for the extraction of a given target compound (Ribeiro et al. 2013; Liu et al. 2018). Limitations related to NADESs also include their high viscosity and non-volatility. The viscosity of natural deep eutectic solvents can be lowered by water addition (Dai 2013). Future developments on DESs and NADESs will depend on their basic characteristics, such as phase behavior of the compounds that constitute these solvents. Compared to ILs, DESs and NADESs are less toxic solvents, exhibit biodegradability and have less impact on the environment. DESs can donate and accept electrons and protons, which means that they can mold hydrogen bonds thus enhancing their dissolution capacity and consequently extraction effectivity (Paiva et al. 2014). While there is a need for conducting additional researching regarding NADESs, these solvents will contribute significantly to the development of a more sustainable industry in the future. There are many documented cases on the use of DESs and NADESs for extraction of bioactives which cannot be extracted using water and organic solvents (Brandt et al. 2011; Liu et al. 2011; Liu et al. 2012; Jin et al. 2016; Liu et al. 2018).

9.3 Bioavailability of Bioactives

9.3.1 Basic Definitions

As stated before, there is numerous scientific evidence that food components possess bioactive properties which include anti-inflammatory, anti-cancer, neuroprotective and blood pressure-lowering properties (Manach et al. 2004; Teodoro 2019; Kris-Etherton et al. 2002; Bishayee and Sethi 2016), thus contributing to the wellbeing and the proper function of the human immune system (Patil et al. 2009). However, there are scientific papers which emphasize that the evidence for the bioactive properties are often demonstrated in laboratory tests, but the health benefits evidence is often difficult to assemble for the in vivo experiments. The reason for that is because often single compound—single effect relation cannot be explored due to many possible interactions of bioactive with the gut microbiota (Weaver 2014). To have a positive effect on health, a bioactive compound needs to remain undamaged through the whole food processing chain, be metabolized and bioaccesible and reach the targeted tissue without damage and changes to its bioactivity (Rein et al. 2012). This whole process is described as bioavailability. Bioavailability represents the part of a bioactive compound eaten together with its food matrix, which retains its bioactivity and is available for utilization at the site of action. (Alegría et al. 2015; Guerra et al. 2012). The terms bioavailability, bioaccessibility,

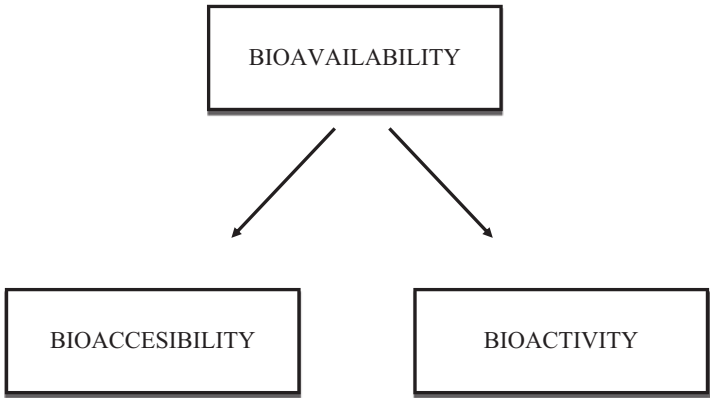

Fig. 9.1 Connection between bioavailability, bioaccessibility and bioactivity (Carbonell-Capella et al. 2014)

and bioactivity are often used together without any distinction among those terms, when, in fact, the term bioavailability is a broader term which includes bioaccessibility and bioactivity. The differences and the correlation among those terms are shown in Fig. 9.1.

As visible from Fig. 9.1, bioavailability is a broader term that includes bioaccessibility and bioactivity. Bioavailability includes digestion, absorption, metabolism, tissue distribution and bioactivity, and the methods used for the assessment and analysis of bioavailability include in vivo assays (Carbonell-Capella et al. 2014). Bioaccessibility defines a part of a bioactive compound which, when released from the food matrix, becomes available for absorption in the intestinal parts of the human digestion system. Bioaccessibility is analyzed by in vitro procedures (Parada and Aguilera 2007). It can be observed through three different steps: (1) release from the ingested food, (2) transformation during digestion and (3) adsorption and transformation through epithelium (Carbonell-Capella et al. 2014). Bioactivity, on the other hand, includes what happens after the assimilation through the epithelium: tissue uptake, metabolism, and physiological response. Bioactivity can be analyzed by a much broader range of methodologies: in vitro, ex vivo and in vivo (Carbonell-Capella et al. 2014).

9.3.2 Methods Used to Asses Bioavailability of Bioactives

There are three most commonly used methods that asses bioavailability, bioaccessibility, and bioactivity of bioactive components: in vivo, in vitro and ex vivo.

The basic idea behind the in vivo experiments is that all the testing is done in a live subject, e.g. when an individual has ingested a bioactive compound and the compound further goes through the digestion process and adsorption. After the pure

form of a nutrient has been consumed, its concentration in blood plasma is measured. In vivo methods enable the collection of a great amount of direct data about the bioavailability of bioactive compounds, and there is also a lot of research available on the use of in vivo methods for the analysis of bioavailability of nutrients originating from foods. E.g. Yuwen et al. (2015) compared the in vitro and in vivo models for bioavailability of nutraceuticals and concluded that the in vivo models, despite their high price and ethical issues, are still considered to be able to predict the bioavailability of bioactives with high precision and accuracy. Fuller and Tome (2005) analyzed the in vivo bioavailability of amino acids and emphasized the importance of the proper selection of sampling. Namely, to properly analyze the loss of amino acids, samples should be taken after the ileal digestion step, while for reliable analysis of nitrogen losses, samples taken after the fecal step are the most representative. Numerous in vivo experiments were also performed on polyphenols and concluded that the oral bioaccessibility of polyphenols is very low. Furthermore, they concluded that bioavailability is greatly dependables on the composition of the food digested with the polyphenols (Olivero-David et al. 2018; Scholtz and Williamson 2007). Some of the conclusions drawn by those authors can be considered as main drawbacks of in vivo methods: different individuals have different physiological states and the overall diets of the individuals greatly influence the results of in vivo testing (Parada and Aguilera 2007).

Compared to the in vivo methods, the in vitro methods are fast, safe and have no ethical constrictions. The analysis is done in a test tube, in which the physiological conditions (e.g. pH, temperature, and salt concentrations) are simulated to be as similar as possible to the conditions in a real living organism. They simulate either the digestion or adsorption process and measure the final concentration of a bioactive compound after the end of the simulation. Adsorption or transport is usually measured using the Caco-2 cells (Nowak et al. 2019). On the other hand, digestion is measured by simulating the conditions of gastric and intestinal fluids, and a bioaccessible compound is a compound that is found undamaged after the small intestinal digestion stage (Nowak et al. 2019). Examples of in vitro digestion applications are present in numerous studies (Pavan et al. 2014; Celep et al. 2015). Further examples include in vitro bioaccessibility of carotenoids, for which has been reported that only a small fraction is bioaccessible (Courraud et al. 2013) and can be improved by the addition of fat and oils (Fernández-García et al. 2012). Similar to carotenoids, vitamin E also has to be packed into micelles to facilitate adsorption (Carbonell-Capella et al. 2014), and the in vitro studies have shown that β-tocotrienol had higher bioaccessibility in comparison to α-tocotrienol (Werner and Böhm 2011). Vallejo et al. (2004) reported a high loss of glucosinates under in vitro gastric conditions of homogenized fresh broccoli, while Alemany et al. (2013) analyzed the bioaccessibility of sterols in fruit-based milk beverages and reported that sterols have a very low bioaccessibility of 2–6%. The drawbacks of the in vitro studies are the inability to simulate the effects of the human microbiota, as well as the possibility of transformation of bioactive to other metabolites which are also considered to be biologically active and can be further absorbed (Parada and Aguilera 2007).

The ex vivo experiments use tissues or cells extracted from the living organism to perform experiments in the laboratory, outside the living organism. Those tests are usually considered to be more accurate than the in vitro experiments since some of the interactions present in a living organism can be detected, but still have some ethical issues present. An example of the ex vivo study can be found in a paper by Vinson et al. (2006), where epicatechin originating from chocolate inhibited plasma lipid oxidation.

9.3.3 The Food Matrix Effect and Interactions

The food matrix is a complex combination of nutrients and non-nutrients, which interact with each other and subsequently influence the bioavailability of food compounds (Crowe 2013). The food matrix directly influences the digestion and absorption of food compounds in the gastrointestinal tract and can be classified into different types such as liquid, emulsion, gel, cellular, network exocellular, fibrous extracellular, viscoelastic, dense, porous and artificial (Aguilera 2019).

Carotenoids are fat-soluble plant pigments that can be used to prevent cardiovascular and eye diseases. As mentioned previously, only a small fraction of carotenoids is bioaccessible (Courraud et al. 2013) and a minimal amount of fat is necessary for absorption (Fernandez-Garcia et al. 2012). However, carotenoids can only become bioaccessible after their release from the food matrix, which greatly limits their general bioavailability. Furthermore, its bioaccessibility, as well as the bioaccessibility of fat-soluble vitamins is also dependent on the presence of phytosterols and phytostanols in the food matrix, which is known to have the potential to reduce plasma concentrations of fat-soluble vitamins (Fardet et al. 2017).

Another example of the food matrix effect is visible for vitamin E. In their review paper, Carbonell-Capella et al. (2014) list several examples of this effect: bioaccessibility of vitamin E for apple sauce was 11%, for beef 86%, for bananas and bread 100%, for cheese and milk 22% and only 0.5% for apples. Parada and Aguilera (2007) stated that folate bioaccessibility is also influenced by the food matrix: folate binding proteins present in fortified milk products decrease the bioaccessibility of folate. Impact on polyphenols is also well documented: reported bioavailability is highly dependent on their structure and conjugation, mostly to sugars, fibers, and proteins, as well as to other factors such as the overall diet, and therefore, the foods most abundant in polyphenols do not necessarily cause the highest increase in polyphenol concentrations in target tissues (Balasundram et al. 2006).

Amino acids and polyunsaturated fatty acids bioaccessibility has also been investigated and is influenced by the food matrix. Domoto et al. (2013) concluded that the bioaccessibility of polyunsaturated fatty acids originating from phospholipid-rich foods was higher in comparison to the ones originating from mono- and triacylglycerol rich foods, while Afonso et al. (2017) concluded that the bioaccessibility of fatty acids greatly depends on the overall diet. Peptides and amino acids bioaccessibility in yogurt formed with different constituents (starch, pectin or β glucan)

were studied by Rinaldi et al. (2015), who concluded that the nature of added ingredient modulates the kinetics of proteins gastric digestion. Bioaccessibility of minerals was also studied: Vitali et al. (2008) analyzed the bioaccessibility of Ca, Mg, Mn and Cu from biscuits prepared from whole grain flour by an in vitro digestion model and concluded that the bioaccessibility was dependant on the protein content, phytic acid, and polyphenols present in the samples.

9.3.4 Optimization and Improvement of Bioaccessibility

As mentioned earlier, to have beneficial effects on human health, bioactives have to be bioaccessible and delivered undamaged to the target tissue. Methods for bioaccessibility and bioavailability improvement include the use of nanosystems, design of colloidal systems and modifications of bioactives to improve their solubility at the targeted site (Rein et al. 2012). The use of nanosystems is extensively explored nowadays. Namely, nanosystems enable bioactives to pass through biological barriers and, at the same time, avoid being modified through metabolic pathways which could lead to low absorption. Examples of nanosystems include curcumin bound to poly (lactic-co-glycolic acid) nanoparticles and the implementation of curcumin in an organogel (Rein et al. 2012). The design of colloidal systems includes the design of micelles and vesicles for nutrient delivery, while technological and chemical modifications often include the encapsulation of bioactive ingredients through coacervation, inclusion complexation, liposome entrapment, spray drying, cocrystallization, nanoencapsulation, freeze-drying and emulsification (Fang and Bhandari 2010), which are explained further in this chapter.

Another interesting aspect of bioavailability improvement is the entrapment of bioactives in vegetable matrices (fruits after processing, spent grain and similar), which not only improve bioavailability but also offer the opportunity to develop novel food products which are interesting to the consumers. Vacuum and/or atmospheric impregnation introduces nutrients into pores present in fruits and vegetables (Parada and Aguilera 2007). For example, lycopene bioaccessibility has been improved by processing raw tomatoes into a paste. Namely, from one side, mechanical forces used in processing cause a release of lycopene from the cells, but also the trans lycopene polymerizes during processing into *cis* form which has higher bioavailability (Porrini et al. 1998). Also, entrapping lycopene using whey proteins enhances its bioaccessibility, as well as entrapment of zeaxanthin in hot milk (Richelle et al. 2002; Benzie et al. 2006; Rein et al. 2012). Anino et al. (2006) used fresh apple pieces for calcium impregnation, resulting in apple pieces that contained 23–62% of the daily needed calcium.

