Functional Foods and Their Implications for Health Promotion

Edited by
Ioannis Zabetakis
Alexandros Tsoupras
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Editor dedications

Ioannis Zabetakis:
To the memory of my Dad (Αριστοτέλης—Aristotle), who taught me how to be practical and innovative and always look into the bigger picture.

Alexandros Tsoupras:
To my wife, Maria, and my family for their love and continuous support, and to the memory of my brothers George (Γεώργιος) and Nestor (Νέστωρ), who inspired me during their short presence, on how to be innovative, after thorough studying/evaluating of what has been found so far and viewing things from all possible angles, through their deep knowledge in chess and history.
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After an academic career in the Universities of Leeds (UK) and Athens (Greece), Ioannis joined the University of Limerick (UL) in Ireland, in 2015. Ioannis is a Senior Lecturer in Food Lipids and also the Head of the Department of Biological Sciences, in the UL. The ongoing focus of his work is on the cardioprotective properties of food lipids, with a particular emphasis on dairy and marine foods. With more than 110 scientific publications and 2 patents, his quest is toward a healthier diet and lifestyle that would render us less dependent on medicines.

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Alexandros is currently an Assistant Professor of Biochemistry and Food Biochemistry at the Department of Chemistry of the International Hellenic University of Greece Alexandros studied chemistry and biochemistry (BSc, MSc, and PhD) with Scholarships and had postdoc experience in Greece (University of Athens), United States (Albany Medical College, NY), and Ireland (Department of Biological Sciences, University of Limerick), where he was also a Lecturer in Food Science and Health for the last 5 years.

His current research is focused on the isolation and characterization of bioactive molecules, compounds, and extracts from sustainable natural sources, such as several foods and food products, byproducts, fermented products, food micro-organisms of biotechnological interest, medicinal plants, etc., for utilizing them on producing novel functional foods, food supplements/nutraceuticals and drugs. Alexandros has also been studying the structure activity relationships of these natural bioactives and the molecular mechanisms of their health benefits against key cellular processes during inflammatory manifestations and inflammation-related chronic disorders (i.e. atherosclerosis and cardiovascular diseases, renal disorders, cancer and metastatic procedures, persistent infections, neurodegenerative disorders, allergies and autoimmune diseases, etc.), with the goal of identifying new preventative and/or therapeutic pathways.

Alexandros's research has been funded by grants/fellowships/scholarships from several nonprofit institutions, while he has also developed several novel separation techniques, bioanalytical methods, inventions and participated in more than 70 peer-reviewed research outputs, and in several conferences, within the areas of biochemistry, food science, and health, with some of these outputs having been awarded.

His motto is "*As long as we have the opportunity, let's do our best for the good of all in the SHORT time given to us*."

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Dr. Ronan Lordan is a Researcher of circadian biology, inflammation, and nutrition at the FitzGerald Laboratory at the Institute for Translational Medicine and Therapeutics, Perelman School of Medicine, at the University of Pennsylvania. He received his BSc (Hons) in Biological Sciences with concurrent Education at the University of Limerick, Ireland. There he continued his studies and obtained a PhD at the Department of Biological Sciences and a Postgraduate Diploma in Teaching, Learning, and Scholarship at the University of Limerick. There he was a lecturer of nutrition, genetics, anatomy, and physiology for modules in the School of Natural Sciences and the School of Allied Health. Dr. Lordan has published over 50 peer-reviewed articles and book chapters. He is an active editor board member and reviewer for several peer-reviewed journals in the field of nutrition, medicine, and biochemistry for Elsevier, Frontiers, and MDPI. Dr. Lordan is a member of the University of Limerick Autism Special Interest Group and the University of Pennsylvania and the

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Preface

Chronic noncommunicable diseases (NCDs) including cardiovascular diseases (CVDs) are the leading causes of death and disability worldwide. Trends indicate that the prevalence of NCD is on the rise with a growing incidence of obesity, diabetes, hypertension, and many other metabolic disturbances in Western and developing populations. The cause of this shift in health is no secret. It has long been known that maladaptive diets and lifestyle are key contributory factors to the development of NCD, in particular, atherosclerosis and CVD. Poor diet and lifestyle in conjunction with our ever-worsening 24-hour lifestyles have meant that NCDs are responsible for 71% of all adult deaths according to the World Health Organization (WHO).

Diet, exercise, and changes to various lifestyle habits are known modifiable risk factors for the prevention of NCD and CVD. Dietary patterns such as the dietary approaches to stop hypertension (DASH diet) or the Mediterranean diet have even been implemented successfully in trials to reduce the burden and risk of CVD. Nutrition as a preventative to disease is not a new idea and has been well documented throughout the centuries. What defines a functional food is its capacity to promote health beyond its basic nutritional value. This may be a food product that is designed to be high in vitamin D, such as fortified milk or irradiated mushrooms. A functional food could be produced from animals by the feed they are given. For example, fish fed olive pomace in their feed have increased bioactive lipids in their flesh or hens fed flax seeds have increased omega-3 fatty acids in their eggs. Indeed, the development of dairy products with specific probiotics or the use of fruit and vegetable wastes for the production of a functional ingredient like a powder high in anti-inflammatory compounds, may all be classified as functional foods due to the additional benefits beyond their simple nutrient contents. Consuming such foods can confer additional benefits to humans. With the knowledge that dietary modifications can reduce the risk of CVD, food manufacturers and supplement developers have become increasingly interested in the development of foods that can contribute to health and wellness. What makes a functional food different from any other type of food is that functional foods are purposely designed to beneficially impact human health.

In the pursuit of health and wellness, sales of products that may beneficially affect health such as dietary supplements, nutraceuticals, and functional foods have grown over the last two decades. Indeed, it is forecasted that the functional foods market will be worth approximately $267,920 million USD by the year 2027. With this level of growth ahead, regulatory agents such as the European Food Safety Authority (EFSA) have implemented legislation and pathways for the regulation and oversight of health claims in a move to protect the consumer and ensure efficacy of functional foods and novel ingredients that may one day help fight the scourge of NCD.

In this book, we have invited experts with a collective interest in functional foods to contribute their knowledge on a wide variety of topics in chapters that detail the latest developments in the field. These chapters focus on the main functional foods under development, the processes involved, the challenges faced, and how the recovery and valorization of food industry byproducts can create value-added products. These chapters also detail the benefits of functional foods with respect to the prevention of metabolic and cardiovascular diseases. Topics discussed in the chapters include the development of functional foods from berries, milk, fruit pomaces, vegetables, the alcoholic beverage industries, and the byproducts and bycatch of seafood and shellfish industries. We hope this book will be useful to food and nutrition, nutraceutical, and pharmaceutical scientists and aspiring developers of functional foods to maintain the health of future generations to come.

Ioannis Zabetakis
Alexandros Tsoupras
Ronan Lordan
Dipak Ramji

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Ioannis

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Alexandros

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I am also grateful to my previous PI, Prof. C.A. Demopoulos, Biochemistry and Food Chemistry Lab, Chemistry Department, University of Athens, for introducing me to the research "trip" of Biochemistry, Food Science, and tackling inflammation-related disorders by utilizing nature's "weaponry."

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I wish also to express my gratitude to my wife, Maria (Μαρία), my parents Vasilios and Georgia (Βασίλειος and Γεωργία), my big brothers Nestor (Νέστωρ) and George (Γεώργιος), who passed away too early, and to all the other members of my family, Ioanna (Ιωάννα), Christos (Χρήστος) and their children, for their constant love, support, and encouragement.

I also feel eternal gratitude to the *Holy Trinity (Αγία Τριάδα—"Agia Triada")*, and especially to *"Logos" (Λόγος—"The Word")*, for always lighting the fire within me for Science and Research, for the good of all: "*ἐρευνᾶτε τὰς γραφάς, ὅτι ὑμεῖς δοκεῖτε ἐν αὐταῖς ζωὴν αἰώνιον ἔχειν" (Research and investigate the Scriptures diligently, because you believe and hope that within them you will find eternal life); St. John's Gospel 5:39*.

Ronan

I would like to thank my co-editors, Dr. Zabetakis, Prof. Ramji, and Dr. Tsoupras, for their collaborative efforts editing this book. I am grateful to the Institute for Translational Medicine and Therapeutics at the Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA, for their continued support. In particular, I would like to thank Dr. Garret A. FitzGerald and the FitzGerald laboratory team with whom it has been an inspiration to work. I would also like to acknowledge my former colleagues at the Department of Biological Sciences, University of Limerick, Ireland, who have supported my career at every step. I would like to thank my siblings Anthony and Lorraine and their families for their love, and the craic. I also want to express my sincere gratitude to my partner Samantha and my parents John and Margaret, for their unwavering love and support.

Dipak

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Part I

Plant-derived functional foods

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Chapter 1

Vegetables as functional foods against cardiovascular diseases

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1. Nutritional quality of vegetables

Vegetables are an important part of the human diet worldwide and are a source of vitamins (C, A, B1, B6, B9, E), minerals (Fe, Zn), fiber, and phytochemicals (Ryder, 2011). Some vegetable phytochemicals, such as flavonoids, isoflavones, carotenoids, anthocyanins, and phenolics are powerful antioxidants and protect against chronic diseases by treating free radical damage, altering the metabolic activation and detoxification of carcinogens, or even affecting processes that alter the course of cancer cells (Herrera et al., 2009; Slimani & Margetts, 2009). Vegetables' intake has been linked to good health, improved gastrointestinal health and vision, and reduced risk of certain cancers, heart disease, stroke, diabetes, anemia, gastric ulcers, rheumatoid arthritis, and other chronic diseases (Keatinge et al., 2010). A high-vegetable diet has been associated with a lower risk of cardiovascular diseases in humans. When a diet is low in vegetables, it is estimated that it causes about 31% of ischemic heart diseases and 11% of strokes worldwide. According to the 2007 World Health Report, unbalanced diets with low vegetable intake and low intake of complex carbohydrates and dietary fiber are estimated to cause approximately 2.7 million deaths each year and were among the top 10 risk factors contributing to mortality (Silva Dias, 2010).

Most vegetables are marketed fresh. Consumption shortly after harvest guarantees the best quality of vegetables. Vegetables in all their forms ensure adequate intake of most of the vitamins and nutrients, dietary fiber, and phytochemicals that help protect health. There is a growing awareness among consumers about the benefits of vegetable-rich diets to ensure adequate intake of most vitamins and micronutrients, dietary fiber, and health-promoting phytochemicals. Interest in whole foods with improved nutritional value is high and most consumers choose foods based on their functionality and health benefits. It is important to note that the health benefits of vegetables should not be limited to one substance or type of vegetable, but a balanced diet that includes more than one type of vegetable is likely to provide better results (Dias, 2012). Most vegetables help protect against chronic diseases. Their bioactive ingredients include vitamins, fiber, selenium, folic acid, carotenoids, and polyphenols, such as flavonoids. The main difference is that each group of vegetables contains a unique combination and amount of these substances.

For example, the Apiaceae family (celery, parsley, carrot) is rich in flavonoids, carotenoids, vitamins C and E. Celery and parsley are among the best sources of apigenin and vitamin E (Nielsen et al., 1999). While carrots are rich in carotenoids and also have a unique combination of three flavonoids: campferol, quercetin and luteolin (Horbowicz et al., 2008; Lila, 2004). The family Asteraceae or Compositae (lettuce, chicory) is rich in conjugated quercetin, flavonoids and tocopherols (Crozier et al., 2000). The Cucurbitaceae family (pumpkin, melon, cucumber) is rich in vitamin C, carotenoids and tocopherols (Dhillon et al., 2012). The family Chenopodiaceae (spinach, beet greens) is an excellent source of folic acid and has been shown to inhibit DNA synthesis in the proliferation of human gastric adenocarcinoma cells (He et al., 1999; Scott et al., 2000). Also all legumes (family Fabaceae or Leguminosae) (beans, peas, soybeans, chickpeas, lentils), apart from being very good sources of plant-based protein intake, they are also good sources of dietary fiber and isoflavones (Misra, 2012). Some legumes are also rich in iron (Trinidad et al., 2010). Another category of vegetables are cruciferous vegetables (Brassicacea or Cruciferae family), which include cabbage, broccoli, cauliflower, Brussels sprouts, cabbage, Chinese cabbage, turnip, radish, arugula, cardamom, and mustard. These vegetables are also rich in

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vitamins (C, E, β-carotene), contain significant amounts of dietary fiber, many are good sources of calcium and are able to accumulate significant amounts of selenium and contain many antioxidant flavonoids (myrictin, lucetin, quercetin, apigenin, campferol) (Banuelos & Meek, 1989; Hertog et al., 1992; Kurilich et al., 1999; Miean & Mohamed, 2001; Nielsen et al., 1993). Even vegetables of the Alliacea family (garlic, onion, leek, chive) are rich in a wide variety of thiosulfides, which have been linked to the reduction of various chronic diseases (Kubec et al., 2000). They also contain two types of flavonoids, anthocyanins and flavonols (quercetin and campferol) (Miean & Mohamed, 2001). They are an excellent source of calcium, selenium, potassium, manganese and chromium, as well as dietary fiber and especially inulin, a polyfructose (Ritsema & Smeekens, 2003; Wang et al., 2005). The therapeutic value of these vegetables is confirmed by multiple epidemiological and experimental studies, and the prevention of cardiovascular diseases has been attributed to the regular consumption of garlic and onions that also contain a number of bioactive molecules that can reduce the risk of cardiovascular diseases (Osmont et al., 2003; You et al., 2005).

Tomato is the second most popular vegetable widely consumed and cultivated in the world after the potato. It is consumed both fresh and in many processed forms (ketchup, canned whole or in pieces, puree, sauce, soup, juice, or sun-dried). It has a unique nutritional and phytochemical profile. The main phytochemical products of tomatoes are carotenoids consisting of 60%−64% lycopene, 10%−12% phytoen, 7%−9% neurosporen and 10%−15% carotene. Processed tomatoes (sauce, paste, juice and ketchup) contain 2−40 times higher lycopene than fresh tomatoes. Tomatoes and tomato-based foods are the richest sources of lycopene (Gerster, 1997; Tonucci et al., 1995). Tomatoes also contain significant amounts of α-, β-, γ-, δ-carotene (0.6−2.0 mg/kg), which is the fourth leading food for provitamin A and vitamin A. In addition to lycopene, tomatoes are rich in potassium and ascorbic acid (200 mg/kg), while they also contain small but significant amounts (1−2 mg/kg) of lutein, α-, β- and γ-tocopherols and flavonoids (Abushita et al., 2000; Leonardi et al., 2000; Rao & Rao, 2007). The flavonoids in fresh tomatoes are present only in conjugated form as quercetin and campferol, while processed products contain significant amounts of free flavonoids (Stewart et al., 2000).

Potato is not only perceived as a source of carbohydrates, but it is also an excellent source of essential amino acids. Potato contains a small amount of protein (less than 6%), but the biological value of potato protein is the best among plant sources and comparable to cow's milk (Dias, 2012). Studies in human nutrition have shown that potato proteins are of very high quality, probably because they are rich in essential amino acids, such as lysine and other metabolites, that can enhance protein utilization (Friedman, 1996). The lysine content of potatoes complements cereal-based diets, which are deficient in this amino acid. In addition to high-quality protein, potato tubers accumulate significant amounts of vitamins (C, B6, thiamine, riboflavin, folic acid, niacin) and minerals (K, Mg, P, Fe, Se, and Zn), as well as a variety of phytochemicals such as phenolics (90% chlorogenic acid), phytoalexins and protease inhibitors (Friedman, 1997). Almost 50% of the total phenolic compounds in the potato are found in the skin and adjacent tissue but are reduced to the center of the tuber. Other antioxidants found in potatoes are α-tocopherol, lutein, and β-carotene (Lachman et al., 2000).

Peppers have a range of colors and shapes. All fresh peppers are excellent sources of vitamins A, C, K, carotenoids, and flavonoids (Bosland, 1996, pp. 479−487). Red peppers are a good source of lycopene, while β-cryptoxanthin, another carotenoid in red peppers, has anticancer effects (Dias, 2012). In addition to being rich in phytochemicals, peppers provide a decent amount of fiber. Also, the levels of carotenoids of provitamin A (α- and β-carotene) in some varieties of hot peppers reach 12 mg/kg (Howard et al., 1994, 2000). The main flavonoids in peppers are quercetin and luteolin, their content varies between varieties (Lee et al., 1995). Red peppers also contain lycopene, which helps protect against cancer and heart disease. Like other nutrient-dense vegetables, peppers contain many different potent phytochemicals. Peppers have also been shown to prevent blood clots from forming and reduce the risk of heart attacks and strokes, possibly due to their content of substances such as vitamin C, capsaicin and flavonoids. The main phytochemicals in hot peppers are capsaicinoids (capsaicin, dihydrocapsaicin). Capsaicin in hot peppers has been shown to lower blood cholesterol and triglycerides, boost immunity and reduce the risk of stomach ulcers. They can help kill bacteria in the stomach that can lead to ulcers. Capsaicin also has analgesic, antibacterial and antidiabetic properties. Finally, hot chili peppers contain a good amount of minerals such as potassium, manganese, iron and magnesium.

Another important vegetable, eggplant, is grown in many countries in all subtropical, tropical, and Mediterranean areas, as it requires a relatively long period of hot weather to give good yields. In addition to being rich in vitamins (K, C, B6, folic acid, and niacin) and minerals (Mg, Cu, K), eggplant also contains important phytochemicals that have antioxidant activity. The phytochemicals contained in eggplant include phenolic compounds, such as caffeic and chlorogenic acid, and flavonoids, such as nanusin (delphinidin-3- (coumaroylrutinoside) -5-glucoside), which is the major phytochemical in eggplant. Nasunin is part of the anthocyanin pigment found in eggplant peel, purple radish, red turnip, and red cabbage. Nasunin is an antioxidant that effectively removes reactive oxygen species, as, by chelating iron, nasunin reduces the formation of free radicals with many beneficial effects, including protecting blood cholesterol from peroxidation that can prevent promote cancer and reduce free radical damage in the joints, which is a primary factor in rheumatoid arthritis

(Noda et al., 1998, 2000). Also, the predominant phenolic compound is chlorogenic acid, which is one of the most powerful free radical scavengers found in plant tissues (Matsuzoe et al., 1999). The benefits attributed to chlorogenic acid include antibacterial, anticancer, antimicrobial, and antiviral activities. In addition to their nutritional value, the phenolic acids in eggplant are responsible for the bitter taste of some eggplants. Eggplant also contains many other antioxidants, such as the carotenoids lycopene, lutein, and α-carotene, as well as the flavonoids myrictin and campferol (Ben-Amotz & Fishier, 1998). Eggplant is an excellent source of dietary fiber and manganese. Finally, studies have shown that eggplant is effective in treating high blood cholesterol (Jorge et al., 1998). Kwon et al. (2008) presented eggplant phenols as inhibitors of key enzymes associated with type 2 diabetes and hypertension.

2. Cardiovascular diseases

Cardiovascular diseases (CVD) are prevalent worldwide and their levels are increasing dramatically (Celermajer, 2012; Liu, Wang, et al., 2013). CVD have become one of the biggest threats to human health, according to the World Health Organization (WHO, 2017), after causing 17.9 million deaths in 2016, accounting for 31% of all global deaths. Of these deaths, 85% are due to heart attack and stroke. In addition to high morbidity and mortality, CVD also lead to severe disabilities and reduce patients' standard of living (Praveen et al., 2013; Yazdanyar & Newman, 2009; Zaina & Lund, 2011). Therefore, it is of major importance and worth exploring ways to prevent and treat CVD as Walker (2013) points out.

CVD are a category of chronic noncommunicable diseases that are significantly associated with complex and dangerous risk factors such as high blood pressure, hyperlipidemia, diabetes, obesity, metabolic syndrome, smoking, excessive alcohol consumption, unbalanced diet, and lack of physical activity (Anthony et al., 2014; Gomez et al., 2003; Li et al., 2014; Tam et al., 2005; Wens et al., 2013; Zhou et al., 2016). Many scientists argue that effective strategies can be applied to prevent and treat CVD to eliminate these factors, such as lowering blood pressure, regulating blood lipid profile, reducing oxidative stress, and regulating inflammatory state, inhibition of thrombosis and attenuation of myocardial damage (Gan et al., 2010; Kones & Rumana, 2014; Mozaffarian, 2016; Singh et al., 2015; Uthman et al., 2015; Zhang et al., 2016; Zhou et al., 2016). Meanwhile, a healthy lifestyle, consisting of a balanced diet, physical exercise, minimal alcohol consumption and smoking cessation, is beneficial to people at high risk of developing cardiovascular diseases (Dutton et al., 2014; Funtikova et al., 2015; Kwok et al., 2014; Naja et al., 2014; Zhou et al., 2016). Among these methods, establishing and insisting on a healthy diet would be an essential, sustainable and economical choice. It has been shown by epidemiological studies (Alonso et al., 2006; Khosravi-Boroujeni et al., 2012; Park, 2010; Pollock, 2016; Wang et al., 2014; Zhang et al., 2011) that vegetable consumption is associated with reduced appearance of CVD.

In addition, research data (Hu et al., 2013; Matori et al., 2012) have shown that many vegetables have been effective in preventing and treating CVD. Such vegetables are potatoes, soy, tomatoes, yams, onions, celery, broccoli, lettuce, and asparagus. The cardioprotective effects of vegetables are due to the bioactive ingredients they contain such as vitamins, essential elements, dietary fiber, protein, and phytochemicals (Armoza et al., 2013; Deng, Lin, et al., 2013; Karimi et al., 2005; Li et al., 2010; Zhang et al., 2013; Zhang, Yu-Jie et al., 2015). Possible mechanisms of action could include antioxidant, antiinflammatory and antiplatelet action, regulation of blood glucose, lipid profile and blood pressure and reduction of myocardial damage (Ademiluyi & Oboh, 2013; Ojewole et al., 2006; Robert et al., 2006; Rodrigues et al., 2005). Finally, clinical trials have shown that eating vegetables was beneficial for cardiovascular health (Hanachiphd et al., 2012; Iua et al., 2013;Miraghajani et al., 2013; Wong et al., 2012).

3. Epidemiological studies of vegetables and cardiovascular diseases

Numerous epidemiological studies have shown that increased vegetable consumption was related to a reduced incidence of CVD and lots of varieties of vegetables like tomatoes, potatoes, onions, cereals, and cruciferous vegetables showed cardioprotective activity (Alonso et al., 2006; Khosravi-Boroujeni et al., 2012; Park, 2010; Pollock, 2016; Zhang et al., 2011). Also, a variety of bioactive ingredients in vegetables have been shown to be beneficial to health, contributing to both the prevention and treatment of CVD (Alonso et al., 2006; Jacques et al., 2013; Park, 2010).

3.1 Cross-sectional studies

Several studies have evaluated the association between vegetable intake and the risk of developing CVD (Khosravi-Boroujeni et al., 2012; Medina-Remon et al., 2013; Sesso et al., 2012). Total cholesterol (TC), TC/high-density lipoprotein (HDL-C) cholesterol ratio, and hemoglobin A1c were found to improve significantly in women who ate more than

10 servings of tomato-based foods per week compared with those who ate fewer from 1.5 servings/week (Sesso et al., 2012). Specifically, the following differences were observed in women with higher consumption compared to those with lower consumption:

- TC was 5.38 mmol/L versus 5.51 mmol/L, $P = .029$ respectively,
- the TC/HDL-C ratio was 4.08 versus 4.22, $P = .046$
- and hemoglobin A1c was 5.02% versus 5.13%, $P < .001$

and consumers of higher vegetable intake were 31% less likely to have elevated above factors.

Another study of 4774 people in Iran found significant correlations between potato intake and diabetes. High levels of fasting blood sugar and low levels of serum HDL were observed (Khosravi-Boroujeni et al., 2012). These results indirectly showed a possible effect of potato consumption on CVD, as high fasting blood glucose levels combined with low serum HDL and diabetes are recognized as risk factors for CVD. In addition, another study of 3995 participants from Mediterranean countries found that in people at high risk for CVD, gazpacho, a Mediterranean cold vegetable soup, had antihypertensive activity (Medina-Remon et al., 2013). It was found that both systolic and diastolic blood pressure of the participants decreased by an average of 1.9 and 2.6 mm Hg respectively, in moderate gazpacho intake (1−19 g/day) and 1.5 and 1.9 mm Hg in high intake (over 20 g/day). Finally, the effects of hypertension were significantly reduced after weekly consumption of 250 g gazpacho.

3.2 Case-control studies

Similar findings were made in case-control studies investigating the relationship between vegetable intake and the incidence of CVD (Galeone et al., 2009; Khosravi-Boroujeni et al., 2013; Lian et al., 2015; Park, 2010). One study analyzed the relationship between onion intake and the incidence of acute myocardial infarction (MI) in Italy (Galeone et al., 2009). Compared with the control group, the risk of acute myocardial infarction (MI) was significantly reduced, both for the group consuming less than one onion serving per week and for the group consuming more than one serving per week (OR = 0.90 and OR = 0.78) respectively. Another study in Korea showed that eating vegetables helped minimize the risk of stroke (Park, 2010). Participants who ate four to six servings of vegetables a day and more than six servings a day were 32% and 69%, respectively, less likely to have a stroke. The researchers also found that the intake of vitamins B1, B2, B6, niacin and folic acid, as well as calcium and potassium were significantly associated with a reduced risk of stroke. Also, the effects of vegetable consumption on the association between hypertension and the relative telomere length of peripheral leukocytes were measured in a study (Lian et al., 2015). On the one hand, it was reported that longer telomere lengths adapted to age were associated with higher vegetable consumption. On the other hand, people with longer age-related telomere length were 30% less likely to suffer from hypertension. This significant relationship was observed only in those with a higher vegetable intake (above 150 g/day) and not in those with a vegetable intake below 50 g/day. Interestingly, in a study conducted in central Iran, data showed that there was a significant correlation between potato consumption and the risk of stroke (Khosravi-Boroujeni et al., 2013). As compared to those with the lowest (5.3 ± 0.4 g/day) consumption, those with the highest (60.0 ± 6.1 g/day) potato consumption were more likely to possess a stroke.

3.3 Cohort studies

It has been confirmed by cohort studies that the consumption of vegetables is inversely proportional, both in the occurrence of various CVD such as hypertension, stroke, and coronary heart disease, and in cases of death (Alonso et al., 2006; Jacques et al., 2013; Zhang et al., 2011). Vegetables had a protective effect in patients with CVD. A study in Spain reported that protein and fiber in cereals helped reduce hypertension (Alonso et al., 2006). Protein and fiber significantly reduced hypertension in participants with the highest intake compared to those with the lowest. The researchers also found that the risk reduction was more significant in the elderly than in the young, in men compared to women, and in obese patients compared to people of normal body weight. In another study, lycopene consumption had a cardioprotective effect and helped reduce the incidence of CVD after 9 years of follow-up and CHD after 11 years of follow-up (Jacques et al., 2013). In addition, researchers have found that eating vegetables could help reduce overall mortality (Zhang et al., 2011). Thus, in their findings, they supported the idea that increasing the consumption of vegetables, especially crucifers, reduces the risk of CVD. However, some studies have not found a significant relationship between vegetable consumption and protection against CVD (Lin et al., 2007; Sesso et al., 2003).

On the one hand, researchers focused on the link between plant-based flavonoid intake and protection against cardiovascular diseases in women, only to find that there was no significant linear trend toward five-fold consumption of both plant-based flavonoids and individual flavone or flavonole (Sesso et al., 2003). Also, consumption of broccoli did not significantly reduce the risk of developing cardiovascular diseases and no significant correlation was observed between the consumption of broccoli flavonole or flavone and nonlethal MI or fatal risk of CHD in US women (Lin et al., 2007). On the other hand, harmful ingredients were found in a few types of vegetables or poorly cooked vegetables (Borgi et al., 2016; Yu et al., 2015). For example, in one study the findings showed that, regular high consumption of soy isoflavones can increase the risk of ischemic stroke in women moderately but significantly, in contrast to the findings of studies in Table 1.1, where soy isoflavones have beneficial effects (Yu et al., 2015). HRs from lowest (mean intake: 6.0 mg/day) to highest intake (median intake: 53.6 mg/day) were 1.00, 1.05, 1.10, 1.11 and 1.24, respectively. In another study, the researchers found that HRs for people who ate four or more servings of baked, boiled or mashed potatoes per week was 1.11, while for French fries it was 1.17 and 0.97 for crisps, compared to with those consuming less than one serving per month (Borgi et al., 2016). These results showed that higher intake of poorly cooked potatoes may increase the risks of developing hypertension.

3.4 Other epidemiological studies

In addition to the above studies, there are a number of epidemiological studies aimed at finding a correlation between vegetable consumption and cardiovascular diseases, the results of which are shown in Table 1.1.

In summary, data from most epidemiological studies have shown the significant contribution of vegetables in reducing the incidence and limitation of CVD. Such vegetables are tomatoes, potatoes, onions, carrots, soybeans, and crucifers. Various types of ingredients, such as vegetable protein, fiber, vitamins (B1, B2, niacin, folic acid), calcium, potassium, and various phytochemicals (lycopene), can contribute to the cardioprotective effect of vegetables. However, in some studies, no such relationship was observed between the risk of developing CVD and the intake of broccoli and vegetable flavonols, and in other studies, eating potatoes, especially poorly cooked, could even increase the risk of developing CVD.

4. Bioactive vegetable ingredients and mechanisms actions

4.1 Soy

Soy is a common vegetable that can be used to extract oil and produce soy milk. Polyphenols, including mainly phenolic acid and flavonoids such as flavones and flavonols, are among the most important bioactive ingredients extracted from soy. Numerous studies have reported that phenolic acid has mainly contributed to the antioxidant capacity of many natural products (Fu et al., 2010, Fu, Xu, Gan, et al., 2011; Guo et al., 2012; Li et al., 2008; Song et al., 2010; Xia et al., 2010).

TABLE 1.1 Other vegetables related to CVD.

Type of vegetable	Subjects	Results	References
Vegetables with carotenoids	US Men 40–84 years old (n = 15,220)	Reduced risk of coronary heart disease (CHD)	Liu et al. (2001)
Onion crecetin	-	It was incorporated into the atherosclerotic area and acted as a complementary antioxidant	Terao et al. (2008)
Soy isoflavones	Chinese adults (n = 572)	Decrease in serum TAGs and thickness of carotid artery media, increase in HDL-C	Cai et al. (2012)
Soy foods, Isoflavones	(Meta-analysis)	Reduced ischemic heart disease, reduction of LDL-C in the blood, improvement of endothelial function, slowing the progression of subclinical atherosclerosis	Messina (2014)
Green leafy vegetables	(Meta-analysis)	The incidence of CVD decreased significantly (15.8%)	Pollock (2016)
Vegetables containing nitrates	Nonhypertensive individuals 20–70 years, (n = 1546)	Antihypertensive action	Golzarand et al. (2016)

Many researchers have suggested that polyphenols have antioxidant and anti-inflammatory effects, which provide cardiovascular protection (Ademiluyi & Oboh, 2013; Deng, Xu, et al., 2013; Fu, Xu, Xu, et al., 2011; Li An-Na et al., 2014; Li et al., 2013; Rodrigues et al., 2005; Zhang, Yu-Jie et al., 2015). An *in vitro* study found that extracts rich in phenolics from soybeans inhibit the activities of the enzymes converting α-amylase, α-glucosidase and angiotensin-I, which are key enzymes associated with diabetes and hypertension (Ademiluyi & Oboh, 2013). Thus, the researchers concluded that soy has the ability to promote health and help prevent and treat diabetes and hypertension. In another study it was found that saponin, which is one of the main soy flavonoids, has a beneficial effect on glucose tolerance and risk factors for atherosclerosis (Rodrigues et al., 2005). As in animals treated with saponins, the LDL-C/TG ratio increased and the proportion of TG, very low density lipoprotein cholesterol (VLDL-C), lipid hydroperoxides and the TC/HDL ratio increased. However, no effects on glucose tolerance, LDL-C, dismutase peroxide (SOD) and glutathione peroxidase (GPx) were found in the experimental groups. These observations suggest that soy saponin may improve serum lipid profile due to its direct antioxidant activity.

Soy has also been reported to contain important phytoestrogens, such as isoflavones and lignans, which are safe and natural alternatives to estrogen receptor modulator versus hormone therapy and have antioxidant and cardioprotective effects (Hu et al., 2013; Matori et al. et al., 2012). The researchers analyzed the functional and anatomical pathological effects of soy extract and isoflavone in post-MI (Miguez et al., 2012). A protective effect was found in the soybean extract group 30 days after MI. In another study, the cardioprotective effects of genistein (isoflavone) from soy extract on H9c2 cardiomyoblast cells treated with isoproterenol (Hu et al., 2013) were investigated. The results showed that genistein administration could down-regulate the expression of mitochondrial proapoptotic proteins such as Bad, caspase-3, caspase-8, and caspase-9 in H9c2 cells. In addition, several survival proteins were expressed in H9c2 cells, including phosphorus p-Akt, p-Bad, and p-Erk1/2. In addition, the researchers reported that genistein had cardioprotective effects in part due to the regulation of Erk1/2 proteins, Akt and the activator of nuclear factor activated B cells (NF−B) by inhibiting the relevant pathways. It was also noted that soy genistein not only reversed preexisting severe pulmonary hypertension, but also prevented its progression to heart failure (HF) (Matori et al., 2012). When genistein and daidzein were administered, significant neuroprotective effects and antioxidant effects were observed in both *in vitro* and *in vivo* ischemia/reperfusion (I/R) assays (Valeri et al., 2012). In addition, the effects of soy genistein on fructose-induced blood pressure were evaluated in hypertensive rats (Palanisamy & Venkataraman, 2013). The results showed that genistein administration could lower blood pressure and restore the expression of ACE, protein kinase C-β II and nitric oxide synthase (NOS). Soy protein is a well-known vegetable protein that is considered a complete protein, valuable for health (Luo et al., 2012; Marsh et al., 2011). The cardioprotective effects of soy protein have been demonstrated by evaluating the relationship between dietary protein source, protein level and serum lipid profile in male rats (Luo et al., 2012). Total serum TGs were found to decrease significantly after long-term soy protein intake, indicating the possibility of reducing the risk of atherosclerosis. Soy protein has also been reported to have cardioprotective effects, in part by improving serum lipids by altering the expression of protein-2 that binds to the sterol regulator and its downstream genes (hydroxymethylglutaryl-coenzyme A) increase the antioxidant effects of SOD and catalase (Marsh et al., 2011).

It has been reported that soy products could be improved in nutritional value after fermentation (Cai et al., 2017). For example, doenjang (a type of fermented soy bean paste) was more effective in preventing the accumulation of visceral fat caused by diet than nonfermented soy in rats, by stimulating the activity of carnitine palmitoyltransferase-1 and by suppressing the activity of fatty acid synthase, possibly due to the higher content of aglycone isoflavones (Kwak et al., 2012). In addition, it has been estimated that regular intake of miso soup (Japanese soy paste) could alleviate the salt-induced stimulation in mice with chronic hypertension by inhibiting the hypothalamic MR-AT1R route (Ito et al., 2014). In addition, the effects of genetically modified soy milk with probiotics (GM) on hamster hypercholesterolemia (Tsai et al., 2014) were investigated. The observations showed that the total serum TGs decreased significantly after treatment with four types of soy milk (GM or nonGM, with or without probiotic fermentation), compared with the control group on a high cholesterol diet. In addition, there was a significant difference between GM and non-GM soy milk groups in overall TGs levels. In addition, GM soy milk has been found to reduce the risk of atherosclerosis by reducing oxidative stress and the formation of atherosclerotic plaque in the aorta. There are other bioactive ingredients in soy, such as nonsaponifiable and oligosaccharides, which are beneficial to the cardiovascular system (Eser et al., 2011; Zhang, Meng, et al., 2015). The protective effects of nonsaponifying soy ingredients on the prefrontal cortex after general I/R brain injury were investigated in rats (Eser et al., 2011). The results showed that levels of malondialdehyde (MDA) and tumor necrosis factor-α (TNF-α), as well as the number of apoptotic neurons, decreased significantly, while SOD activities increased, indicating that nonsaponifiable soy substances had antioxidants and neuroprotective effects. In addition, the protective effects of soy oligosaccharides on cardiac function against I/R myocardial injury were evaluated in rats (Zhang, Meng, et al., 2015). MDA levels were up-regulated, while the antioxidant activity of enzymes and the expression of p-JAK2 and p-STAT3 proteins were increased in the soybean oligosaccharide-treated group. When rats were fed soy oligosaccharides, cardiac contractile function was significantly restored, infarct size was reduced, and creatine kinase, aspartic transaminase, and lactic dehydrogenase activities were also reduced.

4.2 Tomato

Tomatoes were considered to have an important protective role against CVD, in particular, their bioactive ingredient, lycopene, was found to have significant antioxidant, antihypertensive, hypolipidemic, and antiatherogenic effects in *in vivo* and *in vitro* tests (Armoza et al., 2013; Karimi et al., 2005). In one study, it was shown that the increase of MB-isoenzyme in creatine phosphokinase serum (CPK-MB) was inhibited and cardiac injury was improved by lycopene (1.7 and 3.5 mg/kg, intravenously) and tomato extract (1.2 and 2.4 g/kg, intravenous), respectively (Karimi et al., 2005). These results showed that lycopene from tomato extract inhibited doxorubicin-induced cardiotoxicity and could be used in combination with doxorubicin to relieve organ damage caused by free radicals. In another study, researchers investigated the effects of tomato extracts and carotenoids, such as lycopene and lutein, on normal function and NF-κB signaling in endothelial cells (Armoza et al., 2013). All carotenoids could cause a significant improvement in primary endothelial function, which is associated with increased nitric acid and decreased endothelin release. In addition, carotenoids effectively attenuated inflammatory NP-κB signaling, including decreased TNF-α-induced leukocyte adhesion, expression of adhesion molecules (AM) such as intracellular adhesion molecule 1 (ICAM-1) and the vascular cell adhesion molecule 1 (VCAM-1), the nuclear displacement of NF-κB components, and the restoration of the κB uptuctinase inhibitor. In addition, carotenoids played a role in inhibiting NP-κB activation in infected endothelial cells. In addition, lutein in combination with oleoresin synergistically blocked leukocyte adhesion. Sapogenol, another important bioactive ingredient in tomatoes, has been shown to have antiatherogenic activity by providing cardioprotective effects (Fujiwara et al., 2007, 2012). Esculeogenin A, a new tomato sapogenol, has been reported to improve hyperlipidemia and atherosclerosis in ApoE-deficient mice by inhibiting cholesterol acyl transferase (Fujiwara et al., 2007). Esculeogenin A significantly inhibits the accumulation of cholesterol ester, caused by acetylated LDL in macrophages, derived from human monocytes and Chinese hamster ovary cells, depending on the dose. In addition, esculeogenin A prevented the expression of acyl co-enzyme A: cholesterol acyltransferase (ACAT) -1 protein, and suppressed the activities of ACAT-1 and ACAT-2. Serum cholesterol levels, TGs, LDL-C, as well as the rate of atherosclerotic lesions in mice with ApoE deficiency were significantly reduced by oral administration of esculeogenin A, with no detectable side effects. In a similar study, tomatatidine, a tomato sapogenol, was reported to significantly suppress cholesterol acyltransferase activity and lead to reduced atherogenesis (Fujiwara et al., 2012).

In addition, tomato n-toxan extract exerted a protective effect against adrenaline-induced MI in rats (Parvin & Akhter, 2008). MDA levels in the heart and serum aspartate aminotransferase were significantly reduced in rats receiving adrenaline, pretreated tomato extract (1 mg/kg, 2 mg/kg) and vitamin E (50 mg/kg), which also significantly ruled out myocardial necrosis. It could be concluded that tomato n-toxan extract had antioxidant activity, which in turn could prevent catecholamine-induced MI. In addition, the antihypertensive effects of a gamma-aminobutyric acid (GABA) rich tomato variety (DG03-9) were investigated in spontaneously hypertensive rats (SHRs) (Yoshimura et al., 2010). Tomato variety DG03-9 caused a significant reduction in systolic blood pressure with both single and chronic administration compared to the control. In addition, the researchers found that DG03-9 had a higher antihypertensive effect than the common variety (Momotaro), and GABA had a similar effect to DG03-9 at a comparable dose. In addition, it was reported that eating cooked tomato sauce could maintain coronary endothelial function, as it improved the profile of HDL, apolipoprotein A-I and apolipoprotein J. It enhanced endothelial NOS transcription and activation and reduced coronary artery DNA damage in dyslipidemic animals (Vilahur et al., 2015). These bioactivities were responsible for the beneficial effects of cooked tomato sauce. That is, reducing lipid peroxidation, increasing the potential of the antioxidants HDL and preventing the diet-induced attenuation of coronary vasodilation.

4.3 Potato

People all over the world consume a large number of potatoes every year. Potatoes have been found to benefit the cardiovascular system, so it is worth investigating for the treatment and prevention of CVD (Ojewole et al., 2006; Robert et al., 2006). Researchers have focused on the possible effects of an aqueous extract of African potato bulb on the cardiovascular system of experimental animals (Ojewole et al., 2006). First, the aqueous extract (APE) showed negative inotropic effects on isolated electro-motor preparations of left guinea pig heart muscle and negative chronotropic effects on spontaneous right beats, respectively, significantly and depending on the concentration. Second, depending on the concentration of APE, the positive inotropic and chronotropic reactions of noradrenaline and calcium-induced guinea pig muscle strips were reduced or eliminated, which were not modified by exogenous atropine administration to atropine. Third, it caused a reduction or cessation of rhythmic, spontaneous, myogenic contractions of the venous gates in rats, significantly and depending on the concentration. In addition, APE reduced blood pressure, as well as the heart rate of hypertensive rats, significantly and dose-dependently. Overall, APE may be a natural candidate for the treatment of heart failure and hypertension. In another study, plasma cholesterol and triglyceride (TGs) levels and liver cholesterol levels were significantly reduced in rats after a 3-week potato-fortified diet (Robert et al., 2006). The antioxidant activity was also increased due to potato intake. In addition, thiobarbituric acid (TBARS) levels in the heart decreased and the plasma vitamin E/TG ratio improved. These results showed that eating boiled potatoes could be a way to prevent CVD. However, when investigating the effects of soluble fiber extracted from potato pulp on risk factors for diabetes and CVD in rats, no difference in hematological parameters was found. The plasma concentration of TGs in rats decreased only moderately after propagation (Laerke et al., 2007). These findings could lead to the conclusion that plasma cholesterol or glycemic response could not be reduced by increased fermentation and production of dietary fiber propionate.