9.4 Microencapsulation of Bioactives for Improvement of Bioaccessibility and Protection of Functional Properties

According to Marisa Ribeiro et al. (2019), due to their poor bioavailability, low water solubility, fast catabolism and excretion and weak stability in environmental processing and gastrointestinal environments, there are many disadvantages connected with the use of bioactive compounds. Also, bioactives lose their activity during storage and in contact with oxidants (Rein et al. 2012). Therefore, there is an increasing concern in designing encapsulation systems to safeguard the advantages of bioactives. Bioactive compounds encapsulation in the food industry is used to (1) maintain functional properties, (2) boost the durability of low solubility compounds, (3) disguise unwanted flavors, (4) enhance health benefits of food products, (5) control the release of bioactive compounds and (6) increase bioavailability of the bioactive compound (Silva et al. 2014; Bourbon et al. 2016). Encapsulation is defined as the methodology for enclosing substances in solid, liquid or gaseous states in matrices that may release the target component at regulated levels and regulated locations (Bratovcic and Suljagic 2019). The component inside the capsule is called the central layer, inner phase, encapsulant, payload phase, or cover, while the surface is often referred to as sheet, coating, wall material, membrane sheet, carrier layer, encapsulating agent, external phase, or matrix (Hassan et al. 2016; Tangsiriratana et al. 2019). It is important to emphasize that encapsulating material must be "generally recognized as safe" (GRAS) for use in the foodstuffs sector (Singh et al. 2018). Therefore, many of the components used for encapsulation in the food sector are carbohydrates (starches, maltodextrins, etc.), proteins (gelatin, casein, etc.), lipids and other organic and inorganic materials (Shishir et al. 2018, Trifković et al. 2015). Encapsulated particles with a diameter of less than 800 μm can be described as microparticles, while the ones with diameters to 1000 nm can be described as nanoparticles (Lengyel et al. 2019).

9.4.1 Microencapsulation

According to Tayagi et al. (2011), microencapsulation methods can be divided into physical, physicochemical and chemical methods.

Spray drying is one of the most commonly employed physical microencapsulation method since it allows accelerated water evaporation and enables retention of low temperatures in the particles which are being dried. As described by Assadpour and Jafri (2019), the feed pump introduces the feed into the atomizer. Liquid feed is disrupted into droplets that are further dried in the drying chamber. Drying gas is introduced into the drying compartment in parallel to the droplets and after a few seconds, dried droplets drop to the bottom of the dryer. After that, they are drawn into a cyclone where dried particles are isolated from the drying gas and deposited

at the bottom of the compartment. Water separation by spray drying guarantees the microbiological consistency and enables the delivery, dosing, and preservation of the bioactive (Correa-Filho et al. 2019; Sosnik and Seremeta 2015). The biggest limitation is the selection of wall material suitable for use in the food industry.

There are numerous examples of using spray drying technology in the microencapsulation of bioactives in order to preserve their functional properties. Rigon and Norena (2016) described the application of spray drying technology of bioactive substances derived from blackberries. They obtained powders with high solubility and preserved functional properties. Rezende Abrahao et al. (2019) studied microencapsulation of bioactive compounds form espresso spent coffee where they used whey protein as wall material in combination with maltodextrin, arbic gum, and inulin. Da Rosa et al. (2019) presented the microencapsulation of anthocyanin compounds extracted from blueberry by spray drying using different process conditions.

As described by Ravichai and Muangrat (2019), microencapsulation by lyophilization is a process where a mixture to be dried is first chilled to -50 °C and dried by the transition of ice to gas under decreased pressure. The cryodesiccation is known to be a quick and effective procedure for the preparation of microcapsules of bioactives which are unstable at high temperatures and oxidative stress (Sanchez et al. 2013; Wilkowska et al. 2015; Murali et al. 2019). Nougeira et al. (2017) described the preparation of microcapsules containing tetrapenoids from *Phaffia rhodozyma* by lyophilization were 65% encapsulation efficiency was obtained. Bellesteros et al. (2017) used freeze-drying for preparation of microcapsules of bioactive molecules derived from spent coffee material, Tumbas Šapnjac et al. (2017) encapsulated tart cherry marc extract using freeze-drying; Papoutsis et al. (2018) used freeze-drying for preparation of microcapsules of lemon secondary product extracts and El-Messery et al. (2019) analyzed the microencapsulation of natural polyphenolic compounds extracted from apple peel by freeze-drying.

Supercritical fluids have been also used for bioactives encapsulation due to their specific physical properties dependent on temperature and pressure (Budisa and Schultze-Makuch 2014). According to Cocero et al. (2009), when working with sc-CO2 the process can be performed at temperatures that are similar to the atmosphere temperature. As described by Ozkan et al. (2019) supercritical fluid precipitation is focused on ensuring the interaction of the supercritical fluid with microencapsulating solutions. Visentin et al. (2012) presented the use of SC-process to prepare particles of rosemary leaves bioactives that can be efficiently suspended in water. Meozzomo et al. (2016) investigated the use of the SC-process for the preparation of microparticles of bioactives derived from grape marc and showed that the proposed technology was highly efficient. Quintana et al. (2019) developed the process using SC-CO_2 for stabilization of bioactive molecules derived from rosemary.

According to Barin et al. (2019), coacervation offers many benefits like simplicity, adaptability, low cost, etc. As described by Eghbal and Choudhary (2018), coacervation is known as the separation of the colloidal system into two liquid phases and the coacervate refers to a phase that is more concentrated in the component. Some of the examples of using coacervation for bioactives encapsulation are as

follows: Jain et al. (2016) studied the preparation of microcapsules of provitamin A; de Souza et al (2018) described the preparation of microcapsules of bioactives derived from cinnamon based on the formation of polymers; Rudke et al. (2019) applied coacervation for the microencapsulation of provitamin D2 derived from *Agaricus bisporus* L. and provitamin D.

According to Emami et al. (2016), liposomes are useful for the supply of both bioactives able to dissolve in lipid and bioactives able to dissolve in water media. Liposomes have a spherical shape cover that protects the molten center and the phospholipids that are included in the liposome cover form two-layer protection for the bioactives (Mignet et al. 2013). Chen et al. (2019) presented the liposomes co-loaded with epigallocatechin-3-gallate (EGCG) and quercetin, and El-Said et al. (2018), described the encapsulation of powdered doum extract in liposomes with high encapsulation efficiency.

According to Perignon et al. (2015), interfacial polymerization was firstly described in 1960. The basic principle of interfacial polymerization is that two reactants dissolvable in their unmixable solvents connect, which leads to polymerization at the contact area. According to Ozkan et al. (2019), interfacial polymerization method has feasible benefits like the potential to govern particle average dimensions, great capacity for entrapping bioactives, adaptable and persistent membrane properties, low cost and simplicity, but it is also important to mention that there are great difficulties with the production of a large interface were polymerization occurs.

9.4.2 Nanoencapsulation

As stated by Berekaa (2015), nanotechnology is emerging as a field with a lot of interest, mainly due to the possibilities of its applicability in science and technology. In the field of food technology, nanotechnology found its application in the area of nanoencapsulation. Nanoencapsulation (NE), as Assadpour and Jafri (2019) and Paredes et al. (2016) described, is a process for miniature packaging of substances that provides the final product functionality and managed core release. When the particle size is reduced from micro to nano, bioactivity, bioavailability, solubility, and delivery is more efficient since the ratio of area and volume is higher (Pissoschi et al. 2018). In the food sector, NE technology allows targeted site transition of the functional ingredient while also protecting it from degradation during manufacturing processes, storage and utilization (Bratovcic and Suljagic 2019).

Due to the relative novelty of nanotechnology in the field of food, it is important to precisely define the legal aspects of nano-size material containing food (Quintanilla-Carvajal et al. 2010). The European Food Safety Authority (EFSA) states that all the actions have to be taken to assure that food which contains nanoparticles is non-hazardous. Some of the uses of nanotechnology for encapsulation of bioactives and protection and improvement of their functional properties are given in the following text. Pulcharla et al. (2016) prepared nanoformulations containing polyphenols from strawberry and chitosan with an encapsulation efficiency of 60%

and with particle sizes that were in the range from 300 to 600 nm. At pH 7.4 they observed an increased release of bioactive compounds in vitro and based on the obtained results, the authors proposed the adaptation of developed formulation for oral and external applications. Peng et al. (2018) emulsified tea polyphenols using high-pressure and obtained droplets with uniform diameters. It is also imported to emphasize that the prepared emulsions were stable for twenty days of storage. Meng et al. (2019) prepared oil-in-water (O/W) nanoemulsions for stabilization of bioactives derived from tea and analyzed their stability at three temperatures.

Huang et al. (2019) developed liposomal nanoencapsulation to improve the antioxidant effects of curcumin and resveratrol and showed that changes in the ratio of selected bioactive compounds had a significant effect on both physical and chemical properties of the prepared nanoparticles. Bhushani et al. (2017) studied the application of zein for the preparation of nanocapsules of catechins derived from green tea and showed that the addition of zein in the concentration of 5% ensured the formation of particles with a diameter around 160 nm. Pereira et al. (2018) presented the nanoencapsulation of bioactives form guabiroba fruit and showed that prepared nanocapsules possess higher antimicrobial activity compared to liquid extract. Delfanian et al. (2018) prepared water/oil/water emulsion nanoparticles of polyphenols from the *Pistacia atlantica* subsp. *Mutica* and showed that the described system ensured the encapsulation efficiency of over 90%.

9.5 Bioactives Fortification in Foods as a Strategy to Reduce the Occurrence of Chronic Illness

The definition of food fortification is presented by the United Nations Food and Agricultural Organization and the World Health Organization as "deliberately increasing essential micronutrient content in food" WHO (2017). The main aim of food enrichment is the improvement of the nutritional quality of human food to achieve a benefit for the general population with minimal risk to their health. It should also be noted here that enrichment and fortification are synonyms. Enrichment/fortification is also the macronutrient supplementation that is otherwise lost during food production or processing (Allen et al. 2017). Food is a source of macro- and micronutrients (fats, carbohydrates, protein, vitamins and minerals), as well as a source of small quantities of bioactives, which are not essential for life and the body, can function properly without them (like caffeine, polyphenols, flavonoids, fatty acids, etc.).

Major global health problems caused by insufficient intakes of vitamins (most common deficit; vitamins A & D) and minerals (the most common deficits: calcium, iron, and zinc) can be alleviated by food fortification (Knijnenburg et al. 2019). One example of fortified food used daily is iodized salt where the ingestion of iodine prevents iodine intake deficit which affects almost 30% of the population (the third being of school age) and is the leading cause of developmental and intellectual dis-

ability, worldwide. The mandatory fortification was initiated in 1924 in Switzerland and Michigan (United States) when iodine was added to salt to reduce the incidence of endemic goiter (Dwyer et al. 2015; Chadare et al. 2019). Salt iodization is an easy and inexpensive way of adequate iodine intake measurement as applied in over 100 countries through salt-iodization programs, among which 34 include a complete salt iodination program. EuSalt (European Salt Producers' Association) strongly advocates the implementation of the Universal Salt Iodization (USI) system in the European Union (Tareen et al. 2005).

The European public health alliance (EPHA) proposed the EU Regulation on food fortification which contains a table of approved food supplement micronutrients (vitamins and minerals) (Table 9.1) as well as the micronutrients that can be added to food (as vitamins and/or minerals within a compound) because fortification of food often requires a specific form of substance (where Chromium can be added as (1) chromic chloride or (2) chromic sulfate and/or in a form of their hexahydrates). However, as the fortification has an impact on human health, care must be taken because the excessive intake of micro-nutrients has unintended health consequences and therefore maximum amounts of their addition to foods should be determined (Regulation (EC) No 1925/2006 2006).

Micronutrients used for food fortification can be added to foods individually or as a combination of multiple vitamins and minerals. Selenium is an example of separately added trace element and essential micronutrient for humans, whose daily recommendation for a grown person is sixty micrograms (60 μg) (Gao et al. 2011).

Table 9.1 Micronutrients allowed to be used in food enrichment (1st Appendix*, (EC) No 1925/2006)

Vitamins[c]	Example of enriched food[a]	Minerals[c]	Example of enriched food[b]
Fat-soluble		Calcium[b]	Dairy products, biscuits
Vitamin D	Milk, margarine	Magnesium	Flour, pasta
Vitamin E	Fruit juice	Iron[b]	Sauce, curry powder
Vitamin K	Olestra	Copper	NA
Vitamin A	Rice, milk	Iodine	NA
Water-soluble		Zinc	Rice, whole cereals
Vitamin B1	Rice		grains
Vitamin B2	Flour, bread	Sodium	Fish sauce
Niacin (Vitamin B3)	Rice	Potassium	NA
Pantothenic acid	Cereals	Selenium	Salt, yogurt
Vitamin B6	Cereals	Chromium	NA
Folic acid	Wheat flour	Molybdenum	NA
Vitamin B12	Dairy	Fluoride	NA
Biotin	Beverages	Chloride	Biscuits
Vitamin C	Cereals, fruit juice	Phosphorus	Milk-based beverages

[a]Liberato and Pinheiro-Sant'ana (2006)
[b]Vlaic et al. (2019)
[c]Clarke (1995)
NA not available

Selenium plays an important role in glutathione peroxidase, a well-known antioxidant known to suppress cellular oxidative destruction (Vlaic et al. 2019). It also plays an important role in thyroid function by catalyzing the production of its active hormones (Wojciechowska-Durczynska and Lewinski 2017; Stuss et al. 2017). In the epidemiological studies, selenium deficit is positively correlated to the incidence of cancer (Rayman 2005). The most widely used method of selenium supplementation is through yogurt (Alsuhaibani 2018) and table salt. As confirmed by the study of Cheng and Qian (1990), a significant reduction in the prevalence of Keshan disease in China has been documented, due to selenium addition to table salt.

Multiple vitamins and/or mineral insufficient intakes are more frequent in those whose diets do not contain specific foods such as meat, eggs, and other food originated from animals, resulting in insufficient intakes of bioavailable iron and zinc, calcium, vitamins A, B2, B6, and B12. Insufficient intake of folic acid, β-carotene, and vitamin C is the result of diets with insufficient intake of fruits and vegetables (Vlaic et al. 2019). Even simple food processing as grain milling reduces the amount of several nutrients such as folates, iron, zinc, thiamine, riboflavin, and niacin. The risk of missing more micronutrients is increasing especially in people with higher intakes of food from refined cereals and grains (Lindsay et al. 2006). Thus, fortifications are performed in combinations with several vitamins and minerals: a combination of vitamin A and iron in fortified foods will often be found, as well as combinations of different B vitamins or calcium in combination with vitamin D. Food enrichment can be achieved through the addition of a variety of materials from which the final product will have multiple benefits. So, e.g. enriching bakery or meat products with mushrooms will result in a product that ultimately has increased fiber and protein content and thus has increased its nutritional value (Nagy et al. 2017), while another example would be bakery products to which nut paste was added, resulting in a product with increased content of so-called healthy fats, dietary fibers and valuable minerals (Păucean 2017). Considering people with lactose intolerance who have reduced calcium intake due to the lack of dairy products in their diets, enriching foods with vegetables that are a rich source of calcium will certainly be extremely beneficial. However, one should not forget that calcium bioavailability depends on the presence of other bioactive components such as fiber, or phytic and oxalic acid (Vlaic et al. 2019).

Another example of food fortification with bioactives, which are, in this case, water-insoluble, is enrichment with essential fatty acids. This enrichment was introduced to battle one of the world's leading public health problems, related to overweight and obesity (as insulin resistance, abdominal obesity, hypertension, dyslipidemia), that contribute to an increase in diseases with a particular focus on cardiovascular diseases and an increase in mortality as a result of their complications (Nagao and Yanagita 2008).

It is recommended that the ratio: ω-6/ω-3 fatty acids (FA) is around 1, while average values of diets in western countries have undesirable ratios because the ω-6 range from 15–16.7 to 1 ω-3 FA, which represents deficient inputs of ω-3 FA, and a sufficient input of the ω-6 FA (Simopoulos 2002). Fatty acid-enriched products of the desired ratio include foods from the meat and oil group. The anti-tumor effect of

bioactive components associated with FA has been studied, but the results of studies conducted on human populations are not straightforward. It is thus difficult to define guidelines/recommendations for daily intake of ω-3 FA, which would be necessary for the prevention and/or treatment of an illness. Knowledge of the molecular-level mechanisms by which ω-3 FA inhibits cancer is crucial in the definition of "needed" intake and would lead to insights that will be further used in human trials and further clarify their nutrition potential and health benefits (Wang et al. 2014).

Carbohydrates are a source of energy if they are in a form of simple sugar (which consists of 1–2 sugar molecules or oligosaccharides (3–10 glucose molecules) or starch (>10 glucose molecules). Fibers are largely carbohydrates, but can also be non-starch polysaccharides, oligosaccharides, lignin (cellulose, hemicellulose, and lignin) and associated plant substances (Lunn and Buttriss 2007). Dietary fibers are food components not rich in the nutritive sense, but crucial bioactive compounds whose deficit will negatively affect the life quality, e.g. in terms of irregular bowel movements (Dahl et al. 2003). Dietary fiber can be divided into those that are soluble and insoluble, and consumption of any of them has many positive health effects such as maintaining proper bowel function but also, general good health. The ability of fibers to create volume, giving a feeling of satiety (thereby reducing the intake of food in general) is extremely helpful during weight loss programs (because the fibers can replace fat—the dominant calorie donor in food). Research shows that enriching food with dietary fiber in amounts of 2–3 g per serving has a positive effect on health (Besbes et al. 2008; Yilmaz and Gecgel 2009) controlling the levels of blood sugar and cholesterol. Fiber-enriched foods have advantages such as the mentioned fat replacement and thus a product of lower-calorie value; their swelling results with higher water retention and upgraded oxidative stability (Sayago-Ayerdi et al. 2009; Mudgil et al. 2006).

The last macronutrient group whose components are used in food fortification are proteins, macronutrients that are necessary for the proper body growth, development of cells and body tissue and their repair. Proteins play a key role in a range of body functions: coagulation of blood, immune system reaction, vision function, fluid balance, production of various enzymes and hormones, etc. (Vlaic et al. 2019). The human body cannot produce essential amino acids by itself, and they must be secured from a food of plant or animal origin or fortified food (e.g. wheat flour enrichment with legumes) (Păucean 2017).

Bioactives such as flavonoids, carotenoids or bioactive lipids have been validated through a series of epidemiological studies as factors that positively influence human health status, minimizing the risks of the modern age diseases (cardiovascular diseases, Alzheimer's, diseases resulting from metabolic syndrome, etc.) (Siriwardhana et al. 2013; Hellgren 2010). The reason for this lies in the capability of bioactive compounds to modulate biochemical pathways (Carbonell-Capella et al. 2014; Septembre-Malaterre et al. 2018). The beneficial effects of bioactives, on human health, depends on their stability in the process of digestion, which consequently affects their biological availability and accessibility (Carbonell-Capella et al. 2014). Food enrichment seems to be the most efficient method to prevail

impacts of diseases caused by the lack of certain bioactive compounds, particularly those which have taken on epidemic proportions.

9.6 Future Challenges and Development

Since the bioactives implementation in foods over the past decade has increased and consumers are more involved in learning about their benefits, the scientist is more eager to find new bioactives and to find out in which way they influence human health. One of the most important things for consumers is to realize that foods enriched with bioactives have a positive effect on their health. Although throughout history bioactives were used for treatment because of their therapeutic effects, today the first main goal is to extract bioactives, which have physiological effects on the living organisms (Phillipson 2001). The creation of an efficient and ecologically friendly extraction process is, therefore, the first future challenge. Not only are scientists searching for new bioactives, but to preserve the environment, they are also trying to re-use by-products from the food industry to find functional compounds that have benefits for human health and also some compounds that could replace the synthetic additives (Fărcaş et al. 2015). As Bonifácio-Lopes et al. (2019) demonstrated in their review of current extraction techniques used to extract bioactives from brewer's spent grain, various methods for extraction can be applied to extract a certain compound. These methods are also used for the extraction of bioactives from different sources. In order to implement a certain method, time and money are some of the most important things that influence that decision. Pretreatment, as the first step, which is used for material structure break down could be done with acid hydrolysis, microorganisms and inorganic acids (especially sulfuric acid known for one of the highest efficiency for hemicellulose degradation) and also with hydrolytic and oxidative enzymes which sometimes, although it simplifies the process, is still not commonly used (Hosseini Koupaie et al. 2018; Zhang et al. 2018). According to del Campo et al (2006) not only does the pretreatment help minimize energy and cost but also preserves pentose fractions and reduces chances of fermentative microorganisms development. For the destruction of the plant cell walls pretreatment with dilute acid is often used, especially sulfuric acid, which is commonly used for the production of bioethanol from agricultural waste (Bonifácio-Lopes et al. 2019). Hydrothermal treatment (autohydrolysis) which is used for monosaccharides, oligosaccharides and acetic acid procurement does not use any chemical agents. The main drawback is the optimization of reaction conditions which Meneses et al. (2013) described for the process of extracting aroma compounds from brewer's spent grain. Supercritical carbon dioxide extraction that Kitrytė et al. (2015) used to determine the antioxidant potential of malt and brewer's spent grain indicated that they could be used in the food industry as an antioxidant source. Spinelli et al. (2016) who also worked with supercritical carbon dioxide managed to obtain high phenolic and flavanoid content and also good antioxidant properties. This technique which is mainly used for extraction of phenolic and flavanoid compounds although

selective and fast still has a high process cost and is mainly used for targeted compounds that are of high value. There are numerous ways of bioactives extraction ranging from classical solid-liquid extraction to cold atmospheric plasma assisted extraction but the process cost of obtaining certain bioactive is still the main issue. For many extraction processes that have been developed in recent years, the cost of the process is sometimes more expensive than the price of obtained purified bioactives leading to the use of older techniques with minor modifications. Solvent extraction which is influenced by temperature, time and proper solvent selection is still the most common method for recovering antioxidants. One has to take into consideration that, depending on the certain antioxidant procurement, the process has to be optimized to ensure maximal yield as demonstrated in the work of Jurinjak et al. (2018).

The problem of how to implement bioactives in food in the sense that they preserve their functionality remains. As functional foods include vitamins, minerals, phenols, bioactive peptides, etc. (Bao et al. 2019; Day et al. 2009) most of the bioactives still come from medicinal plants. One of the most important steps is the identification of a certain compound that is added to food and its influence of health in order to prevent certain diseases. There are many claims from the food manufacturers that certain foods have some sort of beneficial attribute and as Weaver (2014) states these claims need to have evidence. There has not been such an elaborate study conducted for bioactives used in the food sector like it is the case for active ingredients in the pharmaceutical industry, where each contribution to health and side effects is known since they have to be tested in vivo. Except for the detailed study of vitamin D bioavailability (Nowak et al. 2019), one of the bigger successes in that field was recently developed flavonoid database by the USDA (Bhagwat et al. 2013; Haham et al. 2012; Cohen et al. 2017). The main concern of the food industry is for bioactives that are added to the food to preserve the quality of the product in terms of not changing the color, taste or odor (Champagne et al. 2018) of the original product and maintain all the qualities that bioactives provide. Since bioactives are added during the food production process in order to preserve their bioavailability and stability, certain systems for their delivery are required which not only protects them in the food matrix but also protects them during consummation (Bao et al. 2019). Bilia et al. (2018) suggest that a reduction in particle size, as well as formulations that have lipid or biopolymer delivery system, can increase bioavailability and solubility. Since there is a lack of human clinical studies that were conducted with different delivery systems, a possibility of investigating this field opens, where interdisciplinarity between scientists is of the highest importance. To get the full picture of various fields of science like nutrition, food technology, biology, biotechnology, chemistry, and others have to work together. As Nowak et al. (2019) mentioned, nutraceutical delivery systems that are used in the food industry are similar to the drug delivery systems meaning that they have the purpose of (1) increasing the solubility of a bioactive compound to reach their targeted goal (intracellular or systemic circulation); (2) increase of bioactives stability—whether it is during the production process, in final food form, shelf life and also from physical, chemical and biochemical degradation during the consummation process

(Bruno et al. 2013); (3) masking the undesirable tastes; (4) controlling the release rate like the PEGylated forms in term of drugs and (5) targeting specific areas where bioactives are adsorbed. For that reason, researchers are investigating different delivery systems which are composed of different material and structures that could protect bioactives from certain chemical and enzyme degradation to reach the desired place in the gastrointestinal tract. Some of the well-known formulations of lipids or biopolymers are being redesigned into emulsions that contain lipid and protein, different kinds of gels with implanted droplets and covalently bound polysaccharides (McClements 2017). As Nowak et al. (2019) state, amongst the new materials that are tested, are low molecular weight surfactants and their structures such as micellar, micro and nanoemulsions, solid lipid particles, etc. Also, new surfactants are being proposed such as saponin derived from tea, liposomes derived from sunflower or eggs, PEGylated liposomes and organogels. Some examples of developed delivery systems like calcium alginate microparticles for oral administration can be found in work of Acartürk and Takka (1999), soy protein cold-set hydrogels as controlled delivery devices for nutraceutical compounds (Maltais et al. 2009), use of resistant starch as a carrier for oral colon-targeting drug matrix system (Chen et al. 2007), new biopolymers for bioactive delivery of targeted acid (Chen et al. 2019); testing of new polymers that are covalently conjugated (McClements 2018) and many others making this field very interesting for further studies.

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Chapter 10
Requirements of Bioactive Compounds for Health Claims

Stephen Adeniyi Adefegha

Abstract Bioactive compounds are extra nutritionally active ingredients in food from plant and animal origin. They include polyphenols, saponins, alkaloids, vitamins, minerals, terpenoids, dietary fibers, omega, and poly saturated fatty acids) from vegetables, fruits, spices, nuts, cereals, herbal products, legumes medicinal plants, probiotics, prebiotics as well as those from fungal, algal and animal sources, and other natural antioxidants. In recent times, there is growing evidence from epidemiological and experimental data that bioactive compounds in foods have positive health benefits. These bioactive compounds include, are capable of managing weight, modulating genes, enhancing good health as well as preventing diseases such as cancer, diabetes, cardiovascular disease, stroke, erectile dysfunction, endothelial dysfunction, heart and respiratory infections to mention a few. This fact has propelled a diligent review of the requirement for these health claims. This chapter discusses the need and regulatory aspects of bioactive compounds from food for health claims. It compiles the fundamental processes that should be considered by researchers on the health claims for bioactive compounds. These requirements are meant to protect consumers from frauds perpetrated by manufacturers on nutraceutical products. Bioactive compounds' requirements for health claims may originate from laboratory findings and proceeds to systematic clinical trials to guarantee safety, provide information on bioavailability and efficacy of nutraceutical products.