4.4 Dioscorea

Dioscorea is a common vegetable that is widely used in traditional Chinese medicine and contains a variety of bioactive ingredients, such as saponins, diosgenin, and flavonoids. Saponins have been shown to have antithrombotic activity (Li et al., 2010; Zhang et al., 2013). In one study, total steroid saponins, derived from *Dioscorea zingiberensis* roots, blocked platelet aggregation, leading to prolonged partial thromboplastin activation time (APTT), thrombin time (TT), and prothrombin time (PT) in rats and prolonged bleeding and coagulation time in mice, suggesting the ability to reduce CVD (Li et al., 2010). In another study, researchers evaluated the antithrombotic effects of four types of diosgenyl saponins (Zhang et al., 2013).

The observations showed that diosgenyl β-D-galactopyranosyl-(1 → 4)-D-glycopyranoside, a new saponin disaccharide, showed excellent efficacy in prolonging bleeding time. In addition, it could significantly and dose-dependently inhibit platelet aggregation, prolong APTT, and inhibit factor VIII activities in rats. Overall, it could be concluded that diosynyl β-D-galactopyranosyl-(1 → 4)-D glucopyranoside had significant antithrombotic activity. In addition, the beneficial effects of total saponins on isoprenaline-induced ischemia, derived from three medicinal species of yam (*Dioscorea nipponica Makino, Dioscorea panthaica Prain et Burkill and Dioscorea zingiberensis*), were further investigated (Tang et al., 2015). Total saponins from the three yam species were found to significantly reduce creatine kinase, lactate dehydrogenase and aspartate aminotransferase activities. The concentration of MDA also decreased and the activities of total SOD, catalase, GPx and total antioxidant capacity increased, which was comparable between these three types of yams. In addition, cardiac tissue showed less histological damage. These results may partly explain why total saponins have a cardioprotective effect on myocardial ischemia. In addition to the aforementioned effects, saponins exerted a strong neuroprotective effect and helped to attenuate injury caused by transient focal cerebral I/R through a mechanism involving antiinflammatory and antiapoptotic action (Zhang et al., 2014). Moreover, they significantly reduced the results of the neurological deficit, the volume of the cerebral infarction and cerebral edema in rats, while increasing neuron survival (Nissl bodies) and reduced caspase-3 in the hippocampus Cornu Ammons one and in the cortex of lateral ischemic cortex.

In addition, preadministration of saponins significantly reduced inflammatory cytokines in the serum caused by cerebral artery occlusion and significantly inhibited the antiregulatory, antiapoptotic Bcl-2 and upregulation of Bax proapoptotic proteins. It has been reported that Dioscorea and in particular its bioactive compound diosgenin, exerts an action against thrombosis, possibly by promoting anticoagulation function and blocking platelet aggregation (Chen et al., 2015; Gong et al., 2011). In one study, it was found that platelet aggregation, thrombosis, and APTT, TT, and PT times, in rats were inhibited, as well as bleeding and clotting time was prolonged dose-dependently in mice (Gong et al., 2011). As a result, diosgenin extracted from *Dioscorea zingiberensis* has antithrombotic activity and may contribute to the treatment of CVD. In another study in mice, diosgenin was found to reduce doxorubicin-induced cardiotoxicity (Chen et al., 2015) as it reversed the reduced activities of the antioxidant enzymes and GPx in cardiac tissue. In addition, diosgenin significantly reduced serum cardiotoxicity markers, cardiac levels of TBARS and reactive oxygen species (ROS), activating caspase-3, mitochondrial dysfunction, and NP-κB expression. Finally, diosgenin increased the levels of cyclic guanosine monophosphate in the heart, modifying phosphodiesterase-5 activity and reducing myocardial fibrosis. In the meantime, it has been confirmed that the regulation of protein kinase A and *P*38 could be included in heart health benefits. These results suggest that diosgenin has antioxidant, antiapoptotic activities and protects against doxorubicin-induced cardiotoxicity.

There are other studies focusing on the beneficial effects of yam on the heart and other bioactive compounds of yam have been identified that protect against myocardial infarction and atherosclerosis (Jayachandran et al., 2010; Koo et al., 2014). In a study, the results showed that flavonoid-rich *Dioscorea bulbifera Linn.* could mitigate lipid peroxidation due to its ability to scavenge free radicals and regulate energy-producing mitochondrial enzymes, suggesting a cardioprotective effect on isoproterenol-induced myocardial infarction (Jayachandran et al., 2010). In another study, the researchers concluded that a Chinese yam extract, rich in sitosterol and ethyl linoleic acid, had the ability to prevent atherosclerosis, so it could be a candidate for functional foods. It has been reported that such extracts could inhibit the expression of inflammatory mediators, including TNF, nitric acid, and inducible NOS, and the development of atherosclerotic lesions (Koo et al., 2014).

Also, several studies have suggested the cardioprotective effects of dioscorea, of which bioactive compounds may not have been identified (Amat et al., 2014; Chang et al., 2005). One study confirmed that dioscorea root had antioxidant and antiatherogenic effects in hyperlipidemic rabbits, suggesting that using the root of this vegetable may be a possible way to reduce oxidative stress and treat atherosclerosis (Chang et al., 2005). In another study, *Dioscorea opposita Thunb.* was found to show antihypertensive effects in hypertensive rats through inhibition of endothelin conversion enzymes as well as its antioxidant activity (Amat et al., 2014). Following treatment, this type of yam caused significant reductions in mean arterial pressure, endothelin concentration, MDA, plasma angiotensin-II activity, left ventricular hypertrophy, and cardiac mass index, while increasing SOD activity.

4.5 Onions

Onions are a type of vegetable that is consumed worldwide and contains a variety of bioactive ingredients. Onion extracts showed strong antiatherogenic effects associated with a variety of activities (Jaiswal & Rizvi, 2014; Li et al., 2011). In a study, onion extracts (*Allium cepa L.*) as well as the bioactive ingredients quercetin and catechin were observed to potentiate paraoxonase one activity and radical scavenging activity, which in turn prevented LDL oxidation and lipid peroxidation in male Wistar rats subjected to oxidative stress induced by mercury chloride (Jaiswal & Rizvi, 2014). In another study (Li et al., 2011) onion extract was found to reduce atherosclerotic lesions, increase the production of endogenous aortic hydrogen sulfide (H_2S) and reduce the plasma adrenomedulin content, the aortic adrenomedulin content, the aortic calcitonin-like receptor, and the protein that modifies the activity of the receptor/two mRNA. In addition, plasma GPx levels, SOD activity, endothelial plasma NOS activity, and nitric acid content increased, while MDA and inflammatory response decreased with onion extract. All of these results made onions a potential candidate for antiatherogenic therapy. Some experimental studies have shown that onions have antithrombotic effects by inhibiting platelet response and inhibiting mitogen-activated protein kinase (MAPK) activation. Therefore, onion intake may have the potential to prevent CVD caused by platelet abnormalities (Briggs et al., 2001; Lee et al., 2013; Yamada et al., 2004). One study showed that onion could inhibit platelet-induced thrombosis in dogs (Briggs et al., 2001). Periodic platelet-induced thrombus formation following embolization has been shown to reduce cyclic flow. However, five dogs were given intravenous onion juice (0.09 ± 0.01 mL/kg), reducing circular flows within 20 min and by $60 \pm 14\%$ platelet aggregation. In addition, in five of the six dogs that received 2.0 g/kg of homogenized onion intragastrically, the cyclic flows decreased within 2.5−3 h, accompanied by a $44 \pm 24\%$ reduction in aggregation. Still, as measured by rodent thrombosis/thrombolysis models in another study, a variety of onion crops showed natural antithrombotic effects (Yamada et al., 2004). First of all, the researchers confirmed that Toyohira exerted strong antithrombotic activities as well as antiplatelet effects accompanied by thrombolytic action. While the Super Kita Momiji, 2935A and K83211 showed only thrombolytic activity. In addition, the researchers did not find a significant relationship between quercetin concentration and antithrombotic activity. Interestingly, the antithrombotic effects of quercetin-rich onion peel (OPE) extracts were reported in another study (Lee et al., 2013). OPE significantly reduced TGs and blood glucose, without affecting cholesterol levels. In addition, *in vivo* arterial thrombosis was significantly eliminated in groups receiving 2 and 10 mg OPE. Moreover, thrombin-induced expression of tissue factor in human umbilical vein endothelial cells, a coagulation primer, was greatly reduced by OPE. Finally, the extracellular kinase (ESRK) and c-Jun N terminal kinase (CJNK) signaling pathways activated by thrombin treatment are blocked by pretreatment with OPE.

Onions have also been found to have antihypertensive effects in some other experiments (Naseri et al., 2008; Sakai et al., 2003). For example, onion reduced plasma TBARS in N(G)-nitro-L-arginine (L-NAME) methyl ester, in induced hypertensive rats and SHRs prone to stroke (Sakai et al., 2003). Moreover, onions improved urinary nitrate/nitrite excretion and NOS activity in the kidneys in SHRs prone to stroke but not in L-NAME-induced rats. These results could partly explain the mechanisms by which the onion exerted an antihypertensive effect on these hypertensive rats. In addition, the antihypertensive effects of onion were observed by different mechanisms (Naseri et al., 2008). OPE has been shown to reduce aortic contractions induced by KCl or phenylephrine depending on the concentration. Also, OPE activity could not be attenuated by removing the aortic endothelium or inhibiting the synthesis of nitric acid, cGMP and prostaglandin induced by L-NAME (100 M), methylene blue (10 M) and indomethacin (10 M), respectively. Furthermore, phenylephrine-induced aortic relaxation induced by OPE was not eliminated by atropine, which inhibited acetylcholine-induced relaxation. Finally, after 3 weeks of OPE intervention, a reduction in blood pressure was observed in hypertensive rats receiving fructose.

4.6 Other vegetables

Except for the vegetables mentioned above, there are others that have beneficial effects on the cardiovascular system. Evidence from experimental researches has shown that different vegetables have a cardioprotective effect through various mechanisms (Table 1.2). In summary, many studies have shown that eating vegetables is potentially beneficial in preventing and treating CVD. Vegetables such as potatoes, soy, tomatoes, yams, and onions have been shown to have cardioprotective effects, which are responsible for a variety of bioactive ingredients, including vitamins, essential nutrients, dietary fiber, and plant proteins, and phytochemicals. Cardioprotective effects may include antioxidant activity, antiinflammatory, antithrombotic, lowering blood pressure, modifying lipid metabolism, regulating blood glucose, improving endothelial function, and attenuating myocardial damage. In conclusion, mechanisms of action may include modulation of related enzyme activity, gene expression, and signaling pathways, as well as some other biomarkers associated with the risk of CVD (Table 1.3).

5. Clinical findings

5.1 Whole soy and soy milk

Except for the findings of the aforementioned studies, soy has a variety of bioactive activities in the prevention of cardiovascular diseases, as the results of many clinical trials have suggested that soy consumption may be a way to reduce the incidence of CVD and maintain cardiovascular health (Hanachiphd, 2012; Iua et al., 2013; Miraghajani et al., 2013; Wong et al., 2012). Soy had an effect on CVD biomarkers in older women with metabolic syndrome (Hanachiphd, 2012). Compared to the mean changes from baseline in the control group, LDL-C, VLDL-C and apolipoprotein B100 levels were significantly improved in the whole soy intake group (35 g/day), while less significant improvements were observed in the vegetable protein intake group with texture (35 g/day) (TVP, also known as soy protein texture (TSP), soy meat or soy pieces), a degreased soy flour product, a by-product of soybean oil extraction. Similar results were observed for apolipoprotein A-I in the treatment groups, in which serum TC was significantly reduced. Overall, soy and soy protein texture could improve the lipid profile, but the former caused significant improvements over the latter. In addition, consumption of soy foods was found to improve the lipid profile in patients with hyperlipidemia (Wong et al., 2012).

It is very important to mention that the consumption of soy milk compared to the consumption of cow's milk, significantly reduced systolic blood pressure in patients with type II diabetes and kidney disease (Miraghajani et al., 2013). It also significantly reduced serum TGs (percentage change: 15.22% vs. 2.37%, $P = .02$), although these effects were not significant after adjustment for carbohydrate intake. In addition, data from another study showed that the use of soy products in integrated early rehabilitation therapy in patients with macrophysical MI significantly reduced the risk of arrhythmia (Iua et al., 2013).

5.2 Soy protein

Soy protein has been shown to improve serum lipid profiles as well as other risk factors associated with CVD, as confirmed in a study of 90 moderately hypercholesterolemic Chinese adults (Ma et al., 2011). In a randomized controlled trial (RCT), soy protein supplementation caused a significant mean net change in plasma E-selectin of 3.93 ng/mL compared to milk protein and 2089.8 pg/mL plasma leptin compared to carbohydrates (Rebholz et al., 2013). These observations showed that soy protein supplementation could reduce plasma E-selectin and leptin levels. However, ingestion of either cow's milk or soy protein drink for 8 weeks did not alter soluble AM cell concentrations in prehypertensive individuals or in stage 1 hypertensives, indicating that no beverage has reduced the risk of developing atherosclerosis in people with mild hypertension, improving however, the concentrations of circulating AM cells (Dettmer et al., 2012).

TABLE 1.2 Other vegetables and the cardioprotective actions of the options.

Vegetables	Subjects	Results	References
Celery (seeds)	Rats	Decreased blood pressure, increased heart rate	Moghadam et al. (2013)
Celery (seeds)	RAW264.7 macrophages	Decreased lipid droplets and TC content, decreased secretion of inflammatory cytokine TNF-α and interleukin (II) -6, promoted cell viability, inhibited apoptosis, suppression of NF-κB, *P*65 and notch1 protein expressions	Si et al. (2015)
Celery (leaves)	Rats	Reduced systolic blood pressure, cholesterol, TG, LDL, and VLDL	Dianat et al. (2015)
Asparagus	SHRs	Reduction of systolic blood pressure, urinary protein/creatinine excretion ratio, creatinine clearance and ACE activity	Sanae and Yasuo (2013)
Lettuce	Rats	Decreased LDL/HDL ratio and cholesterol levels in the liver, increased total fecal steroid excretion, depressive apparent absorption of dietary cholesterol, improved plasma VE/TG ratio, limited lipid peroxidation in the heart	Nicolle, Cardinault, et al. (2004)
Green vegetables	SHRs	Formulated fatty acid composition of the liver, protection against atherogenic fatty acid increases	Johnson et al. (2013)
Cabbage	*In vitro* thrombolytic model	Show thrombolytic activity	Emran et al. (2015)
Olive rapeseed	SHRs	Suspended ACE, dilated mesenteric artery	Yamada et al. (2010)
Olive rapeseed	SHRs	Inhibited ACE and renin activities, lowered blood pressure	He, Malomo, et al. (2013)
Olive rapeseed	SHRs	Decreased surface hydrophobicity, scanned oxygen radicals, ACE inhibition, lowering of blood pressure	He, Alashi, et al. (2013)
Spinach	Mice	Decreased catalase, increased SOD activity, protection against doxorubicin-induced heart damage	Breitbart et al. (2001)
Spinach	SHRs	Antihypertensive activity	Yang et al. (2003)
Spinach (leaves)	SHRs	Inhibited ACE, antihypertensive activity	Yang et al. (2004)
Pumpkin	*In vitro*	Antioxidant, inhibited-glucosidase and ACE, antidiabetic and antihypertensive action	Kwon et al. (2007)
Wild carrots	*In vitro*, rats anesthetized under normal pressure	Blood pressure is lowered, guinea pig spontaneous heart rate and rabbit aortic contractions caused by K + are inhibited	Gilani et al. (2000)
Lyophilized carrot	Mice	Increased total neutral fecal excretion, increased antioxidant status and VE/TG ratio, decreased lipemia, regulated cholesterol metabolism	Nicolle, Gueux, et al. (2004)
Carrot	*In vitro*, mice	Antithrombotic action	Yamamoto et al. (2008)
Broccoli	SHRs prone to stroke	Reduced oxidative stress, hypertension, and inflammation	Wu et al. (2004)
Broccoli	Rats	Mammalian heart protection, survival protein activation, improved postischemic ventricular function and precaspase 3 activities and thioredoxin cycle recycling, reduction of myocardial infarction, cardiomyocyte apoptosis, and cytochrome *c* release	Mukherjee et al. (2008)
Broccoli	Rats	Protection against oxidative damage to the myocardium and cell death during I/R, inhibition of necrosis and apoptosis markers, reduced oxidative stress	Akhlaghi και Bandy (2010)
Broccoli	Rats	Improved postischemic ventricular function, reduced MI and cardiomyocyte apoptosis	Mukherjee et al. (2010)

Continued

TABLE 1.2 Other vegetables and the cardioprotective actions of the options.—cont'd

Vegetables	Subjects	Results	References
Corn	SHRs, *in vitro*	Inhibition of ACE, reduction of systolic blood pressure	Yang et al. (2007)
Corn	*in vitro*	Antioxidant, antidiabetic and antihypertensive action, α-glucosidase inhibition and ACE	Kwon et al. (2007)
Purple corn	SHRs	Reduction of blood pressure and heart rate	Shindo et al. (2007)
Maize	Rats	Reduced heart attack size, increased myocardial glutathione levels, formed antioxidant cardiac defenses	Toufektsian et al. (2008)
Pea	Rats	Decreased MDA, tissue calcium concentration, myeloperoxidase, and caspase-3 apoptosis index, protection of the heart from I/R injury	Masini et al. (2003)
Latyrus cicera	Rats	Prevention of tissue injury caused by free radicals, endothelial dysfunction, and leukocyte recruitment, protection against visceral damage caused by I/R	Masini et al. (2007)
Pea	*In vitro*	Inhibited α-amylase, α-glucosidase and ACE	Burguieres et al. (2008)
Pea	Rats	Lowered serum creatinine and renal chemokine receptor 2 level	Aukema et al. (2011)
Pea	Rats	Reduced plasma TC concentrations, affected cellular cholesterol homeostasis	Parolini et al. (2013)

5.3 Soy isoflavones

Soy isoflavones, especially genistein and daidzein, are common phytoestrogens recognized as selective estrogen receptor modulators that have cardioprotective effects *in vitro* and *in vivo* tests. In one RCT, pure daidzein showed no significant effects on body weight, body mass index, waist, and hip circumference, waist to hip ratio, body fat percentage, fat mass, and free fat mass in postmenopausal women who were in the early stages of hypertension or had hypotension (Liu, Ho, et al., 2013). In the same study, pure daidzein was found to have no significant effect on either blood pressure or vascular function (Liu et al., 2015). However, in the above two studies, urinary isoflavones showed good patient compliance within the interventions.

5.4 Combination of soy isoflavone and soy protein

Results from clinical trials have shown that a combination of isoflavones and soy protein may not be an effective intervention for the prevention of CVD (Hodis et al., 2011; Liu et al., 2012). In an RCT, isoflavone soy protein supplementation (ISP) did not result in a significant reduction in the progression of subclinical atherosclerosis in postmenopausal women (Hodis et al., 2011). While subgroup analysis showed that ISP supplements could reduce subclinical atherosclerosis in healthy young women (median age: 53) less than 5 years after menopause who had a low risk of developing CVD. In a double-blind, randomized, placebo-controlled trial of 180 postmenopausal women in China, soy protein in combination with isoflavones at the supplied dose (15 g soy protein, 100 mg isoflavones in patients with no side effects), including serum, HDL-C, LDL-C, TC, TGs and the highly sensitive C-reactive protein (Liu et al., 2012).

5.5 Other vegetables and their bioactive ingredients

Various studies have also been done on vegetables and their bioactive ingredients, such as tomatoes, broccoli, and onions, some of which have been found to have promising properties for the prevention and treatment of CVD. Information regarding these surveys and their findings follows in Table 1.4 below.

In addition, healthy eating patterns characterized by high vegetable content were important in reducing the incidence of CVD (Li et al., 2015; Rodriguez-Monforte et al., 2015). Such dietary standards are the recommended dietary approach to the cessation of hypertension (DASH) and the Mediterranean diet.

TABLE 1.3 Mechanisms involved in the cardioprotective actions of vegetables.

Cardioprotective effects	Responsible molecules	Mechanisms	References
Low blood pressure	Quercetin, phenolics, genistein, daidzein, lycopene, nitrate, potassium	Inhibits ACE activity and the MR-ATIR hypothalamic pathway, relieves sympathoxidation, improves C-βII protein kinase activity, modifies the relative telomere length of peripheral leukocytes, increases NOS expression, inhibits the inflow of Ca^{2+} and contractions caused by K^+.	Sanae and Yasuo (2013) He, Malomo, et al. (2013) He, Alashi, et al. (2013) Ito et al. (2014) Palanisamy and Venkataraman (2013) Golzarand et al. (2016)
Regulation of lipid metabolism	Esculeogenin A, tomatidine, saponins, carotenoids, isoflavones, soy protein, flavonoids	Reduction of TC, TG, TAG, VLDL-C, TC/HDL-C ratio and atherosclerotic plaque formation, increase of LDL-C/TG and VE/TG ratio, inhibition of fatty acid synthetase and ACAT activity, formation of mitochondrial enzymes producing energy, modifying the expression of protein-2 that binds ACAT elements and sterol and its downstream genes.	Fujiwara et al. (2007, 2012) Rodrigues et al. (2005) Nicolle, Cardinault, et al. (2004), Nicolle, Gueux et al. (2004) Cai et al. (2012) Marsh et al. (2011) Ryder (2011) Luo et al. (2012)
Antioxidant action	Myrictin, lucetin, quercetin, catechin, apigenin, campferol, tocopherols, lutein, β-carotene, lycopene, nonsaponifiable saponins, n- toxan, diosgenin, daidzein soy protein	Scanning free radicals (nitric acid, peroxide, hydroxyl, TBARS), increasing the production of endogenous aortic H_2S, and improving the action of SOD, catalase, GPx, carnitine palmitoyltransferase-1, and paraoxonase 1.	Jayachandran et al. (2010) Kwak et al. (2012) Sakai et al. (2003) Robert et al. (2006) Jaiswal & Rizvi, 2014 Zhang, Meng et al. (2015) Li et al. (2011) Chen et al. (2015) Tang et al. (2015) Marsh et al. (2011)
Improving endothelial function	Lycopene, lutein, quercetin, n- toxan, carotenoids, isoflavones	Reduction of the thickness of endothelin and endothelial arteries, an increase of nitric acid, improvement of protein profiles (apolipoprotein A-I and apolipoprotein J), inhibition of the endothelin conversion enzyme, reduction of DNA damage.	Armoza et al. (2013) Amat et al. (2014) Messina (2014) Yoshimura et al. (2010) Vilahur et al. (2015)
Anti-inflammatory action	Polyphenols, saponins, lycopene, lutein, oleoresin, nonsaponifying ingredients, carotenoids	Reduction of leukocyte adhesion by TNF-α, reduction of NP-κB, IL-6, and inhibition of AM expression.	Eser et al. (2011) Armoza et al. (2013) Si et al. (2015) Zhang et al. (2014)
Antiplatelet action	Quercetin, saponins, diosgenin, diosgenyl saponins	Prolongation of APTT, TT, PT, bleeding time and coagulation time, inhibition of MAPK, ESRK, factor VIII and N-terminal c-Jun kinase signaling pathways.	Lee et al. (2013) Li et al. (2010) Gong et al. (2011) Zhang et al. (2013) Briggs et al. (2001) Yamada et al. (2004)
Reduction of myocardial damage	N- toxan, saponins, diosgenin, lycopene, genistein	Reduction of MDA, leakage of water content and size of the infarct, an increase of cyclic guanosine monophosphate, inhibition of creatine kinase, aspartic transaminase, lactic dehydrogenase, and CPKMB activity, regulation of protein kinase A, *P*38 and phosphodiesterase-5 activity, inhibition of Bad, Bax, caspase-8 activity, expression of caspase-9, caspase-3 and aquaporin 4, increase in phosphorus (*P*) -Akt, p-Bad, *P* -Erk1/2, Bcl-2, p-JAK2 and p-STAT3.	Zhang Meng et al. (2015) Parvin and Akhter (2008) Tang et al. (2015) Zhang et al. (2014) Chen et al. (2015) Karimi et al. (2005) Hu et al. (2013)

Continued

TABLE 1.3 Mechanisms involved in the cardioprotective actions of vegetables.—cont'd

Cardioprotective effects	Responsible molecules	Mechanisms	References
Regulation of blood glucose	Quercetin, saponin, phenolics, lycopene, fibers	Inhibition of α-amylase and α-glucosidase activity, improvement of hemoglobin A1c and high fasting blood sugar.	Ademiluyi and Oboh (2013) Rodrigues et al. (2005) Khosravi-Boroujeni et al. (2012) Sesso et al. (2012) Medina-Remon et al. (2013) Sesso et al. (2012) Lee et al. (2013)

TABLE 1.4 Effects of various vegetables on CVD.

Vegetables	Subjects	Results	References
Tomato	Patients with grade 1 hypertension (n = 31)	Reduced blood pressure and TBARS levels	Engelhard et al. (2006)
Tomato	Healthy women (n = 18)	Improved serum antioxidant status, reduced vascular AM 1	Garcia-Alonso et al. (2012)
Tomato	Healthy people (n = 40)	Decreased plasma TC, TGs and various plasma cellular and inflammatory biomarkers and increased plasma HDL-C and IL-10	Valderas-Martinez et al. (2016)
Tomato	Healthy middle-aged volunteers (n = 225)	No changes in inflammatory markers, insulin resistance and sensitivity, lipid concentrations and arterial stiffness	Thies et al. (2012)
Broccoli	Hypertensive individuals (n = 40)	No significant changes in blood pressure and endothelial function	Christiansen et al. (2010)
Broccoli	Healthy Caucasian volunteers (n = 24)	Urinary concentrations of sulforaphane and vitamin C metabolites and decreased concentrations of tetranor-PGEM, 11β-PGF2α and 11-dehydro-TXB2	Medina et al. (2015)
Onion	Healthy men (n = 23)	Improving vasodilation	Nakayama et al. (2013)
Onion	Overweight-obese patients (n = 70)	Reduced systolic blood pressure for 24 h and did not affect vasoactive biomarkers	Bruell et al. (2015)

- The DASH diet recommends the consumption of low-fat vegetables, fruits, and dairy products and has been found to significantly improve cardiovascular risk factors, including BP, TC, and LDL, and reduce the risk of CVD and mortality (Schwingshackl & HoffmanN, 2015; Siervo et al., 2015).
- The Mediterranean diet is characterized as a well-balanced plant-based diet with a high content of vegetables, as well as fruits and whole grains and has been reported to reduce the incidence and mortality of CVD, such as CHD, MI, and stroke (Grosso et al., 2017; Rees et al., 2013; Sofi et al., 2014). In addition, Mediterranean Diet has shown strong anti-inflammatory and antithrombotic preventative properties against the onset and development of CVD (Tsoupras et al., 2018), which is also associated with the bioactive present in its rich fruits and vegetable content.

In summary, clinical trials have shown that several vegetables have contributed to the prevention and treatment of CVD. Whole soy and its ingredients (such as soy protein) had strong cardioprotective effects. Still, some other vegetables, such as tomatoes, broccoli, and onions, were beneficial to patients with CVD to some degree. On the other hand, no significant change in some biomarkers was observed in people consuming tomato, broccoli, and soy isoflavones in some studies, so further clinical trials on the cardioprotective effects of vegetables are warranted. In conclusion, it is important to emphasize that a healthy diet, which is high in vegetables helps reduce the risk of developing cardiovascular diseases and should be applied by all people of all ages.

6. Conclusions

In conclusion, regular consumption of vegetables has undoubtedly positive effects on human health, as their bioactive ingredients can protect against various diseases. A variety of ingredients contribute to the overall health benefit. Of which phytochemicals with antioxidant properties can act directly by suppressing free radicals or indirectly by participating in cellular signaling pathways in balancing redox. Nutrients such as potassium help regulate blood pressure. The fiber content and type of different vegetables can also contribute to the overall health benefit, such as improving bowel passage, lowering cholesterol, and managing blood glucose levels. In addition, increasing the intake of vegetables throughout the diet can reduce the intake of saturated fats, trans fats, and foods with higher caloric value. Because each vegetable contains a unique combination of phytonutrients (vitamins, minerals, fiber, and phytochemicals), a wide variety of vegetables must be consumed to ensure that the diet includes a combination of phytonutrients and brings all the health benefits.

It should be based on three main areas:

- the identification of the genetic mechanisms that regulate the synthesis of the basic phytochemicals of vegetables, in order to develop varieties rich in phytochemicals
- the study of the possible change in the balance of these compounds (and their possible synergies or interactions)
- and determining the optimal conditions for the management of these phytochemicals after harvest and processing, as studies have shown that the bioavailability of some of the phytochemicals increases dramatically after storage and processing and others degrade rapidly.

Also, the results of many epidemiological studies support the hypothesis that the consumption of vegetables is inversely correlated with the risk of developing CVD. Many studies have suggested that many vegetables, such as potatoes, soy, tomatoes, yams, onions, celery, broccoli, lettuce, asparagus, peas, carrots, pumpkin, corn, etc. contain a variety of bioactive ingredients, including vitamins, essential nutrients, dietary fiber, plant proteins, and phytochemicals, could be considered as candidates for the prevention of these diseases. Cardioprotective effects of vegetables may include antioxidant activity, antiinflammatory, anticoagulant, lowering blood pressure, modifying lipid metabolism, regulating blood glucose, improving endothelial function, attenuating damage to the myocardium, and myocardial infarction. signaling routes. In addition, these cardioprotective effects of vegetables have been observed in clinical trials. Thus, eating vegetables could help maintain cardiovascular health and could be used as an effective, sustainable, and economical strategy for both the prevention and treatment of CVD. Finally, in the future, more vegetables should be evaluated for their protective effects on the cardiovascular system and their main mechanisms of action should be investigated through clinical trials.

7. Functional foods from vegetables

Functional foods are those that when consumed regularly exert a specific beneficial effect on health in addition to their nutritional properties (Gul et al., 2016). Functional foods are similar to conventional foods, which are consumed as part of a normal diet but can improve the health of the consumer beyond the primary nutritional function. In general, functional foods are considered foods that are intended to be consumed as part of a normal diet but contain biologically active ingredients that have the potential to improve health or reduce the risk of disease (Gul et al., 2016). Examples of functional foods include foods that contain specific minerals, vitamins, fatty acids or fiber, and foods with added biologically active substances such as phytochemicals, antioxidants, and probiotics (Gul et al., 2016). According to this definition, unmodified whole foods, such as fruits and vegetables, represent the simplest form of functional food. For example, broccoli, carrots, or tomatoes could be considered functional foods because they are rich in plant-active ingredients such as sulforaphane, beta carotene, and lycopene, respectively. Human health depends to a large extent on the consumption of nutritious foods. The production of food with health benefits offers an excellent opportunity to improve public health. Therefore, these foods have received a lot of attention in recent years from the scientific community, consumers, and food manufacturers. Increasing evidence supports the observation that functional foods containing physiologically active ingredients, from plant sources such as vegetables, can enhance health (Gul et al., 2016). Claims about the health benefits of functional foods should be based on scientific findings and additional research to substantiate the potential health benefits.

An example of functional food is the water-soluble tomato extract that contains all of its bioactive ingredients, under the brand name Fruitflow, which is now an established natural food ingredient available worldwide (O'Kennedy et al., 2017). Human volunteer studies demonstrated the activity and bioavailability of the active compounds in Fruitflow, which became the first product in Europe to receive an approved health claim in accordance with Article 13 (5) of the European Health Claims Regulation 1924/2006 on with nutrition and health claims made in food (O'Kennedy et al., 2017). Thus, Fruitflow is now authorized by EFSA for daily consumption, provided that 3 g of Fruitflow 1 or 150 mg Fruitflow 2 must be included in either food (e.g., fruit juices, flavored beverages or yogurt drinks with total volume up to 250 mL), or as a powder, in tablets or capsules as dietary supplements (to be taken with up to 250 mL of water) (O'Kennedy et al., 2017). Since its discovery in 1999, several mechanistic studies and tests have been performed on humans. The studies included the detection of the antiplatelet activity of the tomato, the modes of action, its stability under different conditions and the identification of the compounds with antiplatelet activity. The presence of a range of compounds suggests that they all have antiplatelet activity but act in different parts of the platelet activation/aggregation pathway (O'Kennedy et al., 2017). The chemical properties of the active compounds indicated their potential suitability as therapeutic agents or as functional food ingredients. Strong antiplatelet agents that inhibit platelet aggregation have been identified in Fruitflow, which significantly inhibited platelet aggregation to reduce the risk of cardiovascular diseases (O'Kennedy et al., 2017). Since hyperactive platelets, in addition to their roles in thrombosis, are also important mediators of atherogenesis (O'Kennedy et al., 2017). Also, antiplatelet drugs are not always suitable for use, especially when the risk of a cardiovascular event is relatively low. As aspirin remains the cornerstone of antiplatelet therapy, it does not benefit all patients equally, as evidenced by the phenomenon of aspirin resistance (Müller et al., 2002). In addition, aspirin therapy is responsible for some serious side effects, making it unsuitable for use in primary prevention of CVD (Cai et al., 2016; Hennekens & Dalen, 2014). In addition to reducing platelet reactivity, Fruitflow contains antihypertensive and antiinflammatory agents, making it an effective and natural cardio-protective functional food (O'Kennedy et al., 2017). Therefore, tomato-grown Fruitflow contains bioavailable cardio-protective compounds and may benefit individuals who are vulnerable to developing CVD (O'Kennedy et al., 2017). Thus it may be useful in the primary prevention of CVD, as a number of extensive basic, mechanistic, synthetic and many human trials testify to its cardioprotective benefits.

7.1 Synthetic and structural aspects of Fruitflow (O'Kennedy et al., 2017)

Fruitflow is made in two forms of ingredients, Fruitflow 1 and Fruitflow 2. The raw material for both forms is high quality, minimally processed tomato products. Fruitflow 1 is a syrup, which contains more than 50% w/w carbohydrates and about 3% w/w known bioactive compounds with measured antiplatelet activity. This form is particularly suitable for use in beverages and foods with a high water content. Fruitflow 2 is a low carb powder, which contains bioactive compounds of more than 55% w/w, dried to produce a tablet powder. Fruitflow 2 can be compressed into tablets and has flow properties that make it suitable for capsule formation or for use in dry food mixes.

Both types of ingredients have lycopene, are fat-free and have low levels of minerals and organic acids. The activity of Fruitflow 1, in terms of content of bioactive compounds/g, is lower than that of Fruitflow 2, due to their different sugar content. Thus, 3 g of Fruitflow one gives an equivalent dose of bioactive ingredients (approximately 65 mg) to 150 mg of Fruitflow 2. Of this amount, 6%–10% are known nucleoside derivatives (F1), 13%–15% are known phenolic conjugates (F2) and 8%–10% of known flavonoid derivatives (F3), including at least 2.4 mg of quercetin derivatives per dose. This amount of bioactive compounds, which includes a single daily dose, is equivalent to that found in about three medium servings of canned tomato soup (Fig. 1.1).

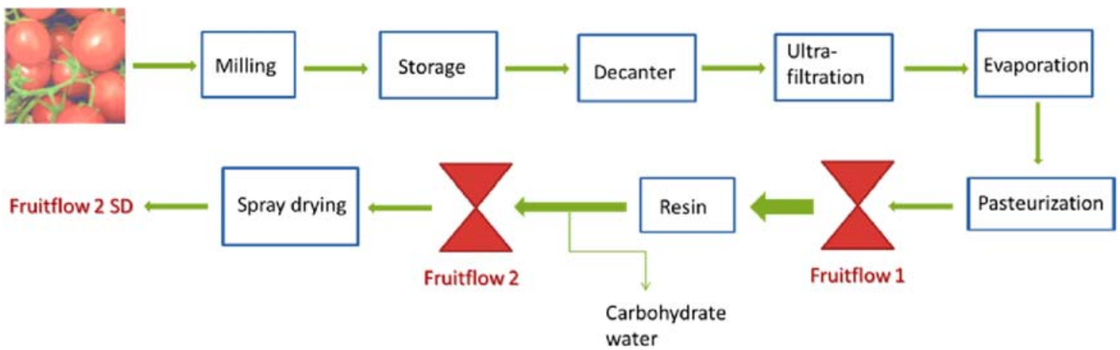

FIGURE 1.1 Production process of Fruitflow 1 and 2.

- Fruitflow one is produced from tomato products with minimal processing of the cold solution in a 5-step process, which uses natural separation methods (centrifugation, filtration) at low temperatures to remove pulp and unwanted insoluble solids from the starting material, which leads to clear yellow juice. The juice is then concentrated by evaporation at low temperature and standardized.
- Fruitflow two is a low-sugar derivative of Fruitflow 1, derived from the same raw material and using the same initial clarification processing steps. After removing the insoluble material, the compound and simple sugars are extracted from the clarified tomato juice using an adsorption column process, in which the sugars, which are the main constituents of the tomato juice, pass through a column of resin without retention, while the remaining components remain in the column. These preserved ingredients, which may represent up to 2% of the fresh weight of the tomato-derived raw material, are then recovered from the column and the concentrate is dried to a powder and standardized.

The resulting Fruitflow two powder contains almost no sugar. The bioactive ingredients are present in concentrations between 28 and 32 times higher than in Fruitflow 1, due to the removal of the sugar and water matrix of the syrup ingredients. However, as the resin column used successfully retains the remaining ingredients of the tomato juice, the relative appearance of these ingredients in Fruitflow two remains similar to that of Fruitflow 1 (i.e., the process does not significantly change the relative proportions of the bioactive ingredients). An industry standard for each ingredient ensures that the end product of each process is an extract whose antiplatelet activity against ADP, collagen, AA and TRAP *in vitro* is above a minimum level and as close as possible to fresh tomato juice. The specification allows a standard use of the ingredients so that Fruitflow one and two can be used alternatively depending on the application. Ingredients Fruitflow one and two are integrated into a range of different food products, from beverages to tablets. Both components are stable, with adequate lifespan as a whole.

7.2 *In vitro* studies

The anticoagulant effects of aqueous tomato extracts (70%−75% inhibitory effect) on human platelets *in vitro* have been published (Dutta-Roy et al., 2001). The nonsugar fraction isolated (total active tomato fraction, tAF) represented 4% of the dry matter of tomato extract and showed strong inhibition of platelet aggregation *in vitro* (O'Kennedy et al., 2017). This was followed by the isolation of many individual components from tAF and it was found that most fall into one of three categories: nucleosides (F1), simple phenolic derivatives (F2) and flavonoid derivatives (F3). All showed antiplatelet activity according to their compounds (O'Kennedy et al., 2017). Proteomic experiments performed to examine the effects of tAF on platelet signaling pathways showed that tAF components altered a range of platelet functions, including those that regulate platelet structure, coagulation, and oxidative status (O'Kennedy et al., 2017). One of the most severely affected proteins was disulfide protein isomerase (PDI), an oxidoreductase that catalyzes the formation and isomerization of disulfide bonds (O'Kennedy et al., 2017). Glycosides (quercetin-related) are present in tAF extracts and have been shown to interact with PDI in this way (O'Kennedy et al., 2017). The interaction of polyphenols with PDI suggested a possible mechanism by which tomato extract components could inhibit different platelet aggregation pathways. The functional effects of tAF components were therefore examined in a series of experiments (O'Kennedy et al., 2017). TAF and its F1, F2 and F3 subunits were observed to inhibit αIIb3 integrin activation (i.e., GPIIb/IIIa). Inhibition of the GPIIb/IIIa activation step, which is common to multiple aggregation pathways, could underlie the broad effects of tAF (O'Kennedy et al., 2006b). This is consistent with the observation that basal platelet concentrations of cyclic AMP are not modified by active ingredients of tomato extract *in vitro*. In addition, tAF decreased the expression of P-selectin (CD62P) on the platelet surface in response to ADP-induced platelet activation in whole blood (O'Kennedy, Crosbie, van Lieshout, et al., 2006). In normal platelets, P-selectin is located in the membranes of α-granule platelets. Upon platelet activation, it redistributes to the platelet surface, where leukocyte adhesion begins. Under conditions of blood flow and shear stress, this glycoprotein promotes platelet cohesion and stabilizes newly formed aggregates (O'Kennedy, Crosbie, van Lieshout, et al., 2006). Thus, tAF components can potentially affect the size and longevity of platelet aggregates (O'Kennedy, Crosbie, van Lieshout, et al., 2006). The tAF components were also found to affect the binding of tissue factor (TF) to activated platelets, at least in part due to their effects on P-selectin (O'Kennedy et al., 2017). In summary, these results demonstrate the effects of tAF on different platelet functions, all of which were consistent with effects mediated in part by polyphenols and PDI and in part by nucleosides that increase platelet cAMP and cGMP levels (Dutta-Roy et al., 2001). Effects on TF binding indicated that tAF components could have a greater effect on certain aspects of the coagulation response, such as thrombin production.

7.3 Additional Fruitflow features

Activation of inflammatory pathways in macrophages plays a critical role in the onset and progression of endothelial dysfunction, which ultimately leads to atherosclerosis (O'Kennedy et al., 2017). The effect of Fruitflow on the inflammatory response of macrophages and endothelial dysfunction in human umbilical vein endothelial cells (HUVEC) has been investigated (Schwager et al., 2016). In this study, Fruitflow reduced the production of inflammatory mediators associated with chronic inflammation and regulated the inflammatory expression of inflammatory mediators via the NP-κB pathway.

Adenosine, chlorogenic acid, and rutin are representative substances of the three main groups of active ingredients in Fruitflow that have been shown to inhibit both platelet aggregation and inflammatory pathways (O'Kennedy et al., 2017). Collectively, the data show that the compounds contained in Fruitflow have the ability to regulate signaling pathways that alter vascular function, the development of atherosclerotic lesions and therefore the risk of CVD by various mechanisms, including antiinflammatory effects (O'Kennedy et al., 2017). Fruitflow also inhibited ACE activity in human serum and rabbit lung in a dose-dependent manner (Biswas et al., 2014). In conclusion, consumption of Fruitflow bioactive ingredients may reduce more than one risk factor for CVD, such as platelet hyperactivity and hypertension.

7.4 Size and variability of the acute antiplatelet effect

Studies have shown inhibition of the platelet response in the ADP agonist by approximately 17%−25% and inhibition of the collagen response by approximately 10%−18% (O'Kennedy et al., 2017). Platelet aggregation caused by arachidonic acid and platelet accumulation induced by thrombin receptor activating peptide (TRAP) has also been shown to decrease after administration of Fruitflow (O'Kennedy et al., 2017). A study in which Fruitflow one was administered to 93 healthy men and women showed that some variability in response may occur, with men responding better than women and people with higher risk factors for developing CVD higher than others (O 'Kennedy, Crosbie, Whelan, et al., 2006). Dose-response studies have shown that a dose of Fruitflow 1 (equivalent to 65 mg tAF or about three bowls of canned tomato soup) has caused platelet inhibition close to the maximum level that can be achieved with this extract and that no significant gain will be achieved in acute dose adjustment (O'Kennedy et al., 2017).