Keywords Bioactive compounds · Health claims · Consumer protection · Food safety · Laboratory findings · Clinical trials

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10.1 Introduction

A bioactive is regarded as a food component with non-nutritional benefits, which may either promote good health or exert a harmful effect upon ingestion (Gry et al. 2007). Recently, increased interests in food bioactive/ bioactive in foods have necessitated a holistic desire to explore the procedures by which they are considered fit for consumption. This fact has informed all stakeholders to understand that proper isolation, elucidation, and characterization, as well as useful biological studies of bioactives, are crucial to identification and recommendation of bioactive compounds in foods for consumption by the public and for public health. Hence, there is need to collate all these requirements necessary for the guidance of food bioactives or bioactive e compounds in food for animal or human consumption (Connie 2014). Particular food containing bioactive compounds and food bioactive have undergone series of approval after passing the stipulated regulatory criteria for health claims and are either been commercialized or about to be marketed. Adoption of these health claims have demonstrated different degrees of success recorded in the area of public health information and management (Connie 2014). Incidentally, very few bioactive compounds/active ingredients in food have successfully passed through the proper regulatory approvals for health claims. Many global agencies have provided rules and regulations for health claims as well as disease claims. According to the 2007 European Union Regulation EC no. 1924/2006, health claims were described extensively under articles 13 for general function claims while article 14 demonstrated disease claims of food bioactives/bioactive compounds in food by the European Food Safety Authority (EFSA) (Connie 2014). In addition, the Food and Drug Administration (FDA) of the United States of America, described claims in three levels namely:

1. Health claims to explain the possible interaction between a bioactive and a disease. In this case, this claim focuses on how the food bioactives/bioactive compounds ameliorate or attenuate a diseased condition. How the food bioactives interact with several biological molecules such as enzymes, hormones, proteins, lipids, peptides, DNA, and RNA.
2. Health claims to describe nutritional content claims that characterize the amounts of nutrients present in food. In this claim, the description of the positive roles of food nutrients ranging from the micronutrients to the macronutrients in promoting good health and wellbeing.
3. Health claims to demonstrate the relationship between the structure and function of food bioactives or bioactive compounds in food. This claim expounds on how the structure of food bioactives or bioactive compounds can influence or alter normal function in animals and humans. It explicitly discusses the possible mechanisms and mode of action of food bioactives or bioactive compounds in food (The Chiropractic Resource Organization 2013) (Fig. 10.1).

These bioactive compounds include peptides, carotenoids, saponins, alkaloids, polyphenols (flavonoids—flavanones, flavones, isoflavones, flavanols, lignans,

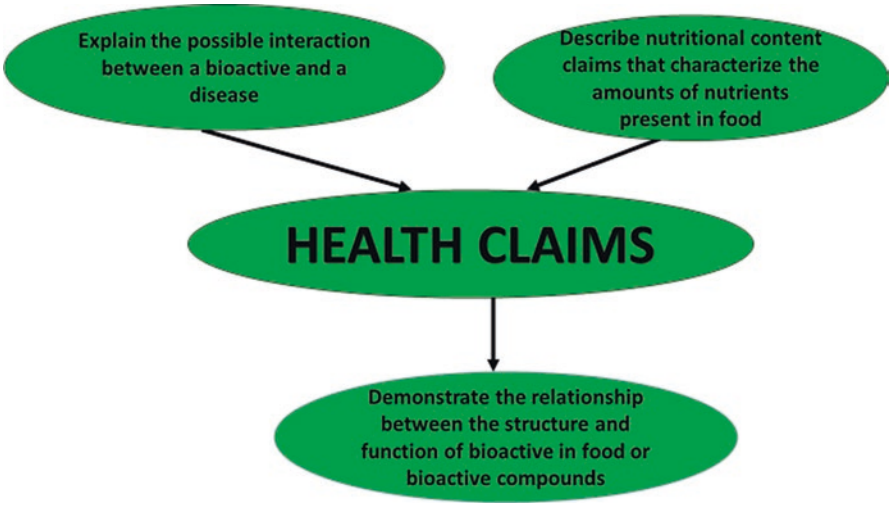

Fig. 10.1 Description of health claims

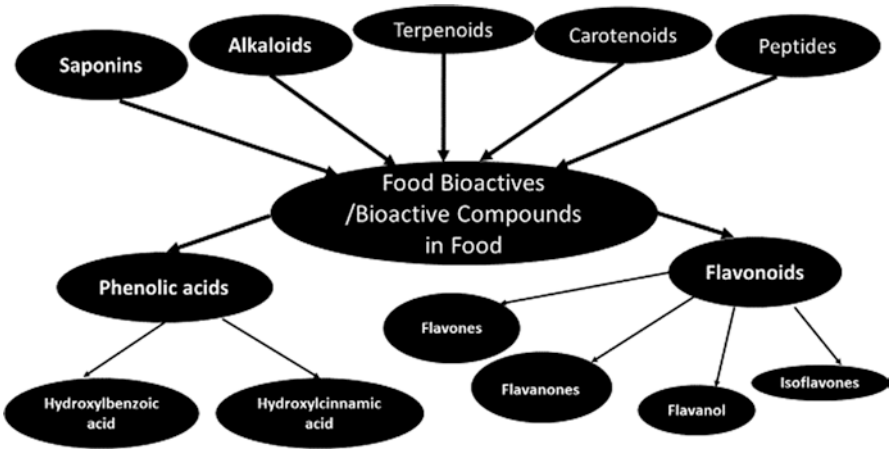

Fig. 10.2 Food bioactives/bioactive compounds in plant foods

proanthocyanidins, and stilbenes and phenolic acids—hydroxycinnamic acids and hydroxybenzoic acids and) terpenoids, omega-3 and polyunsaturated fatty acids (Adefegha 2018) (Fig. 10.2).

Bioactives in food have received enormous attention in that they have shown interesting biological effects including weight management, beneficial against infections ranging from bacterial, viral and fungal, reduction in the cardiovascular and obesity risks, prevention and control of diabetes, endothelial dysfunction, erectile dysfunction, cancer, stroke, arthritis and neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington' s disease etc. (Adefegha 2018) (Fig. 10.3).

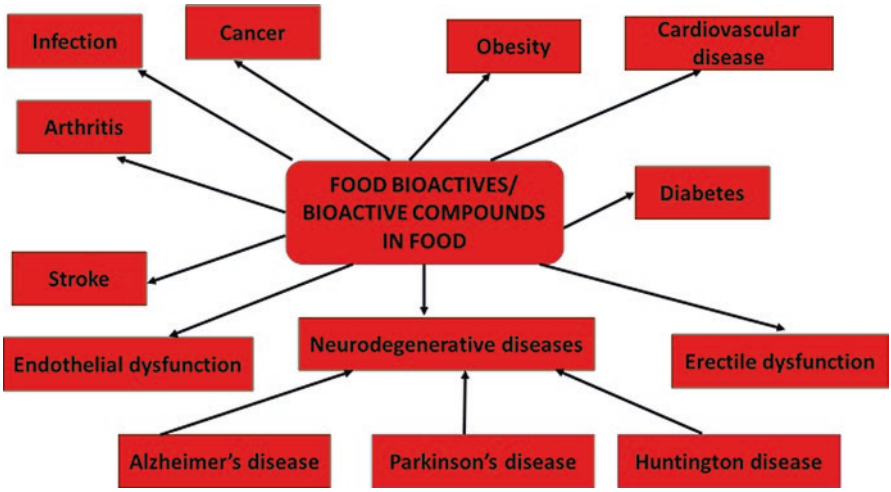

Fig. 10.3 Therapeutic intervention of bioactive compounds against chronic diseases

Slendesta, a product manufactured by Kemin Industries, Inc. and approved by EFSA approval is Slendesta, has an active ingredient, P12, which is a bio peptide that enhances the activation of cholecystokinin. This gut hormone reduces the intake of food. Peptides produced by specialized cells along the gastrointestinal tract, stomach, and pancreas, are potential crucial targets for bioactive in food/bioactive compounds in food to attain satisfaction (Adefegha 2018). Bioactives in foods have a beneficial effect against cardiovascular risks, and these can be attributed to the positive impact on endothelial function (Hooper et al. 2012). Endothelial dysfunction is examined by brachial artery flow-mediated dilation and endothelial pulse amplitude testing (Endo-PAT) (Hooper et al. 2012). Recent findings in bioactive components and their relationship to health are overwhelming, and its ability to maintain and better human health in weight maintenance, management and possible treatment for communicable and non-communicable diseases such as microbial infections, cancer, diabetes, cardiovascular diseases, endothelial and erectile dysfunction, stroke, heart, and respiratory diseases, (Connie 2014) (Fig. 10.3). According to recent developments, bioactive components have been known to have an impact on genes, and the information needed to ascertain health claims are outdated (Connie 2014). Hence, the primary objective of an analyst, those involved in making policies, professional societies is to enhance health (Connie 2014).

The characterization of chemicals is essential in the assessment of risk. Characterization of risk reveals the impact of hazard characterization with analyzed exposure on humans, which is dependent on the information of the chemical or material that has been scrutinized and measured (World Health Organization 2008). Analytical procedures need to be in place concerning the chemical purity and nature of the substance analyzed during in vitro and in vivo hazard, as well as the amount of the chemical in food as regards the required extent or exposure survey (Alder

et al. 2000). Chemicals may find themselves into ingested food in minute or large quantities during processing and preservation (Alder et al. 2000). The characterization of risk of impurities as well as chemicals in food vary from countries, however it is vital to document the stipulated amount of the chemicals that could be termed safe in food as well as the intake needs in reputable databases (World Health Organization 2008).

10.2 Regulatory Programme for Foods

Analyzed information is collated for various reasons, which includes:

- Legal standards needed to ascertain the standard and health benefit of foods that are manufactured within the nation brought into the nation, or exported;
- Examining to ascertain alignment with existing requirements;
- Inspection, mainly for analyzing ingestion or to collate information for quality requirements; and
- Findings for product manufacture, which entails the manufacturing of specifications.

These reasons may have various analytical requirements, mainly in the line of performance characteristics (World Health Organization 2008; Thompson and Wood 1995).

Lack of certainty in analytical assessment, mainly for ingestion measurements, can lead to a lack of assurance in safety and risk measurements (Thompson and Wood 1993). The fitness for purpose of the analytical information in the use of safety and risk measurement should be ascertained on a case-by-case basis, as well as any lack of certainty in the samples should be reported as part of the assessment (Thompson and Wood 1993).

10.3 Quality Management and Quality Control of Bioactive Compounds

Quality management and control of bioactive compounds are two critical aspects that guarantee high level of production standards of bioactive compounds in food for health claims. Quality management of bioactive compounds entails all the procedures involved in the analysis of bioactive compounds in foods while quality control of bioactive compounds gives the perspectives of an expert on the analytical protocols and process for acceptance by the general public (Thompson and Wood 1995). Knowledge of bioactive compounds in foods are essentials in the discovery of their novel therapeutic roles and maintenance of human wellness. The use of different analytical methods/procedure may help to secure, validate, manage and control the

quantity and quality of bioactives/bioactive compounds in food supplements, nutraceuticals and functional foods. The principles of quality control and management often permit policy makers, industrialists, food-drug agencies and other stakeholder to lay hold on the authenticity of data generated from the different analytical methods thus providing valuable information about the products containing bioactive compounds (Thompson and Wood 1993; Thompson and Wood 1995).

Increased awareness on health and nutrition has shifted the focus of food industries and food manufacturers in producing food that can enhance good health and prevent diseases aside supplying additional nutritional benefits (Thompson and Wood 1993). Active ingredients at times act in an additive reaction or synergistic manner in management and fight against pathologies by making changes to metabolic pathways involved in the pathologies or by altering the activities of enzymes (Weaver 2014; Ghanbari et al. 2012; Adefegha and Oboh 2013). Scientifically proven facts are not sufficient for the health benefits of active ingredients or bioactive compounds entailed in foods. randomized controlled trial (RCT) is critical in providing essential data however it is costly and most times need more pre-information for the bioactive compounds in the food which is been analyzed in healthy or clinical patients (Biesalski et al. 2013; Gaine et al. 2013). Shelf-life or stability of a food is defined as the time frame in which a food can be kept or stored under controlled conditions including temperature, light, humidity, moisture content, etc., conditions, alterations which are acceptable by the producers, consumers and within the legal requirements (Sílvia et al. 2017; Moura and Germer 2010). Many modifications and changes occur in food while been processed and stored on exposure to various environmental factors that stimulates reactions that leads to spoilage, degradation of the food, and dissatisfaction by the consumers (Singh 2014). Critical changes in fruit-based liquids can be due to physiochemical, sensory changes, and microbiological alterations usually linked to chemical composition and heat conditions, the quality of the fruit-based drink initially, the quantity of oxygen in the bottle and quantity in open space, the nature of the surface, the package, temperature, etc. (Moura and Germer 2010; Singh 2014).

The quality and quantity of the kind of apparatus used in the processing of food, especially those involved in pumping and liquids affected by temperature, need accurate data of thermal characteristics (thermal conductivity, diffusivity, density, and specific heat). Also, rheological characteristics/properties mainly influenced in pumping and transportation of fluid as while production process is going on and the activity of the properties during production relating to the temperature (Moura and Germer 2010; Singh 2014; Mulvaney et al. 2000; Reuterswärd 2007).

10.4 Health Claim

Regulation (EC) No. 1924/2006 on health claims and nutrition developed on foods defines a health claim as any claim explains, reveals, or implies that a link exists between a food category, a food, or one of the constituents in the food and health (FAO/WHO 1975) (Fig. 10.1).

The significant classifications of health claims are the following:

- Overall or general function health claims (e.g., Ascorbic acid or Vitamin C aids the proper functioning of the nervous system) accepted by the European Commission (EC). There are 229 general function claims except for botanicals, which have been taken/approved by the EC and published on the Community Register. When suggesting such claims, only references linked to relevant scientific justifications are needed (FAO/WHO 1975).
- Proprietary or recent data health claims that are dependent on recently developed scientific data or evidence or/and for which protection of new science data is requested (FAO/WHO 1975).
- The depletion of disease risk claims, which combines the ingestion of food or ingredient with a significant depletion in a risk factor in the development of a disease (e.g. oat beta-glucan), has been known to deplete blood cholesterol. High cholesterol is a risk factor in coronary heart disease development) (FAO/WHO 1975).
- Health claims or childhood development (FAO/WHO 1975).

All food products with health claims require a product-specific HACCP (Hazard Analysis and Critical Control Point) study before it can be considered for marketing or commercialization (FAO/WHO 1975). According to the report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the specifications regarding the safety of food additives, ingredients, components, flavor, bioactive constituents, contaminants, and naturally occurring toxicants and additives must be adhered to, before such products are commercialized and supplied for human consumption and use (World Health Organization 2008). These specifications by JECFA cover the safety, quality, normal stability, and shelf life of the food additive (World Health Organization 2008).

10.5 Method of Analysis for Bioactive Compounds from Plant Sources

Plants consist of bioactive compounds that show a variety of biological functions on human health, including anticancer, antidiabetic, antimicrobial, antioxidant, anti-arthritic, anti-inflammatory properties (Zhao et al. 2015) (Fig. 10.3). Steps involved in the analysis of known and unknown bioactive compounds in plants are rigorous and difficult. It starts with the extraction of bioactive compounds from the source (plants). Extraction procedure can be done on different parts of the plants, including leaves, stem barks, seeds, roots, or the whole section (Altemimi et al. 2017) (Fig. 10.4). This can be done fresh or dried samples. Thus, preparation of the plant samples should commence prior the extraction steps. For new plant samples can be washed with running water, air dried or freeze dried and pulverized or ground into powder. The moisture content of plants before and after drying must be known. The

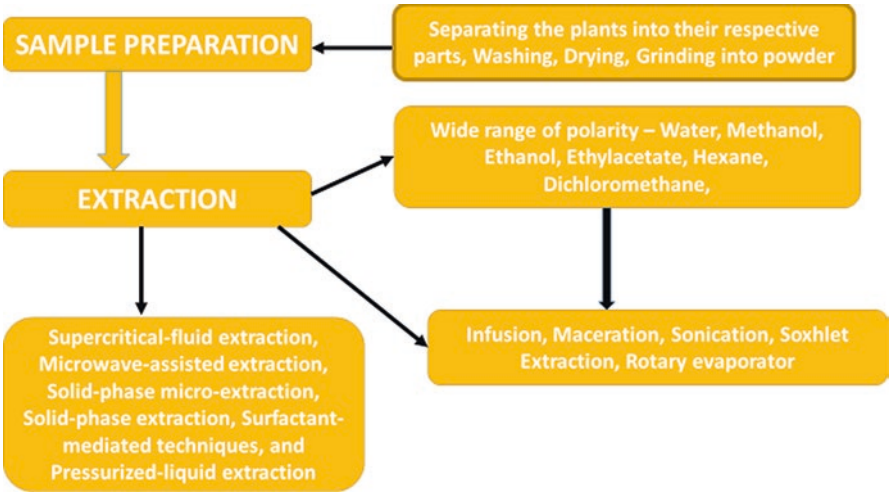

Fig. 10.4 Plant sample preparation and extraction methods for bioactive compounds

large surface area provided by pulverization of the plant samples allows for easy penetration of the solvents used for extraction. Wide range of solvents polarity are often used for the extraction of bioactive compounds from plants. The choice of solvent or solvents, ranging from polar to non-polar solvents, to be used for extraction depends on the bioactive compound or compounds of interest (Sasidharan et al. 2011). The extraction of polar bioactive compounds uses polar solvents such as water, ethanol, ethyl-acetate, methanol or mixture while that of non-polar bioactive compounds, utilizes non-polar solvents such as hexane, dichloromethane, petroleum ether, or their combination (Fig. 10.4). As the target compounds may be non-polar to polar and thermally labile, the suitability of the methods of extraction must be considered. Furthermore, the extraction methods that must be used should take into account the physical and chemical stability of bioactive compounds present in the plants (Zhang et al. 2018a). These plants bioactive compounds include polyphenols, saponins, alkaloids, vitamins, minerals, terpenoids, essential oils, dietary fibers, omega and poly saturated fatty acids, from vegetables, fruits, spices, nuts, cereals, herbal products, legumes medicinal plants, and prebiotics (Zhao et al. 2015; Abuajah 2017) (Fig. 10.2). The plants or plant parts are soaked in the appropriate solvent or solvent mixture before several methods of extraction, including heating under reflux, sonification, soxhlet extraction to mention a few are used (Sasidharan et al. 2011; Zhang et al. 2018a; The United States Pharmacopeia 2002) (Fig. 10.4). The use of water to extract plant herbs in fresh, dried, or powdery forms, has been practiced in folklore medicine from ages. Infusion of plant materials, maceration, addition of cold or hot water to plant materials and the filtrate is consumed as medicinal herbs (Azwanida 2015). It is essential to understand the physicochemical (boiling point, polarity, solubility) and toxicological characteristics of different solvents used for extraction (Pandey and Tripathi 2014). These solvents may include

methanol, water, ethanol, hexane, dichloromethane, ethylacetate, chloroform, and acetone etc. In recent times, researchers and scientists have developed modern day techniques for extraction in small to large scale. These methods include supercritical-fluid extraction, microwave-assisted extraction, solid-phase micro-extraction, solid-phase extraction, surfactant-mediated techniques, and pressurized-liquid extraction (Liu et al. 2008) (Fig. 10.4). The use of rotary evaporation of solvents at specific temperature and pressure as well as freeze-drying of the aqueous portion gives good dried extract.

These methods guarantee solvents removal and prepare samples for further analysis that allows for the identification and characterization of bioactive compounds from plants (Altemimi et al. 2017) (Fig. 10.5). Phytochemicals such as alkaloids, saponins, phenolics, flavonoids, cardiac glycosides, terpenoids) can be screened and quantified in various plants. In addition, chromatographic analyses such thin layer chromatography (TLC), paper chromatography (PC), column chromatography (CC) permit the separation of some bioactive constituents from plants using the stationary and mobile phases (Sasidharan et al. 2011) (Fig. 10.5). The application of some advanced techniques allows further streamlining and characterization of specific bioactive compounds. These advanced techniques include high-performance liquid chromatography coupled with diode-array detector (HPLC-DAD), high-performance liquid chromatography coupled with ultraviolet detector and mass spectrometer (HPLC-UV-MS), high-performance liquid chromatography coupled with electrospray ionization mass spectroscopy (HPLC-ESI-MS), gas chromatography coupled with flame ionization detector (GC-FID), gas chromatography coupled with mass spectrometer (GC-MS), liquid chromatography coupled with mass spectrometer (LC-MS), high-performance thin-layer chromatography coupled with electrospray ionization mass spectroscopy (HPTLC-ESI-MS) and ultra-performance liquid chromatography coupled with mass spectrometer (UPLC-MS) (Fig. 10.5). Fourier-transform infrared spectroscopy (FTIR), Near-infrared resonance (NIR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy are veritable

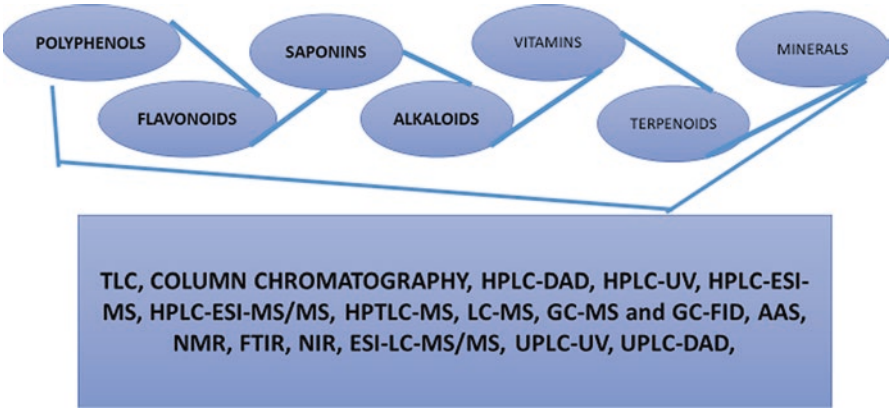

Fig. 10.5 Identification and characterization of bioactive compounds from plants

scientific equipment used in modern science to identify and characterize pure compounds with functional groups (chemical bonds) present in plant extracts with unknown constituents and compounds. Polyphenols, flavonoids, alkaloids, saponins, carotenoids, and peptides can be easily characterized by HPLC-DAD, HPLC-UV-MS, HPLC-ESI-MS, HPTLC-MS, LC-MS, GC-MS, and GC-FID. In contrast, terpenoids, essential oils, and omega-3 fatty acids can be characterized by GC-MS and GC-FID (The United States Pharmacopeia 2002; Azwanida 2015; Pandey and Tripathi 2014; Liu et al. 2008) (Fig. 10.5). Vitamins are nutritive and extra-nutritive components of food. They are regarded as bioactive components because they can elicit disease preventive and health-promoting effects. Vitamins A and E can be detected using the column chromatography and normal-phase HPLC (FAO/WHO 1975; Prashanth et al. 2015). Vitamin D components such as Vitamin D3 and D2 can be identified, determined and characterized by ESI-LC-MS/MS (FAO/WHO 1975; Cortés-Herrera et al. 2018; Zhang et al. 2018b). Vitamin C in foods can be detected, identified, and characterized using HPLC-UV, HPLC-DAD, and UPLC-UV (FAO/WHO 1975; Cortés-Herrera et al. 2018; Zhang et al. 2018b). Vitamin B12 can be analyzed using the LC-UV, LC-DAD and LC-MS (FAO/WHO 1975; Cortés-Herrera et al. 2018; Zhang et al. 2018b) (Fig. 10.5). Minerals are essential food components, which act as cofactors for a number of enzymes. They include potassium, sodium, calcium, magnesium, selenium, iron. Minerals in food can be assayed using the Visible-UV spectrophotometric methods as well as the atomic absorption spectrophotometric method (FAO/WHO 1975; Prashanth et al. 2015). Glucose are monosaccharides that can be obtained from foods such as corn, sugar cane, fruits, vegetables, can be analyzed using polarimeter, Shaffer-Somogyi chromatography, paper chromatography, Sichert-Bleyer modification, Zerban-Sattler modification, glucose oxidase method and spectroscopy (FAO/WHO 1975; Shallenberger and Moores 1957).

10.6 Method of Analysis for Bioactive Compounds from Animal Sources

Animals contain many bioactive compounds that elicit interesting physiological functions. These bioactive compounds include amino acids, peptides, proteins, polysaccharides, and polyunsaturated fatty acids. Due to the biodiversity of animals, a wide range of these animal-derived bioactive compounds can be quickly produced (Zhang et al. 2015). Omega-3 fatty acids are polyunsaturated fatty acids (PUFAs) containing two or more double bonds, with one double bond present at the third carbon atom from the methyl (CH_3) end of the carbon chain. Examples of omega-3 fatty acids found in foods include eicosapentaenoic acid (EPA), docosahexaenoicacid (DHA), α-linolenic acid (ALA) and docosapentaenoic acid (DPA) (Shahidi and Wanasundara 1998). These omega-3 fatty acids can be obtained from animal food sources such as marine foods, fish and seafood products, meat and

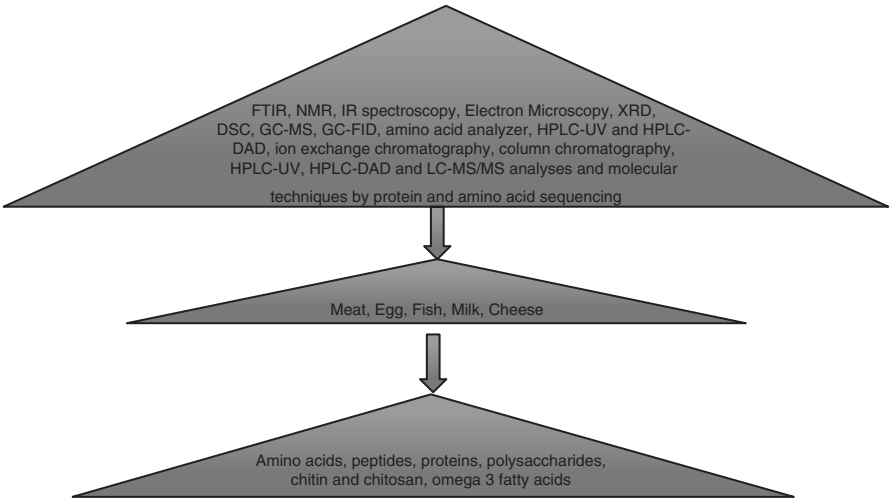

Fig. 10.6 Identification and characterization of bioactive compounds from animal sources

poultry products (Meyer et al. 2003) (Fig. 10.6). The omega 3 fatty acids can be obtained from the animals by using equipment that can press the animals on the surface thus increasing pressure for the release of the oil that will be used for the estimation of omega-3 fatty acid. The oil is hydrolyzed by lipase, subsequently esterified and analyzed using the gas chromatography (GC) for the determination of the omega 3 fatty acid constituents. The physiochemical components (cloudy and turbid point, iodine number, peroxide number, saponification number and free fatty acid content) of the oil obtained can also be determined (Zhang et al. 2015; Schmid et al. 2006). Omega-3 fatty acids can be detected and characterized by GC-MS, GC-FID, ESI-MS and LC-MS (Zhang et al. 2015; Shahidi and Wanasundara 1998; Meyer et al. 2003; Schmid et al. 2006) (Fig. 10.6).