7.5 Effects of the form of ingredients and the food matrix

Studies of the effects of the form of ingredients and the food matrix on the observation of antiplatelet effects, in which 54 people participated, showed that it was not possible to distinguish between the antiplatelet effects observed for the components Fruitflow one and Fruitflow 2 when consumed in doses of 3 g and 150 mg, respectively (O'Kennedy et al., 2017). No difference between the two forms of ingredients could be detected 3 hours after consumption. They also showed that incorporating Fruitflow into beverages, yogurts, and water-based capsules were all viable means of inducing an antiplatelet effect 3 hours after ingestion (O'Kennedy et al., 2017).

7.6 Effects of chronic consumption

Studies have been performed examining the effects of continuous (daily) consumption of Fruitflow (O'Kennedy et al., 2017). These studies showed that the magnitude of the antiplatelet effect observed after consuming one dose of Fruitflow daily for two or 4 weeks was not significantly different from the magnitude of the effect observed after a single dose, i.e., the observed results were not cumulative. The platelet suppression achieved through chronic consumption was continuous measurements of platelet function obtained in fasting individuals in the morning, approximately 24 h after consuming the last dose of Fruitflow, and suppression of initial platelet function was observed after two and 4 weeks (O'Kennedy et al., 2017).

7.7 Security issues

The compounds found in Fruitflow have been shown to affect many aspects of platelet function, including thrombin production (O'Kennedy et al., 2017). Therefore, during all human intervention studies, care was taken to incorporate specific safety-focused measures to examine whether effects on endogenous or exogenous coagulation pathways could be detected (O'Kennedy et al., 2017). An antiplatelet component that has also affected blood clotting pathways could raise safety concerns. However, in all intervention studies performed, coagulation time measurements did not show significant increases from baseline levels (O'Kennedy et al., 2017). Fruitflow does not directly affect blood clotting at any dosage.

Even without directly affecting blood clotting, many antiplatelet drugs, taken on a chronic basis, cause excessive platelet inhibition and are associated with internal bleeding (O'Kennedy et al., 2017). However, Fruitflow differs substantially from antiplatelet drugs in its reversibility of action (O'Kennedy et al., 2017). Widely used antiplatelet drugs have irreversible mechanisms of action. Over 10 days, approximately 90% of the circulating platelet population may be irreversibly affected by the lifespan of these platelets (O'Kennedy et al., 2017). This level of platelet inhibition is then maintained with daily medication. In contrast, the antiplatelet effects of Fruitflow are not irreversible or cumulative and can be overcome with increased concentrations of agonists (O'Kennedy et al., 2017). This very significant difference in mode of action makes Fruitflow suitable for use by the general population as a nutritional functional ingredient, while antiplatelet drugs cannot be used.

As Fruitflow has been designed as a food ingredient, with the potential to be integrated into a variety of food products, a specific study was conducted to examine the potential effects of overconsumption (O'Kennedy et al., 2017). Since the amount of Fruitflow in each serving of a food product is low, equivalent to about three cups of canned tomato soup, and as dose response studies have shown that increasing the dose significantly would not have a much greater effect on platelets, significant risks were expected (O'Kennedy et al., 2017). A study showed that consuming 1L of beverage containing four daily doses of Fruitflow did not dangerously reduce platelet aggregation compared to baseline (O'Kennedy et al., 2017).

In conclusion, as dietary standards are primarily concerned with the proper intake of nutrients, the development of functional foods places particular emphasis on the potential health benefits of phytochemicals. For the development of a final functional food, it is important to ensure stable concentrations and profiles of phytochemicals, supported by studies on digestibility, bioavailability, the bioactivity of bioactive substances, their safety, and their clinical importance, both in the short and long term.

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Chapter 2

Coffee and tea bioactive compounds

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1. Coffee

1.1 Coffee types, main production countries, main chemical composition

Coffee, botanically, belongs to the genus *Coffea* (Hamon et al., 2017) of the Rubiaceae family and the coffee species with the highest commercial interest are *Coffea arabica* (Arabica coffee), *Coffea canephora* (Robusta coffee), and in a very small percent (less than 2%) *Coffea liberica* (Liberian coffee) (Patay et al., 2016). Coffee is produced within the commonly termed "coffee belt" that consists of the band of regions between the Tropic of Cancer (23°26′14″ north of the Equator or 23.4372°) and the Tropic of Capricorn (23°26′14″ south of the Equator or −23.4372°) (Giraudo et al., 2019). According to the International Coffee Organisation (2019), the country with the largest coffee production is Brazil comprising 35% of total world production, followed by Vietnam (18%), Colombia (8%), Indonesia (7%), and Ethiopia (5%). Concerning the species, almost 60% corresponds to Arabica coffee and 40% to Robusta (International Coffee Organisation, 2019). Arabica coffee is the most expensive in the market, due to its demanding production (i.e., high sensitivity to environmental changes), and superior organoleptic characteristics. Arabica coffee is a complex, aromatic beverage with a smooth, sweet, and slightly acidic flavor (Combes et al., 2018). Robusta coffee, on the other hand, has lower production cost, rougher aroma, and bitter, more astringent flavor (Portaluri et al., 2020). Common market practice is to blend Arabica coffee with Robusta in different ratio in order to reduce the cost and achieve the desired and balanced flavor (Couto et al., 2019). In such blends, Arabica coffee is used to enhance the aroma, whereas Robusta is used to increase the body and the foam of the beverage (Colzi et al., 2017).

The organoleptic characteristics of coffee are mainly affected by the chemical composition of the "raw" or "green" coffee beans (Assis et al., 2019). Their chemical composition can vary significantly depending on the growth conditions of coffee trees (Lopes et al., 2016), the harvest and postharvest processing of coffee fruit (depulping, drying, storage, roasting) (de Melo Pereira et al., 2020), and also coffee fruit's genotype (*Coffea arabica, Coffea canephora*). Differences can also occur within the same bean batch (Cagliani et al., 2013). The green coffee beans are mainly composed of insoluble polysaccharides (34%−53%), soluble carbohydrates (6%−12%), lipids (8%−18%), proteins and free amino acids (9%−12%), and minerals (3%−5%) (Hamzalõoğlu & Gökmen, 2020). Between Arabica and Robusta coffee beans, the chemical composition differs significantly; Arabica coffee has a higher concentration of lipids, free sugars, trigonelline and a lower concentration of carbohydrates, caffeine, and chlorogenic acid (CGA) than Robusta coffee (Table 2.1). Among the compounds present in the beans, the biologically active are considered to be the phenolic compounds, alkaloids, and diterpenes (Guercia et al., 2016).

The phenolic compounds that are present in green coffee beans are specific phenols containing more than one phenolic hydroxyl group attached to one or more benzenes. The main ones are CGA, caffeic acid, ferulic acid, and coumaric acid (Quan et al., 2020). Among them, CGA is the most important for its biological activities (Vilas-Boas et al., 2020). Generally, the term CGA refers to the esters of caffeic and quinic acid. The term CGA in coffee is used to define a family of esters of quinic acid and its isomers with caffeic acid, ferulic acid, coumaric acid, and their isomers (Tomac et al., 2020). The 5-O-CGA is the most common isomer found in coffee (Siebert et al., 2019). A typical Arabica green coffee bean comprises approximately 45−50 derivatives of regio-isomeric CGAs, whereas a typical Robusta green coffee bean approximately 80−90 derivatives (Badmos et al., 2019) (Table 2.1). Apart from the phenolic compounds (or polyphenols) in coffee beans, the alkaloids have also a crucial role as bioactive compounds and affect the quality of the final beverage

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TABLE 2.1 Differences in the concentration of the most important bioactive and sensorial compounds between Arabica and Robusta green coffee beans.

Coffee compounds	Arabica (%)	Robusta (%)
Carbohydrates		
Polysaccharides	34–44	48–55
Sucrose	9–13	6–13
Lipids		
Triglycerides-fatty acids	11.53–14.95	6.25–14.64
Linoleic acid (C18:2n-6c)	6.15–8.28	3.04–4.98
Palmitic acid (C16:0)	1.09–1.23	2.72–4.38
Oleic acid (C18:1n-9c)	1.09–1.42	0.88–1.56
Stearic acid (C18:0)	0.87–1.35	0.52–0.84
Arachidic acid (C20:0)	0.15–0.49	0.14–0.36
Linolenic acid (C18:3n-3)	0.20–0.26	0.05–0.10
Diterpenes	1.3–1.9	0.2–1.5
Wax	0.07–0.41	0.01–0.18
Polyphenols		
Chlorogenic acid	7–9	7–12
Alcaloids		
Caffeine	0.7–1.6	1.5–4.0
Trigonelline	0.6–1.2	0.3–0.9

Adapted from Caporaso et al. (2018), Guercia et al. (2016), Jeszka-Skowron et al. (2020) and Romano et al. (2014).

(Stefanello et al., 2019). The most abundant alkaloid in both coffee species is caffeine or 1,3,7-trimethylxanthine. Caffeine belongs to the group of the derivatives of xanthines termed methylxanthines (Jeon et al., 2019). The second alkaloid that is in a very high content in green coffee beans is trigonelline or N-methylnicotinic acid, a pyridine derivative (del Campo et al., 2010), followed by another two methylxanthines; theobromine (3,7-dimethylxantine) and theophylline (1,3-dimethylxanthine) (de Paula Lima & Farah, 2019).

Regarding the lipid content of green coffee beans, it mainly consists of triglycerides (75%), and in smaller concentrations, phospholipids, sterols, diterpenes, and their respective esters with fatty acids, and tocopherols (Williamson & Hatzakis, 2019). Concerning the fatty acid profile, the unsaturated ones outpace the saturated (ratio saturated/unsaturated = 0.86 for Arabica and 0.85 for Robusta coffee). The most abundant polyunsaturated fatty acid is the linoleic acid (18:2n-6), the most abundant monounsaturated fatty acid is the oleic acid (*cis*18:1n-9); palmitic acid (C16:0) is the most abundant saturated fatty acid (Romano et al., 2014). Table 2.1 shows the concentration of the fatty acids with the highest concentration in both Arabica and Robusta coffee beans. Among the lipids, the ones that are well-known for their bioactivities and represent the 15% of the total lipid content are the pentacyclic diterpene alcohols cafestol and kahweol that differ by a double bond in C1 and C2, that presents in kahweol, and their methoxylated compounds, esterified at the C-17 position, with 16-0-methylcafestol to be the most abundant. Cafestol can be found in both Arabica and Robusta coffee beans, kahweol mostly in Arabica, and 16-O-methylcafestol mostly in Robusta coffee beans (Moeenfard & Alves, 2020). As for the tocopherols, they are lipophilic compounds with well-established antioxidant properties and common in vegetable oils (Oliveira et al., 2018).

With regard to mineral composition, coffee beans are a significant source of several macro and microelements like K, Mg, P, Ca, Fe, Mn, Zn, S, and Cu (Debastiani et al., 2019). The content of those elements in the coffee bean depends on several factors such as soil chemical composition, soil management, environmental conditions, processing method, and beverage preparation (Batista dos Santos Espinelli Junior et al., 2020). Thus, the mineral content of coffee beans can be indicative of the origin and the processing steps of coffee beans (Bitter et al., 2020). There are no significant differences among the two different species, Arabica and Robusta coffee beans; the total mineral content is slightly lower in Arabica (3.5−4.4 g/100 g) than in Robusta coffee beans (3.9−4.5/100 g). This content can greatly be affected by the production method applied (Cruz et al., 2015). In general terms, potassium exists in the highest concentration in coffee beans followed by Mg, P, Ca, and S. Slight differences might also appear in the elemental content of same-brand coffee stored produced in different periods of time (Debastiani et al., 2019).

The green coffee beans undergo many chemical, physical and sensorial changes during roasting (Leme et al., 2019). In terms of organoleptic characteristics, the coffee beans increase in volume (50%−80%), become lighter in weight (13%−20%), turn brown and about 800 new compounds responsible for their aroma and taste are formed during the process of roasting (De Luca et al., 2016). From a chemical point of view, many chemical reactions such as Maillard reactions (Badoud et al., 2020), Strecker degradation (Liu et al., 2019), caramelization (decomposition of the sugars) (Bertuzzi et al., 2020), polymer and polyphenol breakdown (Muñoz et al., 2020), and pyrolysis of nonvolatile compounds (Esposito et al., 2020) take place during roasting, resulting in degradation of carbohydrates, phenolic compounds, alkaloids, diterpenes (Wang et al., 2018; Badmos et al., 2019; Barbosa et al., 2019) and formation of mostly melanoidins, that are nitrogenous brown-colored compounds with high molecular weight generated in the final stage of Maillard reaction (Moreira et al., 2017). In addition, other compounds are formed with either a positive (such as simple aldehydes, alcohols, furans, ethers, thiazoles, pyrones, acids, esters, amines, imines, and pyrazines) (Farah et al., 2012) or a negative (such as acrylamide, 5-hydroxymethyl furfural, free radicals) (Bertuzzi et al., 2020; Doğan et al., 2019; Goodman et al., 2011) effect in the organoleptic characteristics and thus, the quality of the roasted coffee beans. Moreover, the chemical changes occurred with the roasting of the coffee beans change the overall antioxidant capacity of them (Priftis et al., 2018). Fig. 2.1.

1.2 Coffee production

After the harvest of the coffee fruit, which is also termed as coffee cherry due to its appearance, three different postharvest procedures that can be followed: the dry/natural, the semi-dry/honey, and the wet processing (Fig. 2.2) that differ in the stage that the outer layers of the coffee fruit (husk, mucilage) are removed. These procedures affect green coffee bean's chemical composition and thus the sensory quality of the final beverage (Tinoco et al., 2019). In the natural/dry processing, the entire coffee fruit is dried prior the removal of its outer layer (Duarte et al., 2010). This type of processing provides a smooth, sweet coffee with heavy body and complex quality attributes (Hadj Salem et al., 2020). In the wet processing, the husk is removed from the coffee beans and the depulped beans are fermented to degrade the mucilage layer. Therefore, the coffee beans are dried without the husk and the polysaccharide rich mucilage (Pereira et al., 2020) resulting in coffee beans with a reduced content in glucose and fructose but no change in the content of caffeine, sucrose, and proteins (Tsukui et al., 2019). The beverage that is provided with the wet processing has lower body, higher acidity, and is very aromatic (de Melo Pereira et al., 2014). The semi-dry/honey processing is a combination of the dry and the wet processing in which coffee fruits are depulped, but the fermentation is occurred as the coffee beans are dried (Evangelista et al., 2014). The golden and sticky mucilage that covers the coffee bean is not removed and is reminiscent of honey, which is where the process gets its name. It adds body and sweet flavor after roasting (Tsukui et al., 2019).

1.3 Coffee bioactive ingredients: Protective effect on health

1.3.1 What are they and how they are affected by processing and type

A great number of the minor compounds present in coffee beans are considered to have significant bioactive potential; they can reduce inflammation by influencing metabolic processes. Coffee bioactive compounds can protect against chronic diseases; many chronic diseases such as diabetes, arthritis, atherosclerosis, and cancer have some degree of inflammation in their genesis (Esser et al., 2014; Greisen et al., 2020). The most influential are the phenolic compounds (mostly CGAs and derivatives), methylxanthines (mostly caffeine, theophylline and theobromine), followed by diterpenes (including cafestol and kahweol), nicotinic acid (vitamin B3), and its precursor trigonelline as well as potassium and magnesium (Jeszka-Skowron et al., 2020).

The content of coffee bioactive compounds can either be maintained or change during the cultivation period or during coffee bean processing. The roasting, however, causes the most profound changes in the bioactive profile. CGAs are greatly affected by the duration and intensity of coffee bean roasting. More specifically, the greater the extent of the roast the lower the CGA content in the coffee bean afterward (Macheiner et al., 2019); around 45%−55% of CGAs can be lost within light roasting (e.g., 230°C, 12 min), whereas more than 99% can be lost within intense roasting (e.g., 250°C, 21 min) (Liang & Kitts, 2015). The high-temperature roasting either converts some CGAs into flavor and aroma compounds or makes them react with other chemical coffee bean or brew compounds through multiple reaction pathways such

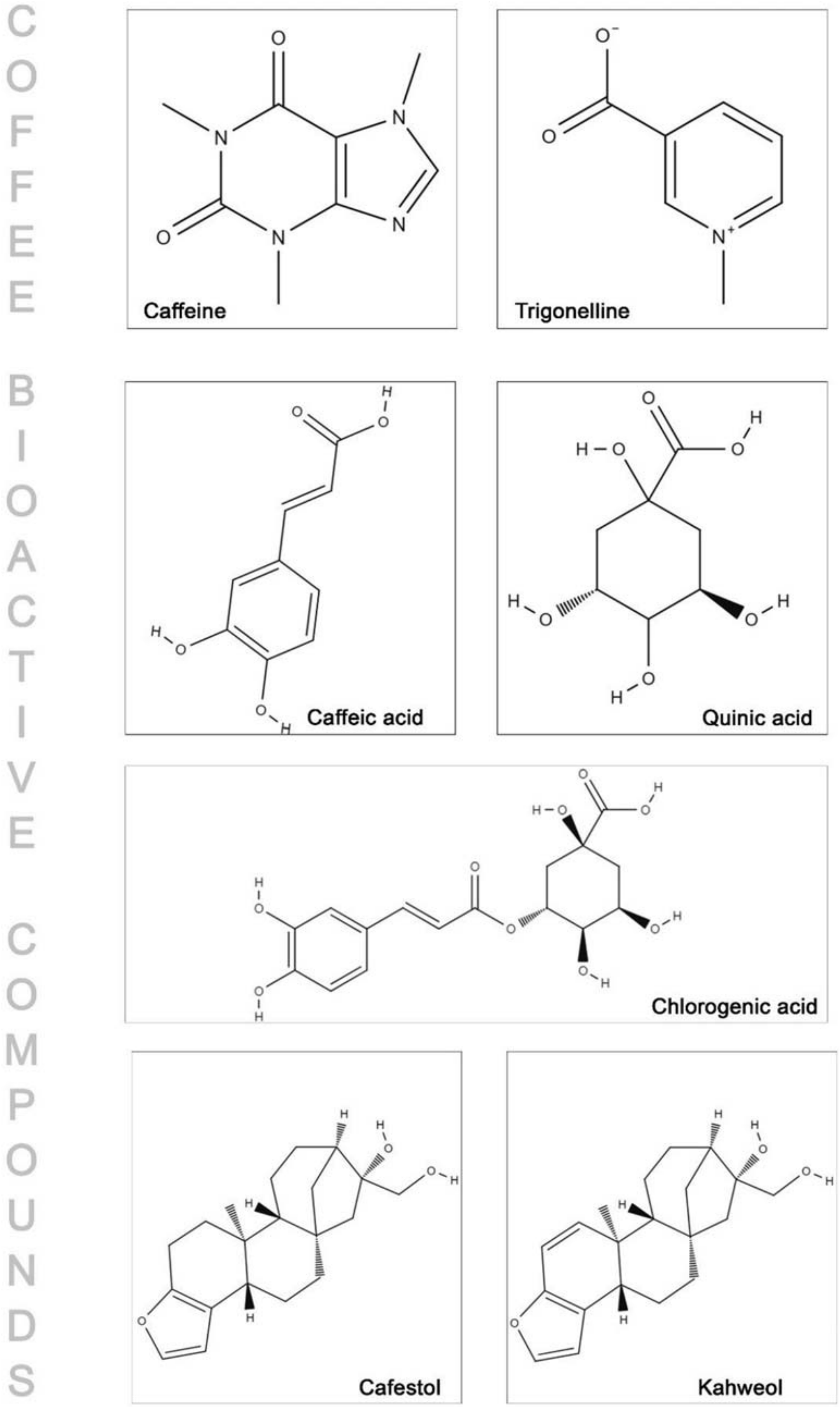

FIGURE 2.1 The chemical structure of the main bioactive compounds in coffee beans.

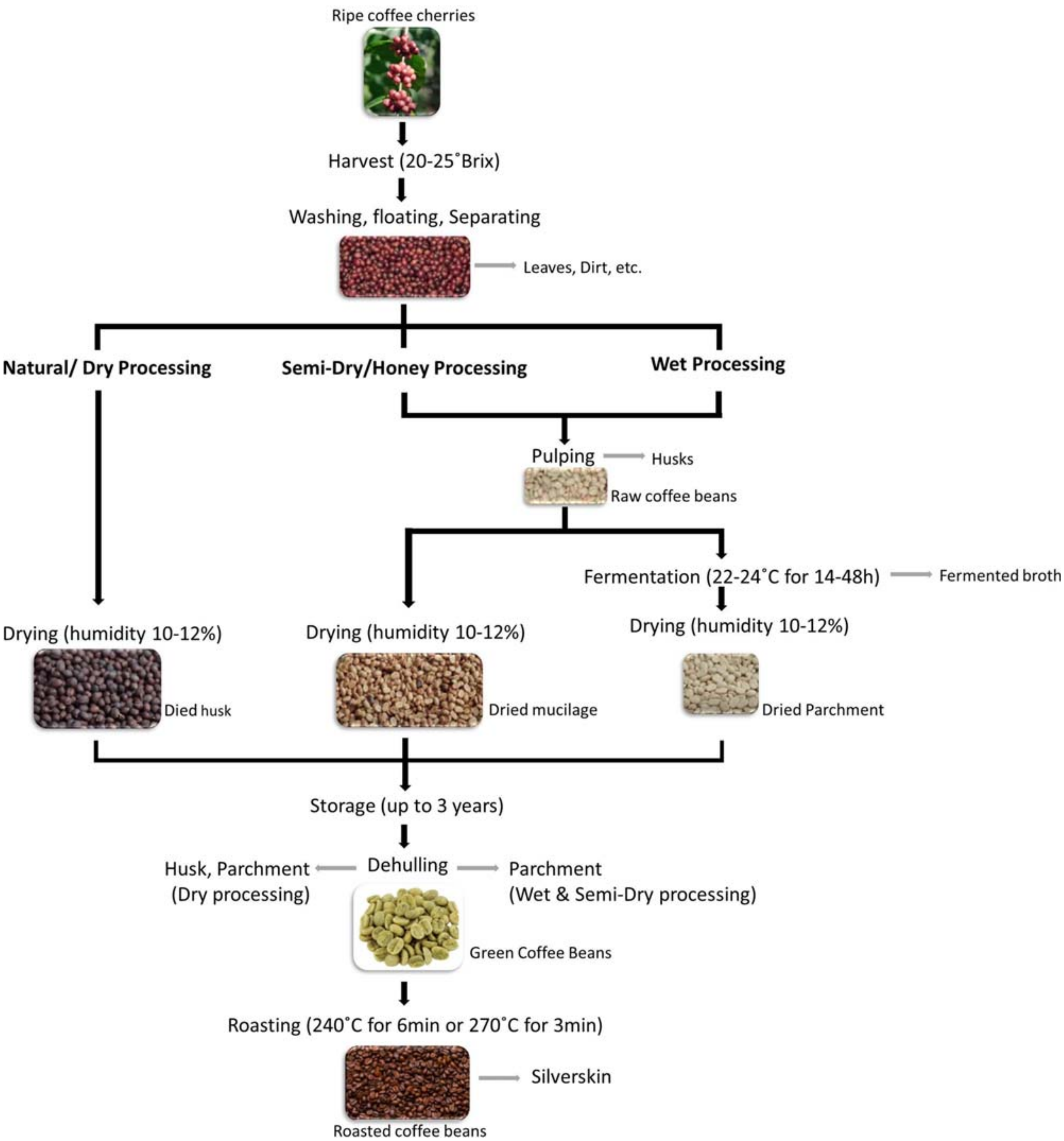

FIGURE 2.2 Coffee production flow diagram. *Adapted from de Melo Pereira et al. (2014) and Hadj Salem et al. (2020).*

as epimerization, dehydration, decarboxylation, lactonization, and acyl mitigation. The nature of these products varies from simple decarboxylazed quinic and cinnamic acids or even more simple phenolic acids, to complex chlorogenic lactones, that are derived from the dehydration of quinic acid moiety (Liang & Kitts, 2015; Mills et al., 2013).

Although intensive roasting decreases the CGAs, the caffeine content isn't altered with the roasting process (Jeon et al., 2019), due to its thermostability (Vignoli et al., 2014). Trigonelline though, which is the second most abundant alkaloid in the green coffee beans, is demethylated during coffee roasting and generates other compounds including nicotinic acid (or niacin) which is a water-soluble B vitamin (Perrone et al., 2008; Stefanello et al., 2019). The concentration of diterpenes is also greatly affected by roasting. More specifically, the high temperature roasting leads to the formation of dehydrated products such as dehydrocafestol and dehydrokahweol. Several other diterpene decomposition products as cafestal, kahweal, isokahweol, dehydroisokahweol, and secokahweol have also been identified (Moeenfard & Alves, 2020).

Concerning the minerals presenting in the coffee bean, they are typically absorbed from the soil and enhanced by fertilizers (Cervera-Mata et al., 2020). Although coffee trees are cultivated in equatorial regions, the soil composition is naturally differentiated; it can vary from volcanic soils, which is characterized from high K concentration (e.g., Indonesia and Central America), to soils rich in basal and granite, characterized from low K concentration (e.g., Brazil, West Africa or India) (Cruz et al., 2015). These soil elements influence coffee beans' mineral content, providing a basis for the evaluation of the authenticity and the geographical origin of coffee bean samples (Oliveira et al., 2015). During roasting, with temperatures reaching typically up to 250°C, the mineral quantity is maintained. By the end of the process, as the bean's mass is reduced, the mineral content increases proportionally on a dry basis (Oliveira et al., 2012).

1.3.2 Evidence for the protective effects of coffee

1.3.2.1 Coffee and inflammation

Inflammation is the array of immune system biological responses that can be triggered by a wide range of factors, such as a physical injury, an ischemic injury, radiation, an infection, or other types of trauma (Fleit, 2014) even a phycological (Rohleder, 2019). Depending on the factors that can cause inflammation and the duration of the biological repairing mechanisms that are activated, the inflammatory responses can be divided to acute or chronic. Acute inflammation has a short duration depending on the extent of the injury; it can last from some minutes to a couple of days. It is characterized by exudation of plasma proteins, fluid and emigration of leukocytes (Arulselvan et al., 2016). Chronic inflammation on the other hand may last from a couple of weeks or months to even a lifetime. It is referred to continuous recruitment of mononuclear leukocytes followed by tissue injuries due to the uninterrupted inflammatory response (Fougère et al., 2017). Both acute and chronic inflammation can largely be prevented by following a healthy diet. Coffee is termed as a potential functional food as it arguably is a source of bioactive compounds with antiinflammatory and antioxidant properties, such as phenolic compounds (mainly CGAs), alkaloids (mainly caffeine and trigonelline), diterpenes (mainly cafestol and kahweol), and melanoidines (Ciaramelli et al., 2019). For this reason, it is of great interest to analyze further the specific health benefits of coffee bioactive compounds.

CGA has known immunoprotective, antiinflammatory, and antioxidant properties. Animal experiments showed that treatment with CGA can prevent inflammation, diabetes and diabetic neuropathy, damage of kidney tissue and oxidative stress by increasing the nuclear translocation of nuclear factor erythroid-delivered 2-related factor 2 (Nrf2) and the heme oxygenase-1 (HO-1) expression and by reducing the phosphorylation of IκB and subsequent nuclear translocation of nuclear factor kappa beta (NF-κB) (Bao et al., 2018). In human carcinoma cells, CGA can evoke up-regulation of cellular antioxidant enzymes and suppress reactive oxygen species (ROS)-mediated nuclear factor kappa B (NF-κB), activator protein-1 (AP-1), and mitogen-activated protein kinase activations (MAPKs) too. Administration of CGA can down-regulate the production of interleukin (IL)-6, IL-1β, and tumor necrosis factor-alpha (TNF-α), in a dose-dependent manner. In addition, CGA showed antihepatotoxic effect on lipopolysaccharide (LPS)-treated mice by suppression of the mRNA levels of TLR4 (Palócz et al., 2016; Tian et al., 2019). The transformation of CGA into quinolactones and melanoidines within roasting provides a greater antioxidant potential (Frost-Meyer & Logomarsino, 2012).

Caffeine has a molecular structure quite similar to adenosine's structure. This causes most of its biological actions by antagonizing all the different types of adenosine receptors (A1, A2A, A3, A2B) leading to stimulation of the central nervous system, prevention of central nervous system dysfunctions, elevation of blood pressure, and increase in metabolic activity (Moases Ghaffary & Abtahi Froushani, 2020; Ribeiro & Sebastio, 2010). Furthermore, dose-dependent caffeine validation experiments showed downregulation at the mRNA levels of key inflammatory genes including signal transducer and activator of transcription 1 (STAT1), TNF, and peroxisome proliferator-activated receptor gamma (PPRAG). Tumor necrosis factor (TNF) and PPRAG genes are suppressed even with quite low caffeine dosage corresponding to the serum concentration of caffeine after one cup of coffee. Cytokine levels of IL-2, IL-4, IL-6, IL-8, IL-10, granulocyte macrophage colony stimulating factor (GM CSF), macrophage inflammatory protein (MIP)-1β, monocyte chemoattractant protein (MCP)-1 can significantly be decreased with caffeine treatment too (Iris et al., 2018). The reduction of pro-inflammatory cytokine levels in mice caused by caffeine, such as the levels of TNF-α and IL-1β, is associated with memory deficits improvements (Barcelos et al., 2020). Caffeine can also inhibit phosphodiesterase (e.g., PDE1, PDE4, and PDE5), which leads to calcium release from the intracellular poles. The crosstalk between mesenchymal stem cells and their associated cells like immunocytes can be altered too (Moases Ghaffary & Abtahi Froushani, 2020). Noticeably, caffeine is also known for its antioxidant activities as it decreases DNA degradation and reduces hydroxyl radicals (Vieira et al., 2020).

Trigonelline has been shown to exhibit various medicinal benefits; it is neuroprotective with cognition improvement potential as it triggers neurite outgrowth of axons and reverses the memory impairment that is caused by amyloid beta (Chowdhury et al., 2018). According to studies in mice models, trigonelline can additionally protect against diabetes by protecting diabetic β-cells (a feature of diabetes is the progressive decline of the β-cells) (Zhou et al., 2017). Furthermore, trigonelline exhibits antioxidant potential as it can inhibit the generation of ROS or ROS-mediated signaling pathway (Qiu et al., 2020).

The diterpenes, cafestol and kahweol, exhibit numerous medicinal activities, including antiinflammatory, chemoprotective, antitumor, and antioxidant activities. Studies on LPS-activated macrophages demonstrated that cafestol and kahweol significantly inhibit the LPS-induced production of prostaglandin E2 (PGE2) and cyclooxygenase-2 (COX-2). Furthermore, these diterpenes can block the LPS-induced activation of NF-κB by preventing IκB degradation and inhibiting IκB kinase activity (Shen et al., 2010). Glutathione transferase (GST) activity can be increased by cafestol and kahweol as well as several GST classes in the intestine and the liver. Coffee diterpenes are also responsible for the elevation of glutathione (GTH) levels by inducting the c-glutamylcysteine synthetase, which is the rate-limiting enzyme of GSH synthesis (Ren et al., 2019). Kahweol also can induce apoptosis and inhibit metastasis by signaling the transducer and inactivating the activator of transcription 3 (STAT3) of cancer cells (Oh et al., 2018). Both diterpenes scavenge ROS which leads to the protection against H_2O_2-induced oxidative stress and stimulate heme oxygenase-1 control for ROS levels (Gökcen & Şanlier, 2019). Finally, bioactive coffee compounds may have a protective effect on the gastrointestinal tract and its particular connection with the brain, known as the brain−gut axis. However, the mechanisms of action of certain health-promoting properties of coffee have yet to be fully understood (Iriondo-dehond et al., 2021).

1.3.2.2 Coffee and cardiovascular diseases

Cardiovascular diseases (CVD) is the general term for heart or blood vessels disorders such as coronary heart disease (CHD), strokes and transient ischemic attack (TIA), peripheral arterial disease (PAD), and aortic disease. It is commonly associated with fatty accumulation inside the arteries (atherosclerosis), amplified risk of blood thrombus or arteries damage in organs such as heart, brain, or kidney (World Health Organisation, 2020). CHD is due to disrupted or decreased flow of blood with high oxygen content to the heart muscle leading to an increased strain in the heart which can cause angina, heart attacks, and heart failure (Wirtz & von Känel, 2017). Stroke is due to the cessation of the bloody supplementation of a part of the brain, which can lead to brain damage and even death. TIA is a similar condition with the stroke characterized from temporarily disrupted blood flow to the brain (Sarikaya et al., 2015). PAD is due to arteries blockage of the body limbs (Nakamura et al., 2017). Aortic disease includes the conditions affecting the aorta; the largest blood vessel in the body carrying blood from the heart to the rest of it (Bicknell & Powell, 2015).

CVD is one of the main causes of death and disability worldwide (World Health Organisation, 2020), but it can often largely be prevented by leading a healthy lifestyle. The effect of coffee on cardiovascular health is an ongoing controversy and depends on the clinical state of every individual, the age, the weight, and of course the levels of consumption. Furthermore, the acute and chronic effects on cardiovascular health caused by coffee consumption in the same individual can significantly differ. Reviews of case-controlled and epidemiological studies demonstrated that a moderate coffee consumption (<300 mL/day) can reduce the risk of CVD by approximately 30%, whereas the abstinence of coffee consumption or the extreme opposite; the overconsumption of coffee (>600 mL/day) can cause detrimental effects in cardiovascular health (Bonita et al., 2007). With regard to blood pressure (BP), acute coffee consumption generally increases the BP in about 30 min after coffee consumption, reaches a peak after 60−90 min, and returns to baseline after 2−4h, which is consistent with the pharmacokinetics of caffeine (Turnbull et al., 2017). On the contrary, systematic reviews and meta-analysis clinical trials in hypertensive individuals found that there is no association between longer term coffee consumption and increased BP or between habitual coffee consumption and increased risk of CVD (Bidel & Tuomilehto, 2013). However, animal experiments demonstrated that the short-term oral administration of green coffee bean extract rich in GCA decreases BP levels in a dose dependent manner, while the long-term administration of the same extract decreases the systolic BP dose dependently (Tajik et al., 2017). Concerning cafestol and kahweol, meta-analysis studies demonstrated that they are both responsible for increasing the levels of low-density lipoprotein (LDL) and total cholesterol. However, with coffee filtration, there is a significant reduction of cafestol and kahweol concentration and thus the consumption of filtered coffee is not associated with an increase on serum cholesterol levels (Gökcen & Şanlier, 2019).

In conclusion, although there are many studies focusing on the effect of bioactive coffee compounds on specific CVD (Table 2.2 offers a summary), their direct contribution is still debated. The weight of the evidence, however, suggests that only the heavy consumption of coffee (>600 mL/day) can be harmful for the heart and the blood vessels, whereas moderate coffee consumption (<300 mL/day) provides some cardiovascular benefits.

TABLE 2.2 Summary of select studies demonstrating coffee bioactivity against inflammation and CVD.

Coffee compounds	Key mechanism involved	Resulted health benefits	References
Chlorogenic acid (CGA)	Modulation of Nrf2/HO-1 and NF-κB pathways	Inhibition of oxidative stress and inflammation, prevents from diabetic neuropathy	Bao et al. (2018)
	Inhibition of NF-κB and JNK pathway	Cardioprotective effects	Tian et al. (2019)
	Down-regulation of IL-6, IL-1β, and TNF-α	Protective effect against LPS-induced inflammation and oxidative stress	Palócz et al. (2016)
Caffeine	Antagonizing A1, A2A, A3, A2B receptors Reduction of: TNF-α, IL-1β	Stimulation of central nervous system, memory improvement, prevention of central nervous system dysfunctions, elevation of blood pressure, and increase of metabolic activity	Moases Ghaffary and Abtahi Froushani (2020), Ribeiro and Sebastio (2010)
	Inhibition of STAT1 signaling and downregulates inflammatory pathways involved in autoimmunity	Protection against autoimmune diseases	Iris et al. (2018)
	Decrease of DNA degradation Reduction of hydroxyl-radicals	Antioxidant properties	Vieira et al. (2020)
Trigonelline	Triggers neurite outgrowth of axons, reverses the memory impairment caused by amyloid beta	Neuroprotective properties, cognition improvement potential	Chowdhury et al. (2018)
	Protection of diabetic β-cells	Protection against diabetes	Zhou et al. (2017)
	Inhibition of ROS generation or ROS-mediated signaling pathway	Antioxidant potential	Qiu et al. (2020)
Cafestol and Kahweol	Inhibition of the LPS-induced production of PGE_2, and COX-2; block LPS-induced activation of NF-κB	Antiinflammatory effects	Shen et al. (2010)
	Signaling the transducer and inactivate the activator of STAT3 of cancer cells	Induce apoptosis and inhibit metastasis	Oh et al. (2018)
	Scavenging ROS	Antioxidant potential	Gökcen and Şanlier (2019)

2. Tea

2.1 Tea types, main production countries, main chemical composition

Tea is produced from the leaves and buds of the plant *Camellia sinensis* and belongs to one of the most consumed beverages in the world thanks to its rich flavor, and potential health benefits (Fang et al., 2019). According to Food and Agriculture Organization (FAO), tea is originated in northeast India, north Burma, and southwest China. The country with the largest tea production is China comprising 41% of total world production, followed by India (21%), Kenya (7.8%), Sri Lanka (4.8%), Vietnam (4.3%), and Turkey (4.3%) (FAO, 2018). Based on the degree of fermentation during tea processing, tea is generally classified into three main groups: green tea (nonfermented), oolong tea (semifermented, 30%−60% fermentation), and black tea (fully fermented, 80%−100% fermentation). In a much smaller scale, white tea, yellow tea (lightly fermented, 10%−20% fermentation), and dark tea (100% fermentation) are produced. Among tea types, black tea is the most widely consumed accounting almost 78% of worldwide consumption, followed by green tea (20%), and the rest comprise only 2% of worldwide consumption (Vuong & Roach, 2014). Tea, in its dry form, has a complex chemical composition (Table 2.1) and contains a profusion of secondary metabolites, such as free amino acids, polyphenols, alkaloids, and terpenes, which mainly contribute to its organoleptic quality and demonstrated health benefit (Jia et al., 2021).

Discussing tea composition, phenolic compounds should be mentioned first as they are the most abundant compounds in dry tea leaves and arguably have great potential as bioactive compounds (Table 2.3). Fresh tea leaves are rich in flavonoids, a specific type of polyphenols. The flavonoids, such as flavanols, flavonols, flavones, flavanones, and anthocyanidins, represent around 30%−42% of the tea leaves' dry weight. Among them, flavan-3-ols, mainly the catechins, exist in the highest concentration (Liu et al., 2020). Green tea is characterized by a significantly higher catechin content compared to oolong and black tea (Donlao & Ogawa, 2019). The major green tea catechins are (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin-3-gallate (EGCG), and its corresponding stereoisomers (see Fig. 2.3). Among them, ECGC is the most abundant (50%−80% of total catechins) and biologically active (Cheng et al., 2020). Catechins contribute to the characteristic astringency and bitterness of the green tea

TABLE 2.3 Main chemical composition of black tea and green tea.

Tea compounds	Black tea (% dry weight)	Green tea (% dry weight)
Polyphenols (total)	25−30	30−40
Flavanols	3−10	30−35
Simple polyphenols	3	2
Flavonols	1	2
Oxidized polyphenols	23−25	4−6
Alkaloids (total)	4−6	4−6
Caffeine	2−5	2−5
Theobromine	0.2−0.4	0.2−0.4
Peptides/proteins (total)	6−16	6−16
Amino acids (total)	1.6−1.7	2.9−3.3
Theanine	0.89−0.92	1.89−2.18
Lipids/organic acids (total)	2−8	2−8
Chlorophyll and other pigments	0.5	0.5
Carbohydrates (total)	10−15	10−15
Pectins	3−4	3−4
Minerals/ash (total)	4−10	4−10
Potassium	5	5
Volatile compounds	0.01−0.02	0.01−0.02

Adapted from Salman et al. (2020), Sharma and Rao (2009), Singh et al. (2017).

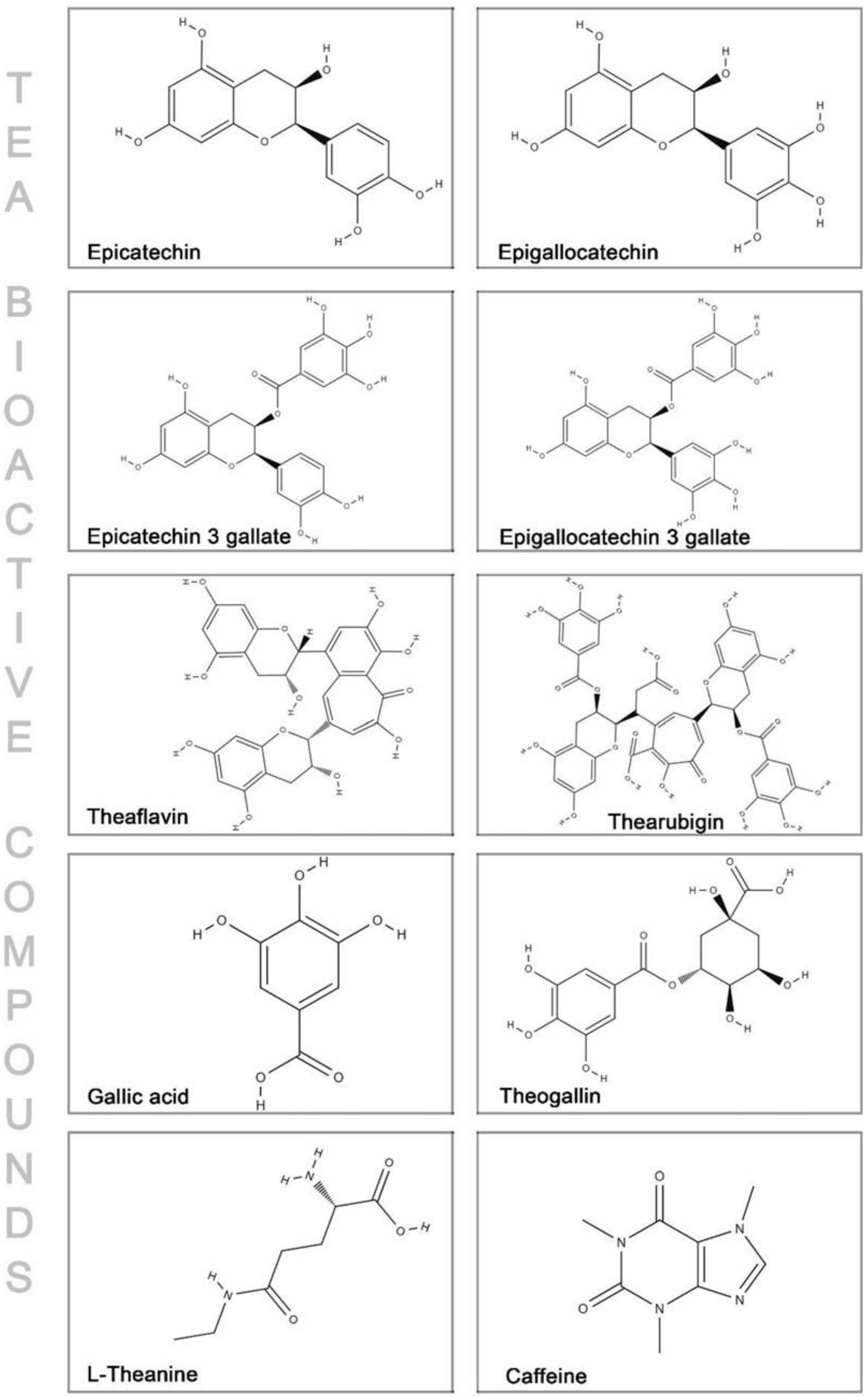

FIGURE 2.3 The chemical structure of the main bioactive compounds of tea.