Chitin and chitosan can be obtained from the exoskeletons and shells of invertebrates, such as crustaceans, mollusks, crabs, and shrimp. They are biopolymers consisting of amino acids and polysaccharides (Zhang et al. 2015). This can be achieved by the removal of calcium carbonate by acid hydrolysis with subsequent solubilization of proteins by alkaline hydrolysis. Chitin and chitosan possess multiple functional properties such as chelation of metal ion, biocompatibility, low immunogenicity, nontoxicity along with antioxidant and antimicrobial activity, biodegradability, optical structural characteristics and, formation of polyoxysalt, ability to form films, and, hence they are of great medicinal and industrial relevance (Pillai et al. 2009). The processes of demineralization, deproteination and deacetylation can be used for the extraction of chitin and chitosan (Younes and Rinaudo 2015; de Queiroz et al. 2017). The following analyses can be carried out to detect and characterize chitin and chitosan in animal samples by the methods of ninhydrin test, infrared spectroscopy, near infrared spectroscopy, linear potentiometric titration, nuclear magnetic resonance spectroscopy, fourier transforms

infrared spectroscopy (FTIR), colloidal titration, circular dichroism, acid hydrolysis, gel permeation chromatography ultraviolet spectroscopy, pyrolysis-gas chromatography, hydrogen bromide titrimetry, thermal analysis, X-ray diffraction (XRD), elemental analysis, scanning differential scanning calorimetry (DSC) and electron microscopy (SEM) (Zhang et al. 2015; Pillai et al. 2009; Younes and Rinaudo 2015; de Queiroz et al. 2017; Abdel-Rahman et al. 2015) (Fig. 10.6). These methods reveal the spectral, crystalline and band structure as well as vibration of different bonds (CO, O–H and N–H) in chitin and chitosan (Zhang et al. 2015; Pillai et al. 2009; Younes and Rinaudo 2015; de Queiroz et al. 2017; Abdel-Rahman et al. 2015).

10.7 Peptides

Peptides are bioactive compounds with different amino acid residues and specific fragments of protein. They have been reported to show additional health benefits aside from their nutritional properties. Bioactive peptides can be found in various animal sources such as milk, fishes such as salmon, herring sardine tuna, eggs, and meat (Möller et al. 2008). Whey protein obtained from milk and milk products such as cheese are new sources of bioactive peptides (Livney 2010). Bioactive peptides from whey proteins are essential probiotics. The amino acid residues in proteins are held together by peptide bonds, hence peptides are formed in this process. Bioactive peptides remain inactive unless they are released from the sequence of protein via acid and enzymatic proteolysis or fermentation, thus modulating human health in different biological systems including the digestive, endocrine, cardiovascular, immune and nervous systems (Zhang et al. 2015; Möller et al. 2008; Livney 2010; Abuine et al. 2019; Colegate and Molyneux 2007; Bhat et al. 2015). Several reports have been shown that lysine, phenylalanine and tryptophan containing peptides have elicited physiological roles. These bioactive peptides are often liberated through *in vitro* or *in vivo* models of animal proteins thus exerting a number of physiological benefit including antihypertensive or blood pressure-lowering (ACE inhibitory) effects, cholesterol-lowering ability, antidiabetic, antimicrobial, cytomodulatory, immunomodulatory antithrombotic, antiobesity, antigenotoxicity and antioxidant activities, increasing mineral absorption property and bioavailability (Zhang et al. 2015; Livney 2010; Abuine et al. 2019; Colegate and Molyneux 2007) (Fig. 10.6). The amino acids residues in the peptides and proteins by amino acid analyzer, HPLC-UV and HPLC-DAD. These bioactive peptides can be analyzed using different chromatographic techniques including ion exchange chromatography, column chromatography, low-resolution (LR), HPLC-UV, HPLC-DAD and LC-MS/MS analyses and molecular techniques by protein and amino acid sequencing (Zhang et al. 2015; Bhat et al. 2015) (Fig. 10.6).

10.8 Examining/Testing of Bioactive Compounds for Recommendation for Health Claim

In the development of bioactive compounds in food for the health claim, certain tests ranging from non-clinical to clinical tests are required before health claims are made on such food products or food bioactive compounds (Motilva et al. 2015) (Fig. 10.7). Once the bioactive compounds are identified and characterized from their various sources (plants and animals), there should be need for testing and validation of their biological significance (both pharmacological and toxicological roles). The *in vitro* analysis, *in silico* assays, *in vivo* animal models and *ex vivo* model/cell-based model) are necessary tests that should be done in the laboratory as well as human trials/models or clinical trials in the clinics and hospitals (Motilva et al. 2015; Jean-Quartier et al. 2018; Curtis et al. 2008; Gil et al. 2015; Gomes et al. 2018). (Fig. 10.7). Preliminary investigation using *in vitro* analysis provides information on the concentration and dose of food bioactive compounds that may elicit a therapeutic effect. Furthermore, these concentrations are tested in different animal models (Curtis et al. 2008; Gil et al. 2015). The animals often used as models include rats, mice, rodents, and rabbits. The relevance of validated and predictive animal models selection, as well as the correct use of animal tests in experimental

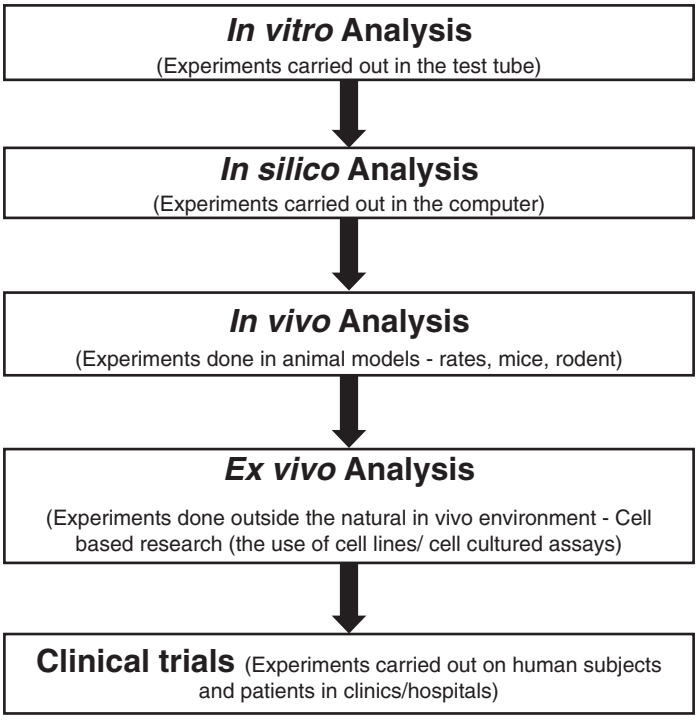

Fig. 10.7 Examining/testing of bioactive compounds for recommendation for health claim

design, execution, and interpretation, may affect reproducibility, quality, and reliability of non-clinical studies necessary to translate to and support clinical studies (Curtis et al. 2008; Gil et al. 2015; Gomes et al. 2018; Lindequist 2016) (Fig. 10.7). Guiding principles on scientific studies are essential for the design and development of nutraceuticals from bioactive compounds. Before the administration of bioactive compounds to human beings, all bioactive compounds should have the following characteristics (Motilva et al. 2015; Jean-Quartier et al. 2018; Curtis et al. 2008; Gil et al. 2015; Gomes et al. 2018; Lindequist 2016; Malve 2016; Nair et al. 2015; Choudhary et al. 2017) (Fig. 10.7):

1. Analysis of chemical composition.
2. Preparation method.
3. Purification method to ascertain the degree of purity.
4. Toxicity test—acute, subacute, subchronic and chronic toxicity tests will be determined at varying doses to ascertain safety in different animal species.
5. Histopathology analysis in several animal organs, especially in liver, kidneys, hearts, testes, and brains.
6. Examine the absorption and pharmacokinetics of these bioactive compounds and their known possible interactions with other substances, drugs, and food.
7. *In silico* studies reveal the structure-function relationship of bioactive compounds as well as provide information on the toxicology and pharmacokinetic studies of bioactive compounds. It validates the *in vivo* and *in vitro* models as well. *in silico* study also provide the information for the creation of computational models or simulations that can be used to make predictions suggest hypotheses, and ultimately provide discoveries or advances in medicine and therapeutics. Amino acid sequences, which provide information about the structural and functional similarities. It covers the area of molecular docking, three-dimensional structures, and interaction of target–ligand binding in bioactive compounds.
8. Assessment of bioactive compounds using several molecular methods including genomics (DNA/RNA) and proteomics (protein) and tools such as immunoblotting, microarrays, polymerase chain reaction (PCR), western blotting and protein sequencing.
9. Cell-based experiments cover a wide range of biochemical cell-free and cell culture assays. In cell-based assays, alteration in the function of the target protein and biological significance of the protein in many diseased states, including cancer and neurodegenerative diseases. Cell differentiation, apoptosis, growth and proliferation, membrane transport, metabolism, cytotoxicity, signal transduction pathways, reporter gene, agonists, and antagonists' identification, can be assessed in cell-based assays and cell culture experiments.
10. Bioactive compounds can be evaluated in *in vivo* and *ex vivo* models, in which more complex structures are examined. In these models, a small number of animals (blood vessels, brain, cardiac muscle, endocrine glands, liver, spleen, smooth muscle of the gastrointestinal tract, airways, urinary tract, among others) are used for biological experiments.

11. Biological techniques, which range from *in vitro* tools to the use of whole animal models, aid the validation of clinical trials, and permit the modulation of a desired target in diseased patients. Scientific evidence in animal models can also be validated in transgenic and gene knockout animals, using small molecule inhibitors, antisense oligonucleotides, and small interfering RNA (siRNA). Information or data generated from animal models may predict the efficacy of bioactive compounds in alleviating or promoting the signs and symptoms of human diseases. This fact can be validated and confirmed after the completion of clinical trials (Fig. 10.7). Nevertheless, animal tests are essential to guide the early stages of development, particularly for making decisions regarding whether to such bioactive compounds be tested in human models or clinical trials be performed.
12. The initial results obtained from *in vitro, in vivo*, *ex vivo* studies, as well as clinical trials in normal subjects and diseased individuals on the therapeutic effects, the clinical indication and the pharmacokinetic profile of bioactive compounds are essentials for the confirmation of the efficacy of bioactive compounds in many biological models (Motilva et al. 2015; Jean-Quartier et al. 2018; Curtis et al. 2008; Gil et al. 2015; Gomes et al. 2018; Lindequist 2016; Malve 2016; Nair et al. 2015; Choudhary et al. 2017) (Fig. 10.7).

10.9 Functional Food and Food Processing

Food or diet are susceptible to alterations in the processing and composition of nutrients, and hence, understanding of nutrients that improves health should be instilled in manufacturing novel products (Hasler 2002). Functional foods are food and food products, which contains bioactive compounds in their natural forms or processed style, and can supply health benefit in addition to the primary role of providing essential daily nutritional needs (Abuajah et al. 2015; Picó et al. 2019). Plant foods such as spices, grains, cereals, legumes, nuts, fruits and vegetables are often considered as functional foods. Bioactive compounds and bioactive ingredients can be extracted, purified, incorporated into other food products as supplements and in tablet form (nutraceuticals) (Picó et al. 2019; Varzakas et al. 2016). In addition to the extraction and purification of bioactive compounds in functional foods and food products, food processing methods may alter the nutritional, sensory and biological properties of food products as well as quantity and quality of bioactive compounds in food (Abuajah et al. 2015; Wang and Bohn 2012). The significance of traditional and modern food processing techniques in preservation and deactivation of bioactive ingredients/compounds have been reported in literature (Hasler 2002; Abuajah et al. 2015; Picó et al. 2019; Varzakas et al. 2016; Wang and Bohn 2012). The fortification of food is a well-developed production technique and can be seen in application of many products, for example infant meals which are often fortified with minerals and vitamins mineral (e.g Vitamin A, B, C, D, E, K, calcium and iron), fruit juices with added omega 3 fatty acids, breakfast cereals with

fortified vitamin (e.g. folic acid) and mineral (e.g calcium and iron) (Betts et al. 2014). Manufacturers need to consider if the product is able to take the added ingredient within its natural matrix simply, or there is a need for further process alterations (e.g. encapsulation) (Wang and Bohn 2012; Betts et al. 2014). This way could include administration of the protected bioactive ingredients to their specific site and release under certain trigger factors (enzymes, pH, salts, etc.) (Wang and Bohn 2012; Betts et al. 2014).

Technologies of some food processing are listed below:

Mechanical Processes

- Size designation: particles are separated according to size by filtering and size classification. This is used in grain processing, application of milling. Examples of apparatus are air separators, sifting machines (Wang and Bohn 2012).
- Sorting: separation of particles from each other. This is used in separation according to density, susceptibility, magnetic, electricity conductivity differences. Examples of apparatus are separation of stones, magnets (Wang and Bohn 2012).
- Filtration: filtration of liquids, solids, which is used in the separation of solid particles. They are used in dairy industries, beverage industries, ingredient manufacturing. Examples of filtration apparatus include fixed bed filtration, membrane filtration unit (Wang and Bohn 2012).
- Centrifugation: separation of particles by suspension by centrifugation forces. This is used in the dairy industry, beverage industry, processing of vegetables and fruits, production of oils. Examples of apparatus include a separator, centrifuge (Wang and Bohn 2012).
- De-foaming: division of non-needed stable foam during processing by the use of mechanical fixtures to de-stabilize foam, division of liquid, gas. This is used in dairy industries, beverage industries—examples of apparatus process machinery within mechanical fixtures, tanks (Wang and Bohn 2012).
- De-dusting: extraction of solid particles from the gaseous phase, e.g., prevention of dust explosions by centrifugation forces, filtration medium. This is used in milling powder, baking powder. Examples of apparatus are air separator, aerocyclone (Wang and Bohn 2012).
- Floatation: division of solid particles from liquids by linking particles to the gas bubble, and then foam separation. This is used in beverage industries. An example of an apparatus is the floatation reaction vessel (Wang and Bohn 2012).
- Agglomeration: production of larger particles from a mixture of powder by the affinity of particles, used in ingredient industries, pellet production, and tablet production. Examples of apparatus palletization drum, tablet press (Wang and Bohn 2012).

Thermal Processes

- Heating: When heat is introduced to food in various applications and methods (boiling, steaming, roasting, indirect heating, microwave, sterilization, pasteurization, drying etc), it alters chemical or rheological properties. Example of apparatus includes cooking vessels, autoclaves, reaction vessels, continuous liquid sterilization (UHT), drying machines (Wang and Bohn 2012).

- Cooling: control of the temperature of products by extraction of heat energy via passive or active cooling. This is done through the processes of food production. The apparatus used is similar to the ones used in heating (Wang and Bohn 2012).
- Evaporation: extraction of liquid or moisture content, elevated reliable content by application of heat (under controlled conditions) to evaporate liquid or solvent (water etc.). used in beverage industries, powder and ingredient manufacture. Example of apparatus used is evaporation tower (Wang and Bohn 2012).
- Crystallization: division of solids from liquids. Alteration in temperatures stimulates crystallization of solid in high concentration. They are used in sugar industries, ingredient industries. An example of the apparatus used is the crystallization reactor (Wang and Bohn 2012).

Various production processes are needed to produce food from unprocessed materials, reconstruct the rheological and physical appearance of the product to make sure the food is healthy and safe, with equal and consistent quality, stability, and supply (Wang and Bohn 2012). One of the most used procedures in many liquid and food products is treated with heat, which is used in processing the product (i.e., handling the product in order to enhance the bioavailability of nutrients, reconfigure carbohydrates, starches, and protein denaturation), in order to produce the required taste, smell, appearance (e.g. Maillard reaction), alter the structure of the food (e.g. changes in texture as a result of changes in ingredients or the process of drying), or to store, preserve or disinfect the food by inactivating the microorganism, enzymes, and toxins by heat (e.g. canned foods been sterilized by heat, vegetable blanching to cause the enzymes to be inactive) (Wang and Bohn 2012). Procedures of heat treatment as well as other major food processing procedures may often lead to reduction of bioactivities of resident ingredients, which are crucial to human diet (Wang and Bohn 2012). Significant sources of essential phytochemicals like vegetable and fruit products need to be preserved while been processed, stored and packaged so that they remain available for human diet (Reuterswärd 2007). Recently, there has been development in novel food processing procedures:

- High-pressure treatment/Ultra-high-pressure treatment: It is one of the ways by which hygienic food products can be obtained. Most microbes cannot survive the high pressure in which food is subjected to, hence it reduces microbial load and provides safety against microorganisms. It is also beneficial as the one of the best preservative methods of natural and high quality nutritious and sensory (appearance, flavor, taste and texture) values. It is applied in the sterilization of products that easily or quickly spoil and damage through processing (e.g. meat, dairy products, fruits, sea foods and vegetables). Although, it is limited due to the high cost of processing (maintenance and investments), and the available apparatus are mostly batch processes (Wang and Bohn 2012).
- Freeze drying: freeze-drying is an excellent preserving procedure of products characteristics and can be used in a vast range of products. It is applied with products that have a fragile texture in which there is a need to protect the naturally occurring ingredients (e.g. flavors). E.g. fruits having high application values. It is limited due to the fact that there can be formation substances that are heat

stimulated, also there is lesser protection from microorganisms, and the cost of production is also high (Wang and Bohn 2012).
- Ultrafiltration/membrane filtration: unwanted products like products formed in heat treatment are not developed. It is applied as additional treatment in the pasteurization of milk (it is possible to treat in overall smaller temperature). It is limited because it can be used in only liquid products, and the energy cost used is high (Wang and Bohn 2012).

10.10 Determination of Shelf Life (Stability)

Shelf life (Stability) of a product is defined as the time frame in which a product is still acceptable for ingestion or consumption at specific storage temperatures and other conditions. For products having health claims, it is expedient that shelf-life is considered:

- Shelf-life and continuity or consistency of the food/bioactive compound, which is expected to portray the acclaimed effect is ascertained in the final product as ingested (Betts et al. 2014);
- Still hold nutritional parameters to align with any label declaration of nutritional information (Betts et al. 2014).

As shelf-life analysis proceeds, the parameters of a product with a health claim, the consistency and stability of food/bioactive compounds for which the demand is expected in the product as ingested, sensory microbiological, chemical and biochemical properties and ingestion of the product are examined (Betts et al. 2014). The point in a product becomes unacceptable from one or more of these aspects is the expiry of shelf-life (Betts et al. 2014).

The stability of active ingredients or bioactive compound(s) represents an additional, important factor affecting the shelf-life of food products that have health claims. In certain situations, it could represent the shelf-life limiting factor (Betts et al. 2014).

The shelf life of food product depends on the method of preservation used and the nature of the food products (Betts et al. 2014; IFTS 1993). The kind of packaging employed in containing the food will also have a substantial effect; hence, the producer of the food can decide and assigns the shelf life of the food, whilst noting the requirements of relevant legislation (IFTS 1993; Campden and Chorleywood Food Research Association Group 2004). Many factors influence the shelf life of a product, some include raw materials, proper treatment or hygiene, formulation of product, intrinsic properties of the product, such as salt content, water activity, pH, preservatives, procedure steps and parameters, packaging, including gas atmosphere, oxygen content, distribution time and temperature, handling of the consumer (Betts et al. 2014).

Intrinsic and extrinsic factors affect the stability/shelf-life of a product (Betts et al. 2014).

10.10.1 Intrinsic Factors

These are the characteristics of the food itself.

- The quality and nature of the ingredients, raw materials, constituents, and bioactives: The stability of the food constituent has to be controlled during the shelf life, and the deterioration curve of the compound examined (Betts et al. 2014). It is expedient to be able to state the targeted shelf life. Decent quality raw materials with less amount of microorganisms present should result in products with a consistently approved shelf-life (IFTS 1993; Campden and Chorleywood Food Research Association Group 2004). For raw materials with several impurities/ dirts and high microbial load, further treatment or washing of plant material is required to remove the impurities/ dirts and minimize the amount of microorganisms that can lead to spoilage thus extending the shelf-life of the food products (Betts et al. 2014; IFTS 1993; Campden and Chorleywood Food Research Association Group 2004). In such scenario it is expected to set specifications (microbiological limits) on raw materials. The stability of the constituent/bioactive should be analyzed while the processing is going on and the storage to know whether the beneficial health effect has been altered or not (IFTS 1993; Campden and Chorleywood Food Research Association Group 2004).
- Formulation of products as well preservative use: The extraction of fluid content can hinder mold and bacterial deterioration or spoilage.
- Structure of the product: Fluids and semi-foods usually have a homogeneous composition, unlike fast foods that do not have a similar arrangement (Betts et al. 2014; IFTS 1993). Moisture and flavours movement through layers, coatings and surface treatments will hinder or aid the spoilage potential. The structure of the product can influence the bioavailability (Campden and Chorleywood Food Research Association Group 2004).
- Availability of oxygen and redox potential within the food: This can exhibit a crucial impact in which microorganisms that cause spoilage and pathogenic organisms can develop and survive on the food (Betts et al. 2014). This can also influence the oxidation-reduction reactions which leads to rancidity, vitamins loss, cause browning effect, and changes to flavour. Moulds require oxygen to develop and as such are typically found on the surface of food (Campden and Chorleywood Food Research Association Group 2004).

10.10.2 Extrinsic (External) Factors

- Procedures applied to food: The impact of technology needs to be examined on the stability of bioactive compounds.
- Canning: The process of canning inactivates most organisms that are heat-resistant. However, milder heat procedures will lead to the inactivation of some

bacteria, and a higher number will survive (Betts et al. 2014). The greater the raw materials present, the higher the amount of microorganism that will withstand and lessen the shelf-life. The more the procedures, the lengthier the shelf-life generally. In addition, the bioactive compounds in canned foods are grossly affected by the heat treatment thus enhancing or reducing the potency of the bioactive compounds.

- The kind of packaging, including the gaseous environment: Packaging has a primary objective of shielding or protecting food after been manufactured and, as such, can be used to lengthen the shelf-life. However, if the gaseous environment is altered (for example, gas flushing or vacuum packing), this will add to the development of some microorganisms that cause spoilage and pathogens, as well as aiding the growth of microorganisms that need oxygen (including moulds) (IFTS 1993). It should be noted that specific attention needs be given to psychotropic pathogens, pathogens that can develop at lessened temperature of the cold chain (Betts et al. 2014; IFTS 1993; Campden and Chorleywood Food Research Association Group 2004). Aside the microbial action, the preservation of bioactive compounds present in packed foods depend on the materials used for packaging as well. Exposure of packaged foods to several environmental conditions such as temperature, pressure, may alter the physicochemical and biological activities of the bioactive compounds in food.
- The temperature of Storage (Ambient, chilled, or frozen): As storing in cold conditions will inhibit the development of microorganisms, some specific pathogens and microorganisms cause spoiling, that freezing can only reduce the growth speed but not outrightly stop their growth (Betts et al. 2014; IFTS 1993; Campden and Chorleywood Food Research Association Group 2004). A lot of spoilage microorganisms and specific important pathogens will grow actively because they are psychotropic (cold-tolerant), however their development will generally be slower unlike the growth rate in ambient storage (Betts et al. 2014; IFTS 1993; Campden and Chorleywood Food Research Association Group 2004). In addition, consistent alteration in the temperature of bioactive compounds in stored food may affect the structure –function relationship of these bioactive compounds. It can lead to breaking and/or formation of certain bonds and rings.

10.10.3 Recommended Practices for Shelf-Life Testing

Examination of stability or shelf-life is essential when products are formulated again; for example, less critical alterations done when products are reformulated may have a crucial influence on growth of microorganisms, or on texture and stability of outcome (IFTS 1993; Campden and Chorleywood Food Research Association Group 2004). An alteration in formulation of product will cause re-examination of the shelf-life of the products. If there is an alteration in parameters, procedures used

in the development of the product, the originality of the previous shelf life/stability data needs to be elucidated (Betts et al. 2014). The resultant effect of these alterations on the content of the bioactive constituent, sensory and other properties, food safety can be assessed reliably if only the shelf-life analysis were manufactured on the same product, in the same packaging, processed with the same technology (Betts et al. 2014; IFTS 1993).

Before the shelf-life examination, HACCP analysis must be carried out to evaluate and ascertain the significant factors influencing the safe shelf-life, the safety of the sensory assessors also involved in the evaluation of the shelf-life is a prerequisite (Betts et al. 2014). Hence, it is expedient that the safety of products must be ascertained before they are evaluated. The quantity of analysis carried out is dependent on the target shelf-life of the product, sampling should be done at the inception of shelf life, at the end of the target shelf life and minimum three times in between (IFTS 1993).

For temperature-sensitive constituents, like bioactives, it is better to carry out analysis at maximum and higher temperatures. Evaluation at peak and higher temperatures will reveal the influence of the changes from the optimal storage temperature (Betts et al. 2014; IFTS 1993; Campden and Chorleywood Food Research Association Group 2004). The product needs to be stored at the specific storage temperature, for those products that need to maintain cold chain during their storage and handling it is also expedient to carry out another evaluation to ascertain the influence of the abuse of temperature (Betts et al. 2014). Some of the tests for checking the shelf life (mostly sensory and microbiological ones) can be made more times to monitor and improve the understanding of the possible changes in time (IFTS 1993; Campden and Chorleywood Food Research Association Group 2004).

Extra information can be gotten for evaluation of the shelf-life:

- a complete analysis of the shelf-life of like-products already being manufactured;
- the use of visible microbiological models for visualizing when the growth of microorganisms (pathogens and organisms that cause spoilage) may get to a critical point and render the product unacceptable (IFTS 1993);
- forced storage, for example, increased temperatures could be used to improve the rate at which deterioration occurs, and hence lessen the length of shelf life evaluation, which is of excellent benefit is marketed after production (Campden and Chorleywood Food Research Association Group 2004). However, it is not all reactions as food spoils that follow Van't Hoff rule (which states that increase in temperature by 10° generally increases the rate of chemical reactions by a factor of 2–3), this procedure is constricted, but can however be used in getting results faster as regards to shelf life behaviour of food product (Betts et al. 2014); and
- use of storage at different temperatures.

In manufacturing, all these procedures have limitations, and using all together can be used for some food products to get an accurate result (Betts et al. 2014; IFTS 1993). In examining the shelf life of food, it should be pointed out what characteristics of the food are to be the restricting factor (Betts et al. 2014).