(Ye et al., 2018). During oolong and black tea fermentation, flavan-3-ols are degraded up to 90% or more. Catechins are oxidized under the action of polyphenol oxidase (PPO) and peroxidase (POD) to form polyphenols with high molecular weight; the theaflavins (TFs) and the thearubigins (TRs).

The main theaflavins present in oolong and black tea are: a) theaflavin (TF1), and its galloylated forms, b) theaflavin-3-gallate (TF2A), c) theaflavin-3′-gallate (TF2B), and d) theaflavin-3,3′-digallate (TF3). Theaflavins have orange-red color (Tao et al., 2016). Thearubigins are oligomeric and polymeric compounds with red-brown color formed by the further oxidation and polymerization of theaflavins. The TFs/TRs ratio can be used to evaluate the fermentation process of tea leaves. Theaflavins and Thearubigins can furtherly be oxidized, coupled, and polymerized with other substances (polysaccharides, proteins, lipids, etc.) to form dark brown pigments termed as theabrownins (TBs) (Wang et al., 2016). Theaflavins, thearubigins, and theabrownins determine the color and influence the taste intensity and briskness of the tea infusion (Dong et al., 2018). Concerning the tea flavonols, they are mainly present in tea leaves in the form of *O-glycosides* with a glycoside moiety at the position C-3 of aglycones (e.g., myricetin, kaempferol, and quercetin). Flavonols account approximately 13% of the total polyphenols in fresh tea leaves (0.5%−2.5% of the dry weight of tea) (Guo et al., 2021) and their quantity is maintained during the processing (Sharma & Rao, 2009). Flavones and their glycosides, such as apigenin, have also been detected in tea but exist in trace levels (He et al., 2020). The major simple phenols (i.e., one phenol ring) in tea are gallic acid and theogallin (5-galloylquinic acid). Fresh tea leaves contain higher amount of theogallin than gallic acid. During fermentation, the concentration of gallic acid is increased, whereas the concentration of theogallin is decreased. However, in most black and oolong teas, the concentration of theogallin is still higher than of gallic acid.

Fresh tea leaves contain a considerable amount of free amino acids (up to 50 mg/g of dry weight), mostly in their L-form. L-Theanine (L-γ-N-ethyl glutamine), is the most abundant and biologically active free amino acid of fresh tea leaves; it accounts 60%−70% of their total free amino acid content (Jiang et al., 2019). The amino acid concentration is increased during tea leaves processing due to protein and peptide proteolysis. The Maillard reaction also leads to degradation of the amino acids giving rise to aroma compounds, pigments, polymeric molecules, and polyphenols. During tea leaves storage, the deactivation of the enzymes and the reduction of the water content make the decrease in amino acid concentration more evident. Another change that occurs in amino acids during the processing and storage of the tea leaves is the enantiomeric isomerization, that is, some amino acids convert their L-form to their D-form, decreasing their bioactivity. The D/L ratios of each amino acid vary depending on the conditions of tea leaves processing and storage (Xu et al., 2020). Referring specifically to L-theanine, its concentration in the final infusion varies with the cultivar type, the processing steps and conditions, and the way the infusion is prepared. Normally, a standard size cup of tea (200 mL) brewed at 80°C for 2 min contains 7.9 and 24.2 mg of L-theanine in green and black tea, respectively. Addition of sugar doesn’t affect its content whereas addition of milk seems to decrease it (Sharma et al., 2018). For reference, L-Theanine is the main compound responsible for the umami taste of tea as well as glutamic acid (Glu) and aspartic acid (Asp) but in a lesser extent. Glycine (Gly), alanine (Ala), threonine (Thr), proline (Pro), and serine (Ser) contribute to the sweet taste. Methionine (Met), arginine (Arg), valine (Val), histidine (His), leucine (Leu), isoleucine (Ile), phenylalanine (Phe), and tryptophan (Trp) contribute to the bitter taste (Ye et al., 2018).

Fresh tea leaves are also a significant source of alkaloids, whose concentration remains unaffected during tea processing. The alkaloids presenting in tea are: caffeine (2%−5% of the dry weight), and in smaller amounts theobromine (0.2%−0.4%) and theophylline (0.02%) (Vuong & Roach, 2014). Caffeine contributes to the astringency and bitterness of the tea infusion (Ye et al., 2018). The main pigments in the fresh tea leaves are chlorophylls and carotenoids. Chlorophylls are oxidized during tea oxidation of leaves to pheophytins and pheophorbides. Together with the oxidized polyphenol forms present in the dried leaves (theaflavins, thearubigins, theabrownins), they contribute to the characteristic brown-black color of black tea infusion (Li et al., 2019).

Fresh tea leaves and the resulting infused beverage contain trace minerals. The mineral composition of the final tea is largely associated with the mineral composition of the soil where the tea shrub grew, the ability of the plant to selectively bio-accumulate certain elements, and the processing and packaging conditions. Tea shrubs normally grow in acidic soils, which are rich in trace elements for root uptake (Ma et al., 2019). Fresh tea leaves contain trace elements such as Al, Ca, K, Mg, Mn, Na, P, and S in a scale of mg/g of dry weight, and others like Cu, Fe, Zn in μg/g to mg/g. They also contain toxic elements such as As, Cd, Pb but in a very low level (Długaszek & Kaszczuk, 2020). The concentration of trace elements increases during tea processing. Trace elements are essential for the healthy growth of the plant and very beneficial for human nutrition. However, bioaccumulation of trace elements (e.g., Al) in the tea infusion might be harmful to human health (Zuziak et al., 2018). Tea composition as reported in the literature is summarized in Table 2.3.

2.2 Tea production

Many high-quality tea infusions consist of the apical bud, and two to three leaves just below the bud. The first postharvest step is withering; the freshly harvested shoots are spread in suitable withering systems until processing. In green tea production, the tea shoots undergo a slight withering, whereas in oolong and black tea production, the withering time is more extended. During this process, moisture is continuously removed from the leaves and they become soft and pliable. Chemically, the content of the most amino acids, caffeine, and gallic acid is increased, whereas the content of catechins is decreased due to the activation of oxidative and hydrolytic enzymes (Ye et al., 2018). The processing steps occurring after withering are different for each tea type. In green tea production, "fixing" follows withering. Fixing is a thermal process that aims at the deactivation of endogenous enzymes. More specifically, PPO and POD are deactivated resulting in the prevention of enzymatic oxidation and browning reactions (Li et al., 2021a). After fixing, the tea leaves are rolled under light pressure in order to disrupt the green cell membrane that promotes the outflow of water from the tea leaves (Miyagishima et al., 2011). Finally, the tea leaves are dried; almost the 70% of the moisture content is reduced, shelf-life is extended and the formation of flavor and color is promoted (Qu et al., 2019). With regard to black tea production, maceration follows withering. Maceration is the breaking of tea shoots into several shapes and sizes of tea particles depending on the level of cell damage, and on the method that is adopted. That processing step involves the intermixing of chemical constituents (mainly polyphenols) with enzymes (PPO) and results in the formation of compounds greatly contributing to the organoleptic quality of tea (Yõlmaz et al., 2020). Tea maceration can either be accomplished by the orthodox method; the tea shoots are rolled either by hand or by a rolling machine, or by the CTC method; tea shoots are crushed, teared, and curled by a CTC machine (Shevchuk et al., 2018).

So called "fermentation" is the next and most crucial step in black tea manufacture. Although this term in common in the industry, the correct terminology should be is "tea oxidation" because it only involves the enzymatic oxidation of polyphenols (namely, catechins). No microorganisms are taking part in this stage in all common tea types (black, oolong, green) discussed in this chapter. Therefore, during tea oxidation, the colourless catechins are converted to reddish-brown oxidized polyphenols (theaflavins, thearubigins, and theabrownins) enzymatically catalyzed by PPO and POD enzymes. Furthermore, amino acids, lipids, and terpenoids form volatile organic compounds at this stage. These compounds together influence black tea's distinctive flavor and quality (Jin et al., 2020). Finally, the shoots are dried. In the tea industry, there are primarily two types of dryers that are used: Endless chain pressure (ECP) dryer and fluidized-bet dryers (FBDs). In FBD, the fermented tea leaves are exposed to hot inlet air at 140°C, whereas in ECP dryers, the temperature of drying air is around 100°C (Konar et al., 2012).

Sorting, which follows drying, is a mechanical process in which tea particles are classified according to their size, and unwanted materials (i.e., fibers, stalks) are removed. Finally, the final product, which is completely dried, is packed into suitable containers that are promptly closed (Tea Research Association, 2020). With regard to oolong tea manufacture, the withering process consists of two phases: solar and indoor withering. During the solar withering, except from the moisture loss, the UV radiation from the sun promotes the gene expression of intracellular hydrolytic enzymes, and enables the hydrolysis of volatile-organic-compound precursors, which present in the glycosidic form (Lin et al., 2016). The indoor withering, which follows the solar withering, is combined with turning-over treatments. More precisely, tea leaves are repetitively subjected to turning-over and withering in turn, becoming dehydrated and bruised to some extent. This is the key step for the determination of the characteristic rich flavor of oolong tea (Hu et al., 2018). To control the enzymatic reactions that occurred during withering combined with turning-over processes, high temperatures are applied within a process called firing. Drying follows firing, and in this step the remaining moisture of the tea leaves is removed applying heat as energy input (Wu et al., 2020). The whole process of tea manufacture is illustrated in Fig. 2.4.

2.3 Tea bioactive ingredients: protective effect on health

The major bioactive compounds in tea are polyphenols (catechins in green tea, and oxidized polyphenols such as theaflavins in black tea), theanine, and caffeine. Their bioactive effects and how they are affected by processing in the different types of teas are discussed below.

2.3.1 Tea and inflammation

Inflammation is called the biological response of immune system which is triggered by various factors, such as pathogens, toxic compounds, or damaged cells. These factors might induce acute and/or chronic inflammatory responses in the vital organs of the body including heart, brain, liver, lung, and kidney leading to damaged tissues or disease. Both infectious and

FIGURE 2.4 Tea production flow diagram. *Adapted from Feng et al. (2019), Li et al. (2021b), Lin et al. (2016) and Theppakorn (2016).*

noninfectious agents as well as cell damage, actuate inflammatory cells, and trigger inflammatory signaling pathways (Chen et al., 2018). Tea with its bioactive properties has been shown to act beneficially to these signaling pathways and therefore prevent from multiple inflammatory conditions.

Catechins exhibit great antiinflammatory properties especially in green tea, in which they are present in a very high concentration. A double-blind, placebo-controlled trial demonstrated that green tea rich in catechins improved inflammation and liver fat content by reducing oxidative stress in patients with nonalcoholic fatty liver disease (Sakata et al., 2013). Another randomized controlled trial showed that 12 weeks of consumption of tea extract rich in EGCG (857 mg), can significantly result in weight loss in obese women (Chen et al., 2016). According to another randomized controlled trial, the weight loss is more profound if the supplementation is combined with physical activity (Levy et al., 2017). Treatment with green tea extract of obese mice with nonalcoholic steatohepatitis restores the hepatic metabolome in association with limiting endotoxemia-TLR4-NF-κB-mediated inflammation (Sasaki et al., 2019). Data of other experiments involving mice also suggest that green tea extract protects against obesity by a gut-adipose axis mechanism that limits endotoxin translocation and consequent adipose TLR4/MF-κB inflammation by improving gut barrier function (Dey et al., 2019). An experiment demonstrated that there is a suppressive effect of EGCG on the release of lipid accumulation and pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β), and on the activation of microglial in both cellular and high-fat-diet rodent models (Mao et al., 2019). Green tea catechins are also considered to be beneficial in brain function, stress reduction and sleep improvement according to randomized controlled trials in healthy adults (Unno, Noda, et al., 2017; Unno, Yamada, et al., 2017). A double-blind, placebo controlled, crossover study tested the supplementation of omega 3 essential fatty acids, green tea catechins, and ginsenosides in healthy cognitive adults and found positive outcomes for their brain activation and brain functional connectivity (Carmichael et al., 2018). With regard to experimental studies, EGCG reduces inflammation and prevents chronic diseases by suppressing the NF-κB and AP-1 gene's signaling pathways (O'neill Rothenberg & Zhang, 2019). Finally, mice experiments showed that supplementation with green tea polyphenols can be beneficial against exposure to perfluorodecanoic acid (PFDA), which is a highly toxic food contaminant being used extensively in food applications as surface antifouling agent, by ameliorating hepatic oxidative stress, cell apoptosis, necrosis, steatosis, edema, and degeneration, and reducing hepatic inflammation and NLRP3 inflammasome activation caused by a moderate dose of PFDA (Wang et al., 2020).

Theaflavins exhibit antiinflammatory, antioxidant, and antiapoptotic properties. They diminished significantly the ROS production of steatotic hepatocytes and TNF-α production by LPS-stimulated cells, thus offering protective effects against ischemia-reperfusion injury in fatty liver (Luo et al., 2012). Theaflavin-3,3′-digallate could reduce the production of pro-inflammatory cytokines *in vivo* and *in vitro* and ameliorate acute lung injury (ALI) in a mouse model (Wu et al., 2017). Using a cell model for inflammatory response, it has been showed that theaflavin-2 suppressed the 12-O-tetradecanoyl-phorbol-13-acetateinduced cyclooxygenase-2 (COX-2) gene expression, and also down-regulated TNF-α, iNOS, ICAM-1, and NF-κB (Gosslau et al., 2011). According to animal experiments, theaflavins-enriched black tea infusions have hepatoprotective effects on fibrosis, potentially by inhibiting the TGF-β1/Smad signaling in rats (Weerawatanakorn et al., 2015). Another experiment in mice demonstrated that theaflavins can suppress neural inflammation and prevent the symptoms of inflammation-related brain disorders (Ano et al., 2019). Furthermore, the potential of using theaflavins as chronic airway inflammation treatment has been suggested, as they can inhibit the activation of epidermal growth factor receptor (EGFR), decrease the level of mucin 5AC, and relieve airway mucous hypersecretion via the EGFR signaling pathway in rats (Wu et al., 2012).

L-theanine has been found to have antiinflammatory activity, antioxidative properties, and hepatoprotective effects. According to experiments in mice, pretreatment with theanine significantly decreased the release of IL-1β and TNF-α, inhibited the expression of several inflammatory factors (including IL-1β, TNF-α, and IL-6), and increased the IL-10/interferon (IFN)-γ ratio in the hepatic tissues (Wang et al., 2018). Moreover, L-theanine administration significantly decreased the production of immunoglobulin E (IgE), monocyte chemoattractant protein-1 (MCP-1), IL-4, IL-5, IL-13, TNF-α, and interferon-gamma in bronchoalveolar lavage fluid (BALF). In asthma, L-theanine alleviates airway inflammation, which likely occurs via the oxidative stress-responsive NF-κB pathway in mice (Hwang et al., 2017). With regards to the antioxidant effect of L-theanine, it increased activities of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione POD in aging rats. Thus it enhanced total antioxidant capacity, and decreased malondialdehyde and nitric oxide synthase levels in serum and liver (Zeng et al., 2020). It has also been suggested that L-theanine has a neuroprotective role through regulation of heme oxygenase-1 (HO-1) activation in rats with cerebral ischemia/reperfusion (IR) injury (Zhao et al., 2020). Lastly, combined treatment with L-theanine and caffeine provides neuroprotective potential, as it can attenuate the elevated inflammatory response levels observed in the cortex after cerebral IR (Sun et al., 2013). Except from the compounds that have been mentioned above, tea is also a source of caffeine, a compound with well-demonstrated anti-inflammatory potential mainly for the brain function. Detailed information on caffeine's effects against inflammation can be found in paragraph 1.3.2. Table 2.4 summarizes the main findings of the studies mentioned regarding the bioactivities of tea against inflammation.

2.3.2 Tea and cardiovascular diseases

CVD are a global health issue, as they represent one of the main causes of death and disability among populations. They are a group of heart and blood vessels' disorders including CHD, cerebrovascular heart disease, rheumatic heart disease, and other conditions. According to WHO, four out of five of CVD deaths are due to heart attacks and strokes (World Health Organisation, 2020). Epidemiological studies, *in vivo* and *in vitro* experiments, and clinical traits have demonstrated that frequent tea consumption is greatly associated with the reduction of CVD risk, due to the bioactive compounds presenting in it.

Green tea catechins significantly prevent against CVD. A meta-analysis study suggests that green tea and its catechins may improve blood pressure, and the effect is greater in people with systolic blood pressure $\geq$130 mmHg (Khalesi et al., 2014). Green tea catechins administration is also considered to reduce total, and LDL-cholesterol. However, no significant reduction in HDL-cholesterol or triglyceride levels associated with green tea catechins has been observed (Kim et al., 2011). Furthermore, EGCG treatment of mice reduced nuclear translocation of NF−B p65 in their aortic vessels, decreased their blood pressure, serum concentrations, and triglycerides (Babu et al., 2012). A double-blind, placebo-controlled,

TABLE 2.4 Summary of select studies demonstrating tea bioactivity against inflammation.

Type of study	Focus, target	Key findings/results	References
Epidemiological	Green tea polyphenols	Beneficial against exposure to PFDA, by ameliorating hepatic oxidative stress, cell apoptosis, necrosis, steatosis, edema, and degeneration, and reducing hepatic inflammation and NLRP3 inflammasome activation caused by a moderate dose of PFDA	Wang et al. (2020)
	Green tea rich in catechins	Improvement of inflammation and liver fat content by reduction of oxidative stress in patients with NAFLD	Sakata et al. (2013)
	Tea extract rich in EGCG (857 mg)	Potential for weight loss in obese women	Chen et al. (2016)
	Green tea catechins	Beneficial in brain function, stress reduction and sleep improvement in healthy adults	Unno, Noda, et al. (2017)
In vitro	EGCG	Release of lipid accumulation and pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β), and activation of microglial in both cellular and high-fat-diet rodent models	Mao et al. (2019)
	Theaflavins	Reduction of ROS production of steatotic hepatocytes and TNF-α production by LPS-stimulated cells, thus offering protective effects against ischemia-reperfusion injury in fatty liver	Luo et al. (2012)
	Theaflavin-2	Suppression of COX-2, downregulation of TNF-α, iNOS, ICAM-1, and NF-κB in cell models	Gosslau et al. (2011)
In vivo	Green tea extract	Improvement of gut barrier function in mice providing protection against obesity	Dey et al. (2019)
	Theaflavin-3,3′-digallate	Reduction of the production of pro-inflammatory cytokines and amelioration of acute lung injury in mice	Wu et al. (2017)
	Theaflavins-enriched black tea extracts	Hepatoprotective effects on experimental fibrosis by inhibiting the TGF-β1/Smad signaling in rats	Weerawatanakorn et al. (2015)
	Theaflavins	Suppression of neural inflammation and prevention of the symptoms of inflammation-related brain disorders in mice	Ano et al. (2019)
	L-theanine	Decrease of the release of IL-1β and TNF-α, inhibition of the expression of IL-1β, TNF-α, and IL-6, and increase of IL-10/IFN-γ ratio in hepatic tissues in mice	Wang et al. (2018)
		Antioxidant properties, and decreased malondialdehyde and nitric oxide synthase levels in serum and liver of aging rats	Zeng et al. (2020)
		Neuroprotective role through regulation of HO-1 activation in rats with cerebral IR injury	Zhao et al. (2020)

randomized, cross-over study suggests moderate effects of single doses of catechins on peripheral microcirculation (Fuchs et al., 2014). According to another randomized-controlled trial, ingestion with tea significantly increased serum adiponectin levels, and elevated blood pressure, which led to great improvement of accommodation ability and eyestrain in subjects younger than 45 years old (Maeda-Yamamoto et al., 2018). With regard to epicatechin, a randomized controlled trial showed that in (pre)hypertensive men and women, epicatechin may contribute to the CVD prevention by improving endothelial function and reducing inflammation (Cialdella-Kam et al., 2017). Lastly, an experimental study demonstrated that continual consumption of beverages containing green tea catechins reduced abdominal fat and improved metabolic syndrome, suggesting its potential to prevent against diabetes and CVD (Zheng et al., 2013).

In black tea, which is a great source of oxidized polyphenols (mainly theaflavins), a randomized controlled clinical trial demonstrated that its consumption within a normal diet decreases independent cardiovascular risk factors and improves the overall antioxidant status in humans (Bahorun et al., 2012). Furthermore, chronic black tea extract consumption improves endothelial function in ovariectomized rats providing insights for developing black tea extract as beneficial dietary supplements for postmenopausal women (Leung et al., 2016). In another experiment, by using mice and cell cultures cardiovascular benefits of black tea have been demonstrated in ameliorating vascular dysfunctions. This provides insight into developing black tea into beneficial dietary supplements in hypertensive patients (San Cheang et al., 2015). Further study in rats evaluated the influence of theaflavins on circulation: single oral dose of theaflavins rich fraction (10 mg/kg) caused transient increase in mean blood pressure and heart rate (Saito et al., 2016). Additionally, theaflavins alleviate intracerebral haemorrhage−induced inflammatory responses and brain injury in rats inhibiting NF-κB-related gene pathway (Fu et al., 2018).

L-theanine is another bioactive compound in all tea types which prevents against CVD. L-theanine has a potential as a locally administrable therapeutic agent for acute cutaneous inflammation. More precisely, mice experiments demonstrated that L-theanine ameliorated: TPA-induced erythema; increase of vascular permeability; epidermal and dermal hyperplasia; neutrophil infiltration, and activation. The above take place due to downregulation of the expression of PECAM-1 (a platelet endothelial adhesion molecule-1) in blood vessels, of the production of pro-inflammatory cytokines IL1β, TNF-α, and of mediator cyclooxygenase-2 (COX-2), which is mainly expressed in neutrophils (Zeng et al., 2018). Pairwise comparisons of RNA-sequencing data have showed that L-theanine is able to upregulate a ray of the rhythm genes and differentially expressed genes that are involved in vasoconstriction and actin cytoskeleton regulation pathways. This suggests that L-theanine changes the circadian gene rhythm involving in the process of vascular smooth muscle restructure (Wang et al., 2019). Another important implication of L-theanine against CVD is that it promotes nitric oxide (NO) production in endothelial cells resulting in vascular benefits (Siamwala et al., 2013). Table 2.5 summarizes the main findings of the studies mentioned regarding the bioactivities of tea against CVD.

Overall, bioactive compounds presenting in tea such as catechins, theaflavins, L-theanine, and caffeine have well-demonstrated potential against the generation of numerous diseases and for their prevention. Precisely, the consumption of three to six cups of tea on a daily basis provides the desirable effects for the prevention of inflammatory conditions and CVD (De Koning Gans et al., 2010). However, the consumption of more than six cups of tea per day can bring negative outcomes for health including hepatic injury (Ohishi et al., 2016). To maximize tea's bioactive potential, habitual tea consumption should be followed by a healthy diet and regular physical exercise.

3. Conclusion

Both coffee and tea are rich sources of bioactive compounds. The main bioactive compounds in coffee beans are phenolic compounds (mainly CGA and its derivatives), methylxanthines (mainly caffeine but also theophylline and theobromine), diterpenes (cafestol and kahweol), and trigonelline (the precursor of nicotinic acid). These compounds have demonstrated antiinflammatory, immunoprotective, neuroprotective, chemoprotective, antitumor, and antioxidant activities. Regarding the effect of coffee bioactive compounds against CVD, their contribution is still debated. The weight of the evidence, however, suggests that only the heavy consumption of coffee (>600 mL/day) can be harmful to the heart and the blood vessels, whereas moderate coffee consumption (<300 mL/day) provides some cardiovascular benefits. With regard to the bioactive compounds of tea, the polyphenols (catechins in green tea, oxidized polyphenols such as theaflavins in black tea), L-theanine, and caffeine are the most abundant. The consumption of three to six cups of tea on a daily basis provides the desirable effects for the prevention of inflammatory conditions and CVD. Nevertheless, the consumption of more than six cups of tea per day can bring negative outcomes for health including hepatic injury. To maximize the bioactive potential of coffee and tea, their habitual consumption should be followed by a healthy diet and regular physical exercise.

TABLE 2.5 Summary of tea bioactivities against CVD.

Type of study	Focus/target	Key findings/results	References
Epidemiological	Green tea catechins	Blood pressure improvement, reduction of total, and low-density lipoprotein (LDL)-cholesterol, abdominal fat and improvement of metabolic syndrome	Khalesi et al. (2014), Kim et al. (2011), Zheng et al. (2013)
	Green tea as a whole	Increase of serum adiponectin levels, elevation of blood pressure, improvement of accommodation ability and eyestrain	Maeda-Yamamoto et al. (2018)
	Epicatechin	Improvement of endothelial function and reduction of inflammation in (pre) hypertensive men and women	Cialdella-Kam et al. (2017)
	Theaflavins	Decrease of independent cardiovascular risk factors and improvement of the overall antioxidant status in humans	Bahorun et al. (2012)
In vitro	Theaflavins	Cardiovascular benefits by ameliorating vascular dysfunctions, beneficial for hypertensive patients	San Cheang et al. (2015)
	L-theanine	Changes in the circadian gene rhythm involving in the process of vascular smooth muscle restructure	Wang et al. (2019)
In vivo	EGCG	Reduction of nuclear translocation of NF−B p65 in aortic vessels, decrease of blood pressure, serum concentrations, and triglycerides in mice	Babu et al. (2012)
	Black tea extract	Improvement of endothelial function in ovariectomized rats	Leung et al. (2016)
	Theaflavins	Transient increase in mean blood pressure and heart rate	Saito et al. (2016)
		Protection of rat heart against ischemia/reperfusion injury	Ma et al. (2011)
	L-theanine	Amelioration of TPA-induced erythema, increase of vascular permeability, epidermal and dermal hyperplasia, and neutrophil infiltration	Zeng et al. (2018)

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Chapter 3

Cacoa and cocoa products

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1. Introduction

Cacao (*Theobroma cacao* L.) is a tree that belongs to the Malvaceae family (Fig. 3.1). *Theobroma* genus includes 22 species; nonetheless, *Theobroma cacao* is the only species with global economic significance (Afoakwa, 2014). Its name was given by the botanist Carl Linnaeus in 1937, who proposed the genus *Theobroma* meaning food of the gods (*"Theo"* and "*broma"* are Greek words meaning "God" and *"*food", respectively) (Cuatrecasas, 1964; Lima et al., 2011). The word *cacao* has been reported to surge from the Olmec and Mayan word *kakaw*, and then adopted by the Aztec Nahuatl language with the word *cacahuatl* (Dillinger et al., 2000; Nair, 2010).

Cacao trees grow in tropical lowland rain forests within latitudes approximately of 20°N and 20°S of the Equator (Bartley, 2005; Motamayor et al., 2008; Umaharan, 2018), with temperatures in between 18°C and 32°C. Ideal rainfall ranges from 1500 mm/year and 2500 mm/year with an optimum humidity close to 70%−80% during the day, and 90%−100% during the night. The pH of the soil for adequate growth must be between 5.0 and 7.5. The tree normally reaches 4−8 m tall but can exceed this range especially when grown in wild conditions. Highest crops yields can occur after 3−5 years and the tree has a commercial life span period of 25−30 years (Afoakwa, 2014).

The tree produces hermaphrodite flowers and fruits all year (Bartley, 2005; Motamayor et al., 2008) mostly concentrated in one or two annual peaks. Cacoa fruits achieve ripeness 5−6 months after pollination and fertilization occur. The fruit is called *cacoa pod* and has a length of 15−30 cm, a diameter of 8−10 cm, and a weight ranging from 200 g to 1 kg. In general, the pod has a hard husk of about 10−15 mm thickness, although some pure Criollo varieties are distinguished for their soft husk (Toxopeus, 2001). The fruit houses an average of 30−40 seeds attached to a central placenta (Afoakwa, 2014) (Fig. 3.2). Average annual yields of cacao plantations are close to 300−500 kg/ha, but broad ranges can be found, from yields below 100 kg/ha to values close to 3000 kg/ha (Arevalo et al., 2017; Asare et al., 2019; Motamayor et al.,

FIGURE 3.1 Cocoa trees (left, *property of the authors*), cocoa fruit pod attached to the tree (middle, *property of Cristiana González*, undergrad Food Engineering Student, University of Costa Rica, used with permission) and different harvested cocoa pods (right). *Property of Óscar De La Cruz, Costa Rican cocoa producer, used with permission.*

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FIGURE 3.2 Cacoa fruit pods (left), cacoa fruit fresh seeds (middle), and cacoa fruit placenta (right). *Property of Óscar De La Cruz, Costa Rican cocoa producer, used with permission.*

2008; Orozco & Deheuvels, 2007; Phillips-Mora et al., 2012). This behavior depends on different factors such as the genetics, the environment, the age of the trees, the agronomical practices, among others.

Evidence suggests that the origin of genetic diversity of cacao occurred in the Amazonian Region (Bartley, 2005), and different hypothesis have been proposed to explain its domestication process. One of them suggests that cocoa varieties of the Upper Amazon were introduced into Mesoamerica for further domestication. In contrast, another hypothesis proposes that domestication first occurred in South America, followed by domestication in Mesoamerica, and triggered by commercialization routes of native-American civilizations (Cornejo et al., 2018; Motamayor et al., 2002). Over the years, cacao was spread mostly by European colonizers, first into the Caribbean islands, and followed by other territories in West Africa and Asia (Grivetti & Shapiro, 2011).

The relevance of cacao in society radiated to economic, cultural, political, health, and religious sectors during the pre-Hispanic period of the American continent. Reports evidence that cacao beans were used as exchange currency in commercial activities, as a tribute, and as religious element. The Aztec and Mayan elite used to drink a beverage called *xocoatl* or *chocoatl,* from the Nahuatl language meaning bitter (*-xococ*) water (*-atl*). This was made with roasted and grinded cacao beans mixed with other ingredients such as maize, chili pepper, honey, among other spices (Grivetti & Shapiro, 2011; Lange & Fincke, 1970; Wood, 1985).

Traditional morpho-geographic classification of cacao has been separated in three main groups or cultivars: Criollo, Forastero, and a hybrid product of the latter two called Trinitario. Criollo has been recognized as the most important cocoa group domesticated in Mesoamerica, presumably derived from an ancient Curaray germplasm from South America (Cornejo et al., 2018).

Fruit pods belonging to the Criollo group are usually characterized by deep furrows, a warty and rough surface, and pointy ends. The seeds are large and they normally have white or pale violet seeds, with lower astringency and shorter fermentation times compared to other cacao types. This type of cacao is distinguished by higher quality but tends to give lower yields, be less resistant to diseases or environmental stress, and is usually less adaptable as other common cacao types (Nair, 2010; Wood, 1985).

In contrast, Forastero pods generally have smooth surfaces with rounded ends. The seeds are characterized by their dark violet color, are smaller, flatter, require longer fermentation times, and have a higher fat content than the previously mentioned. Forastero trees are in general recognized for having higher yields and resistance, but beans are not considered to have remarkable sensory quality. Nonetheless, these generalizations must be taken carefully given the large genetic variability of these groups, which leads to a wide spectrum of characteristics of the materials (Nair, 2010; Wood, 1985).

On the other hand, since the Trinitario group surged from hybridization between Forastero and Criollo types, its morphological features, yields, and quality are intermediate and diverse (Cornejo et al., 2018; Nair, 2010; Toxopeus, 2001).

Nowadays, modern cacao classifications propose 10 genetic clusters or groups which are: Marañón, Curaray, Criollo, Iquitos, Nanay, Contamana, Amelonado, Purús, Nacional, and Guiana (Motamayor et al., 2008). In addition, information on more than 14 000 cacoa clones is currently available (Wadsworth et al., 2004), and continuous efforts are made to incorporate new plants into cacao gene banks and breeding programs (Turnbull et al., 2010).

Worldwide cacao production represents more than 4.7 million tons per year, market that is dominated by few countries (Table 3.1). Africa achieves more than 70% of the total world production, with Ivory Coast achieving almost 50% of the world total trade. This country in junction with Ghana, Nigeria and Cameroon sum up more than 95% of the African production and therefore have a significant contribution in the global production (International Cocoa Organization (ICCO), 2020b).

America is the second larger cocoa producing continent, accounting for close to 18% of total global trade. Ecuador is the highest American producer, reaching almost 40% of total cocoa in the continent. Brazil, Peru, and Dominican Republic contribute significantly, reaching together with Ecuador more than 80% of the American cocoa production (International Cocoa Organization (ICCO), 2020b).

TABLE 3.1 World cacoa production in terms of fermented and dried cacoa beans (International Cocoa Organization (ICCO), 2020b).

Region	Production by period (1000 tons)				
	2015/2016	2016/2017	2017/2018	2018/2019	2019/2020[a]
Africa	2922 (73%)	3627 (76%)	3494 (75%)	3644 (76%)	3598 (76%)
America	674 (17%)	767 (16%)	835 (18%)	837 (18%)	849 (18%)
Asia/Oceania	397 (10%)	374 (8%)	319 (7%)	299 (6%)	277 (6%)
World total	**3994 (100%)**	**4768 (100%)**	**4648 (100%)**	**4780 (100%)**	**4724 (100%)**

[a] *Previsions for that period.*

Asia and Oceania reach less than 10% of the total global production with Indonesia, Malaysia, and Papua New Guinea being the principal cocoa producers of both continents, and accounting more than 85% of its production (International Cocoa Organization (ICCO), 2020b).

Industry classifies cocoa beans by means of its quality into two main categories: bulk and fine or flavor cocoa. Bulk cocoa, also named basic or ordinary cocoa, represents almost 95% of total global production. It is characterized by an intense cocoa and chocolate flavor with few other flavors, and it is mainly intended to produce milk and dark chocolates, cocoa mass, cocoa butter and cocoa powder (Dand, 2011; End & Dand, 2015; Fowler & Coutel, 2017).

On the other hand, fine or flavor cocoa is targeted to markets that elaborate products with high cocoa solids or blends that have special aroma notes such as floral and fruity, among others (End & Dand, 2015; Hegmann et al., 2017; Kadow et al., 2013). In particular, the International Cocoa Organization defines fine or flavor cocoa to that which "*is recognized for its unique flavor and color, and produced in countries designated in Annex C of the International Cocoa Agreement*" (Table 3.2) (International Cocoa Agreement, 2010). The list of countries updated to 2020 is shown in Table 3.2, which is dominated by countries of the American continent.

To a large extent, bulk cocoa has been associated with the Forastero group (meaning Amelonado); meanwhile fine cocoa is commonly linked with Criollo and Trinitario groups. However, different exceptions have been found as, for example, the Equatorian genetic group named Nacional which is considered a fine cocoa and the Cameroonian Trinitario cocoa considered as bulk cocoa (Amores et al., 2007).

Nonetheless, although genetics play an important role in the quality, agricultural and postharvest practices and all the elements that compose the *terroir,* or the growing and processing geographic environment also have an effect in the final flavor and in the overall characteristics of the cocoa and subsequently in its derived products (End & Dand, 2015; Hegmann et al., 2017, 2020; Sukha et al., 2014, 2017).

A remarkable aspect of cocoa production refers to the active participation of small farmers in the total global production. Approximately 80%–90% of total global production is generated by smallholders, representing close to 5.5 million producers, with average planting areas of 0.2–0.5 ha. Their activity is circumscribed in family farming model

TABLE 3.2 Producing countries exporting exclusively or partially fine cocoa by September 2020 (International Cocoa Organization (ICCO), 2020a).

Amount of fine or flavor cocoa from total cocoa beans exports	Countries
100%	Brazil, Costa Rica, Dominica, Granada, Jamaica, Madagascar, Saint Lucia, Trinidad, and Tobago
Less than 100%	Colombia (95%), Dominican Republic (45%), Ecuador (75%), Guatemala (75%), Haiti (4%), Indonesia (10%), Nicaragua (80%), Panama (50%), Papua New Guinea (70%), Peru (75%)
Existing, but percentage unknown	Belize, Bolivia, Honduras, Mexico, Saint Thomas and Prince, Venezuela, Vietnam

systems, production units that generate incomes for families, and which may contribute to stability, peace, and reduction of social gaps, encourages rural governance, and contributes to biodiversity, among others (Arevalo et al., 2017).

To illustrate this, for 2014, Danso-Abbeam et al. estimated that *ca*. 800 000 smallholders were involved in cocoa commercialization in Ghana, representing *ca.* 29% of the total income of the country in terms of international exchange. In some developing countries, cocoa trees are grown in as small-scale plantations within regions with limited productive alternatives. To mention, in rural areas of Cameroon and Nigeria, cacao is the main crop produced in up to 65%−75% of households (Degrande et al., 2006).

The development of agroforestry practices in Indonesia and other countries have also contributed to retain biodiversity and ecological interactions, reducing the environmental impact of agriculture practices in comparison to intensive monoculture approaches. The economic benefits represented by cocoa production have been promoting the intensification or improvement of agricultural systems but also the conversion of forests into agricultural areas (Steffan-Dewenter et al., 2007).

However, beyond this economic, social, cultural, gastronomical, and biological relevance, an increasing interest has raised into the potential role of the consumption of cocoa-derived products as part of healthy diets (Aprotosoaie et al., 2016; Barrera-Reyes et al., 2020; De Feo et al., 2020; Flores & Eugenia, 2019; Goya et al., 2016; Ludovici et al., 2017; Magrone et al., 2017; Rimbach et al., 2009). In this sense, previous epidemiological studies (Hollenberg, Fischer et al., 2009; Hollenberg, Gregorio et al., 1997) have motivated the development of more research focused on the comprehension of the biological value of cocoa intake.

The following sections will address the chemical composition of cocoa, the effect of processing and formulation on its chemical composition, and the current evidence on biofunctionality and health impact of the intake of cocoa, cocoa products, and cocoa phytochemicals, with particular interest in cardiovascular health.

In this chapter, the nomenclature suggested by Frank et al. (2019) is used: *phytochemical* will be referred as a compound or metabolite from plant origin, and the concept *bioactive compound* will be used to refer chemical compounds or metabolites that exert a biological effect either positive or negative. The authors of this chapter encourage the reader to consider the term *bioactivity* or *bioactive compound* in a wide spectrum, since *in vivo* systems are complex and dynamic.

2. Cocoa processing

Cocoa processing stages can be classified in two: postharvest processing (or primary processing), and industrial processing (or secondary processing). Postharvest processing is normally conducted at producing sites, it starts with the harvesting of the cacao fruits and ends on the storage of fermented and dried cocoa beans (Fig. 3.3) (Aprotosoaie et al., 2016; Copetti et al., 2011; Nielsen et al., 2013).

Industrial processing includes the transformation of the cocoa beans into cocoa butter, cocoa powder, and chocolate, among other derivatives. These steps include roasting, grinding, alkalization, refining, and conching, among others (Aprotosoaie et al., 2016; Copetti et al., 2011; Nielsen et al., 2013) (Fig. 3.4).

2.1 Postharvest processing

During ripening, cocoa follows a series of changes that have a profound impact on physicochemical characteristics and subsequently on cocoa quality. Morphological parameters like color and size are commonly used as harvest indicators. Biochemical changes also take place in the pulp and in the cotyledons, as, for example, conversion of starch into soluble sugars, and changes in moisture and acidity. Parameters such as moisture, soluble solids, and pH are useful and practical indicators that help in determining the adequate harvesting time. These changes depend not only on the genetic background of the crop but also on factors as the geographic environment and the agronomical practices and have a direct influence on cocoa quality. (Bojacá et al., 2019; End & Dand, 2015; Hegmann et al., 2020; Niether et al., 2017; Packiyasothy et al., 1981; Rojas et al., 2020).

After harvesting, cocoa follows the traditional process which includes preconditioning, fermentation, drying, and storage (Fig. 3.3). The final goal of these postharvest operations is to produce cocoa beans that contain a variety of flavor metabolites as for example organic acids, esters, and alcohols, but also aroma and flavor precursors including reducing sugars, free amino acids, and oligopeptides (Aprotosoaie et al., 2016; Biehl & Ziegleder, 2003; Biehl & Ziegler, 2003, pp. 1448−1463; Perez et al., 2020).