10.10.4 Establishing Specification for Active Ingredients and Ensuring Homogeneity Between Batches (Steps from Prototype Development to Scaling-up to Factory Level)

Usually, the model product evolution entails three stages: small scale bench work-model evolution, pilot scale work, and production scale factory trials (Betts et al. 2014).

10.10.5 Small Scale Bench Work: Prototype Development

The main objective of the small scale benchwork is to ensure proper formulation of food products and analyze the products for physical-chemical and sensory properties. The products should be manufactured in a reproducible and can be done in a way that saves or manages cost (Campden and Chorleywood Food Research Association Group 2004). Samples from this level can be used as a foundation in the next steps. The possibility and viability of the product need to be reassessed (Betts et al. 2014; Campden and Chorleywood Food Research Association Group 2004).

Right from the activities of the small scale bench work to the factory trials the data needs to be gotten, examined, reassessed and corrected systematically for the complete product specification and also in line with the ingredient specifications, recipe, and product properties, feasibility of the quantity of the constituent within a batch and between batches and the stability of the component during shelf life, cause–effect relationship, food safety and HACCP, process ability, costs and consumer approval (Betts et al. 2014; Campden and Chorleywood Food Research Association Group 2004).

In the product development brief, the minimum effective dose of the bioactive compound needs to be clearly stated for the product development team, and gotten at preparation, manufacture of all samples and maintained during their shelf-life (Betts et al. 2014). The nature of the product and the bioactive compound should also be reassessed and evaluated if the planned production process has an altered on their stability/degradation (Betts et al. 2014; IFTS 1993; Campden and Chorleywood Food Research Association Group 2004).

The first draft product specification needs to be set up during the small scale bench work. However, some of the data can only be defined during pilot-scale trials (Abuajah 2017). The sensory and nutritional properties of the test and control product need to be analyzed to ascertain that they match each other, the nutrient composition of the analysis and control product should be assessed to determine they align with all legal requirements (Betts et al. 2014; IFTS 1993).

10.10.6 Pilot-Scale Work

A right product specification entails the data listed below to ascertain standardized properties (IFTS 1993). During the pilot scale work, batch sized products are manufactured with the same type of apparatus or at times with the similar equipment and process like those been used in the in full-scale marketing production. At this level, the process ability of the product can be examined (IFTS 1993). The sensory characteristics, microbiological and chemical composition, physical parameters, shelf life and the HACCP study of the analysis and the model products stated at small scale bench work needs to be ascertained. It should be determined that the data product aligns with the control product for the samples to be made available for the human intervention studies (IFTS 1993). The product properties need to be reviewed with the draft specification to know if it aligns, specifically the weight, microbiological parameters, chemical, physical parameters of the products and sensory properties (IFTS 1993).

For products that have health claims, personal observations have to be made on the accurate description of the following information (Betts et al. 2014; IFTS 1993):

- Name of the product, identification of document: date; and
- Composition of the product: formulation of the product; percent of the ingredient, bioactives in the recipe for standard production volume; ingredients list, ingredients specifications, raw materials; bioactive compounds that have beneficial health effect, bioavailability with the limits of feasibility within the processed product; data for characterization of the bioactive compounds and the food matrix; allergen and sensitivity information; ethical and religious information (Betts et al. 2014; IFTS 1993; Campden and Chorleywood Food Research Association Group 2004).

Another aspect of data needed for the product specification for all new products separately from products with health claims (Betts et al. 2014; IFTS 1993; Campden and Chorleywood Food Research Association Group 2004):

- recognized legal information;
- the kind of additives used;
- small description of the production process: HACCP summary, CCPs;
- quality and quantity parameters: nutritional parameters/labelling nutritional information/nutritional profile (as appropriate) and their maximal approved feasibility; product structure and the bioavailability of food/bioactive constituent; sensory parameters and their maximal approved usefulness; microbiological, chemical, physical properties; quality assurance and food safety limits of feasibility within the finished product; chemical, physical, microbiological; weight filling; packaging, the kind of the primary and secondary packaging, specifications of the packaging materials; shelf life at set condition of the storage of the storage of the storage of the storage of the storage of the storage of the storage of the room; transport requirements; storage requirements; labelling, product label; health claims; nutritional values; allergen, sensitivity information; ethical and

religious information (as appropriate); GMO information; instructions for users; statement of warranty; recommendation by authorized person (Betts et al. 2014; IFTS 1993).

All of the required data on food safety, religious, ethical, nutrition and sustainability information along with a defined preparation/users' manual and storage and handling requirements needs to be listed in the product specifications (Betts et al. 2014; Campden and Chorleywood Food Research Association Group 2004). The specification of the concluded analysis and control products should be made available to the centers working on human intervention studies (HIS) (Betts et al. 2014; Campden and Chorleywood Food Research Association Group 2004). Scientists performing the HIS should also give feedback to the product manufacturing team regarding any needed changes of the product, packaging of the product, the portion size, and the method of preparation as soon as possible to allow smooth administration of the study (Betts et al. 2014). Production timeframe of the analysis and control products for HIS needs to be manufactured by the food manufacturing company and clinical center (Campden and Chorleywood Food Research Association Group 2004).

It is as well a beneficial way of preventing misunderstandings and provide useful data to the consumer/food producers and the food producers/consumers of the food product (Betts et al. 2014; IFTS 1993; Campden and Chorleywood Food Research Association Group 2004).

Process specification (Betts et al. 2014; IFTS 1993; Campden and Chorleywood Food Research Association Group 2004):

- process description;
- process steps description;
- performance criteria ("Fo" for sterilized products, "P" for pasteurized products, uniformity of composition, weight—target and tolerance);
- size of the batch, if relevant;
- process parameters (time, temperature, and pressure): target and acceptance limits;
- the procedure of monitoring of the key parameters, frequency, responsibilities;
- actions at deviations, responsibilities;
- approval, verification;
- HACCP summary;
- CCPs/and CPS, identification, descriptions;
- critical restrictions; procedures of monitoring, frequency, responsibilities; and
- corrective actions, responsibilities.

The process control measures, which has to be implicated for ensuring low feasibility within batches and between batches typically include the following elements (Betts et al. 2014; IFTS 1993; Campden and Chorleywood Food Research Association Group 2004):

- Designing the criteria for performance;
- Stating what has to be achieved at this step considering food safety, quality, legality, and uniform composition and properties;
- Reporting the control process for each level;
- Pointing out the critical control points, where parameters affecting the quantity of the bioactive constituent, legality, food safety hazards and quality attributes, the structure of the food and composition can be and required to be monitored (key control points and CCPs). These controls must be in place permanently (Betts et al. 2014);
- Pointing out the critical process parameters (target values and acceptance limits) (Betts et al. 2014);
- At the chosen key control points bringing up a monitoring system, based on reoccurring checks or continuous evaluations, observations. The results of the monitoring have to be noted. The monitoring activities, their reoccurrence, and responsibilities have to be defined (Betts et al. 2014);
- They are creating corrective actions, which have to be put into effect at deviations. The activities and responsibilities have to be stated and the steps taken have to be noted (Betts et al. 2014; IFTS 1993); and
- Confirmation and attestation of the process performance. This fact can be done by reassessing the process control data and by evaluating the major product parameters and properties such as the quantity of the bioactive compound, by assessing the amount of the parameters of the significant procedure steps ascertaining food safety, sensory evaluation, microbiological testing etc. (IFTS 1993; Campden and Chorleywood Food Research Association Group 2004).

Specific aspects linked to the products having health claims also have the uniform quantity and stability of the bioactive compound and the duplicable structure of the food, including the maximal approved feasibility of the bioactive compound within a batch and between batches (Betts et al. 2014; IFTS 1993; Campden and Chorleywood Food Research Association Group 2004). During the pilot-scale testing, the food products containing bioactive compounds and the materials used in packaging them have to be evaluated and examined. In addition, a targeted cost of the manufacturing process and overall costs have to be assessed (IFTS 1993). Packaging testing should be carried out. It should be ascertained that the test product aligns with the control product for the samples to be made available for the human intervention studies (Betts et al. 2014; IFTS 1993).

10.10.7 Factory Scale Production Trials

It is a standard industrial protocol to evaluate the duplicability of the main parameters as well as the quantity of a specific component such as the bioactive compound found on at least 3–3 representatives/samples taken at various times from varies parts of a batch from three non-dependent manufacturing tests (Campden and

Chorleywood Food Research Association Group 2004; Campden and Chorleywood Food Research Association Group 2007). The point of this level is to be able to manufacture food products on a bigger scale reproducibly to ascertain that the expected concentration of the bioactive compound which is the reason for the beneficial health effect can be continuously ensured within a batch and between different batches (Campden and Chorleywood Food Research Association Group 2007). The entire final version of the product needs to be delivered consistently at the exact cost and exact quality (Campden and Chorleywood Food Research Association Group 2004; Campden and Chorleywood Food Research Association Group 2007). As the product manufacturing procedures occur, the main versions of product specifications have to be reassessed following the alterations and acceptance (IFTS 1993). The growth of the strategy used in marketing begins with noting the needs of those who use the products (consumers) and making available products or services that pleases this request of the consumers (Betts et al. 2014).

10.10.8 Characterization of Active Ingredients/Bioactive Compound

It is expedient for any food or ingredient or bioactive compound in which its health claim is created to be characterized (Marconi et al. 2018). The originator of the bioactive compound and the part which entails it and its specification, and the specification of food category for which health claim is made needs to be made available (Betts et al. 2014; Marconi et al. 2018). For the recognition and enactment of the bioactive compound usually more developed experimental procedures are required, this entails noting new substances, characterizing their structure and mode of action, and also the significant factors of the amount in the standard matrix and controlling the specifications of the product (Marconi et al. 2018).

Experimental procedures need to be fit for the purpose and need to be assessed entirely for the aim. The Experimental methods used in the enactment of the bioactive compounds, food ingredients and nutrient examination of foods and macronutrients needs to be standardized and ascertained in line with the required guidelines (Marconi et al. 2018). According to the EFSA guidance the analysis needs to be carried out in a proper laboratory where the information can be approved and the quality system created in the laboratory is pointed out (Bernal et al. 2011; EFSA 2011). As touching experimental procedures, it is expedient to make use of detectors that are able to detect compounds structurally, and not only to measure them by time of retention, wavelength, etc. (Park et al. 2012). Recently, gas or liquid chromatography tandem mass spectrometry is one of the experimental procedures revealing the highest potential for carrying out these aspects (Park et al. 2012). The experimental procedures made should be ascertained in terms of specificity, accuracy and reliability (Park et al. 2012). Food containing bioactive compounds are raw materials for food and pharmaceutical industries. The food grade delivery systems

provide necessary strategy that alters the food product properties (Rajasekaran and Kalaivani 2013; Coelho et al. 2010). Food and food products containing bioactive compounds navigate the human body when consumed from the mouth through or to the stomach, small intestine or colon (Rajasekaran and Kalaivani 2013; Coelho et al. 2010; Palzer 2009). In the delivery system, encapsulation materials and the food matrix can be altered considerably during storage, processing, ingestion and digestion of bioactive compound in Food (Chen 2004; McClements et al. 2009a). Alterations are caused by ionic strength, pH, surface activities, activities of enzymes (lipases, proteases, amylases), flow and force profiles (disruption, pressure, agitation) linked with chewing, stomach and intestine passage in food bioactive compounds (McClements et al. 2009a; Ubbink et al. 2008; Marques 2014; Van Aken 2007; Pothakamury and Barbosa-Canovas 1995; Siepmann and Siepmann 2008). Therefore, conscious efforts are required to monitor the release, digestion, stability and absorption of food bioactive compounds in order to ascertain the health claims of such food products containing the bioactive compounds and components (Sereno et al. 2009; Augustin et al. 2001; Augustin and Sanguansri 2008; Chen et al. 2006). The manufacturers' knowledge, together with more understanding of the link between food properties and bioactive ingredient adsorption, is beneficial in the design of food materials and encapsulation techniques, which, after protecting the ingredient, give monitored release at target points in the gastrointestinal tract (Weiss et al. 2008; Dziezak 1998; Augustin et al. 2011; Hejazi and Amiji 2003; McClements et al. 2009b).

10.11 Conclusion

This chapter reviews the requirements of bioactive compounds in foods for health claims. Polyphenols, saponins, alkaloids, vitamins, minerals, terpenoids, omega and poly saturated fatty acids, polysaccharides, chitin, and chitosan and peptides are bioactive compounds that are capable of managing weight, modulating genes, enhancing good health as well as preventing diseases such as cancer, diabetes, cardiovascular disease, stroke, erectile dysfunction, endothelial dysfunction, heart and respiratory infections to mention a few. The procedures and criteria for coming up with proofs for health claims must be thorough scrutinized in order to provide the public with the accurate and correct information on therapeutic or/ and nutraceutical properties as well as toxicological effects. Variation in food processing techniques, safety and design of food bioactives/bioactive compound are essentials for laboratory investigation using different models and translation into human clinical trials. Thus, providing evidence-based criteria for possible adoption by industries. All hands must be on deck to ensure that scientists, policy makers, and professional like biochemist, microbiologists, food scientists, food technologists, food chemists and pharmacists follow these procedures and criteria on bioactive compounds for health claims. Many functional foods with these bioactive compounds with scientifically

health claims are currently in the market or under consideration by manufacturers/scientists. However, consumers should therefore adhere strictly to the instructions on these products/labels to avoid possible adverse effects and toxicities.

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