From an industrial perspective, less representative but still relevant, alternative processes can also be done in which cocoa fermentation is not applied. In this case, cocoa beans can undergo drying immediately after cocoa pod opening and seed extraction or extracted seeds can be washed to remove the pulp before drying. The latter is called *Lavado* cocoa, from

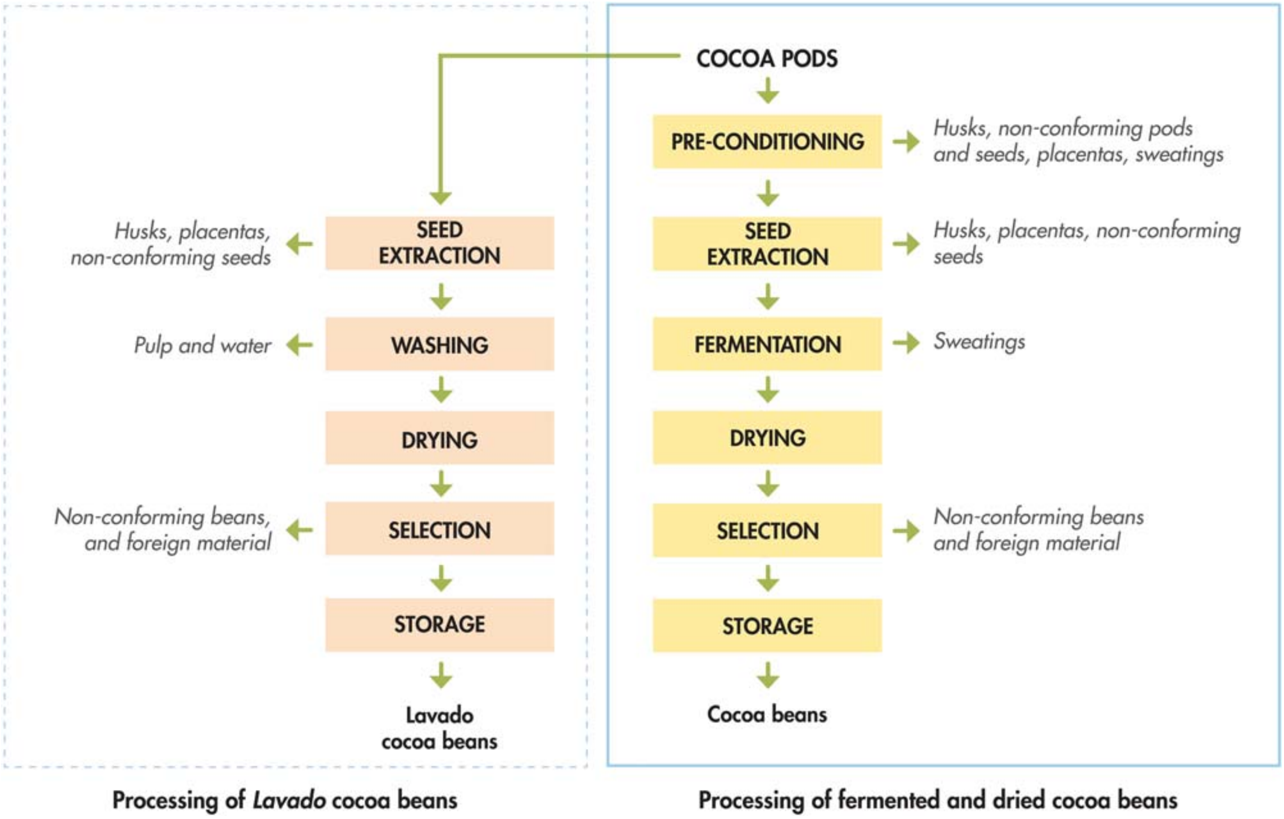

FIGURE 3.3 Postharvest process for obtaining conventional fermented and dried cocoa beans, and Lavado cocoa beans. *Property of the authors, based on Afoakwa (2014), Biehl and Ziegleder (2003), Biehl and Ziegler, 2003, Ciaramelli et al. (2021).*

the Spanish word meaning washed. Unfermented beans have a different flavor profile as well as a characteristic terracotta color compared to fermented cocoa beans (Chin et al., 2013; Ciaramelli et al., 2021; Colombo et al., 2012; Secretaría de Patrimonio y Fomento Industrial, 1979).

Preconditioning. Cocoa preconditioning is an optional step that reduces the total amount of pulp and fermentable sugars, promoting the production of less acidic fermented cocoa beans. Pre-conditioning can be done before or after opening of the fruits. In the first case, the pods are retained or stored for some days prior to fermentation, causing dehydration and biochemical changes in the pulp. In the second case, preconditioning is mainly done by reducing the pulp content employing different mechanical technologies like centrifugation, pressing, hot air drying, draining the pulp by spreading it on a surface, or application of enzymatic hydrolysis (Afoakwa, 2014; Santander Muñoz et al., 2020).

Fermentation. Cocoa fermentation is one of main determinants steps of cocoa quality. Traditional processing starts with the opening of cocoa pods and seed extraction; afterward seeds are placed in a container system where a spontaneous solid-state microbial fermentation occurs. The seeds are inoculated by the microbiota present in the surrounding environment including hands of workers, cutting tools, contact surfaces, and insects, among others (Nielsen et al., 2013; Ozturk & Young, 2017).

Cutting-edge processes apply starter cultures in controlled, non-spontaneous fermentations and are gaining strength to counteract the variability of spontaneous fermentations, to enhance the final quality, and to reduce processing times. *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, *Acetobacter aceti, Acetobacter pasteurianus, Pichia kluyveri, Pichia kudriavzevii, Hanseniaspora uvarum, Hanseniaspora thailandica, Kluyveromyces marxianus,* and *Torulaspora delbrueckii*, among others, have been explored as possible starter cultures (Batista et al., 2015; Crafack et al., 2013, 2014; Magalhães da Veiga Moreira et al., 2017; Ooi et al., 2020; Sandhya et al., 2016; Visintin et al., 2017).

There are several fermentation methods which seek to gather the cocoa seeds in a space that keeps the heat, protects the seeds from the environment, and favors the drainage of the fermentation sweatings. Traditional containers used for these purposes are (Fig. 3.5): heaps, boxes, trays, baskets, and rotatory drums, but many other configurations have been designed

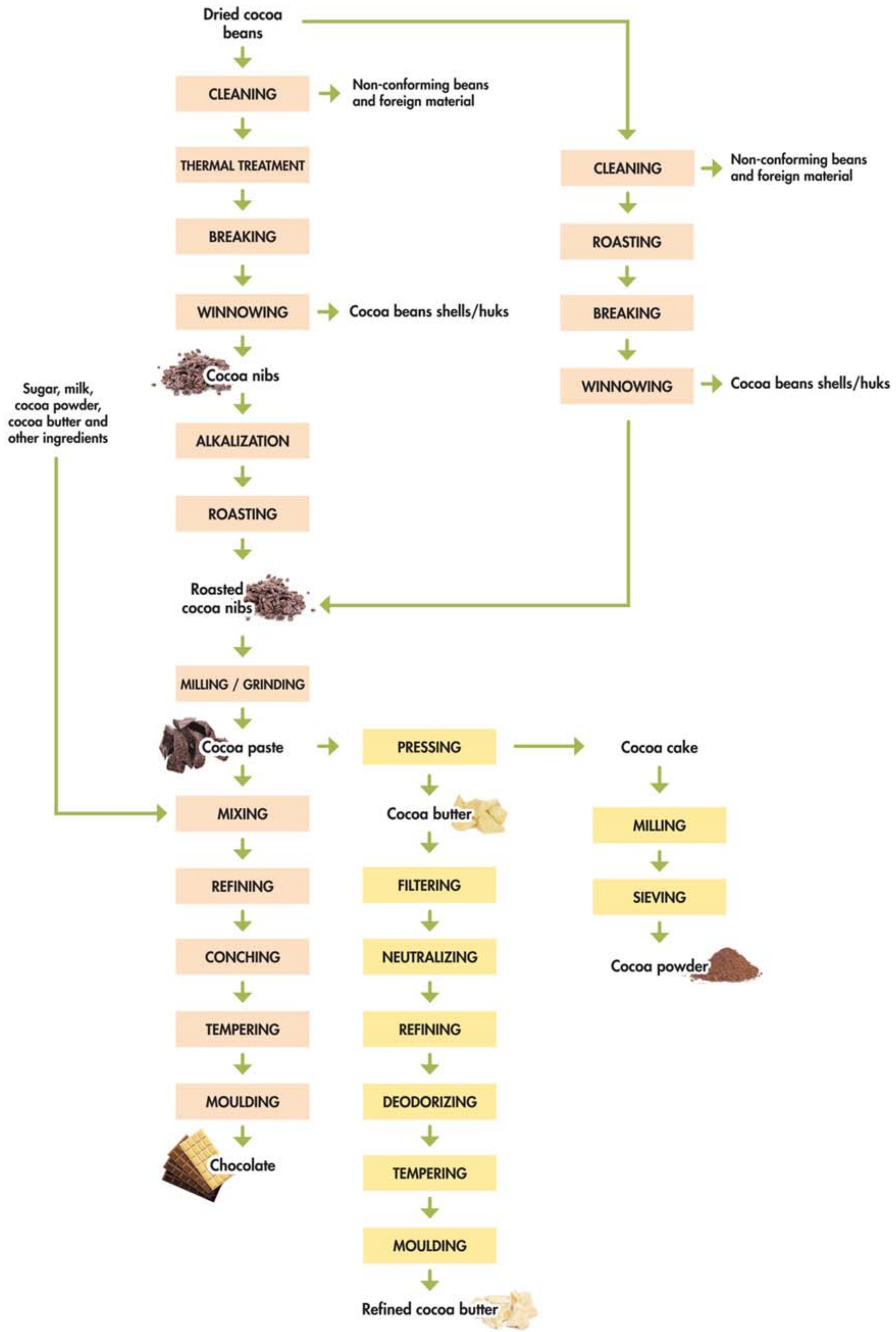

FIGURE 3.4 Industrial processing of fermented and dried cocoa beans. *Property of the authors, based on Afoakwa (2014), Biehl and Ziegler (2003), Kamphuis and Fowler (2017), Stauffer (2017).*

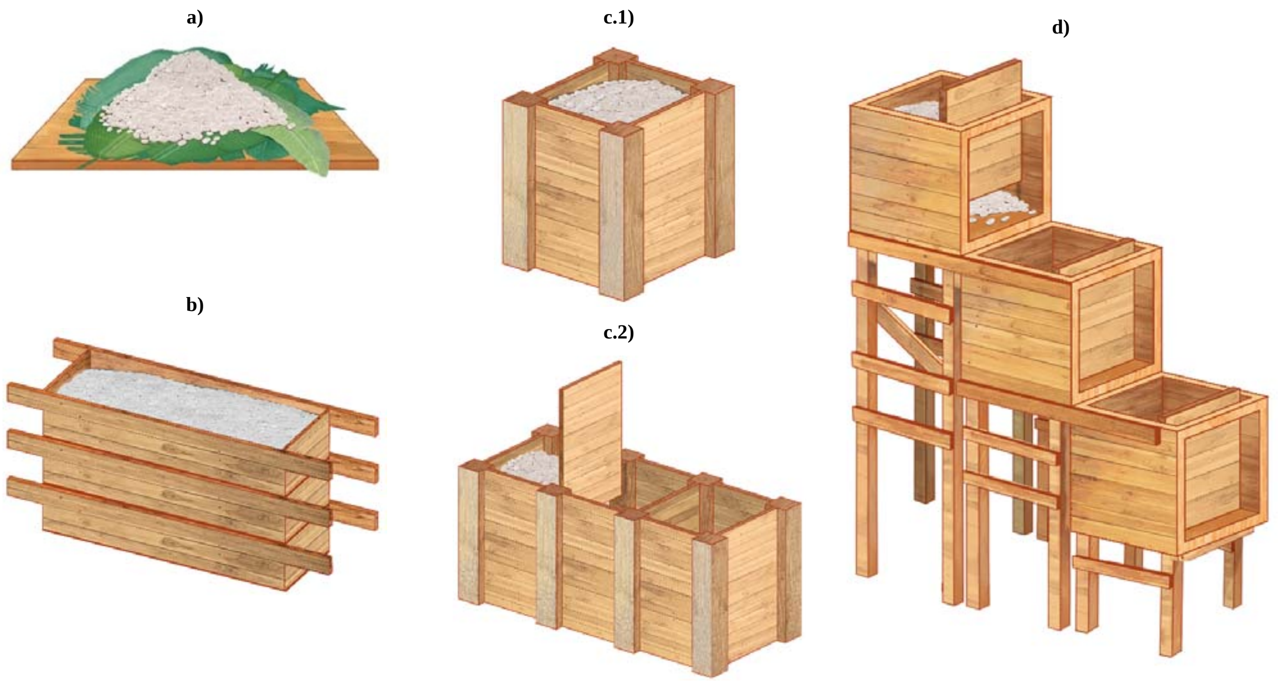

FIGURE 3.5 Cocoa fermentation systems **a:** heap, **b:** tray, **c:** boxes (c.1 single box and c.2. multiple horizontal boxes), **d:** cascade/ladder boxes methods. *Property of the authors.*

and used as sacks and stainless steel tanks (Aprotosoaie et al., 2016; Crafack et al., 2013; de Melo Pereira et al., 2013; Domínguez-Pérez et al., 2020; Santander Muñoz et al., 2020).

In the heap method, the cocoa seeds are stacked on a surface and covered with leaves of the Musaceae family, for example, plantain or banana leaves. The box fermenting systems consist of an individual or multiple wooden boxes that can be arranged in a horizontal or vertical configuration where the seeds are placed and covered by leaves, jute, and/or plastic bags. The tray system is another fermentation method where different wooden trays containing cocoa beans covered with leaves and stacked one over (Fig. 3.5) (de Melo Pereira et al., 2013; Nielsen et al., 2013; Santander Muñoz et al., 2020).

During fermentation, microorganisms metabolize the pulp causing pectin depolymerization, transformation of sugars into ethanol, and subsequently into acetic acid, accompanied by a rise in temperatures around 45−50°C (Ardhana & Fleet, 2003; Mozzi et al., 2013; Vuyst & Weckx, 2016). Fermentation metabolites penetrate the bean through the testa causing changes as, for example, a decrease in the cotyledon pH. In combination with the rise in temperature, acidification of the cotyledon causes the death of the embryo and decompartmentalization of cell structures. These favor the contact between substrates and enzymes initially separated by compartments, promoting a variety of enzymatic and nonenzymatic reactions (Lima et al., 2011).

During this stage, proteases convert proteins into different oligopeptides and amino acids, glycosidases act on glycosides as flavonoid glycosides, and polyphenol oxidases reduce the polyphenol content by producing quinones which proceed nonenzymatic reactions leading to formation of insoluble complexes and polymers (Biehl & Ziegleder, 2003; Febrianto & Zhu, 2020; Mayorga-Gross et al., 2016; Nair, 2010; Sorrenti et al., 2020; Voigt et al., 2018; Vuyst & Weckx, 2016). Migration of aroma compounds present in cocoa pulp into the bean has been also proposed to occur during fermentation (Chetschik et al., 2018).

Drying. After fermentation, drying step is applied to reduce the moisture content of beans from 40% to 60% to approximately 7%. During drying, acetic acid content is reduced by volatilization and Maillard reactions are favored. In addition, microbial growth is inhibited as a consequence of moisture and water activity reduction, which also leads to increase of cocoa beans shelf life (Biehl & Ziegleder, 2003; Crafack et al., 2013; Hii et al., 2009; Santander Muñoz et al., 2020).

Drying rate influences directly the bean quality. If drying is too fast, the testa hardens causing reduced permeability to water, oxygen, and acids. This not only hinders the drying process itself, but reduced oxygen migration into the beans also decreases oxidation reactions required for flavor development. In addition, reduced migration of acids from the beans increase its acidity (End & Dand, 2015; Santander Muñoz et al., 2020). If drying rate is too slow, fungi can grow creating detrimental flavor attributes and safety risks, including the production of mycotoxins like aflatoxins and ochratoxin A (Copetti et al., 2014; End & Dand, 2015; Hii et al., 2019; Kedjebo et al., 2016; Turcotte et al., 2013).

Owing economic reasons, the most common technique is sun drying, but mechanical drying is also been employed. Sun drying can last from 1 to 4 weeks depending mainly on the environmental conditions. Mechanical drying can reduce drying times but temperatures below 60°C are recommended to facilitate flavor development reactions (Hii et al., 2019; Nielsen et al., 2013).

Storage. Cocoa beans final moisture must be around 7% and quality assessments as the cut test must be applied to select the cocoa beans that will be stored. Common materials for storing the beans are bags or sacks made from jute, sisal fiber or polypropylene (Barreiro & Sandoval, 2020; End & Dand, 2015; International Trade Centre UNCTAD/WTO, 2001). Different measures must be taken into consideration during storage and transport to prevent pest infestation (e.g., moths, beetles, rodents, or mold growth) (Abdullahi et al., 2018; Bateman, 2015; Fowler & Coutel, 2017). Inadequate storage practices can increase the free fatty acid content, which subsequently affects the quality of cocoa butter, and can increase the incidence of mycotoxins and debris derived from infestation which threatens product safety (Dano et al., 2013; End & Dand, 2015; Hii et al., 2019).

2.2 Industrial processing

Industrial processing starts with selection and cleaning of the cocoa beans (Fig. 3.4), where nonconforming beans and foreign material are separated, as environmental contaminants, insects, stones, and splinters, among others.

Depending on the industrial scale, beans may receive a thermal treatment with hot air, steam or radiation for safety assurance (Afoakwa, 2014; Gutiérrez, 2017). This is also applied to loosen the shells before breaking and winnowing of the beans. Winnowing refers to the step where the nibs and the shells (also called husks) are separated through airstreams coupled with sieves. Thermal treatment can also be applied after winnowing and is sometimes called sterilization. At a smaller industrial scale, whole dried beans are cleaned and afterward subjected directly to the roasting step, then they are broken, and winnowed (Biehl & Ziegler, 2003, pp. 1448−1463).

Roasting. Roasting is a crucial step for the development of aromas, flavors, and colors, in addition moisture content reduces below 2%, volatiles as acetic acid decrease, and the total microbial load reduces. It can be applied on cocoa beans, nibs, or paste (Afoakwa, 2014; Giacometti et al., 2015; Gutiérrez, 2017). Roasting temperatures usually range from 120°C to 150°C but broader ranges are commonly applied; times depend on the temperature profile applied. The high temperatures promote the development of the typical cocoa and chocolate aroma and flavor due to a series of chemical reactions involving the interaction of flavor precursors formed during fermentation and drying (e.g., amino acids, oligopeptides, and reducing sugars) (Aprotosoaie et al., 2016; Voigt, 2013).

Caramelization, non-enzymatic Maillard browning, and Strecker degradation reactions reduce extensively the concentration of glucose and fructose and promote the formation of wide range of compounds including pyrazines, thiazoles, oxazoles, pyrroles, quinoxalines, pyridines, pyrones, phenylalk-2-enals, lactones, diketopiperazines, derivatives of phenylalanine, furanones, aldehydes, and biogenic amines (Aprotosoaie et al., 2016; Biehl & Ziegleder, 2003; Gutiérrez, 2017; Ioannone et al., 2015; Kothe et al., 2013; Taş & Gökmen, 2016; Voigt, 2013).

Insoluble complexes between polysaccharides, proteins, polyphenols, and Maillard products are also formed (Redgwell et al., 2003). Epimerization of flavanol monomers, and procyanidin dimers and trimers have been suggested to occur during this step (Kothe et al., 2013).

Alkalization. Alkalization is also known as "Dutching" and is an optional step in the cocoa processing, commonly applied in large scale industries. Here, the cocoa is mixed with an alkali solution (potassium or sodium carbonate) at high temperatures and pressures generating changes in the cocoa pH. As a consequence, solubility is augmented and flavor is improved as acidic and astringent notes decrease (Afoakwa, 2014; Biehl & Ziegler, 2003, pp. 1448−1463; Fowler & Coutel, 2017; Valverde García et al., 2020; Ziegleder, 2017). Alkalization can be done in different stages, before or after roasting, and applied to nibs, cocoa paste, or more frequently to cocoa powder (Aprotosoaie et al., 2016; Kamphuis & Fowler, 2017; Valverde García et al., 2020).

Milling/grinding. Milling step also called grinding is where nibs are subjected to a series of size reduction or grinding operations, leading to the production of a cocoa mass or paste also called cocoa liquor. This is frequently done by two or three consecutive steps in different mills obtaining different granulometry (Stauffer, 2017).

Cocoa butter and cocoa powder processing steps. Cocoa butter, can be obtained by cocoa liquor mechanical pressing. The pressing cake produced in this step is subsequently milled and sieved, leading to cocoa powder. Cocoa butter can further follow a series of refining steps such as filtering, neutralizing, refining, deodorizing, and tempering (Gutiérrez, 2017; Kamphuis & Fowler, 2017; Nair, 2010).

Refining, conching, and tempering. Cocoa mass, cocoa butter, and cocoa powder are basic ingredients for chocolate production, which can be mixed with sugar, and milk, among other ingredients. This mixture is further refined by mechanical forces to reduce the granulometry of solids and is afterward conched. During conching, temperatures range normally between 55 and 90°C. Here, the chocolate becomes fluid, a reduction of particle agglomerates is reduced, and coating of nonfat solids with cocoa butter is promoted. This step also reduces significantly the total moisture content, and the concentration of volatile compounds such as acetic acid, and short chain fatty acids (Ziegleder, 2017). The conched chocolate is then tempered, where cocoa butter is crystalized into stable polymorphs which lead to optimal appearance (gloss), temperature stability, and texture. The tempered chocolate is then molded, packaged, and stored (Biehl & Ziegler, 2003, pp. 1448−1463).

3. Cocoa composition: main cocoa components and their dependence on processing

Almost 70%−80% of the cocoa fruit consists of the husk, therefore representing a significant by-product of cocoa processing. Inside the fruit cocoa seeds are found; they are surrounded by a pulp or mucilage and the cotyledons are protected by the testa, the latter accounting for 10%−16% of the weight of the dried bean (Álvarez et al., 2010; Andrade-Almeida et al., 2019; Campos-Vega et al., 2018; Fowler & Coutel, 2017; Ouattara et al., 2021).

The following section presents a global and non-exhaustive description of cocoa composition along the transformation chain focusing on composition of cocoa beans and its derivatives. Tables 3.3 and 3.4 show the reported values for different components of cocoa and its more relevant derivatives. Fig. 3.6 show a schematic representation of main phytochemical present in cocoa.

It must be stated that the composition of cocoa is dependent on the genetics, agricultural, postharvest, and industrial processing practices. In addition, it is important to highlight that data reported differs in the analytical methodologies employed, in the basis employed to express the results (e.g., fresh, dry and/or defatted basis) as well in the details regarding the samples and their processing, creating a considerable variability in the available compositional information. Furthermore, composition of cocoa derivatives is highly dependent on the percentage of cocoa ingredients added and the specific formulation. Thus, the data presented must be used carefully.

Moisture. Moisture decreases significantly along processing steps. Fresh cotyledons moisture varies due to several factors, but can be in between 30% and 60% (Graziani de Fariñas et al., 2003; Lares Amaiz et al., 2013; Peláez, Guerra, & Contreras, 2016; Torres et al., 2004). This value drops significantly after drying, and depending on the practices applied, high variability can be found from 3% to 7% or even higher (Andrade-Almeida et al., 2019; Caprioli et al., 2016; Álvarez et al., 2010).

However, international cocoa guidelines and standards recommend a final moisture of 7%. Lower moisture leads to brittle cocoa beans, and higher values can represent a risk for microbial growth (End & Dand, 2015). Moisture content reaches the lowest values after roasting and conching stages, with values close to 1%−2% or lower (Aprotosoaie et al., 2016; Biehl & Ziegler, 2003, pp. 1448−1463).

pH and acidity. Reported pH values of the fresh cotyledons are 5.86−7.00, while values for fermented and dried beans range between 4.93 and 6.49 (Table 3.3), with acetic acid and lactic acid being some of the predominant acids. Regarding transformed cocoa products, pH may vary widely depending on the processing steps, specially if the alkalization step is applied. For example, if the pH is around 5 to 6 the cocoa is classified as dark natural cocoa, but if its pH exceeds 7.6 it is classified as strong alkalized, the latter being the darker, less astringent, and more soluble alkalized cocoa (Valverde García et al., 2020).

Fat. Fat is the main component of the cocoa beans (Padilla et al., 2000), representing almost half of the fermented and dried cocoa beans (Servent et al., 2018) (Table 3.1). It is constituted by about 95% triacylglycerols (TAG), 2% diacylglycerols, 1% polar lipids, and 1% free fatty acids (Biehl & Ziegleder, 2003). It has been reported that fat profiles are strongly dependent on the type of cocoa and its origin. For example, Lipp et al. concluded that, for the samples analyzed, South American cocoa butter showed higher concentration of highly unsaturated TAG, higher than 6%, compared to the Asian cocoa butter which presented contents lower than 4% (Lipp et al., 2001).

The principal fatty acids of cocoa are palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1). Their proportions varie, but in general each of them has a concentration close to 30 g/100 g of the total fatty acid (TFA) content, accounting close to 90% of the TFA. Reported concentrations of linoleic acid (C18:2) are between 1.09 and 3.36 g/100 g

TABLE 3.3 Composition of cocoa pulp, fresh cocoa seeds, and fermented and dried cocoa beans reported by several authors.

Component	Cocoa pulp	Fresh cocoa seeds	Fermented and dried cocoa beans
Moisture (g/100 g)	82.50–85.9 (Lima et al., 2011)	29.12–61.01 (Banboye et al., 2020; Graziani de Fariñas et al., 2003; Lares Amaiz et al., 2013; Ortiz de Bertorelli et al., 2009; Padilla et al., 2000; Sotelo & Alvarez, 1991)	3.21–7.29 (Álvarez et al., 2010; Andrade-Almeida et al., 2019; Caprioli et al., 2016)
Fat (g/100 g)	0.35–0.75 (Lima et al., 2011)	19.56–23.92 (Sotelo & Alvarez, 1991)	24.33–43.7 (Caprioli et al., 2016; Ospina, 2001) and 51.4–57.7 (Perea et al., 2011)
Protein (g/100 g)	0.43–0.57 (peptides included) (Lima et al., 2011)	6.34–9.47 (Agus et al., 2018; Sotelo & Alvarez, 1991) and 17.5 (d b.) (Aremu et al., 1995)	8.08–16.0 (Andrade-Almeida et al., 2019; Caprioli et al., 2016; Hashimoto et al., 2018; Ospina, 2001) and 13.64–15.02 (db) (Verdesoto et al., 2009)
Carbohydrates (g/100 g)	–	3.62–10.50 (Sotelo & Alvarez, 1991)	24.38–43.35 (Andrade-Almeida et al., 2019; Caprioli et al., 2016) and 26.34–33.56 (Verdesoto et al., 2009)
Fiber (g/100 g)	–	3.13–3.17 (Sotelo & Alvarez, 1991)	4.28–7.10 (Andrade-Almeida et al., 2019; Ospina, 2001) and 19.98–20.85 (db) (Verdesoto et al., 2009)
Free or reducing sugars (g/100 g)	6.8–11.13 (glucose and fructose) and 1.35–4.35 (sucrose) (Lima et al., 2011)	2–4 (free sugars, db) (Aprotosoaie et al., 2016) and 50.1 mg glucose eq./g (db, deffated) (De Brito et al., 2001)	0.18–1.85 (db) (Verdesoto et al., 2009)
Oligosaccharide (mg glucose eq./g)	–	7.9 (db, deffated) (De Brito et al., 2001)	–
Starch (mg glucose eq./g)	–	140 (db, deffated) (De Brito et al., 2001)	–
Ash (g/100 g)	3.95 (Agus et al., 2018)	1.41–2.07 and 4.4 (d b.) (Aremu et al., 1995; Sotelo & Alvarez, 1991)	2.22–3.8 (Andrade-Almeida et al., 2019; Ospina, 2001), 2.83–3.84 (db) (Verdesoto et al., 2009; Álvarez et al., 2010)
pH	4.20–4.21 (Peláez, Guerra, et al., 2016)	5.86–7.0 (Graziani de Fariñas et al., 2003; Lares Amaiz et al., 2013; Ortiz de Bertorelli et al., 2009; Peláez, Guerra, et al., 2016)	4.65–6.49 (Álvarez et al., 2010; Andrade-Almeida et al., 2019; Banboye et al., 2020; Verdesoto et al., 2009)
Acidity (g/100 g)	0.77–0.96 (g acetic acid eq./100 g of cocoa) (Peláez, Guerra, et al., 2016) and 1.72 (NaOH meq./g) (Lares Amaiz et al., 2013)	0.45–1.35 (Graziani de Fariñas et al., 2003; Lares Amaiz et al., 2013; Ortiz de Bertorelli et al., 2009; Peláez, Guerra, et al., 2016)	0.61–2.49 (Andrade-Almeida et al., 2019), and 0.37–0.65 (db) (Álvarez et al., 2010)
Theobromine (g/100 g)	–	1.088–2.034 (db) (Peláez, Bardón, & Camasca, 2016; Sotelo & Alvarez, 1991)	0.854–2.01 (db) (Peláez, Bardón, et al., 2016; Verdesoto et al., 2009), 14.5–17.7 (mg/g, db) (Bordiga et al., 2015), and 0.8–1.5 (Hashimoto et al., 2018)

Caffeine (g/100 g)	–	0.182–0.350 (db) (Peláez, Bardón, & Camasca, 2016; Sotelo & Alvarez, 1991)	0.087 and 0.27 (db) (Peláez, Bardón, et al., 2016; Verdesoto et al., 2009), 0.190–1.16 mg/g (db) (Bordiga et al., 2015), and 0.04–0.26 (Hashimoto et al., 2018)
Theophylline (mg/100 g)	–	325.4–473.9 (db) (Sotelo & Alvarez, 1991)	–
Total phenolics	–	–	3.0–7.9 g tannic acid eq./100 g (Hashimoto et al., 2018), and 33.0–52.9 mg catechin eq/g (db) (Bordiga et al., 2015)
Epicatechin (g/100 g)	–	0.055–1.855 (db) (Peláez, Bardón, et al., 2016)	0.033–0.504 (db) (Peláez, Bardón, et al., 2016)
Catechin (g/100 g)	–	0.020–0.065 (db) (Peláez, Bardón, et al., 2016)	0.002–0.014 (db) (Peláez, Bardón, et al., 2016)

db, dry basis; *eq*, equivalents.

TABLE 3.4 **Chemical composition of different cocoa derivatives reported by several authors.**

Component	Roasted nibs	Cocoa powder	Chocolate
Moisture (g/100 g)	3–3.7 (Fowler & Coutel, 2017; Lima et al., 2011)	Maximum 4.5 (Kamphuis & Fowler, 2017), and 3–7 (Oracz et al., 2020)	Milk: 0.8; dark: 0.5 (Montagna et al., 2019)
Fat (g/100 g)	48–57 (Fowler & Coutel, 2017; Lima et al., 2011)	10–24 depending if it is fat reduced or highly fat reduced cocoa powder (Kamphuis & Fowler, 2017; Oracz et al., 2020)	Milk: 36.3; dark: 33.6 (Montagna et al., 2019)
Protein (g/100 g)	11–16 (Fowler & Coutel, 2017; Lima et al., 2011)	20–22 (db) (Oracz et al., 2020)	Milk: 7.3; dark: 6.6 (Montagna et al., 2019)
Carbohydrates (g/100 g)	6.0 (Lima et al., 2011)	16 (polysaccharides) (db) (Oracz et al., 2020)	Milk: 49.7; dark: 50.5 (Montagna et al., 2019)
Fiber (g/100 g)	2.1–3.2 (Fowler & Coutel, 2017; Lima et al., 2011)	33.2–34 (25.5 insoluble and 8.5 soluble) (db) (Oracz et al., 2020)	Milk: 3.2; dark: 8 (Montagna et al., 2019)
Ash (g/100 g)	2.6–4.2 (Fowler & Coutel, 2017; Lima et al., 2011)	5.80 (db) (Oracz et al., 2020)	–
pH	–	5.0–8.2 (Kamphuis & Fowler, 2017)	–
Theobromine (g/100 g)	0.8–1.4 (Fowler & Coutel, 2017; Lima et al., 2011) 12.0–21.6 mg/g (db) (Bordiga et al., 2015), and 6.68–8.52 mg/g (Spizzirri et al., 2019)	19.63–29.38 mg/g (Nazaruddin et al., 2001), and 2,1 (db) (Oracz et al., 2020)	Milk: 0.125 dark: 0.802 (Montagna et al., 2019) dark: 6.14–8.26 mg/g (db) (Bordiga et al., 2015) 0.67–1.14 mg/g (Nazaruddin et al., 2001)
Caffeine (g/100 g)	0.1–0.7 (Fowler & Coutel, 2017; Lima et al., 2011) 0.413–0.894 mg/g (db) (Bordiga et al., 2015) and 1.60–2.81 mg/g (Spizzirri et al., 2019)	3.27–6.58 mg/g (Nazaruddin et al., 2001), and 0,2 (db) (Oracz et al., 2020)	Dark: 0.164–0.347 mg/g (db) (Bordiga et al., 2015) 0.026–0.153 mg/g (Nazaruddin et al., 2001)
Total phenolic content	26.1–49.8 (mg catechin eq/g db) (Bordiga et al., 2015) and 2.37–12.96 mg/g (Spizzirri et al., 2019)	4.0g/100g (polyphenols, db) (Oracz et al., 2020)	Dark: 14.9–22.7 (db) (Bordiga et al., 2015)

db, dry basis; *eq*, equivalents.

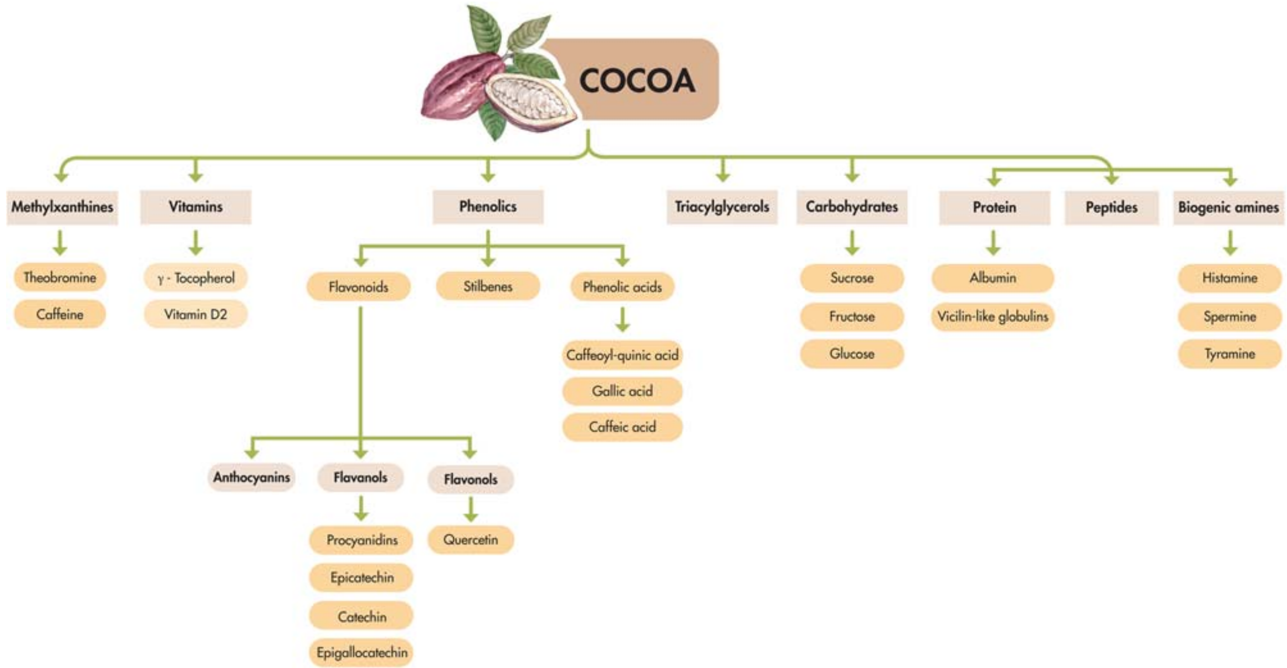

FIGURE 3.6 Schematic representation the main groups of cocoa phytochemicals including some of the representative compounds.

TFA, while eicosanoic acid (arachidic acid) (C20:0) range from 0.82 to 1.1 g/100 g of TFAs. When analyzing the TAG composition, palmitic-oleic-palmitic (ca. 18 g/100 g TAG), palmitic-oleic-stearic (ca. 42 g/100 g TAG), and stearic-oleic-stearic (ca. 26 g/100 g TAG) constitute close to 85% of the total TAG configuration (Lipp et al., 2001; Álvarez et al., 2007).

Fat remains mostly stable during fermentation (Servent et al., 2018). During roasting, oxidation of free fatty acids and fats has been suggested (Afoakwa, 2014). Significant reductions of short chain fatty acids have been reported during conching (Ziegleder, 2017). Also, evidence of fat hydrolysis and saponification of triacylglycerols during cocoa alkalization has been reported (Valverde García et al., 2020).

Proteins. Proteins might reach between 10% and 16% of the dry weight of fresh cocoa seeds, and differences have been observed due to geographical origin. Previous reports indicate that albumins and vicilin-like globulins are the main proteins, each one of them representing close to 50% of the total protein content. Concentration of free amino acids in fresh cocoa seeds around 5 mg/g (dry basis) has been published (Aprotosoaie et al., 2016; Biehl & Ziegleder, 2003; Kumari et al., 2018; Marseglia et al., 2014; Rawel et al., 2019). Other proteins correspond to enzymes that have a relevant role in cocoa fermentation such aspartic and cysteine endoproteases, seryl exopetidases, leucine-*p*- nitroanilides, glycosidades, invertases, polyphenol oxidases, and peroxidases (Biehl & Ziegleder, 2003; Hii et al., 2019; Rawel et al., 2019).

During fermentation and drying proteolytic reactions produce several hydrophilic oligopeptides and hydrophobic amino acids which are flavor and aroma precursors. These react via Maillard reactions during roasting leading to the development of flavor and aroma compounds (Biehl & Ziegleder, 2003; Marseglia et al., 2014; Mayorga-Gross et al., 2016; Voigt et al., 1994, 2018).

D'Souza et al. reported that peptides are not significant in cocoa fresh seeds in contrast to the composition after 24 h of fermentation. During fermentation, D'Souza et al. identified 392 peptides derived from vicilin, 167 from albumins, 26 from peroxidases, 20 from lipoxygenase, 20 from basic chitinase and 241 di-, tri-, and tetrapeptides derived from other proteins. More than 50% of the detected peptides had a length in between 2 and 5 amino acids (D'Souza et al., 2018).

Carbohydrates. In cocoa beans, total free sugar content is in between 2% and 4% dry basis, with sucrose representing close to 90% of total sugars. Fructose and glucose are also present in relatively high concentrations compared to minor sugars and alcohols: galactose, raffinose, verbascose, stachyose, melobiose, sorbose, mannotriose, xylose, arabinose, mannitol, and inositol (Bertazzo et al., 2013; Biehl & Ziegleder, 2003; Cerbulis, 1955; Reineccius et al., 1972). Starch is also present, and the fiber fraction is mostly composed by cellulose, pentosanes, and other carbohydrates (Afoakwa, 2014; Bertazzo et al., 2013).

Reductions during fermentation of more than 80% di- and oligosaccharides such as sucrose, raffinose, 1-kestose and stachyose have been observed. Fructose and glucose otherwise showed increases of 69% and 97% respectively, suggested to be a consequence of the hydrolysis of di-, tri- and tetrasaccharides (Megias-Perez et al., 2020).

The total sugar content has been reported to decrease during drying and roasting of cocoa beans due to Maillard reactions and residual enzymatic activity (Nogales et al., 2006; Voigt, 2013). Alkalization can have different effects on sugars, some reports indicate no change observed during this process meanwhile others highlight a sharp reduction in their concentration (Valverde García et al., 2020).

Minerals. Minerals present in cocoa are related not only with the genetics but with the *terroir*: soil, air, water, geographical location, and agricultural practices employed have an influence, so high variability can be found (Cinquanta et al., 2016; de Araujo et al., 2017; Dion-Laine et al., 2015; FDA, 2020). Besides, mineral content in cocoa derivatives depends on the amount of non-fat cocoa solids used in a specific formulation (Lambert, 2017).

In general, potassium, phosphorus, and magnesium tend to present the highest concentrations in cocoa. Bertoldi et al., analyzed the mineral profile of 61 samples of fermented and dried cocoa beans obtained in four continents and 56 elements were quantified in all samples. Potassium was the element that presented the highest relative concentrations with mean values where between 1200 and 1450 mg/100 g of cocoa. Phosphorus values oscillated between 454 and 583 mg/100 g of cocoa and magnesium range from 3.39 to 3.83 mg/100 g of cocoa (Bertoldi et al., 2016).

In chocolates, calcium content has been associated with the milk content, and general values of different minerals increased as the cocoa content increased in dark chocolates, from lower values in milk chocolate to the highest in 90% cocoa chocolates (Cinquanta et al., 2016). Potassium, phosphorus, magnesium and calcium have been reported to have the highest values in chocolates followed by aluminum, iron and zinc (Bertoldi et al., 2016). Alkalization has been suggested to increase the content of some elements depending on the salts employed for the treatment (Valverde García et al., 2020).

Vitamins. It has been reported that the principal vitamin E isomer in cocoa butter is γ-tocopherol with values ranging from 3.40 to 246.90 mg/g of cocoa butter. Lower concentrations of α-tocopherol, γ-tocotrienol, and δ-tocopherol have been also reported (Lipp et al., 2001). Kühn et al. determined vitamin D in cocoa beans and different cocoa derived products as chocolates and cocoa butter and powder, with highest values for vitamin D2. Fermented and dried cocoa beans had vitamin D_2 contents from 0.04 to 0.54 μg/100 g of fresh weight, roasted cocoa beans between 0.04 and 0.45 μg/100 g of fresh weight, cocoa powder with 20% of fat content had higher values in between 1.42 and 1.82 μg/100 g of fresh weight, and cocoa butter from 0.77 to 4.30 μg/100 g of fresh weight. Chocolates in general presented values from 1.40 to 5.48 μg/100 g of fresh weight. Lower values were reported for vitamin D3 which showed trace levels in just a few samples such as well in cocoa butter, and some chocolates (Kühn et al., 2018).

Nevertheless, vitamin content of final products depends on final composition of cocoa products. For example, in the case of vitamin E, the final content may vary depending on the particular list of ingredients used in the formulation due to the presence of vitamin E congeners at varied extent in industrial oil and fats (Schwartz et al., 2008) and nuts (Stuetz et al., 2017).

3.1 Other phytochemicals

Cocoa has a diverse profile of secondary phytochemicals, many of them recognized due to their biofunctionality (Fig. 3.6). Phytochemical content and profile depend on multiple factors as genotype, origin, postharvest and industrial processing of cocoa (D'Souza et al., 2017; Giacometti et al., 2015; Urbańska et al., 2019). Polyphenols, in particular, flavonoids as procyanidins and flavan-3-ols, phenolic acids, stilbenes, methylxanthines, peptides, N-phenylpropenoyl-L-amino acids, among others have been object of research and commercial interest (Cádiz-Gurrea et al., 2014; Hurst et al., 2008; Jerkovic et al., 2010; Ortega et al., 2010; Sorrenti et al., 2020).

Polyphenols. Polyphenol content in nonfermented cocoa beans is close to 15%−20% in fat free dry basis (Fowler & Coutel, 2017). Elwers et al. obtained values of polyphenol content for un-fermented cocoa samples between 6.93% and 17.96% of fat free dry basis (Elwers et al., 2009; Hii et al., 2009). Fermented and dried cocoa can have values in the range of 4%−14% in fat free dry basis (Fowler & Coutel, 2017). Hashimoto et al. (2018) reported an average of total polyphenols representing 3.0%−7.9% in total weight from 81 analyzed samples from Brazil and Ivory Coast.

For chocolates, polyphenolic concentrations depend in a great extent in the percentage of cocoa solids. Evaluation of the total content of polyphenols in chocolates produced from beans of five different countries reported contents from 910 mg/100 g to 4055 mg/100 g of chocolate, expressed as equivalents of gallic acid (Urbańska et al., 2019). Higher values of total phenolic content have been reported for dark chocolate compared to milk and white chocolate (average concentration of 578.64 mg; 160.46 and 126.39 mg catechin equivalents/100 g chocolate, respectively) (Meng et al., 2009).

From the total polyphenols, proanthocyanidins have been reported to represent approximately 58%–65%, followed by catechins (29%–38%) and anthocyanins (1.0%–4.0%) (Biehl & Ziegleder, 2003). The main flavan-3-ols are (−)-epicatechin and (+)-catechin, dimers and higher polymerization degree procyanidins have been reported, to mention procyanidins B1, B2, B5 and C1 (Hurst et al., 2011; Mayorga-Gross et al., 2016). Total procyanidins have been quantified on different cocoa derivatives. Values for dark chocolate ranged from 8.5 mg/g to 19.8 mg/g, with lower contents in milk chocolate (2.16–3.14 mg/g) (Gu et al., 2006).

In general, polyphenol levels tend to decrease along the different processing steps. Oxidation, hydrolysis, complexing, polymerization and diffusion have been suggested during fermentation and drying in several studies (Mayorga-Gross et al., 2016; Salvador et al., 2019; Santander Muñoz et al., 2020). Epimerization, oxidation, and complexing mechanisms during roasting and alkalization stages might also play a role in modifying the polyphenolic profile, normally resulting in a reduction of the polyphenols (Aprotosoaie et al., 2016; Giacometti et al., 2015; Ioannone et al., 2015; Lambert, 2017; Oracz & Nebesny, 2019; Salvador et al., 2019).

Losses over 60% of total polyphenols have been reported during alkalization (Urbańska et al., 2019), where monomerization of procyanidins and glycosylation of flavanols has been also observed (Valverde García et al., 2020). Non-alkalized cocoa has been reported to have higher contents of cathechin, epicathechin, procyanidin B2, and procyanidin C1 (1.3; 5; 7; and 14 times, respectively) compared to alkalized cocoa (Ellinger et al., 2020).

Nonetheless distinct results have been found, which might derive from differences in methodologies employed. To illustrate this, D'Souza et al. revealed an increase of almost the double of the total polyphenol content in cocoa beans after fermentation. In this study, metabolites such as epicatechin, proanthocyanidin dimers, and trimers, among others, increased during cocoa fermentation meanwhile procyanidin B-type oligomers from 4 to 11 monomers and procyanidin A-type glycosylated oligomers decreased (D'Souza et al., 2017). In contrast, Pallares Pallares et al. did not observe significant changes in epicatechin and procyanidin B1 concentrations along cocoa fermentation (Pallares Pallares et al., 2016). Aqueous extracts obtained from raw cocoa shown ca. 14% higher content of the total phenolic acids in comparison to an aqueous extract obtained from roasted beans. However, extracts from roasted beans show double the content of (+)-catechin but just *ca*. 76% and 25% of content (−)-epicatechin and epigallocatechin observed in raw cocoa extracts (Żyżelewicz et al., 2020).

Other minor flavonoids constitute the phenolic fraction of cocoa, like flavonol glycosides of quercetin and kaempferol, flavones like luteolin and apigenin glycosides, and flavanones as naringenin. Reported glycosides are formed with sugars as glucose, rutine, ramnose, galactose, and arabinose (Lamuela-Raventos et al., 2013; Sánchez-Rabaneda et al., 2003).

Cyanidin-3-galactoside and cyanidin-3-arabinoside are the major contributors in the anthocyanin fraction of cocoa beans (De Taeye et al., 2016). In what respects to genetics some types of cacao differ significantly in the phenolic profile, for example Criollo type cocoa has low or non-detectable concentrations of anthocyanins, whereas Forastero- and Trinitario-type cocoas have large amounts of these compounds (Elwers et al., 2009). An extensive list of different polyphenols detected in cocoa has been reported by D'Souza et al. (D'Souza et al., 2017).

Besides flavonoids, stilbenes have also been identified in cocoa: *trans*-resveratrol and the glucoside *trans*-piceid. In one study, 22 cocoa liquors obtained from different cocoa types were analyzed, and both stilbenes were detected with variable concentration ranges (Jerkovic et al., 2010).

Another study quantified both stilbenes in various derivatives of cocoa: cocoa powder, different chocolates chocolate syrups, and cocoa powder. The highest values were observed for *trans*-piceid for all the analyzed products. In particular, cocoa powder presented the highest values for both stilbenes: *trans*-piecid values were in between 6.23 and 8.17 μg/g and *trans*-resveratrol from 1.25 to 2.27 μg/g (Hurst et al., 2008).

Phenolic acids. A variety of phenolic acids have been identified in cocoa like, for example, caffeoyl quinic acid, vanillic acid diglucoside, protocatechuic acid, vanillic acid glucoside, gallic acid, caffeic acid, ferulic acid, *p*-coumaric acids, and chlorogenic acid (Belščak et al., 2009; Cádiz-Gurrea et al., 2020; D'Souza et al., 2017). Their presence and concentration are dependent on the matrix analyzed, the processing steps applied, the type of cocoa, among other factors.

N-phenylpropenoyl-L-aminoacids (NPAs). These are classified as polyphenol-amino acid conjugates which might contribute to the cocoa sensory profile, and might have functional properties. Reported concentrations reach 2.31 mg/g in defatted raw cocoa beans. Aspartic acid amide of caffeic acid (Caff-Asp) has been reported to be the major NPA, with values of 0.34–2.42 mg/g. Other identified NPAs are clovamide (Caff-DOPA), dideoxy-clovamide (pC-Tyr), 2-O-caffeoltartaric acid, N-[3′,4′-dihydroxy-(E)-cinnamoyl]-L-aspartic acid and N-[3′,4′-dihydroxy-(E)-cinnamoyl]-L-tyrosine (Lechtenberg et al., 2012; Oracz et al., 2020; Stark et al., 2008; Stark & Hofmann, 2005).

Methylxanthines. Theobromine and caffeine are the main methylxanthines in cocoa, representing 1%–2% and lower than 2% of total percentage in dry weight, respectively. Trace concentrations of theophylline and 7-methylxanthine have also been reported (Biehl & Ziegleder, 2003; Franco et al., 2013). Methylxanthines contribute to the bitterness of cocoa (Stark et al., 2006; Trognitz et al., 2013) and can be reduced by 30%−45% during fermentation buy diffusion mechanisms (Fowler & Coutel, 2017; Lima et al., 2011; Vuyst & Weckx, 2016). Reduction of methylxanthines during alkalization has been suggested as methylxanthines can produces salts with the alkaline bases (Valverde García et al., 2020).

Biogenic amines. These metabolites derive from the decarboxylation of amino acids produced naturally by the metabolism of the food matrix, by contamination or by microbial activity (Ruiz-Capillas & Herrero, 2019). In the latter, substrate-specific reactions can occur mediated by amino acid decarboxylases from microorganisms of genus like *Bacillus*, *Citrobacter*, *Clostridium*, *Escherichia*, *Lactobacillus,* and *Pediococcus* (ten Brink et al., 1990). In cocoa, formation of biogenic amines is promoted by an increase in the content of free amino acids due to protein acid hydrolysis and by the reduced pH that enhances microbial decarboxylation (Karovičová & Kohajdová, 2005). Some biogenic amines reported in cocoa are histamine (Restuccia et al., 2016; Spizzirri et al., 2019), spermine (Dala-Paula, Deus, et al., 2021), spermidine, putrescine, tyramine, tryptamine, phenylethylamine (Dala-Paula, Deus, et al., 2021; Deus et al., 2021; Spizzirri et al., 2019), cadaverine (Spizzirri et al., 2019), and serotonin (Deus et al., 2021).

Deus et al. (2021) indicate that total and single biogenic amines (serotonin, spermidine, putrescine, tyramine, tryptamine) tend to be reduced along fermentation time of cocoa beans, with phenylethylamine showing an opposite behavior and being detectable just after 60 h of fermentation (Deus et al., 2021). Also, Spizzirri et al. (2019) reported differences in the total biogenic amine contents and profiles in fermented but non-roasted beans of different regions. Authors also report an increase in total and single amines after roasting process, with a direct relationship to roasting temperature (Spizzirri et al., 2019).

Variable total biogenic amine contents have been observed in cocoa derivatives as cocoa powder, and different types of chocolates, with values ranging from 5.7 to 72.3 μg/g. For example, histamine levels in a 70% cocoa content dark chocolates reached up to 35.0 ± 0.4 μg/g (Restuccia et al., 2016). In a commercial dark chocolate containing 70% cocoa, Dala-Paula et al. detected cadaverine, 2-phenylethylamine, putrescine, spermine, spermidine, tryptamine, and tyramine for a total amine content of 8.67 mg/100 g with no detectable amounts of other biogenic amines like histamine, agmatine, and serotonin (Dala-Paula, Deus, et al., 2021). Beyond differences in total content, variation in amine profile has been also reported.

Differences regarding type of formulation have been also reported in the biogenic amine content in cocoa products, with no specific relationship to cocoa content. While values lower as 0.57 ± 0.02 mg/100 g have been reported for cocoa powder with 80% cocoa content, amine contents reaching up to 3.81 ± 0.30 mg/100 g have been observed for cocoa powder containing just 19% cocoa. Histamine levels in commercial cocoa products have been reported to reach up to 4.72 ± 0.05 and 4.54 ± 0.04 mg/100 g in white and milk chocolates, respectively (Restuccia et al., 2016).

4. Bioavailability and metabolism of cocoa phytochemicals

Beyond the effect of processing and of the final product preparation that influences the content of secondary metabolites, it is relevant to consider the metabolic handling of these compounds that may determine the final activity of cocoa derived products.

Phytochemicals are susceptible to transformation in the body by enzymatic processes involved in the transformation of xenobiotics, as it occurs with drugs and other external substances (Holst & Williamson, 2008). Oxidation and conjugation reactions occur both in the intestines and liver. Oxidation reactions (Phase I reactions) mainly involve the incorporation of reactive hydroxyl-groups, while conjugation (Phase II reactions) implies the addition of a wide variety of chemical moieties including sulfate, glucuronide and acetyl groups (Neilson & Ferruzzi, 2013). In the case of prescribed drugs, the existence of those transformations is determinant for activation, excretion but also conversion to potential toxic substances (Jackson et al., 2018; Testa et al., 2012). At the same extent, the activity of phytochemicals is modulated by xenobiotic transformation either in positive or negative way.

Phytochemicals present in cocoa are propense to transformations in the intestinal lumen prior to absorption. For example, flavonoids are generally present in food plants in glycosylated form (Lamuela-Raventos et al., 2013; Sánchez-Rabaneda et al., 2003). Hydrolyzation of glycosylated flavonoids occurs in the brush border before absorption. Lactose-phlorizin hydrolase (LPH) located in the luminal membrane of enterocytes is involved in the hydrolysis of flavonoid glucosides. However, intracellular CBG (broad specificity cytosolic β-glucosidase) also possess the ability to hydrolyze phenolic-glucosides within the enterocyte. Nevertheless differences up to 87-fold times in the activity of hydrolysis of quercetin-4-glucoside have been reported in human intestine samples (Németh et al., 2003).

Metabolites and biomarkers of cocoa and chocolate intake**.** After consumption of cocoa or cocoa products, increased plasmatic concentrations of methylxanthines, phenolic acids, and native and conjugated flavonoids, among others have been detected.

In the group of methylxanthines, increased plasmatic concentrations of theobromine (Costa-Bauza et al., 2018; Martínez-López et al., 2014; Ptolemy et al., 2010; Richelle et al., 1999; Rodriguez et al., 2015; Stellingwerff et al., 2014), caffeine (Martínez-López et al., 2014; Ptolemy et al., 2010), 3-methylxanthines and 7-methylxanthines (Martínez-López et al., 2014) have been reported. Other metabolites observed in urine after cocoa intake are theophylline, paraxanthine, 1-methylxanthine, and different isomers of dimethyluric acids (Mayorga-Gross & Esquivel, 2019).

In the case of phenolic acids, ferulic acid has been also observed in plasma after the administration of a cocoa procyanidin concentrate (Wiese et al., 2015). A wide diversity of phenolic acids and their metabolites have been detected in urine such as 3-hydroxyphenyl acetic acid, 3,4-dihydroxyphenyl acetic acid, 3-methoxy-4-hydroxy-phenylacetic acid, hippuric acid, *p*-hydroxyhippuric acid, phenylacetic acid, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, protocatechuic acid, and vanillic acid after the intake of different cocoa derivatives (Mayorga-Gross & Esquivel, 2019).

Among the family of flavonoids, different phytochemicals and metabolites have been reported in plasma after intervention with cocoa products. Catechin (Heiss et al., 2005; Holt et al., 2002; Keogh et al., 2007; Neilson et al., 2009; Ottaviani et al., 2012, 2011; Schroeter et al., 2006)), epicatechin (Baba et al., 2000; Barnett et al., 2015; Campbell et al., 2007; Heiss et al., 2005; Holt et al., 2002; Keogh et al., 2007; Neilson et al., 2009; Ottaviani et al., 2011; Richelle et al., 1999; Rodriguez-Mateos et al., 2012; Roura et al., 2005; Schroeter et al., 2006; Wang et al., 2000), 4′-O-methyl-(−)-epicatechin, 3′-O-methyl-epicatechin (Rodriguez-Mateos et al., 2012; Schroeter et al., 2006), procyanidin B2 (Holt et al., 2002; Ottaviani et al., 2012), epicatechin-glucuronides (Actis-Goretta et al., 2013; Barnett et al., 2015; Neilson et al., 2009; Ottaviani et al., 2012; Schroeter et al., 2006), epicatechin-sulfate (Actis-Goretta et al., 2013; Barnett et al., 2015; Neilson et al., 2009; Ottaviani et al., 2012), 4′-O-methyl-(−)-epicatechin-glucuronide (Actis-Goretta et al., 2012; Heiss et al., 2005; Ottaviani et al., 2012; Schroeter et al., 2006), and 4′-O-methyl-(−)-epicatechin-glucuronide-sulfate (Ottaviani et al., 2012) are the main flavonoid forms reported in human plasma and/or urine.

A wide range of phenyl-γ-valerolactone (PVL) derivatives, phenyl valeric acid (PVA) derivatives, and other microbial associated metabolites have been also reported in both plasma and urine of humans after ingestion of cocoa products (Gómez-Juaristi et al., 2019; Urpi-Sarda et al., 2009). It is relevant to mention that microbial-associated metabolites identified include chemical compounds directly produced by microbiota like the PVA and PVL, but also phase I and II metabolites of those compounds (e.g., glucuronidated and sulfated metabolites).

Gómez-Juaristi et al. reported molecules derived from PVL and PVA but also other microbial associated metabolites in both plasma and urine of humans after ingestion of soluble cocoa products. Among the PVL group, 5-(3′,4′-dihydroxyphenyl)-valerolactone, 5-(4′-hydroxyphenyl)-valerolactone-3′-glucuronide; 5-(hydroxyphenyl)-valerolactone-sulfate; 5-phenyl-valerolactone-methoxy-glucuronide and 5-phenyl-valerolactone-3′-sulfate have been reported in urine and plasma while 5-phenyl-valerolactone-3′-glucuronide, 5-(3′-hydroxyphenyl)-valerolactone, 5-phenyl-valerolactone-methoxy-sulfate and 5-(3′-hydroxyphenyl)-valerolactone-4′-glucuronide have been reported in urine (Gómez-Juaristi et al., 2019).

Within the group of phenyl-valeric acids 4-hydroxy-5-(3′,4′-dihydroxyphenyl)-valeric acid; 4-hydroxy-5-(hydroxyphenyl)-valeric acid-glucuronide has been reported in urine while 4-hydroxy-5-(hydroxyphenyl)valeric acid-sulfate was observed in both plasma and urine. Minor microbial associated metabolites including 3,4-dihydroxyphenylpropionic acid, hydroxybenzoic acid, 3-hydroxyhippuric acid, 3-methoxy-4-hydroxyphenylpropionic acid, 3,4-dihydroxyphenylpropionic acid have been also reported among others in plasma and urine (Gómez-Juaristi et al., 2019). However, it is important to mention that microbial metabolites including hydroxyphenyl propionic acids hydroxybenzoic acids are generally associated to transformation of a wide range of phytochemicals (Serra et al., 2012).

Different biomarkers of cocoa consumption have been proposed, as for example 6-amino-5-[*N*-methylformyl amino]-1-methyluracil (AMMU), 3-methyluric acid, 7-methylxanthine, 3-methylxanthine, theobromine, 3,7-dimethyluric acid, hydroxyphenylacetic acid, and 5-(3,4-dihydroxyphenyl)-γ-valerolactone with its glucuronides and sulfates (Mayorga-Gross & Esquivel, 2019).

In the next sections, a brief description of the metabolism of cocoa phytochemicals will be presented with emphasis in flavonoids biotransformation. For more detailed description, a comprehensive review of metabolic transformation and bioavailability of epicatechin and epicatechin derivatives has been published by Borges and collaborators (Borges et al., 2018). A schematic representation of the transformation of cocoa phytochemicals with emphasis in metabolic handling of epicatechin is shown in Fig. 3.7.

Bioavailability and metabolism of flavonoids. Bioavailability studies of cocoa phytochemicals and derivatives lead to the general understanding that flavonoids from cocoa are quickly absorbed and susceptible to phase II metabolism (Barnett

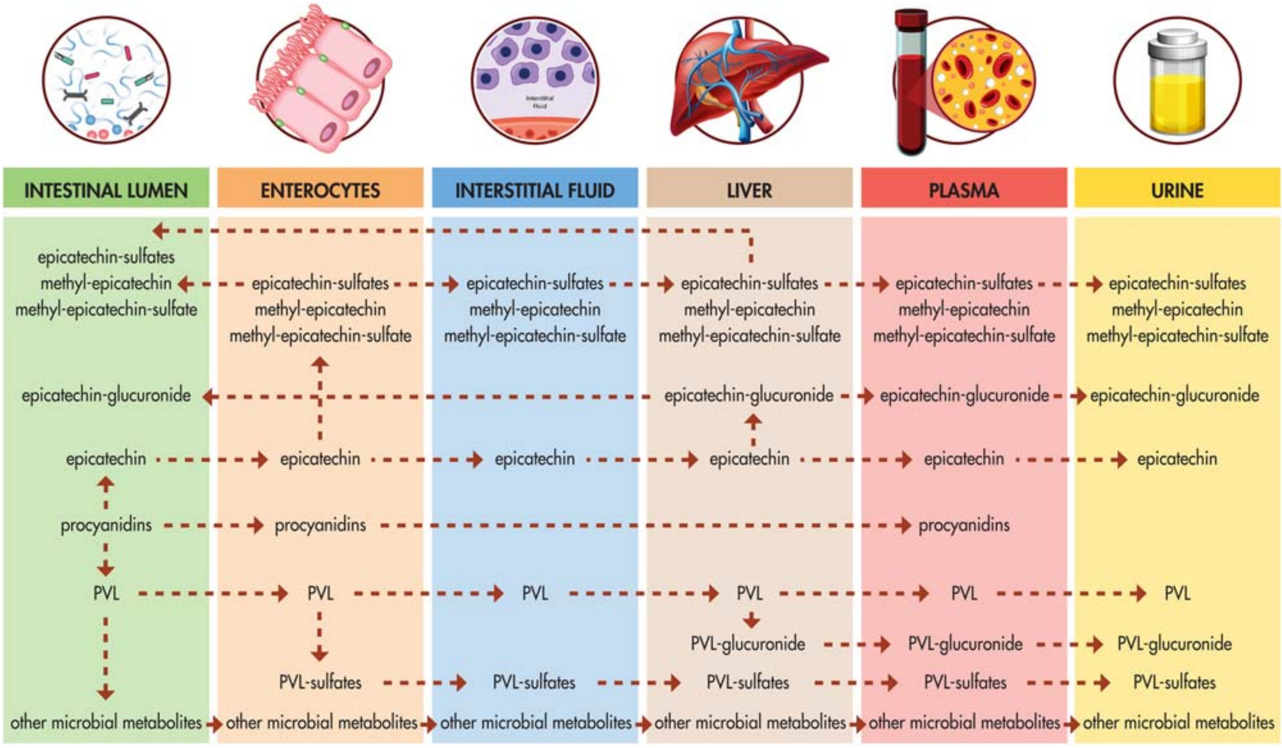

FIGURE 3.7 Schematic representation of metabolic transformation and transport of epicatechin and epicatechin metabolites including transformation to γ-phenylvalerolactones (PVL) and other microbial metabolites. *Authors based on Actis-Goretta et al. (2013) and Gómez-Juaristi et al. (2019).*

et al., 2015). The quick appearance of phytochemical derived byproducts in human urine reflects a rapid turnover of potentially bioactive compounds. For example, acute administration of cocoa products has been related with increased urinary concentration of epicatechin already 30 min after administration (Neilson et al., 2009).

Direct intestinal infusion of (−)-epicatechin showed the appearance of metabolites in the intestinal fluid as fast as 30 min, suggesting that this flavonoid is quickly absorbed and transformed by cells of the intestine. The rate of metabolism of (−)-epicatechin has been reported to be strongly determined by interindividual variation since not just the total amount but also the profile and proportion of different metabolic derivatives varies among subjects (Actis-Goretta et al., 2013).

Significant biliary excretion of 3′-O- β -D-glucuronide-epicatechin, 3′-O-sulfate-epicatechin, and 3′-O-methyl-5-O-sulfate-epicatechin has been observed after direct intestinal perfusion of (−)-epicatechin, already during the first 2.5 h after infusion, suggesting a rapid transport of metabolites along the enterohepatic system strongly affected by interindividual variations (Actis-Goretta et al., 2013).

It has been reported the appearance of 3′-O-sulfate-epicatechin, 3′-O-methyl-4′-O-sulfate-epicatechin, 3′-O-methyl-5-O-sulfate-epicatechin, and 3′-O-methyl-epicatechin in both apical and basal compartments in cultured intestinal cells treated only with pure (−)-epicatechin (Actis-Goretta et al., 2013). These results suggest two relevant points: first, the variety of transformations occurring to flavonoids already at intestinal level, and second, the existence of transport mechanisms for metabolites in both directions, to the plasmatic compartment (basolateral) but also a retrograde efflux in the direction of the luminal compartment (apical).

Nonetheless, results regarding metabolism and intestinal transport differ when comparing *in vitro* (cultured cells) and *in vivo* analyses. For example, in vitro experiments indicate that Caco-2 cells exposed to (−)-epicatechin preferentially transport the aglycone form to the basolateral space in comparison to metabolites. In contrast, direct infusion of pure (−)-epicatechin in human intestine using an *in vivo* approach reported higher presence of metabolites in plasma and urine compared to the aglycone form (Actis-Goretta et al., 2013). Moreover high interindividual variation in epicatechin kinetics among subjects has been observed after intervention using cocoa products (Ellinger et al., 2020).

Intervention with flavonoid rich cocoa preparation in humans has been also reported to be mainly associated with higher plasmatic presence of epicatechin metabolites instead of the parent un-metabolized compound. Epicatechin-3′-β-D-glucuronide is reported as major glucuronidated metabolite while epicatechin-3′-sufate has been reported as the main sulfated form. However, despite lower maximal plasmatic concentration for epicatechin has been reported compared to epicatechin-3′-β-D-glucuronide (4 ± 1 nM versus 589 ± 85 nM, respectively), parent compound shows faster appearance of maximal plasmatic concentrations (Ottaviani et al., 2012).

Comparing the effects of the administration of 40 g of cocoa containing skimmed milk versus skimmed milk as control, an increase up to 45% in the concentration of phase II metabolites derived from epicatechin were quantified in a 24-h urine collection. In addition, plasma samples obtained after an overnight fasting period show not detectable amounts of phase I/phase II flavanol metabolites (Urpi-Sarda et al., 2009) indicating a rapid plasmatic clearance of cocoa phytochemicals.

Plasmatic concentration of epicatechin is higher than catechin concentration after acute intervention with cocoa products (Ellinger et al., 2020). Also, a preferential plasmatic concentration of specific enantiomeric forms has been suggested for cocoa flavonoid derivatives. While (−)-epicatechin derived forms are reported to be higher for epicatechin-3′-β-D-glucuronide and epicatechin-3′-sufate in human plasma after a cocoa containing preparation, a predominant (+)-epicatechin derived form is mainly detected as the the epicatechin-5′-sufate metabolite (Ottaviani et al., 2012).

Acute administration of high procyanidin chocolate (4.0 g total procyanidins/kg) in healthy volunteers has been reported to generate plasmatic epicatechin concentrations *ca.* 20 times higher compared to low procyanidin chocolate (0.09 g total procyanidins/kg). However, plasmatic concentration of epicatechin recovered its baseline values already 6 h after intervention. Moreover, observed differences between low and high procyanidin chocolates were observed at 2 but not at 6 h after consumption, suggesting that rapid acute responses are predominantly observed after chocolate intake (Schramm et al., 2001).

In the same direction, the concentration of (−)-epicatechin derivatives in urine has been reported to correlate with the rate of intestinal absorption, and the most predominant metabolites in urine correspond with the most predominant ones observed in plasma after administration (Actis-Goretta et al., 2013). These results may imply that increased absorption of cocoa metabolites could also promote a higher excretion rate, suggesting once again that physiological effects of cocoa phytochemicals may be exerted on a limited time window after consumption.

However, Jang et al. demonstrated accumulation of *O*-methyl-epicatechin-glucuronide conjugates in a dose dependent manner in adipose tissue of pigs exposed during 29 days to cocoa. This behavior was observed despite an increased dose dependent excretion of the same metabolite in urine (Jang et al., 2016). These data suggest that despite the rapid turnover and excretion of cocoa phytochemicals, bioaccumulation may occur during long term exposure and for instance be associated with biological activities in the organism.

Quercetin has been also reported to be metabolized already at the level of gastrointestinal tract. In animal models, conjugated quercetin metabolites of hepatic origin can be also founded in luminal content, suggesting enterohepatic recirculation. It has been also observed a differential transport and bioavailability for quercetin and its metabolites. While in stomach, colon and cecum just *ca*. one-third of the total quercetin content corresponds to conjugated forms, in inner organs like liver, kidney, and plasma 92%, 96%, and 100% of quercetin equivalents are reported to occur in conjugated form (Graf et al., 2006).

Microbial associated metabolites, including hydroxyphenyl valerolactones such DHPV, DHPV *O*-glucuronides and MHPV-*O*-sulfate have been reported to increase significantly in urine of humans after intervention with cocoa in comparison with control conditions (Urpi-Sarda et al., 2009). While phase I and phase II metabolites like epicathechin-3′-glucuronide and epicatechin-methoxy-sulfates increase in plasma between 0 and 4 h after intake of cocoa, microbial related metabolites like PVL-associated metabolites require colonic absorption and its appearance in plasma tend to be delayed (4−8 h after intake) (Gómez-Juaristi et al., 2019).

After intake of cocoa products containing low and high cocoa flavanol content (1.32 ± 0.17 and 2.73 ± 0.18 mg/g dry matter, respectively), Gómez-Juriasti et al. reported no significant differences in the plasmatic concentrations of glucuronidated, sulfated, and methoxy-sulfated metabolites obtained from the direct transformation of cocoa phytochemicals by human phase I and phase II metabolism. In contrast, increased plasmatic concentration of microbial byproducts was observed after high flavanol-containing products (Gómez-Juaristi et al., 2019). These data suggest that increased dietary concentration of phytochemicals is not necessarily related with increased bioavailability of cocoa phytochemicals but may determine the number of compounds available for microbial transformation. This can be relevant when considering that in intestinal administration of purified (−)-epicatechin, up to 45% of the total flavanol can avoid absorption and metabolism at the intestine (Actis-Goretta et al., 2013) and can be therefore available for colonic microbial transformation.

Microbiota has therefore a preponderant role in cocoa phytochemical metabolism, but in addition the intake of cocoa products has been also related with modulation of gut microbiota in mammals (Jang et al., 2016; Żyżelewicz et al., 2019). Cocoa administration in pigs modulate the microbial profile at gut level (Jang et al., 2016) while inclusion of roasted cocoa extracts in the diet of laboratory rats is related with modulation of microbial enzymatic activity. Reduced activity of fecal microbial β-glucosidase, β-galactosidase and β-glucuronidase has been reported after exposure to cocoa bean extracts in comparison with control diets (Żyżelewicz et al., 2019).

Also, modulation of the volatile fatty acid profile in rat cecum content after exposure to diets enriched with cocoa bean extracts suggest together with the change and enzymatic activity a variation either in microbial population or its metabolic rate (Żyżelewicz et al., 2019). The change in glucosidase activity can have an impact on microbial metabolism of phytochemicals (Németh et al., 2003) while the change in fatty acid profile can eventually have systemic consequences due to the current evidence or metabolic modulation by this microbial derived short chain fatty acids (Chambers et al., 2018).

In addition, microbial by-products may also exert modulation of xenobiotic metabolism (Clarke et al., 2019; Dempsey & Cui, 2019). An integrative and detailed perspective of food-derived phytochemicals including colonic transformation and xenobiotic metabolism has been summarized previously by Crozier et al. (2010). A review on the interaction and cross modulation between cocoa phytochemicals and microbiota has been addressed by Sorrenti et al. (2020).

Bioavailability and metabolism of methylxanthines. Methylxanthines are rapidly absorbed mostly at intestinal level, but absorption in stomach is also possible for caffeine. Pharmacological administration of caffeine can lead to a maximal plasmatic concentration already 30 min after intake (Arnaud, 2011); however, the maximal plasmatic concentration after intake of a cocoa beverage can be delayed up to 1.5−2 h after intake (Munford et al., 1996). Theophylline is absorbed in the intestine with high efficiency. Theobromine is also highly absorbed but absorption rate is reduced with increased doses (Arnaud, 2011). For theobromine higher maximal plasmatic concentrations has been observed after intake of chocolate compared to theobromine capsules. After chocolate intake, acute plasmatic concentration (0−3 h) of theobromine has been reported to be higher than plasmatic concentration of caffeine, paraxanthine, and theophylline (Munford et al., 1996).

Caffeine can be metabolized in the liver to paraxanthine as main metabolite, but also to theophylline and theobromine at minor extent by N-demethylation reactions (Nehlig, 2018). Additionally, dimethylxanthines (paraxanthine, theobromine, and theophylline) are susceptible to C-8 oxidation reactions leading to the formation of dimethyluric acids (Oracz et al., 2020). Excretion of methylxanthines and derivatives occurs rapidly and mainly through urine, but fecal excretion also occurs. Most of absorbed methylxanthines are cleared from the body after 24 h (Arnaud, 2011). A comprehensive review of methylxanthines bioavailability has been addressed by Oracz et al. (2020).

Bioavailability and metabolism of biogenic amines. Biogenic amines are reported to be increased during simulated digestion of chocolate, indicating that generation of biogenic amines from cocoa could not just only occur during processing but also along the gastrointestinal tract (Dala-Paula, Deus, et al., 2021). Putrescine concentration in fecal samples correlates significant with microbial composition, suggesting that microbiota may also play a relevant role in metabolism of biogenic amines (Matsumoto & Benno, 2007).

Absorption of biogenic amines in the intestine occurs fast with evidence suggesting that its intestinal transport follows a passive diffusion mechanism (Milovic, Faust, et al., 2001; Milovic, Turchanowa, et al., 2001). Finally, biogenic amines are also synthetized in certain amount in the human body through normal metabolism and increased by inhibition of monoamine oxidase enzymes involved on their degradation (Berry, 2004).

Effect of matrix composition and interaction with other chemical compounds. The bioavailability of phytochemicals is affected by its original concentration in the food matrix but also by matrix effects (Dudonné et al., 2016). Food-derived phytochemicals may exert complex interactions with other xenobiotic (drugs, environmental, or dietary elements) that should be carefully assessed when describing bioavailability and biological activities of the food matrix as a whole (Deng et al., 2017; Koziolek et al., 2019; Li et al., 2017). For example, phytochemicals (Li et al., 2017) and other xenobiotic like drugs (Liu et al., 2020; Xu et al., 2018, p. 4), can modulate the rate of phase I and phase II transformations involved in xenobiotic metabolism. Then, it may be expected that bioavailability of food derived phytochemicals in chocolate and other foods can be also affected by concomitant presence of other foods, additives or food-derived compounds.

The presence of milk protein influences pharmacokinetics of epicatechin uptake from chocolate in humans. Despite total epicatechin absorption did not differ among formulations in a 6-h kinetic evaluation, the maximal plasmatic concentration of epicatechin has been reported to be reduced in subjects eating products containing milk protein compared to dark chocolate (Neilson et al., 2009).

Human intervention studies indicate that the use of maltitol as sweetener is associated with reduced plasmatic concentration of un-methylated epicatechin metabolites, 3′-O-methyl-epicatechin, 4′-O-methyl-epicatechin, and total flavanols observed 1 and 2 h after consumption when compared to products using sucrose as sweetener. However, despite the changes observed in the first 2 hours after intake, the parameters used to estimate the total absorption and maximal plasmatic concentration (AUC and Cmax, respectively) in a total 4-h blood collection period were significantly reduced for un-methylated epicatechin but not for the methylated metabolites 3′-O-methyl-epicatechin and 4′-O-methyl-epicatechin

(Rodriguez-Mateos et al., 2012). These results suggest that maltitol might eventually affect the absorption kinetics but not necessarily the total amount of phytochemicals obtained from cocoa.

Co-administration of methylxanthines has been reported to be associated with an increase of circulating plasmatic levels of flavanol metabolites. Cumulative plasmatic concentration of flavanol metabolites increased 37 $\pm$ 17% when using a concomitant dose of 122.4 mg/75 kg of total methylxanthines compared to control conditions (using standardized doses of 820 mg of total cocoa flavanols/75 kg body weight) (Sansone et al., 2017).

Regarding other potential concomitant effects, EGCG present in tea (*Camellia sinensis*) has been reported modulate the intestinal expression of phase II enzymes and receptors involved in response to xenobiotics *in vivo* (Li et al., 2018), and administration of noni juice has been described to increase the urinary concentration of isoflavone derivatives in rats (Fallas-Ramirez et al., 2018). In the same direction, co-administration of grape and blueberry has been reported to have an effect on bioavailability of phenolic compounds (Dudonné et al., 2016).

As mentioned previously, administration of cocoa extracts in animal diets has been reported to modulate microbial enzymatic activity in gut (Żyżelewicz et al., 2019). Since microbial glycosidases and other enzymes are related with intestinal transformation of phytochemicals (Németh et al., 2003), long-term exposure to cocoa products or derivatives might also have the potential to exert an effect on bioavailability of other phytochemicals.

For further details on bioavailability and metabolic conversion of cocoa phytochemicals, a comprehensive description of these processes has been addressed by Oracz et al. (2020). For a closer detail on biomarkers of intake after exposure to cocoa and cocoa products, a comprehensive summary of metabolites observed in human plasma and urine after consumption has been reported both by Mayorga-Gross and Esquivel (2019) and Oracz et al. (2020).

5. Cocoa phytochemicals and cardiovascular performance: Biochemical and physiological-associated mechanisms

As addressed in the previous sections and in Fig. 3.6, chemical composition of cocoa and products containing cocoa includes a wide variety of nonnutritive phytochemicals (Fanton et al., 2021) (see Sections 2 and 3, and Fig. 3.6) as phenolic compounds, methylxanthines, peptides, essential fatty acids, vitamins, and minerals, among others.

Cocoa products and phytochemicals derived from it is consumption have been suggested to be involved in the modulation of the health status, including cardiovascular protection (Aprotosoaie et al., 2016; Flores & Eugenia, 2019; Ludovici et al., 2017; Magrone et al., 2017; Rimbach et al., 2009), modulation of inflammation processes (De Feo et al., 2020; Goya et al., 2016; Magrone et al., 2017), and in the improvement of cognitive performance (Barrera-Reyes et al., 2020). The mechanism through which cocoa phytochemicals modulate cellular responses has been described to involve the regulation of the oxidative mechanism and inflammation, but also the balance of satiety promoting hormones, the inhibition of digestive enzymes, and the regulation of glucose balance. A compressive literature review on mechanism involved in the response to cocoa phytochemicals has been reported by Strat et al. (2016). For the purpose of this chapter, the biological activity focused on cardiovascular performance and inflammation will be described further.

Cardiovascular diseases (CVD) are a group of pathologies that affect the heart and the vascular systems, including arrythmia, stroke, myocardial infarction, and peripheral arterial diseases among others (Shah et al., 2015). Together with other noncommunicable disease, cardiovascular pathologies have risen in the last decades in high, but also middle- and low-income countries. CVD have been reported to cause more than 50% and up to 70% of total deaths by noncommunicable diseases in high and middle/low-income countries, respectively. With some few countries as exceptions, CVD are considered the leading death cause worldwide (Benziger et al., 2016) reaching an estimated proportion of more than 30% of the total death causes worldwide for 2017 (Roth et al., 2018).

Different process including altered plasmatic lipid profiles (Nordestgaard & Varbo, 2014), hypertension (Yusuf et al., 2020), altered function of the vascular endothelium (Eroglu et al., 2020) and unresolved proinflammatory environments have been associated (Alfaddagh et al., 2020; d'Alessandro et al., 2020) as increased risk or promoting factors of CVD. Thus, inflammation and its related manifestations have been proposed as one of the main causative factors for the onset and development of CVD (Tsoupras et al., 2018).

Flavonoids. In particular, flavan-3-ols effect on health has led to numerous investigations, and a health claim related to cocoa flavanols accepted by the European Food Safety Authority (EFSA). The EFSA panel concluded that "c*ause and effect relationship has been established between the consumption of cocoa flavanols in a high-flavanol cocoa extract (*i.e., *in capsules or tablets) and maintenance of normal endothelium-dependent vasodilation*" when the dose is 200 mg of cocoa flavanols consumed daily (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2014, p. 3654).

Flavanol and flavanol metabolites are reported to be involved in nitric oxide (NO)-dependent vasodilatation in isolated vascular endothelium suggesting its potential role in the improvement of vascular function (Schroeter et al., 2006). In addition, cocoa phytochemicals have been suggested to reduce platelet activation and aggregation (Garcia et al., 2018), while they also act as modulators of the synthesis of inflammatory mediators of the leukotriene and prostaglandin families (Schewe et al., 2002). Leukotrienes and prostaglandins are mediators of inflammatory process, and its regulation has been suggested to have a role in cardiovascular health and disease (Sonnweber et al., 2018). A cocoa extract containing 49% total procyanidin per weight reduced by 16% the production of leukotrienes while increasing up to 200% the production of prostaglandin PGI_2 in endothelial cultured cells (Schramm et al., 2001).

Inflammatory mediators derived from arachidonic acid like prostaglandins and leukotrienes are synthetized by enzymatic reactions at cellular level. Currently, cellular models have provided evidence that phytochemicals presented in cocoa have an inhibitory effect over the activity of mammalian enzymatic systems involved in the transformation of arachidonic acid into pro-inflammatory mediators (Schewe et al., 2001, 2002).

In transfected *Escherichia coli* expressing the human enzyme 5-lipoxigenase, (−)-epicatechin has been reported to reduce the conversion rate of arachidonic acid into 5-HPETE and LTA4 (members of the leukotriene cascade) in a dose dependent manner. In the same direction, procyanidins isolated from cocoa has been reported to reduce the activity of human 5-lipoxygenase with higher activity exerted by dimer in comparison to higher monomeric forms. Dimeric procyanidins are reported to possess similar activities than (−)-epicatechin while conjugated procyanidins with more than 6 units show almost not apparent activity. Quercetin has been also reported to inhibit human 5-lipoxygenase with 30 times lower IC_{50} values in comparison to (−)-epicatechin (0.6 and 22 μmol/L, respectively). Despite that the presence of redox active phytochemicals may modulate some side reactions, the inhibition of enzymatic activity suggests that mechanisms of flavanol and flavonol activity may involve mechanism beyond radical scavenging activity (Schewe et al., 2002).

Additional studies suggest that both epicatechin and epigallocathechin inhibit the activity of rabbit 15-lipoxygenase in a dose dependent manner but do not exert inhibition over soybean 15-lipoxygenase (Schewe et al., 2001) bringing evidence that inhibition of inflammation mediators might be due to enzymatic inhibition instead of direct radical scavenging activity.

In vitro inhibition of 15-lipoxygenase by epicatechin gallate is one order of magnitude stronger compared to epicatechin and a similar behavior has been reported for epigallocatechin-gallate and epigallocatechin (Schewe et al., 2001). Therefore, hydrolytic cleavage that occurs in intestine (Németh et al., 2003) will cause a reduction of the biological activity regarding anti-inflammatory modulation.

A more complex scenario can be explored when the degree of oligomerization is analyzed. When analyzed the inhibitory activity of procyanidins over human 5-lipoxygenase, higher activity was observed for trimers and tetramers when comparing to hexamers or higher oligomers. At oligomerization degrees from 6 to 9 units, practically no inhibitory activity was detected (Schewe et al., 2002). In contrast, when analyzing the inhibitory activity of procyanidins on rabbit 15-lipoxygenase, an increased inhibitory activity was observed as function of oligomerization degree. In contrast to human 5-lipoxygenase, in this second approach higher activities were observed in hexamers and nonamers compared to trimers and tetramers (Schewe et al., 2001).

As discussed previously, the degree of oligomerization is affected by processing techniques (D'Souza et al., 2017) and high degree phytochemicals can be observed in plasma but they are hydrolyzed and metabolized already at level of the gastrointestinal track by endogenous and/or microbial enzymes (Actis-Goretta et al., 2013; Gómez-Juaristi et al., 2019).

Methylxanthines. Methylxanthines have been also related with modulation of parameters involved in cardiovascular performance. Methylxanthines have been widely related with inhibition of adenosine receptors in the central nervous system and therefore related to cognitive functions (Daly et al., 1981). However, adenosine receptors present a broader distribution and are also present in heart and blood vessel endothelial cells, where they modulate different processes including heat rate and vascular tone (Headrick et al., 2013).

Acute administration of caffeine causes an increase in central systolic pressure in humans (Karatzis et al., 2005; Waring et al., 2003). Interestingly, despite structural similarities different biological effects are observed when evaluating the acute effect of theobromine, caffeine or theobromine plus caffeine intake. Caffeine increased blood pressure, theobromine reduced blood pressure, while the intake of both theobromine and caffeine did not show significant differences when compared to placebo administration (Mitchell et al., 2011).

In a similar direction, caffeine administration has been reported to reduce heart rate (Mitchell et al., 2011; Waring et al., 2003) while theobromine at high doses causes increased heart rate (Mitchell et al., 2011). As reported for blood pressure, the intake of both methylxanthines together did not alter the heart rate compared to placebo (Mitchell et al., 2011). These data are quite relevant and suggest both the possible role of methylxanthines in cardiovascular performance, but also the relevance of evaluate total exposure to this substance.

Methylxanthine interventions use doses that may reach up to 300 mg caffeine (Waring et al., 2003) or 700 mg theobromine (Mitchell et al., 2011), doses that might be difficult to reach through consumption of cocoa products and therefore, the potential effects of consumption will be most likely observed in association with other foods or supplements. Karatzis et al. (2005) have reported effects over blood pressure already after intake of a cup of coffee containing just 80 mg caffeine. Moreover, paraxanthine, a metabolite from caffeine metabolism in the body, can exert more potent effects over adenosine receptors (Nehlig, 2018).

Biogenic amines. Biogenic amines have been also related to modulation of cardiovascular parameters. Tryptamine has been reported to possess a dose dependent vasoconstrictor effect in isolated mesenteric arteries in rats (0.1−1000 nmoles doses). Interestingly, in isolated mesenteric vessels that have been previously pre-vasoconstricted using pharmacological treatments, tryptamine shows a vasoconstrictor effect at low doses (0.1−10 nmoles) but a predominant vasodilatation effect at higher doses (25−1000 nmoles). Also, a vasodilatation behavior in preconstricted vessels is reported for tyramine and phenylethylamine (Anwar et al., 2012). These data is considered relevant since inhibition of physiological process associated with vasoconstriction has been suggested as therapeutic strategies for cardiovascular improvement (Eroglu et al., 2020).

Nonetheless, direct intravenous administration of tyramine is related to an increase in systolic blood pressure, diastolic blood pressure, and heart rate in humans (Schäfers et al., 1997). Also tyramine has been reported to induce vasoconstriction in isolated brain arteries in a dose-dependent manner (Marín et al., 1981), and increased blood pressure and hypertension has been classically defined as risk factor for CVD (Yusuf et al., 2020).

Cardiovascular effects exerted by tyramine are disrupted by different inhibitors of the adrenergic receptors (Schäfers et al., 1997) indicating a pharmacological relevance and the close relationship with noradrenaline signaling. However, additional studies also indicate that vasoconstriction mediated by biogenic amines can be independent from adrenergic signaling (Broadley et al., 2013). In a more complex scenario, biogenic amines are involved in mechanisms mediated by 5-HT receptors (Anwar et al., 2012; Broadley et al., 2013). Physiological experiments assessing endothelium response to biogenic amines (p-tyramine and phenyl-ethylamine) and the influence of adrenergic and 5-HT antagonists, suggest that additional trace amine-associated receptors (TAARs) may play a role on the observed responses (Herbert et al., 2008). Recently, it has been reported that the vasoconstrictor effect of phenylethylamine in isolated rat aorta is potentiated by inhibitors of NO synthesis (Broadley & Broadley, 2018) a mechanism previously discussed to be involved in the response to other cocoa phytochemicals.

Tyramine and phenylethylamine are long-lasting considered as sympathomimetic amines, what means that promote biological functions exerted by catecholamines (Knoll et al., 1996). Catecholamines (adrenaline/epinephrine, noradrenaline/norepinephrine, and dopamine) regulate a wide and complex variety of physiological process, many of them involved in cardiovascular physiology. Cathecolamines can activate a diverse group of α- and β-adrenergic receptors with different ligand affinities, tissue distribution and physiological responses. Catecholamine-mediated responses include dilatation of peripheral blood vessels (β2), but also promotion of increased heart rate and contractility (β1), coronary artery vasoconstriction (α1) and peripheral vasocontraction (α1) in a process regulated by the concentration of specific catecholamine presented and modulation of other physiological controlling systems (Motiejunaite et al., 2020).

EFSA has estimated the toxicological threshold in 50 mg per person for histamine, while in the case of tyramine a 600 mg per person has been defined as toxicological threshold for healthy subjects and 50 mg per person as threshold for patients taking monoamine oxidase inhibitors due to the increased synthesis of biogenic amines in the body under these pharmacologic treatments (EFSA Panel on Biological Hazards (BIOHAZ), 2011). Since the content of biogenic amines has been related to allergies, food intoxication, and other negative health outcomes, and having that an elevated content of biogenic amines may also imply a negative microbiological control along the food chain, an increased concern regarding biogenic amine content has raised (Gardini et al., 2016; Ruiz-Capillas & Herrero, 2019). Optimization of starting cultures and fermentation has been suggested as mechanisms to improve biogenic amine control in foods (Naila et al., 2010).

Essential fatty acids. Cocoa and cocoa products also contain the essential omega-6 fatty acid, linoleic acid (C18:2) (Álvarez et al., 2007; Lipp et al., 2001). Both omega-3 and omega-6 fatty acid are relevant in human nutrition due to its involvement in the synthesis of modulators of inflammation including prostaglandins and thromboxanes (Simopoulos, 2016). Despite omega-6 has been related with pro-inflammatory process (Marchix et al., 2015), its moderate consumption is still considered as part of a balanced diet due to its implication in physiological process (Innes & Calder, 2018). However, since a daily omega 6/omega 3 ratio lower than 2 or 1 has been recommended for some authors for healthy cardiovascular performance (DiNicolantonio & O'Keefe, 2018; Simopoulos, 2016) new formulation strategies have been applied in the last years with the aim to increase omega-3 content in cocoa products (Jeyarani et al., 2015) due to the pro-inflammatory potential observed for omega-3 fatty acids (Tan et al., 2018). In addition, the form that these fatty acids are present in cocoa is also of importance and should also be studied, since the polar forms such as glyco-lipids found in other beverages like tea, cider and beer, have shown strong anti-thrombotic and anti-inflammatory properties, in contrast to their neutral lipid forms (as esters and triglycerides) (Tsoupras et al., 2021; Tsoupras, Lordan, Harrington, et al., 2020; Tsoupras, Lordan, O'Keefe et al., 2020).

Vitamins. Vitamin E is an essential micronutrient that has been considered to be essential to keep membrane integrity (EFSA, 2015). However, in the last years, a wide range of biological activities including modulation of inflammatory mechanisms has been proposed for different vitamin E congeners and their metabolites (Wallert et al., 2021). γ-Tocopherol, the main vitamin E form reported in cocoa butter (Lipp et al., 2001) has been related with inhibition of cyclooxygenase, and synthesis of proinflammatory mediators (PGE2, PGD2 and 8-isoprostane) in cultured cells (Jiang et al., 2000) and reduction of the production of proinflammatory mediators PGE2 and LBT4 in subcutaneous tissue of rats (Jiang & Ames, 2003).

Role of redox active phytochemicals. In general, an association has been made among the bioactivity of phytochemicals or phytochemical rich foods with *in vitro* antioxidant activity. However, simplifying biological activity of phytochemicals to its *in vitro* antioxidant activity may under or overestimate some biological processes (Pompella et al., 2014). Recently a study with rats evaluated diets enriched with extracts from cocoa beans and similar or higher values of antioxidant cellular parameters (GSH, GSSG, TBARS) where observed when lower redox active compounds were present in the extract (Żyżelewicz et al., 2020). Nevertheless, it has been reported the modulation of endogenous mechanism related to the cellular redox regulation by some phytochemicals present in cocoa. Procyanidin B2 has been reported to protect cultured cells from ROS by promotion of the expression of enzymes regulated by the Nrf2 pathway (Rodríguez-Ramiro et al., 2012). Activation of Nrf2 pathway in cultured cells has been also reported to be increased after exposure to monomeric catechin-rich fractions extracted from cocoa (Rowley et al., 2017). However, in contrast to this result, epicatechin and cocoa phenolics have been reported to protect cultured liver cells from high glucose-induced oxidative stress damage by avoiding the activation of Nrf2 (Cordero-Herrera et al., 2015) suggesting a complex regulatory scenario.

Nrf2 is a transcription factor that translocates from the cytoplasm to the nucleus, causing the activation of the expression of genes involved in the cellular endogenous antioxidant defense by modulation of the so-called antioxidant response elements (ARE) at DNA level. Through this mechanism, cells promote the synthesis of different enzymes involved in detoxification of compounds but also elimination of oxidative species, and therefore considered as a protective mechanism (Niture et al., 2014). Nevertheless, under certain specific conditions, Nrf2 activation may promote deleterious physiological process. The hyperactivation of Nrf2 has been associated with tumor progression and resistance of tumoral cells to different therapeutic interventions (Menegon et al., 2016). Regarding the complex nature of cellular responses, further evidence may be required regarding the role of phytochemicals from cocoa and other plant foods in Nrf2 regulation.

The emerging interest in raw cocoa. Having that the biological activity of cacao has been strongly associated to secondary non-nutritive phytochemicals (Fanton et al., 2021) and since changes in these metabolites are dependent of cocoa processing (Section 3), alternative cocoa processes have been proposed, for example, the production of raw cocoa beans (Żyżelewicz et al., 2019, 2020).

The term raw cocoa beans can be used for non-fermented and non-roasted cocoa beans (De Taeye et al., 2017) or fermented but non-roasted cocoa beans (Assi-Clair et al., 2019; Kedjebo et al., 2016; Żyżelewicz et al., 2019, 2020). Cocoa bean extracts have been reported to exert modulation on fecal microbial enzymatic activity and the volatile fatty acid profile in cecum of laboratory rats beans (Żyżelewicz et al., 2019, 2020). The reported differences between the effect of raw cocoa extracts, roasted cocoa extracts, and monomeric flavan-3-ol fractions suggest that chemical composition of the different matrix might possess biological significance (Żyżelewicz et al., 2019). However, there is still scarce evidence regarding biological activity and safety parameters should be further analyzed.

Limitations of the assessment of biological functions by isolated compounds. Biological activities have been suggested for isolated compounds that may be present in cocoa and cocoa products; however, it is relevant to consider that concentration, identity, and therefore biological activity of these phytochemicals are modulated by different factors including the processing of the raw beans, the final preparation and consumption of the food item but also by the effect of other dietary elements, metabolic transformations by the human body or microbiota. In the previous sections, the effect of processing on phytochemical content and diversity but also factors influencing bioavailability of cocoa phytochemicals have been discussed. Since these factors may influence the final biological effects exerted by food items, in the next section, current evidence based on interventional studies evaluating the use of cocoa or cocoa products rather than isolated compounds will be addressed.

6. Clinical interventions with cocoa and cocoa products: addressing cardiovascular performance

As mentioned previously, wide interindividual variation is observed for metabolic conversion and bioavailability of phytochemicals including those in cocoa. Interindividual variations in the assessment of the biological function of phytochemicals have gained attention in the last years and its assessment is currently considered a relevant challenge to address when analyzing results derived from clinical interventions involving food and food derived compounds (Morand & Tomás-Barberán, 2019).

Analysis of the results regarding the cellular mechanisms that may be involved in biological activities of phytochemicals must be carefully addressed considering the biotransformation of native phytochemicals during digestion and absorption (Hollman, 2014). *In vitro* approaches using native instead of metabolized compounds may have limited capacity to reproduce the physiological role of phytochemicals and therefore is relevant to validate results from *in vitro* models by *in vivo* approaches using animals and/or humans (Schroeter et al., 2006).

Following, we address a brief discussion of available literature regarding *in vivo* modulation of physiological parameters linked to cardiovascular performance by cocoa products and phytochemicals. Studies evaluating the effect of cocoa administration on cardiovascular performance have been focused on the evaluation of different parameters including body weight, blood pressure, blood vessel vasodilatation, and the modulation of inflammation mediators.

Non-interventional dietary assessments have been associated free-willing consumption of cocoa-based products with an improvement in platelet aggregation related parameters, suggesting a better cardiovascular performance in chocolate casual consumers compared to non-consumers. These data indicate that commercially available products may have short-term effects in human health. However, dietary intervention using free dietary access implies reduced control over concomitant intake of other sources of bioactive compounds that may limit an accurate data interpretation. Also, free-willing selfreported consumption may be sensitive to under or over estimation of consumption, moreover when an exhaustive classification of the wide variety of products available in the market is possible (Bordeaux et al., 2007).

Serum lipid profile is a common indicator of cardiovascular performance. High LDL and low HDL plasmatic concentrations of cholesterol are considered factors that increase cardiovascular risk (Langsted & Nordestgaard, 2019). Elevated plasmatic triacylgliceride concentrations have been also associated with higher LDL cholesterol concentration and cardiovascular risk (Nordestgaard & Varbo, 2014).

An intervention using a high polyphenol cocoa product in type 2 diabetic (2 weeks) did not change the glycosylated hemoglobin status of patients but improved the serum lipid profile by increasing the HDL content and reducing the cholesterol/HDL ratio (Mellor et al., 2010). A placebo control intervention in prediabetic patients (59.3 ± 7.1 years) using 83.3 ± 2.7 mg procyanidin per day for 6 weeks (two daily doses of 41.6 ± 1.4 mg/dose) showed slight improvement in blood pressure after intervention with cocoa phenolics in women but not in men (Shiina et al., 2019). A similar dark chocolate intervention (540 mg total polyphenols/day) showed moderate improvement in blood pressure parameters (Matsumoto et al., 2020).

Regulation of inflammatory mediators has been suggested to have a role in cardiovascular health and disease mostly related to the beneficial effect of the promotion of an antiinflammatory environment in vascular endothelial (Sonnweber et al., 2018). Schramm et al. evaluated the effect of the consumption of 37 g of low or high procyanidin chocolate (0.09 and 4.0 g total procyanidins/kg, respectively) on the concentration of inflammation mediators in plasma (Schramm et al., 2001). After 2 hours postingestion, a significantly lower plasmatic concentration of leukotrienes (6.53 ± 0.83 vs. 9.18 ± 0.75 nmol/L) and significantly higher plasmatic content of prostacyclins (554 ± 37 vs. 397 ± 40 pmol 6-keto-PGF_1/L) was observed in subjects consuming high procyanidin chocolate compared to low procyanidin chocolate. A reduction in the ratio leukotriene/prostacyclin suggests a positive outcome for cardiovascular performance (Schramm et al., 2001).

The maintenance of endothelial function and vasodilatation capacity (Eroglu et al., 2020) and the reduction in blood pressure (Yusuf et al., 2020) have been addressed in different ways in order to dimmish cardiovascular risk. Low dose daily consumption of dark chocolate (6.3 g/day for 18 weeks) is related to a time-dependent reduction in both systolic and diastolic blood pressure in prehypertensive or moderate hypertensive patients. In contrast, white chocolate consumption in the same study did not show significant effects on these parameters. White chocolate possess significant lower content of phenolic bioactive compounds like catechin, epicatechin, procyanidins, and other flavonoids compared to dark chocolate, suggesting a biological effect mediated by phenolic phytochemicals (Taubert et al., 2007).

A 3-month intervention study evaluating the effect of daily intake of dark chocolate in blood pressure parameters assessing both a low (6 g chocolate/day) and a high dose (25 g chocolate/day) indicates that long-term intake of dark chocolate significantly reduces the mean and systolic 24 h blood pressure in both schemes of intervention in comparison with pretreatment values. Additionally, diastolic 24-h blood pressure was significantly reduced in comparison with pretreatment conditions after low doses but not at high doses. These data suggest that an increase in daily of cocoa products may not necessarily correlate with an higher positive impact on physiological parameters (Desch et al., 2010).

Flow-mediated dilatation (FMD) is a parameter that positively correlates with cardiovascular performance (Balzer et al., 2008). The aortic augmentation index (*IAx*) and pulse wave velocity (*PWV*) are both parameters used for the estimation of arterial stiffness, an aging related physiological process suggested as risk factor for cardiovascular performance (Mackenzie et al., 2002).

In diabetic patients, flow-mediated dilation has been reported to be significantly improved after 30 days intervention of a high cocoa drink (3 doses of 321 mg flavanol per day). However, nonsignificant effects were observed when consuming a control cocoa drink (3 doses of 25 mg flavanol per day). A significant reduction in plasmatic LDL and glycosylated hemoglobin was also observed after consumption of a high flavanol drink (Balzer et al., 2008).

Plasmatic appearance of flavanol and flavanol-metabolites is statistically associated with flow-mediated dilatation. Moreover, plasmatic concentration of flavanols in humans correlate to NO signaling and the flavanol mediated effect over vascular function is reduced by pharmacological inhibition of the enzyme NO synthase, with similar effects obtained also in isolated endothelium in *ex vivo* analysis (Schroeter et al., 2006). The endothelial NO synthase (eNO) promotes vasodilatation of blood vessels by local production of NO and therefore its activity is involved in vascular function and blood pressure and its regulation has been associated with progression and treatment of CVD (Daiber et al., 2019).

Acute administration of cocoa flavanols in patients with varied symptoms of CVD has reported an increase in FMD of blood vessels, but also a reduction of the braquial PWV. In the case of FMD, the positive effect follows a dose-dependent behavior determined by total flavanol intake and is increased by co-administration of methylxanthines. Interestingly, no effect on these parameters was observed when methylxanthines were administrated alone suggesting: (1) a potential synergistic effect or (2) an effect of methylxanthines over the bioavailability, rate of metabolic transformation and/or excretion of flavanols and flavanol metabolites (Sansone et al., 2017).

A double-blind, crossover intervention study using a low and high flavanol cocoa preparation (9 vs. 375 mg total flavanols) in senior volunteers receiving current treatment for coronary artery disease showed a positive effect of the high dose flavanol-containing formulation on endothelial function parameters over a 30-day period. Reduced systolic pressure, increased plasmatic nitrite concentration, and FMD were variables positively affected by high flavanol intervention (Heiss et al., 2010).

Biogenic amines modulate biological processes, including the promotion of vasodilatation of blood vessels (Anwar et al., 2012) but also modulation of the heart rhythm, and induction of hypo- and hypertensive effects (Anwar et al., 2012; Schäfers et al., 1997; ten Brink et al., 1990). Despite bioactive amines have been suggested to be compounds that can potentially be used as health promoting elements due to their neuroactive potential, the existence of a mixture of negative side effects suggests the need of further research on the topic (Yõlmaz & Gökmen, 2020).

Vasodilatation potential of biogenic amines might be considered as a positive modulator of cardiovascular performance; however, this kind of compounds have been also linked to negative cardiovascular outcomes including acute arrhythmia (Gammone et al., 2018; Hansson et al., 2004) and supraventricular tachycardia (Parasramka & Dufresne, 2012). The previous mentioned studies directly linked chocolate consumption as the main, or one of the most probable cause of the observed negative effects. Despite that an unbiased direct connection to a single food product might be difficult to be established in acute events, and even that epidemiological analysis did not report an increased risk of arrythmia related to chocolate consumption as independent risk factor (Larsson et al., 2018), it may be relevant to consider the content of bioactive amines in cocoa products as a risk factor for susceptible populations.

The European Food Safety Authority consider bioactive amines a health risk factor for sensitive populations including histamine sensitive persons and patients using monoamine oxidase inhibitors (EFSA Panel on Biological Hazards (BIOHAZ), 2011) a group of prescription drugs frequently used in treatment of neurological disorders (Deshwal et al., 2017). Interestingly, research focused of natural sources for monoamine oxidase inhibitors in herbal extracts have reported that diverse phytochemicals presented also in cocoa like (+)-catechin (Hou et al., 2005), (−)-epicatechin (Hou et al., 2005; Samoylenko et al., 2010), quercetin (Lee et al., 2000), and procyanidin B2 (Samoylenko et al., 2010) exert *in vitro* inhibitory activity over this type of enzymes.

Other phenolic compounds like the common Tea phenolic compound EGCG has been also reported to possess *in vitro* inhibitory activity over monoamine oxidases (van Diermen et al., 2009). These results point the relevance of an integrative evaluation of the food content, bioavailability, and biological roles of biogenic amines and its possible biological consequences on sensitive populations, moreover when exposed to prescribed or naturally occurring monoamine oxidase inhibitors. Currently, a comprehensive review about monoamine oxidase inhibitory activity of phenolic compounds has been published by Dhiman et al. (2019) (Dhiman et al., 2019).

Fermented foods, fish, and wine are the most common sources of biogenic amines (Doeun et al., 2017), but also fresh plant origin like eggplant and bean sprouts have been reported to contain up to 15.5 and 329.9 mg total amines/100 g, respectively. The histamine content in these two products has been reported to reach up to 12.5 and 8.75 mg/100 g, respectively (Dala-Paula, Starling, & Gloria, 2021). In commercial mature soft cheese, tyramine content has been reported to reach up to 130.6 mg/100 g while histamine levels can reach up to 102.5 mg/100 g during storage (Dabadé et al., 2021).

Also, it is relevant to notice that the caloric value of different cocoa products must be taken in consideration when discussion a dietary improvement of cardiovascular performance. CVD are multifactorial pathologies related with several interconnected factors including obesity (Cercato & Fonseca, 2019) and the prevalence of overweight and obesity has been associated to dietary patterns including high proportion of caloric-dense meals (Vernarelli et al., 2018).

Despite the current challenge to accurately assess the individual effect of food groups or specific food items on weight gain, elevated consumption of foods with high caloric value has been associated with increased weight and obesity (Schlesinger et al., 2019). In this context, even when cocoa products possess phytochemicals related with positive cardiovascular effects, the total caloric value of the food matrix containing cocoa or cocoa phytochemicals must be also considered in order to promote a healthy lifestyle. The caloric value of cocoa products varies significantly from dark to sweetened chocolates or from pure cocoa powder to cocoa butter or cocoa flavored beverages (Hashem et al., 2019) and must be considered when promoting a healthy chocolate consumption.

Desch et al. indicate that a 3-month high dose chocolate intervention (25 g/day) correlates with significant increases in body weight in contrast to a low dose intervention (6 g/day) where no effects in body weight were observed (Desch et al., 2010). In a similar direction, when analyzing chocolate consumption indistinctly from the product type or formulation, Greenberg et al., 2013 have reported a significantly higher long-term weight gain in subjects consuming chocolate frequently. Moreover, body weight gain correlates in a dose-dependent manner to chocolate consumption, suggesting a potential role of chocolate intake in weight gain (Greenberg & Buijsse, 2013).

Certainly, intricate background can be presented in epidemiological data assessing dietary assessment (Illner et al., 2012). Dietary assessment indicates that subjects with more frequent consumption of chocolate can also be suitable to dietary patterns characterized by higher caloric intake or lower dietary inclusion of vegetables, two factors that could be related to weight gain independently from cocoa consumption. Also, in patients with prediagnosed obesity-related diseases a negative correlation between chocolate intake and body mass index (BMI) has been observed (Greenberg & Buijsse, 2013). Similar, a Swedish cohort study indicates that subjects with higher chocolate consumption tend to be less overweight despite higher calorie intake in their diets (Larsson et al., 2018).

Golomb et al. reported an inverse correlation between the frequency of chocolate consumption and BMI while no correlation was observed between total chocolate consumption and BMI (Golomb et al., 2012). Despite BMI may not necessarily be always an accurate indicator of overweight (Romero-Corral et al., 2008; Yoon et al., 2015) it can be used as simple parameter to determine when a subject suffers from obesity or overweight (Romero-Corral et al., 2008).

Altogether, data reflects the complexity of epidemiologic evidence regarding specific food item consumption and body weight gain and suggest that correlation analysis between health status and food intake must be carefully interpreted both when analyzing positive and negative effects of food consumption.

7. Closing remarks

Cocoa and cocoa products have had a significant role in societies since ancient times, contributing to the economy, culture, and food security. In the last years, a wide variety of phytochemicals have been identified in cocoa, including polyphenols as flavan-3-ols, methylxanthines, fatty acids, phenolic acids, vitamins, minerals, and peptides, among others. The concentration and distribution of these compounds is strongly dependent on the genetics of the cocoa, as well as on the processing stages, including the formulation of final products.

There is increased evidence of the biological activity of cocoa products and its phytochemicals, suggesting a potential role in health promotion. Current evidence suggests that acute positive effects over vascular parameters are observed after intake of cocoa products containing significant amounts of flavonoids, and this evidence has been supported by evaluation of mechanisms using *in vitro* approaches.

Scientific evidence regarding *in vivo* modulation of cellular pathways by phytochemicals may not be enough to unequivocally consider foods as therapeutic elements, therefore, its use as replacement of clinical therapeutic regimens must be strongly discourage. However, the increased evidence of biological activity of cocoa phytochemicals suggests its consideration as part of an integrative approach for sensitive populations such as those undergoing therapeutic interventions.

Therefore, the evaluation of the role of cocoa and cocoa phytochemicals on physiological regulation points toward the need of an integrative evaluation of its nutritional value. The modulation of cellular mechanisms in the metabolism of exogenous compounds including drugs or those related to inflammation and the development of chronic diseases may imply a health promoting potential of consumption, but also the possibility of eventual deleterious side effects, like interactions with specific prescription drugs or interference with metabolic regulation in certain specific contexts.

Based on current evidence and food safety recommendations, and despite the vasodilatory potential of biogenic amines, it is recommended to address a control in cocoa process in order to maintain total and single levels of these compounds below the suggested toxicological thresholds. Moreover, it should be considered that as part of a diverse diet, the consumption of cocoa products may be also associated with the consumption of other sources of biogenic amines like fermented foods and fish.

The effect of complex matrices in processed products but also the effect of concomitant intake with other sources of biologically active compounds may be further analyzed in the next years since cocoa consumption cannot be considered as an isolated element of human diet. In addition, current evidence also suggests that the observed effects may not necessarily increase with higher cocoa intake, and in contrast an excessive consumption may be potentially associated with negative health outcomes such obesity.

Since complex clinical trials evaluating prescribed drugs include generally bigger and more heterogeneous samples (Coory, 2010) in comparison to clinical interventions assessing nutritional interventions, and having that sample size has a relevant effect on the robustness of statistical inferences (Greco et al., 2013) it would be desirable to perform comprehensive controlled, multicenter studies in the upcoming years, with the aim to provide additional data relevant to the determination of the impact of cocoa intake in promotion of health status.

In this direction, the development of clinical trials using cocoa and cocoa derived products currently faces the challenge of the development of control test substances. The visual, sensory, and/or functional properties of the cocoa products is defined by its complex food matrix and are difficult to reproduce for control conditions (placebo) that might promote accurate blinded studies including human participants (Desch et al., 2010). The current limited availability of standards for metabolite quantification (Gómez-Juaristi et al., 2019; Rodriguez-Mateos et al., 2012) is also an opportunity that can be addressed in the future in order to facilitate more accurate determinations regarding phytochemical bioavailability and the biological activity of derived metabolites.

Finally, nonnutritional topics such as land and water availability as well as changes in climatologic conditions have create new challenges that must be addressed in the future of cocoa production. Marketing of cocoa beans can represent up to 100% of the total income for small cocoa producers (Anim-Kwapong & Frimpong, 2004), and small-scale activities are highly sensitive to environmental stressors such drought, floods, and increased temperatures.

Antwi et al. have reported high levels of food insecurity among cocoa producers. In this study up to 61% of households depending on cocoa production present a low dietary diversity score, implying a low food security status. The challenge of achieving food security among local farmers is significantly higher in households headed by women or persons with lower scholarity level (Antwi et al., 2018). Therefore, promotion of resilient local productive systems is essential for future cocoa production (Jacobi et al., 2015). In this direction, alternative agricultural practices including organic production and agroforestry have been reported to enhance resilience of small-scale cocoa producers (Jacobi et al., 2015; Schneider et al., 2017).

Health promoting effects of cocoa consumption have raised an increased interest in cocoa product intake and marketing beyond its gastronomic value and cultural/ethnic relevance. Nevertheless, socio-economic, and ecological sustainable development of cocoa production and cocoa industry should be encouraged together with the expansion of new marketing opportunities based on biofunctionality of cocoa phytochemicals and food products. Together with health promoting applications and economic growth, the environmental and social sustainability are opportunities to be addressed in current approaches regarding cocoa valorization.

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Chapter 4

Olive oil

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1. Introduction

Olive oil is the product obtained from olives, the fruit of the olive tree (*Olea europaea* L.). The olive fruit is an oily drupe, consisting of a stone (endocarp), the pulp (mesocarp), and skin (epicarp) and is similar in size to a cherry. Olive oil is extracted directly from olives by mechanical or physical means. It has no additives to preserve the quality of its natural components. Olive oil has been produced for millennia by the countries bordering the Mediterranean Sea. Nowadays referred to as a functional food, olive oil is the principal source of lipids in the so-called Mediterranean diet. This diet refers to an eating pattern with ancestral origins. It consists of high consumption of olive oil, fruits, vegetables, legumes and whole-grain cereals, moderate to high consumption of fish, moderate consumption of dairy products (cheese and yogurt mostly), and a small portion of nonfish meat products, nuts, seeds, olives, garlic, aromatic herbs, and spices (Bach-Faig et al., 2011; Trichopoulou et al., 2014). Water and herbal infusions are the drinks of choice, while wine consumption is moderate (Bach-Faig et al., 2011; Trichopoulou et al., 2014).

Large-scale epidemiological studies, such as the Seven Countries study, have correlated diet and lifestyle with cardiovascular diseases (CVD) risk factors in cohorts from different countries (major conclusions found in Seven Countries Study, 2020). One of the main findings of this long-term scientific research was that people who adhered to the Mediterranean diet, such as the Greek cohorts, and had a frequent consumption of olive oil, had a low all-cause mortality rates and low coronary heart disease death rates (Keys et al., 1986). This fact did not happen to populations that followed a Western diet, which is nutritionally more deficient and more processed, having a greater consumption of red meat, dairy products, carbohydrates, and other fats of vegetable or animal origin. Olive oil, then, began to be perceived as a functional food, due to its high content of monounsaturated lipids. Other large-scale randomized trials, in the scope of the PREDIMED study (main findings revised by Perona, 2014), have found a correlation between the Mediterranean diet, where olive oil plays a pivotal role, with a lower incidence on CVD, but also on the prevention of chronic noncommunicable diseases such as diabetes, cancer, hypertension and neurodegenerative diseases.

The beneficial effects of olive oil consumption in human health have been thoroughly researched, mainly because of the lipid components, including lipid vitamin E (tocopherols) (Flori et al., 2019). Meanwhile, some of the minor constituents of olive oil, such as polar phenolic compounds, have been found to have high antioxidant activity, with several positive effects from a biological point of view (Visioli et al., 1998). The phenolic compounds of olive oil have been studied intensively (as reviewed by Bulotta et al., 2014; Farràs et al., 2020; Martín-Peláez et al., 2013; Servili et al., 2014; Souza et al., 2017). However, many randomized clinical trials that aimed to evaluate the health effects of olive oil have been designed in the context of the Mediterranean diet. This design leads to erroneous conclusions about the impact of olive oil on health since various foods included in this dietary pattern have a high concentration of bioactive compounds, including phenolic compounds. Thus, synergies between the different components of the diet are implicit, and the results of these trials can be biased. As such, this chapter will only focus on specific scientific research on olive oil or its components.

2. Production

The European Union is the foremost producer, consumer, and exporter of olive oil. Olive oil is produced mainly by Spain, Italy, Greece, and Portugal, countries of the European Union (EU), and by Tunisia, Turkey and Morocco (non-EU countries) (International Olive Council, 2019a). Due to the acknowledged health benefits of olive oil, olive groves have

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expanded to the five continents, with olive varieties adapted to different climates, cultivated in intensive farming regimes, intending to produce a large number of olives with a high oil yield in less time. In the 2019/2020 campaign, the production of olive oil in the EU was about 2 million tons and, in the world, it was around 3 million tons (European Commission, 2020). Although the worldwide production of olive oil is limited, it has been increasing. Olive oil production is likely to grow 1.1% per year until 2030, in the EU (European Commission, 2019a), and its global market value is estimated at 17.2 billion US dollars by 2028 compared to 11.2 billion US dollars in 2018 (Shahbandeh, 2019).

The high demand for virgin olive oil is because it is a natural food, rich in monounsaturated lipids, and in compounds with cardioprotective, antioxidant, antiinflammatory, and antitumor activity. Several studies over the last decades in cell lines, animals and humans have provided such evidence, as concluded in a recent international consensus report on the health benefits of virgin olive oil (Gaforio et al., 2019). At the same time, demand often exceeds supply, leading to fraud and adulteration of olive oil in the marketing chain. This is a problem that science cannot yet effectively control (Conte et al., 2020), and which is on the agenda of food safety regulators (European Commission, 2019b).

The quality of olive oil is strictly regulated by physical and chemical parameters and by sensory criteria defined by different international standards such as the Codex Alimentarius, the International Olive Council (IOC), and the EU. The physical and chemical quality parameters evaluate the degradation index of the oil. They include the percentage of free acidity, the peroxide index, and the ultraviolet extinction coefficients. Also, the fatty acid content and sterols composition assess its authenticity (European Commission, 1991). Olive oil is the only food product in the world that undergoes a sensory analysis by qualified panels of tasters who assess whether an olive oil should belong to a particular quality category (International Olive Council, 2018). There are, therefore, different categories of olive oil depending on their quality (International Olive Council, 2019). Virgin olive oil is olive oil extracted directly from olives, only by physical means without using any chemical process or solvents, at a maximum temperature of 28°C. Virgin olive oils are a natural product whose lipid degradation was minimized during production and whose sensory attributes (odor and flavor) are intact or practically unchanged. Usually, these oils have a shelf life of about 1 year. Extra virgin olive oil (EVOO) represents the top category of olive oils and belongs to the category of virgin olive oils. EVOO have no sensory defect of any kind (e.g., fusty, musty, winey, acid-sour, rancid, among others) and their degree of lipid degradation is up to 0.8% of free acidity. EVOO is acknowledged worldwide for its beneficial health effects derived from its unique constituents. The high content of oleic acid (C18:1*n*-9) and a wide variety of phenolic compounds distinguish EVOO from other common vegetable oils (e.g., sunflower, soybean, palm, or rapeseed oils), which are extracted with aggressive chemical processes, using hexane and high temperatures. Other lower grade olive oils include lampante olive oil, olive pomace oil, or olive oil. Olive oil refers to a mixture of virgin olive oil and refined olive oil. Refined olive oil has undergone a chemical process that removes unpleasant odors and flavors, and whose high acidity makes it unsuitable for consumption (International Olive Council, 2019).

Olive oil is obtained through a sequence of procedures that begins with harvesting the olives, the transport to the olive mill, and short-term storage of the fruits. The conditioning of the olives after harvesting, during transport, and storage in the mill before oil extraction is crucial. It will reduce the endogenous hydrolytic and oxidative actions by lipases and lipoxygenases, respectively. At the olive mill, the extraction of olive oil comprises three main stages: firstly, the preparation of the olive paste, with milling or crushing followed by malaxation; second, the separation of the solid phase, by pressing or centrifugation; third, the separation of the liquid phase, by decanting and or centrifugation. Finally, the olive oil is stored, conserved, and packaged (Servili et al., 2012). Currently, there are three industrial systems for obtaining virgin olive oil: the classic pressure extraction system, and two centrifugation systems, the continuous two-phase system and the continuous three-phase system, as schematically shown in Fig. 4.1.

Malaxation is a specific process for the production of virgin olive oil and consists of mixing the olive paste slowly and at low temperature (28°C maximum) to avoid excessive lipid oxidation. This process promotes the coalescence of oil droplets from the olive's mesocarp leading to a high extraction yield during the subsequent centrifugation. The duration and temperature of malaxation significantly affect the intensity of the sensory attributes of virgin olive oil, along with the content of phenolic compounds, mainly secoiridoids (Angerosa et al., 2001; Inarejos-García et al., 2009). Malaxation under vacuum conditions significantly increases the total phenolic content compared to malaxation at atmospheric pressure (Miho et al., 2020).

The main goals of the current industrial production technologies of high-quality virgin olive oils are to meet the regulated criteria, to improve the sensory quality, and the health properties of the product, to design olive oils with a high content of bioactive compounds. So, it is necessary to understand how the volatile and hydrophilic phenolic compounds present in olives are formed and degraded. These processes are closely related to the endogenous enzymatic activity of the olive fruit, and their concentration in the oil will be affected by the operating conditions of the mechanical extraction process. Thus, the crushing and malaxation conditions are considered the most critical points in the olive oil production

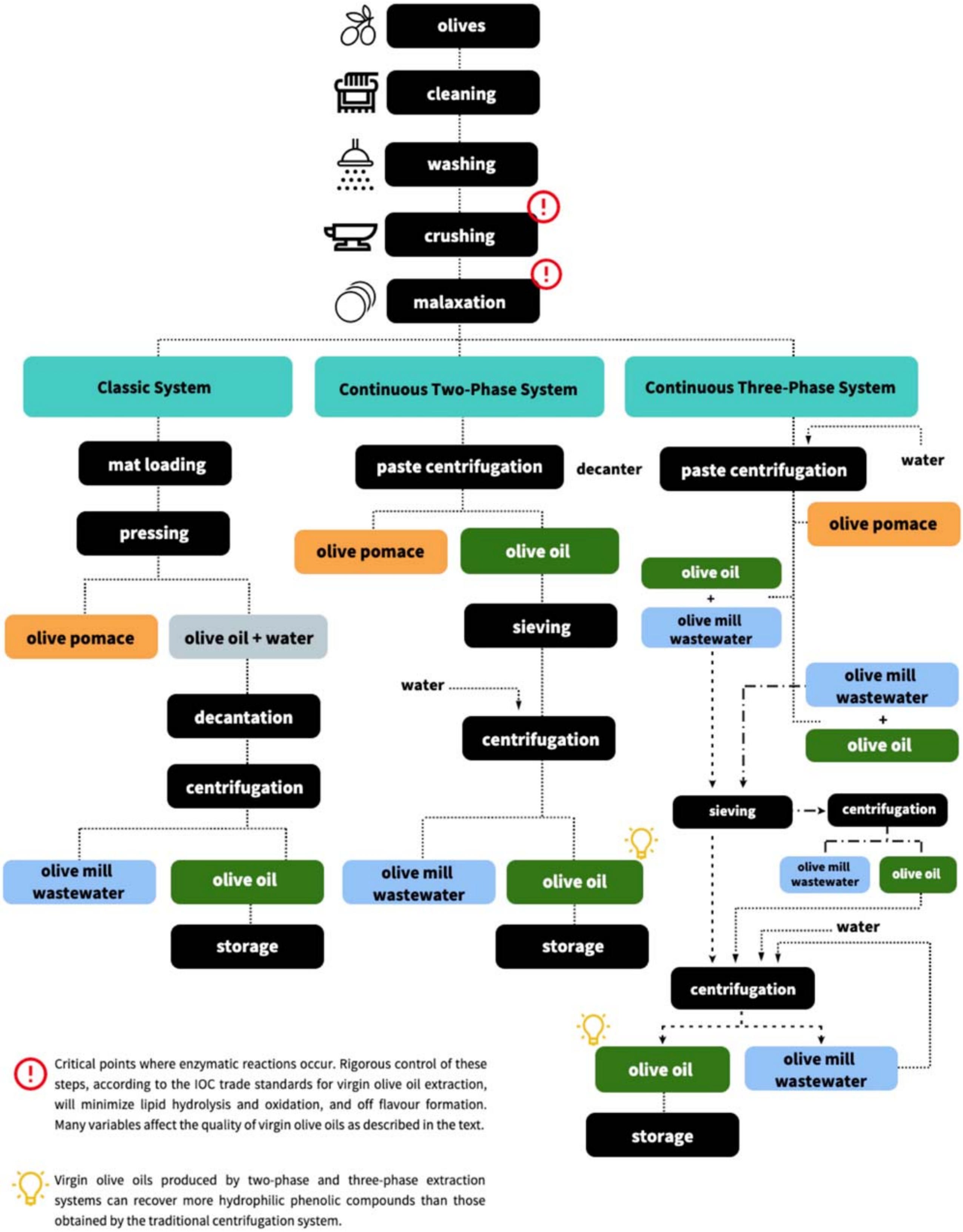

FIGURE 4.1 Flow diagram of current virgin olive oil production systems.

process concerning chemical changes, such as enzymatic action, and the consequent influence on their lipid, aromatic, and phenolic constituents (reviewed by Clodoveo, 2012). The most relevant volatile compounds in high-quality virgin olive oils are the C5 and C6 compounds, particularly the linear saturated and unsaturated esters, alcohols, and aldehydes (Angerosa et al., 2001; Servili et al., 2003). These compounds are produced via lipoxygenases from polyunsaturated fatty acids (PUFAs). Their profile depends on the level and activity of each enzyme involved in the lipoxygenase pathway. Furthermore, two-phase decanters, unlike the three-phase system, which imply adding water, provide virgin olive oils with a higher phenolic concentration since they use a minimal amount of water (Stefano et al., 1999). Thus, the loss of hydrophilic phenolic compounds in the vegetation water is significantly reduced in this system, preventing a higher loss of these compounds which would otherwise occur.

Emerging technologies aim to increase the quality, extraction yield, and working efficiency of the olive mill, such as pulsed electric field (PEF), microwaves, and ultrasound. PEF is an innovative technology that can be applied to the olive paste to significantly improve the extraction yield (Puértolas & de Marañón, 2015; Veneziani et al., 2019). It also increases the oil content in phytosterols, tocopherols, and phenolic compounds (Puértolas & de Marañón, 2015), mainly hydrophilic phenols (up to 14.3%) (Veneziani et al., 2019). It does not produce any off-flavor or sensory changes, so it does not compromise the quality parameters. PEF allows a reduction in malaxation temperature to 15°C without impairing the extraction yield and also saves energy (Abenoza et al., 2013). Microwave treatment can replace traditional malaxation without interfering in extraction yields, which will increase by exposing the olive paste to a megasonic field (Leone et al., 2017). On the other side, the ultrasound-assisted extraction applied to the industrial extraction of virgin olive oils has shown to be a time-saving, environmentally friendly process, which does not compromise the synthesis of aromas and does not favor enzymatic degradation reactions (reviewed by Clodoveo, 2019). This technology has been under intense research. There is a work in progress toward a product ready for the market. Besides technological variables, other variables related to the extraction and storage of virgin olive oils are detrimental to the chemical profile and the quality of the final product.

Factors that influence the chemical profile of virgin olive oils include edaphological and climatic variables (soil, altitude, climate), agronomic and field-related variables (cultivation techniques, phytosanitary conditions, harvesting methods), botanical variables (olive varieties), and geographical variables (olive grove location). Other factors of extreme importance to minimize the hydrolysis and oxidation of olive oil include the following: the presence of oxygen (promotes oxidation); light (ultraviolet radiation triggers auto-oxidation reactions); temperature (high temperature promotes lipid degradation, it cannot exceed 28°C in the extraction of virgin olive oils, and for olive oil conservation the ideal range is between 15 and 18°C); humidity (absence of water from residual vegetation is crucial); and solid residues (promote fermentation in unfiltered or nondecanted olive oil). The olive oil profile changes due to the drastic reduction of compounds derived from the lipoxygenase pathway, during storage. Changes are also due to the formation of new volatile compounds, which give rise to unpleasant sensory defects. Therefore, the oil storage tanks must be made of stainless steel, or vitrified iron and the headspace should be filled with an inert gas to avoid oxidation at the air/oil interface.

Hence, virgin olive oils must be produced no later than 48 h after harvesting the olives. The olives should be in perfect health conditions and taken directly to the olive mill in containers that allow them to aerate. The olive oil is extracted at low temperature. Appropriate quality management practices in the olive mills and oil packaging plants are of high importance. Depending on the olive variety and the type of product to be obtained, oleologists evaluate the virgin olive oil batches considering the compliance with quality parameters. Thus, they can produce monovarietal olive oils (made with only one olive variety) or blends (mixtures of different olive cultivars) that should be well-balanced in terms of the positive sensory attributes, that is, fruity, bitter and pungent flavor, but it also depends on the market niche. Unlike odor and taste, the color, opacity and thickness of virgin olive oils do not interfere with their quality but distinguish them in the chemical profiles. The intensity of the fruity, bitter, and pungent flavors is characteristic of each virgin olive oil, and they define a unique sensory profile. Therefore, it is relevant to know the chemical composition of virgin olive oils and understand why their constituents, as a whole, make this food so valuable from a nutritional and functional point of view.

3. Chemical composition

Olives are composed of water (70%−75% of the mesocarp's weight), lipids (up to 15% in green olives and up to 30% in ripe olives), proteins (1.5%−2.2% of the fruit's weight), free phenols, and their glycosides (1%−3%) (Bianchi, 2003). Free organic acids (1.2%−2.1% of the pulp's dry weight) include oxalic, succinic, malic, and citric acids, together with a small proportion of free fatty acids. The olive pulp also contains sugars such as glucose, fructose, sucrose, and mannitol (3.5%−6.0%) (Bianchi, 2003). The endocarp, or stone, represents 18.0%−22.0% of the total weight of the fruit. The seed is the soft part of the olive, representing 2%−4% of its mass, and it has a considerable amount of lipids (22%−27%)

(Bianchi, 2003). Most of the oil is in the mesocarp as lipid droplets that form during olive ripening. A phospholipid monolayer surrounds the lipid droplets. A little oil (about 5%) comes from the seed.

The chemical composition of olive oil is substantially different from that of the olive, due to the extraction process and to all the variables mentioned above. Olive oil comprises a large glyceride fraction composed of triglycerides (TG, c. 97%), of which the most abundant is triolein (trioleoylglycerol, 40%−60% of total TG). The fatty acids that make up the oil are mostly esterified in TG and other lipids, and a small part is in the free form (up to 2% in virgin olive oils) resulting from lipid hydrolysis. The most abundant types of fatty acids are monounsaturated fatty acids (MUFAs, 72%), mostly C18:1*n*-9 (55%−83%), saturated fatty acids (14%), mostly palmitic acid (C16:0%, 7.5%−20%), and PUFAs (14%), predominantly linoleic acid (C18:2*n*-6, 3.5%−21%) (Aparicio-Ruiz, 2013). The nonglyceridic or unsaponifiable fraction of virgin olive oils (c. 3%) contains several compounds in low concentrations. Many of these components are highly bioactive and are therefore particularly important in terms of health properties (Flori et al., 2019). Besides, some of them provide the sensory properties to olive oil and are indicators of the quality and authenticity, as summarized below. Hydrocarbons are mainly represented by squalene that accounts for a large proportion of the terpenic hydrocarbons in olive oil (200−8260 ppm) and may be an indicator of the quality and origin of olive oil. Triterpenoids are polycyclic compounds originating from squalene and have significant biological effects as antioxidant, antitumor, cardioprotective, and antiinflammatory activities (Sánchez-Quesada et al., 2013). In olive oil, relevant triterpenoid compounds include maslinic acid, oleanolic acid, uvaol, 24-Methylene-cycloartanol, cycloartenol, and α- and β-amyrins. These compounds are also important in detecting low-grade olive oils, as erythrodiol in detecting olive pomace. Tocopherols, in particular α-tocopherol or vitamin E (10.2−208 ppm), are natural antioxidants in olive oil, but several other health-promoting bioactivities have also been attributed to the vitamin E and olive oil tocopherols in general, including antiinflammatory and antithrombotic cardioprotective properties (Flori et al., 2019). Aliphatic and aromatic alcohols, and phytol and geranylgeraniol, respectively, are found in olive oil. Pigments as chlorophylls and carotenoids account for six to 94 ppm. β-carotene (0.15−0.67 ppm), the biological precursor of vitamin A, stands out (Aparicio-Ruiz, 2013; Jimenez-Lopez et al., 2020). Aromatic compounds are low molecular weight compounds (less than 300 Da) that volatilize at room temperature and are responsible for the different odors felt when smelling virgin olive oils. Volatile compounds mainly result from the oxidation of PUFAs and more than a 100 volatiles could be detected in virgin olive oil. The most abundant volatiles (80% of total) found in olive oil include C6 aldehydes such as hexanal, *cis*-3-hexanal, and *trans*-2-hexanal, alcohols such as hexanol, *cis*-3-hexenol, and *trans*-2-hexenol, and esters such as hexyl acetate and *cis*-3-hexenyl acetate (Angerosa et al., 2000; Conde et al., 2008; Sánchez & Harwood, 2002). C6 volatile compounds are the major contributors to the green/fruity odor of virgin olive oils. The accurate profiling of aroma compounds is relevant to establish the interplay between chemical compounds and sensory analysis.

Sterols are the analytical fingerprint that enables the genuineness of olive oil to be checked. The IOC trade standard sets the limits for sterol composition. The most abundant sterol in olive oil is β-sitosterol (530−2639 ppm), which, together with other phytosterols, has plasma cholesterol-lowering effect. Monoacylglycerols and diacylglycerols (0.25% and 1%−2.8%, respectively) are minor lipid components found in virgin olive oils, as are polar lipids (Aparicio-Ruiz, 2013; Jimenez-Lopez et al., 2020).

Polar lipids in olive oil include several classes of compounds such as phospholipids (up to 157 ppm) (Hatzakis et al., 2008) and glycolipids. They have been poorly studied at the molecular level not only because of their trace amounts but also due to their diversity and complexity of analysis (Alves et al., 2018). Besides having great bioactive potential, polar lipids can represent putative markers of identity and traceability for olive oil. They have also been associated with its oxidative stability. Advances in olive oil lipidomics (Alves et al., 2016) and the olive fruit lipidomics (Alves et al., 2019) have provided new insight into their complex polar lipid composition, and subsequently, on their potential contribution to the health properties of virgin olive oils. For example, polar lipid extracts derived from EVOO, have shown strong antithrombotic and antiatherogenic health benefits, both *in vitro* in platelets (Karantonis et al., 2002), but also *in vivo* in models of hypercholesterolemic rabbits (Karantonis et al., 2006).

Finally, phenolic compounds found in virgin olive oils are secondary metabolites that comprise a wide diversity of structures with several reported biological activities (revised by Bendini et al., 2007). They have shown noticeable antioxidant properties, justifying their role in the oxidative stability of virgin olive oil. Some of them confer the characteristic bitter and pungent flavor to virgin olive oils. Total phenolic compounds account for c. 100 mg kg^{-1} up to almost 1000 mg kg^{-1} in virgin olive oils and each virgin olive oil has a different phenolic profile resulting after extraction, that starts changing during storage. These phenolic compounds can be grouped into secoiridoids, phenolic alcohols, phenolic acids, flavonoids, lignans, and hydroxy-isochromans. The most common secoiridoids are demethyloleuropein, oleuropein, ligstroside, and their aglycones. Crushing and malaxation trigger enzymatic reactions on secoiridoids, catalyzed by endogenous β-glucosidases, yielding secoiridoid aglycones. Some secoiridoid compounds, as ligstroside or oleuropein glucoside, suffer spontaneous hydrolysis. Ligstroside undergoes two hydrolysis reactions to give rise to hydroxytyrosol, a compound with high antioxidant activity. Oleuropein is hydrolyzed to give rise to oleuropein aglycone. The bitterness of virgin olive oils is due to the secoiridoids, especially the dialdehydic form of oleuropein aglycone. Major phenolic alcohols found in virgin olive oil are tyrosol (*p*-hydroxyphenyl ethanol, *p*-HPEA) and hydroxytyrosol (2-[3,4-dihydroxyphenyl] ethanol, 3,4-DHPEA) (Aparicio-Ruiz, 2013; Bendini et al., 2007; Jimenez-Lopez et al., 2020). Hydroxytyrosol, tyrosol and their secoiridoid derivatives account for approximately 90% of total phenolic compounds in virgin olive oil (de la Torre-Carbot et al., 2005). The concentration of both compounds tends to increase along the storage process due to the hydrolysis of secoiridoids. The dialdehydic forms of elenolic acid linked to tyrosol (2-(4-Hydroxyphenyl)ethyl (4E)-4-formyl-3-(2-oxoethyl)hex-4-enoic acid, *p*-HPEA-EDA), referred to as (+/−)-Oleocanthal, or linked to hydroxytyrosol (3,4-dixydroxyphenylethanol elenolic acid, 3,4-DHPEA-EDA), also known as oleacein, and their secoiridoid derivatives (ligstroside aglycons) are the most abundant phenolic compounds in EVOO, reaching up to 90% of the total phenolic content (Servili & Montedoro, 2002). Common phenolic acids include hydroxybenzoic, vanillic, caffeic, ferulic, gallic, *p*-Coumaric, o-Coumaric, syringic, and sinapic acids. Major flavonoids comprise luteolin, apigenin and several of their derivates. Main lignans are (+)-pinoresinol and (+)-1-acetoxypinoresinol. Finally, isochromans (mainly 1-phenyl-6,7-dihydroxy-isochroman and 1-(30-methoxy-40-hydroxy)phenyl-6,7-dihydroxy-isochroman) exist in low concentration. Their concentration rises during extraction due to the hydrolytic process that originates carbonyl compounds and hydroxytyrosol, both being isochromans derivatives (Aparicio-Ruiz, 2013; Bendini et al., 2007; Jimenez-Lopez et al., 2020). Some of the phenolic compounds from virgin olive oils with reported health benefits are depicted in Fig. 4.2.

4. Health claim

There is a health claim on olive oil polyphenols approved by the European Food Safety Authority (EFSA) (EFSA, 2011), that is regulated by the European Commission (Regulation No. 432/2012). According to this claim, olive oil polyphenols contribute to the protection of blood lipids from oxidative stress. The claim applies only to olive oils containing at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of oil (i.e., 250 mg/kg). This claim has raised several questions regarding different interconnected viewpoints, as presented next. In the EFSA document, the distinct olive oil categories are not explicitly defined. Olive oil, as referred in the claim, should only designate high-quality fresh virgin olive oils containing significant amounts of oleuropein/ligstroside aglycones and derivatives (Conte et al., 2020). Likewise, the term polyphenols is not accurate to match the basic structure of secoiridoids assigned to in the claim (Conte et al., 2020). Commercial top-quality olive oils, that is, virgin olive oils, usually have lower amounts of the specific phenolic compounds related to the claim. Thus, the minimum concentration of 250 mg/kg is substantially high for

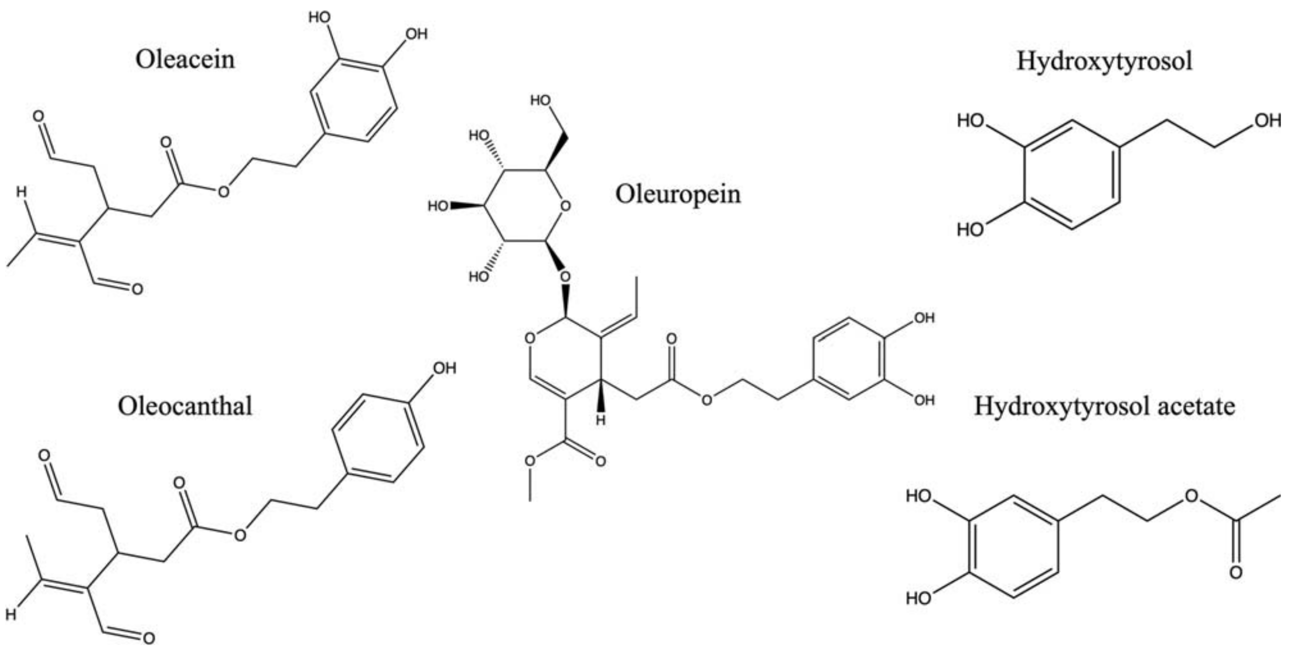

FIGURE 4.2 Main bioactive phenolic compounds of virgin olive oil.

a virgin olive oil to be qualified for the claim. This is a challenge for the olive oil industry since the phenolic content in most of the marketed olive oils does not reach this minimum (Farràs et al., 2020; López-Huertas et al., 2020). Thus, a high intake of olive oil would be necessary to meet the prescription of hydroxytyrosol established by the EFSA, which is problematic within the context of a balanced diet (Robles-Almazan et al., 2018). An alternative to counteract this issue is to enrich naturally high-phenolic virgin olive oils with complementary sources of phenolic compounds. It would increase the daily intake of these compounds without increasing the caloric intake. This approach has been used in dietary interventions of many clinical trials (Farràs et al., 2015; Fernández-Castillejo et al., 2017). Besides, there are only a few olive varieties that may contain such amounts of health-promoting specific compounds. Olive oils containing the minimum required hydroxytyrosol levels and its derivatives to comply with the EFSA's health claim have been shown to depend on the olive varieties and their ripening stage (López-Huertas et al., 2020). Furthermore, the initial phenolic profile will influence the health benefits of EVOOs during storage. As referred previously, several modifications occur during the storage of olive oil. For instance, some phenolic compounds such as oleacein and oleocanthal can decrease the concentration of total phenolic compounds in EVOOs stored for 12 months in the dark. At the same time, hydroxytyrosol and oleocanthalic acid significantly increased in the stored EVOOs (Castillo-Luna et al., 2021). Moreover, there is a lack of standardized analytical methods to quantitatively determine the phenolic compounds belonging to the group of hydroxytyrosol/tyrosol and its derivatives impacting on the minimum limit defined by the health claim (Conte et al., 2020). The simplification of the current analytical methods would involve hydrolysis of the bound forms of hydroxytyrosol and tyrosol, and quantification of their total free forms (Conte et al., 2020; Tsimidou et al., 2018). A validated protocol based on ultra-high-performance liquid chromatography has been proposed for determining the total hydroxytyrosol and tyrosol content (Tsimidou et al., 2019). Despite the claim approval, the unclear terminology and, most importantly, the absence of an appropriate analytical protocol for determining the bioactive compounds are the major current problems upon its implementation.

5. Bioavailability and bioaccessibility

Bioaccessibility refers to a fraction of a compound accessible to be absorbed by intestinal cells after its release from a food matrix in the gastrointestinal tract during digestion. The *in vitro* bioaccessibility of nutritional and bioactive compounds in olive oil digestates is higher compared to other vegetable oils (Alberdi-Cedeño et al., 2020). The *in vitro* digestion of olive oil yielded a high degree of lipolysis, releasing monoglycerides and fatty acids to a great extent. The oxidation extent was pretty small. The concentration of minor components such as squalene, some sterols, terpenes, and sesquiterpenes was not modified during the *in vitro* digestion, being bioaccessible after digestion. Enrichment of olive oil with antioxidant phenolic compounds did not affect the extent of lipolysis but reduced the oxidation degree to minimum values (Alberdi-Cedeño et al., 2020). The antioxidant activity of phenolic compounds from olive oil was negatively affected by the digestion procedure *in vitro*, so only a fraction of these compounds could be considered bioaccessible (Dinnella et al., 2007). Also, a significant loss of phenolic compounds occurred during the digestive process, between the buccal and duodenal steps, so the bioaccessibility of phenolic compounds from virgin olive oil was low (Quintero-Flórez et al., 2018). On the other hand, a considerable recovery of hydroxytyrosol and tyrosol was due to the hydrolysis of secoiridoid derivatives. Also, differences in the phenolic composition of virgin olive oils affect the bioaccessibility of the phenolic compounds. This can be attributed to the initial concentration, stability to gastrointestinal conditions, or the lipid matrix. Besides, some phenolic compounds remaining in the nonabsorbable fraction reach the colon and colonocytes absorb them. Thus, further research is required to determine the biotransformation of the nonabsorbable fraction phenolic compounds by the colonic microbiota to metabolites with potentially protective effects *in vivo* (Quintero-Flórez et al., 2018). Another *in vitro* study has shown that flavonoids (cyanidin and luteolin equivalents) are the most affected compounds by the digestion process (Rocchetti et al., 2020). Tyrosol equivalents recorded high bioaccessibility values (average of 66%) during the pancreatic phase. Most of the phenolic compounds showed a relatively low bioaccessibility toward gastric and intestinal phases. Secoiridoids concentration declined during the digestion process, contrarily to hydroxytyrosol (Rocchetti et al., 2020). Further studies should confirm the bioaccessibility of phenolic compounds and the bioavailability of hydroxytyrosol *in vivo*.

Bioavailability of a nutrient refers to the fraction that was digested and absorbed, reaching systemic circulation after oral administration. It is available for the metabolic functions of the body. Bioavailability of many phenolic compounds of virgin olive oil has been extensively studied and reviewed throughout the years (de la Torre, 2008; Deiana et al., 2018; Vissers et al., 2004), in particular hydroxytyrosol (Robles-Almazan et al., 2018). Phenolic compounds of virgin olive oil are well-absorbed by humans (40%−95%), in a dose-dependent manner, mainly by the small intestine (Deiana et al., 2018). Conjugated forms represent most of the phenolic content found in plasma and urine, namely glucurono-conjugates

(Vilaplana-Pérez et al., 2014). At least 5% are excreted in urine as tyrosol and hydroxytyrosol (Vissers et al., 2004). In general terms, after ingestion of virgin olive oil, phenolic compounds and their metabolites concentrate in the intestinal lumen, possibly playing a significant action there. Some complex phenolic compounds reach the intestine, being directly absorbed or metabolized during absorption. Other phenolic compounds are submitted to high biotransformation in the gastrointestinal tract. These bioactive compounds are concentrated in the small intestine and the large intestine. They have been considered to contribute positively to the maintenance of homeostasis of the intestinal epithelium (Deiana et al., 2018). Therefore, these compounds may help to counteract the onset or delay the progression of several inflammatory diseases (Deiana et al., 2018). Hydroxytyrosol is well-absorbed by the gastrointestinal tract. The absorption mainly occurs by passive transport in the small bowel and the colon. However, its bioavailability is low because the gut and liver metabolism leads to the formation of the sulfate and glucuronide conjugates that provide minimal concentrations of its free form in body fluids (de la Torre, 2008). Hydroxytyrosol has an intense and rapid metabolism. The first stage of its metabolism occurs inside enterocytes, and subsequently, in the liver. Gut microbiota has been identified as a remarkable modulator of absorption both of hydroxytyrosol and its metabolites. The kidneys mainly excrete conjugated catabolites (Visioli et al., 2003). The time required for the complete elimination of hydroxytyrosol and its metabolites from the body is approximately 6 h in humans (Robles-Almazan et al., 2018). Hydroxytyrosol is also a dopamine metabolite. As body fluids contain both endogenous and exogenous sources of hydroxytyrosol, it is difficult to assign a contribution from the virgin olive oil intake (de la Torre, 2008). There are also controversial data on the biological activities of hydroxytyrosol and its conjugated forms. The amount of free hydroxytyrosol in plasma and urine is almost undetectable, so the biological activity is assigned to hydroxytyrosol metabolites (Vilaplana-Pérez et al., 2014). Besides, a correlation could not be made between antioxidant phenolic compounds from virgin olive oil as hydroxytyrosol and protection of low-density lipoprotein-cholesterol (LDL-C) against oxidation because the content of those compounds found in body fluids was too low to produce a measurable effect (Vissers et al., 2004). Despite the large body of evidence on the bioavailability of phenolic compounds from virgin olive oil, there is a consensus that more research is needed to comprehend their antioxidant activity *in vivo* and the potential health benefits derived from their consumption in humans.

6. Data from *in vitro* and *in vivo* experiments

Numerous *in vitro* and *in vivo* studies have been carried out to assess the effects of olive oil and its bioactive compounds in the context of CVD and inflammation. This evidence consolidates the previous knowledge on the cardioprotective, vasculoprotective, antioxidant, and anti-inflammatory effects of olive oil consumption on cellular and animal models, in particular of EVOO (Gaforio et al., 2019; Jimenez-Lopez et al., 2020; Nocella et al., 2018). It also allowed understanding the effect of a specific bioactive compound present in this food (Bermudez et al., 2011; Segura-Carretero & Curiel, 2018; Souza et al., 2017).

The protective effects of EVOO and some of its bioactive components, such as oleic acid, polar lipids, vitamin E, total phenolic compounds, hydroxytyrosol, and oleacein have been studied in the context of CVD (Table 4.1). Studies in rats fed a diet supplemented with 10% olive oil have demonstrated the ability of dietary oleic acid to reduce body weight, lower TG levels as well as increase high-density lipoprotein-cholesterol (HDL-C) and omega-3 PUFA in plasma, thereby reducing the risk of CVD (Nogoy et al., 2020). The low incidence of CVD associated with the consumption of olive oil in Mediterranean countries is also expected to be partly related to platelet-activating factor (PAF) antagonists (Karantonis et al., 2006). Glycerophospholipids and glycolipids (polar lipids) in olive oil have demonstrated *in vitro* antithrombotic activity by promoting potent inhibition against PAF-induced platelet aggregation through competitive binding to the cellular PAF receptor (Karantonis et al., 2008). In rabbits fed with olive oil or their polar lipids, the concentration of PAF-acetylhydrolase in the blood increased platelet aggregation. There was less oxidation in the plasma, a reduction in the thickness of the lesions, and the vessel walls retained their elasticity (Karantonis et al., 2006). Several thrombin and PAF inhibitors and weak PAF-type agonists have been identified in olive oil (Karantonis et al., 2002). Inhibition of PAF activity contributes to increasing the permeability of the endothelium, plays an essential role in thrombotic complications, induces the release of active oxygen species, is produced during the oxidation of LDL-C, and contributes to this same oxidation (reviewed by Demopoulos et al., 2003).

Tocopherols exert a preventive activity against reactive oxygen species (ROS) in biological systems. Several health claims are associated with a positive effect of the consumption of tocopherols on cell aging, certain types of cancer, maintenance of the immune system, and CVD (EFSA, 2010). Vitamin E is a generic name used for a group of fat-soluble plant compounds which include tocopherols and tocotrienols. Virgin olive oil contains α-, β-, and γ-tocopherols, and α-tocopherol accounts for more than 95% of total tocopherols ranging from 50 to 300 mg/kg (Beltrán et al., 2010). α-tocopherol is the most common form of vitamin E in human blood and tissues and exhibits the most significant

TABLE 4.1 ***In vivo*** **and** ***in vitro*** **studies on the effect of olive oil and its active compounds on cardiovascular diseases and inflammation.**

Type of olive oil or active compound	Type of study	Model of disease	Effects	References
Olive oil[a]	*In vivo*	CVD risk in diabetic offspring	↓ deposition of collagen IV and fibronectin in the heart, ↓ prooxidant markers and apoptosis	Roberti et al. (2020)
		Gestational diabetes mellitus	↓ placental MMP2	Capobianco et al. (2018)
		Liver inflammation	↑ iNOS expression	Meidan et al. (2018)
		Metabolic syndrome and inflammation	↑ cannabinoid-2 receptor and NOS1 gene and protein expression	Notarnicola et al. (2016)
		Pressure ulcers	↓ production of pro-inflammatory cytokines, ↓ inflammatory cell infiltration, ↑ angiogenesis	Donato-Trancoso et al. (2016)
Extra virgin olive oil	*In vitro*	Antioxidant effect	↓ NOX2 activation and H_2O_2 production	Carnevale et al. (2018)
		Inflammation	Restoring normal levels of membrane fluidity and counteracting redox imbalance	Bordoni et al. (2020)
		Endothelial dysfunction and atherosclerosis	↑ microRNAs regulating genes involved in atherosclerosis	Santiago-Fernandez et al. (2020)
		Systemic erythematosus lupus	↑ Nrf-2 and HO-1 protein expression, amelioration of activation of JAK/STAT, MAPKs and NF-kB pathways, ↓ production of pro-inflammatory cytokines, ↓ plasma/serum levels of cytokines, ↓ histological damage	Aparicio-Soto et al. (2016)
		Hepatotoxicity	↓ plasma/serum levels of cytokines, ↓ apoptosis in injured cells, ↓ histological damage	Elgebaly et al. (2018)
		Nonalcoholic fatty liver disease	Activation of PPAR-α and Nrf-2 transcription factors, inactivation of NF-kB and SREBP-1c	Hernández-Rodas et al. (2017)
			↓ plasma/serum levels of cytokines, ↓ inflammatory cell infiltration	Jurado-Ruiz et al. (2017)
		Inflammatory bowel disease	↓ production of pro-inflammatory cytokines, ↓ histological damage	Cariello et al. (2020)
	In vivo	Alzheimer's disease	↑ synaptophysin, amelioration of behavioral deficits and activation of cell autophagy	Lauretti et al. (2017)
		Rheumatoid arthritis	↑ Nrf-2 and HO-1 protein expression, amelioration of activation of JAK/STAT, MAPKs and NF-kB pathways, ↓ production of pro-inflammatory cytokines, ↓ plasma/serum levels of cytokines, ↓ histological damage	Rosillo et al. (2016)
	Ex vivo	Metabolic syndrome	Transcriptome modulation of cardiometabolic disease and cancer-associated pathways	D'Amore et al. (2016)

Continued

TABLE 4.1 ***In vivo*** **and** ***in vitro*** **studies on the effect of olive oil and its active compounds on cardiovascular diseases and inflammation.—cont'd**

Type of olive oil or active compound	Type of study	Model of disease	Effects	References
Oleic acid	*In vitro*	Adipose tissue inflammation	Attenuation of JNK-mediated PPARγ suppression	Scoditti et al. (2015)
	In vivo	CVD risk	↓ body weight and triglycerides, ↑ plasma HDL-C and omega-3 PUFA	Nogoy et al. (2020)
		Sepsis	↑ IL-10, ↓ production of pro-inflammatory cytokines, ↓ systemic corticosterone, ↓ inflammatory cell infiltration, ↓ bacterial load, and restored PPARγ expression	Medeiros-de-Moraes et al. (2018)
Phenolic compounds[b]	*In vitro*	Intestinal inflammation	↓ production of pro-inflammatory cytokines, and modulated MAPK—NF—kB pathway	Serra et al. (2018)
			Regulation of IL-8 expression by transcriptional and posttranscriptional mechanisms	Muto et al. (2015)
		Inflammation	↓ visfatin expression, ↓ production of pro-inflammatory cytokines, ↑ IL-10	Martin et al. (2019)[a]
		Oxidative stress	↓ ROS production	Presti et al. (2017)
	In vivo	Hypercholesterolemia	↓ malondialdehyde and TNF-α	Katsarou et al. (2016)
		Hypertension	↓ SBP, cardiac hypertrophy, angiotensin II, total-Chol, urinary endothelin-1, oxidative stress biomarkers, ↑ *ex vivo* aortic endothelial dysfunction, ↔ pro-inflammatory cytokines	Vazquez et al. (2019)
		Adipose tissue inflammation	↓ nitrites/nitrates in the aorta, ↔ atherosclerosis	Luque-Sierra et al. (2018)
		Inflammatory bowel disease	↓ TNF-α gene expression, total-Chol, HMGCR, ↑ PPAR-α hepatic gene expression	Bigagli et al. (2019)
		Nonalcoholic fatty liver disease	↓ liver inflammation and mitochondrial oxidative stress, and restore insulin sensitivity	Lama et al. (2017)
		Rheumatoid arthritis	↓ production of pro-inflammatory cytokines, ↓ COX-2 activity, ↓ MAPK and NF-kB signaling pathways	Rosillo et al. (2019)
	Others	Atherosclerosis *in vitro* and *ex vivo*	↑ LDL and HDL resistance to oxidation, reverse Chol transport, ↑ ABCA1 protein expression	Berrougui et al. (2015)
		Immune response *ex vivo*	↓ production of pro-inflammatory cytokines, ↓ ROS production and MAKP phosphorylation	Souza et al. (2017)
		Systemic erythematosus lupus in humans and animals	↓ production of pro-inflammatory cytokines and nitrite, ↓ iNOS, PPARγ and TLR-4	Aparicio-Soto et al. (2018)

Hydroxytyrosol	*In vitro*	Adipose tissue inflammation	↓ COX-2 activity, ↓ ROS production, ↓ production of pro-inflammatory cytokines, ↓ NF-kB signaling pathway	Scoditti et al. (2019)
			Attenuation of JNK-mediated PPARγ suppression	Scoditti et al. (2015)
		Skin inflammation	↓TSLP, ↓ NF-kB signaling pathway, ↓ expression inflammation-related genes	Aparicio-Soto et al. (2019)[d]
	In vivo	Diet-induced obesity	Mitigation of ER stress aberrant activation, chronic inflammation, IR and hepatic steatosis	Wang et al. (2018)
		Acute liver injury	↓ production of pro-inflammatory cytokines, ↓ histological damage, ↓ ERK signaling pathway	Yu et al. (2020)
		Systemic inflammation	↓ plasma/serum levels of cytokines, ↓ COX-2 activity, ↓ DNA damage	Fuccelli et al. (2018)
		Rheumatoid arthritis	↓ COX-2 activity, ↓ paw oedema and histological damage, ↓ iNOS expression	Silva et al. (2015)
		Neuroinflammation	Modulation of microglia M1/M2 polarization and downregulation of TLR-4 mediated NF-kB activation and ERK signaling pathway	Zhang et al. (2020)
Hydroxytyrosol acetate	*In vivo*	Rheumatoid arthritis	↓ plasma/serum levels of cytokines, ↑ Nrf-2 and HO-1 protein expression, ↑ activation of JAK/STAT, MAPKs and NF-kB pathways	Rosillo et al. (2015)
		Acute ulcerative colitis	↓ COX-2 activity and iNOS protein expression, ↓ histological damage, ↓ JNK phosphorylation	Sánchez-Fidalgo et al. (2015)
Oleuropein	*In vitro*	Acute kidney injury	↓ MPO activity and *Ccl2* expression, ↓ apoptosis in injured cells, ↓ histological damage	Yin et al. (2019)
		Rheumatoid arthritis	↓ production of pro-inflammatory cytokines, ↑ Nrf-2 and HO-1 protein expression, ↓ MAPKs and NF-kB signaling pathways	Castejón et al. (2017)
	In vitro and *in vivo*	Inflammatory bowel disease	↓ production of pro-inflammatory cytokines, ↓ histological damage, ↓ inflammatory cell infiltration, ↓ ROS production	Huguet-Casquero et al. (2020)
Oleacein	*In vitro*	Adipose tissue inflammation	↓ expression of genes implicated in adipocyte inflammation, angiogenesis, oxidative stress, antioxidant enzymes, leukocytes chemotaxis and infiltration, and improved expression of PPARγ	Carpi et al. (2019)[e]
	Ex vivo	Ischemic stroke risk	↓ HMGB1, MMP-9, MMP-9/NGAL complex and TF, ↑ IL-10 and HO-1	Filipek et al. (2017)
Oleocanthal	*In vivo*	Alzheimer's disease	Restored blood–brain barrier function and reduced neuroinflammation	Al Rihani et al. (2019)

Continued

TABLE 4.1 ***In vivo*** **and** ***in vitro*** **studies on the effect of olive oil and its active compounds on cardiovascular diseases and inflammation.—cont'd**

Type of olive oil or active compound	Type of study	Model of disease	Effects	References
Others[c]	*In vitro* and *in vivo*	Subclinical chronical inflammation[f]	↓ production of pro-inflammatory cytokines, ↓ IL-1β, and protected intestinal integrity	Liehr et al. (2017)

Abbreviations: ABCA1, ATP-binding cassette transporter A1; *Ccl2*, chemokine (C–C motif) ligand 2; *Chol*, cholesterol; *COX-2*, cyclooxygenase-2; *CVD*, cardiovascular diseases; *ER*, endoplasmic reticulum; *ERK*, extracellular-signal-regulated kinase; *HDL-C*, high-density lipoprotein-cholesterol; *HMGB1*, high-mobility group protein-1; *HMGCR*, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; *HO-1*, heme oxygenase-1; *IL*, interleukin; *iNOS*, inducible nitric oxide synthase; *IR*, insulin resistance; *JAK/STAT*, Janus kinase-signal transducer and activator of transcription; *JNK*, c-Jun N-terminal kinase; *LDL-C*, low-density lipoprotein-cholesterol; *MAPK*, mitogen-activated protein kinase; *MMP*, matrix metalloproteinase; *MMP-9/NGAL*, matrix metalloproteinase 9 and neutrophil gelatinase-associated lipocalin complex; *MPO*, myeloperoxidase; *NF-kB*, nuclear factor-κB; *NOS1*, nitric oxide synthase 1; *NOX2*, NADPH oxidase 2; *Nrf-2*, nuclear factor E2-related factor 2; *PPAR*, peroxisome proliferator-activated receptor; *PUFA*, polyunsaturated fatty acid; *ROS*, reactive oxygen species; *SBP*, systolic blood pressure; *SREBP-1c*, sterol regulatory element binding protein 1c; *TF*, tissue factor; *TG*, triglyceride; *TLR-4*, Toll-like receptor 4; *TSLP*, thymic stromal lymphopoietin; *TNF-α*, tumor necrosis factor alfa.

[a]*The olive oil grade was not mentioned in these studies.*
[b]*Depending on the study, phenolic compounds may be naturally present or extracted from virgin olive oils or can be pure compounds.*
[c]*Unsaponifiable fraction, phenolic fraction, squalene and hydroxytyrosol.*
[d]*Both hydroxytyrosol and hydroxytyrosol acetate were studied.*
[e]*Both oleuropein and oleocanthal were studied.*
[f]*Olive oil extract of phenolic compounds and triterpenic acids.*

biological activity as it is the only form incorporated in very low-density lipoproteins. After intestinal absorption, tocopherols are secreted in chylomicron particles and transported to adipose tissue, skin, muscles, bone marrow, and brain. β-, γ-, and δ-tocopherols are subjected to ω-hydroxylation, oxidation, and β-oxidation in the liver to generate 13′-hydroxychromanols and carboxychromanols which have potent antioxidant properties and strong radical-scavenging action (Flori et al., 2019). Intake of vitamin E from foods or dietary supplements is associated with a lower risk of CVD. Several cohort studies have demonstrated a reduction in the risk of ischemic cardiomyopathy, coronary heart disease (Muntwyler et al., 2002), myocardial infarction (Stephens et al., 1996), and mortality due to heart failure (Eshak et al., 2018) in individuals with vitamin E supplementation. There was a strong reduction in coronary heart disease mortality (Muntwyler et al., 2002) and a reduced risk of myocardial infarction in patients with coronary atherosclerosis (Stephens et al., 1996). In contrast, controlled clinical trials have not shown significant changes in vascular function or cardiovascular risk factors for palm tocotrienols (Stonehouse et al., 2016). There is also controversial information in different clinical studies which have not demonstrated a positive association between vitamin E or α-tocopherol intake and the incidence of ischemic CVD, mortality from CVD, stroke, and coronary heart disease (Henríquez-Sánchez et al., 2016; Kubota et al., 2011). However, preclinical evidence has shown that α-tocopherol decreases lipid peroxidation and platelet aggregation (Kaul et al., 2001). Several *in vitro* and *in vivo* assays have demonstrated the antiinflammatory effects of α-tocopherol, namely, in the inhibition of NF-κB, the activity of protein kinase C (PKC), and the biosynthesis of adhesion molecules (Cook-Mills, 2013; Rashidi et al., 2017). A modulating effect of α-tocopherol was observed during inflammatory processes by decreasing the cytokines (interleukins (IL)-1β, IL-6, and IL-8), releasing tumor necrosis factor (TNF)-α, and inhibiting the 5-lypoxygenase (LOX) pathway (Mathur et al., 2015). Intake of vitamin E is also associated with a reduction in the apoptotic activity of cardiomyocytes, demonstrating a cardioprotective effect in rabbit models of hypercholesterolemia (Sozen et al., 2018) and rats with streptozotocin-induced diabetic heart failure (Hamblin et al., 2007).

There is a large body of preclinical and clinical evidence on the beneficial cardiovascular effects of vitamin E (reviewed by Flori, 2019). However, most of these studies relied on dietary supplementation with these vitamins, so clinical trials lack a correlation between the health benefits of consuming olive oil and its vitamin E content.

A high-cholesterol diet supplemented with EVOO improved the inflammation associated with hypercholesterolemia in the heart of rats. It reduced the levels of malondialdehyde and TNF-α. This reduction was associated with the antioxidant action of phenolic compounds (Katsarou et al., 2016). Olive oil phenolic compounds improved the prognosis of atherosclerosis (Berrougui et al., 2015). There was evidence of increased resistance to oxidation by LDL-C and HDL-C, and promotion of reverse cholesterol transport via the ATP binding cassette subfamily A member 1 (ABCA-1) pathway (Berrougui et al., 2015). The ABCA-1 protein plays a significant role in HDL biosynthesis and cholesterol cell homeostasis (Phillips, 2018). On the other hand, the antihypertensive effect of EVOOs enriched with phenolic compounds from the olive fruit and leaves (750 mg/kg of oil) has been demonstrated in spontaneously hypertensive rats (Vazquez et al., 2019). There was a decrease in systolic blood pressure, reduction in cardiac hypertrophy, angiotensin II levels, total cholesterol, urinary endothelin-1, and oxidative stress biomarkers. Oleacein has been shown to reduce the risk of ischemic stroke *ex vivo*, by attenuating the destabilization of atherosclerotic plaques in hypertensive patients, after transient ischemic attacks (Filipek et al., 2017). The protective role of EVOO against the atherogenic process has been shown *in vitro*, by studying microRNAs in endothelial cells (Santiago-Fernandez et al., 2020). The effect of TG-rich lipoproteins isolated from human blood after a high-fat meal with EVOO (25 mL) on the microRNA-sequencing profile of human umbilical vein endothelial cells caused upregulation of 22 genes regulating microRNAs involved in atherosclerosis. Besides being involved in cardiovascular protection through cholesterol-mediating effects, EVOO has shown antioxidant activity at the cellular level, in human platelets from healthy subjects. It reduced NADPH-oxidase 2 activation through hydrogen peroxide (H_2O_2) scavenging (Carnevale et al., 2018). This H_2O_2 scavenging property was directly correlated with the concentration of total phenolic compounds, providing the first evidence that EVOO downregulates platelet H_2O_2, reducing oxidative stress involved in the atherosclerotic process. Besides, the phenolic compounds extracted from olive oil have demonstrated *in vitro* resistance to oxidative stress by decreasing the production of ROS in mouse embryonic fibroblasts (Presti et al., 2017). The total phenolic compounds, in particular ligstroside aglycone (aldehyde and hydroxylic form), were responsible for the observed effects.

Several studies have shown the *in vitro* antiinflammatory activity of EVOO and several of its bioactive compounds. EVOO contributed to restore the fluidity of the membrane of THP-1 human macrophages and counter redox imbalance (Bordoni et al., 2020). The unsaponifiable fraction, the phenolic fraction, and hydroxytyrosol from virgin olive oil reduced the inflammatory response in lipopolysaccharide (LPS)-induced inflammation in primary human monocytes. There was a down-regulation of visfatin expression, a decrease in the production of pro-inflammatory cytokines (IL-1β and IL-6, and TNF-α) and an increase in the release and gene expression of the antiinflammatory cytokine IL-10 (Martin et al., 2019).

Inflammation of adipose tissue is one of the root causes of obesity and associated diseases, such as metabolic syndrome (Ellulu et al., 2016). Several bioactive compounds in olive oil have been shown to reduce inflammation in adipose tissue. Oleic acid and hydroxytyrosol prevented TNF-α-induced suppression of total adiponectin secretion and expression of mRNA levels in human and murine adipocytes, under pro-inflammatory conditions induced by the cytokine TNF-α (Scoditti et al., 2015). When combined, oleic acid and hydroxytyrosol inhibit the downregulation of adiponectin by attenuating JNK-mediated PPARγ suppression. Hydroxytyrosol also modulated the expression of adipocyte genes and microRNAs under inflammatory conditions of adipose tissue through mechanisms involving a reduction in oxidative stress and inhibition of NF-κB (Scoditti et al., 2019). Oleacein and oleocanthal extracted from EVOO prevented the expression of genes and microRNAs related to inflammation in adipocytes by attenuating the activation of NF-κB (Carpi et al., 2019). The hypertrophy and inflammation of adipose tissue were reduced in mice fed a high-fat diet supplemented with olive oils with different phenolic content (Luque-Sierra et al., 2018). In an obesity mouse model, hydroxytyrosol (20 mg/kg/day for 10 weeks) was shown to attenuate aberrant activation of the endoplasmic reticulum, thereby improving chronic inflammation, insulin resistance, and hepatic steatosis (Wang et al., 2018). The influence of a diet enriched with olive oil (12%) on the status of inflammation of adipose tissue, in the context of metabolic syndrome, was demonstrated in male C57BL/6J mice by the increased expression of cannabinoid receptor 2 (CB2), which exerts antiobesity effects by silencing activated immune cells, and expression of nitric oxide synthase (NOS)1 protein, which modulates transcription of the CB2 receptor gene (Notarnicola et al., 2016). These effects have been attributed to the high MUFA content of olive oil. A single dose of high-polyphenol EVOO (50 mL) could modify the transcriptome of peripheral blood mononuclear cells by modulating different pathways associated with the pathophysiology of cardiometabolic diseases and cancer (D'Amore et al., 2016). These effects increased in healthy individuals, rather than in patients with metabolic syndrome.

Olive oil has also shown beneficial effects on animal models of diabetes. Studies in the offspring of rats with mild diabetes who developed gestational diabetes mellitus and whose diet was supplemented with 6% olive oil during pregnancy showed attenuated placental dysfunction and attenuated fetal overgrowth (Capobianco et al., 2018). There was also a decrease in cardiovascular risk in a generation of diabetic mice whose maternal diet was supplemented with 6% olive oil, due to a reduction in pro-oxidant markers, and deposition of collagen IV and fibronectin in the heart of mice, prevention of connective tissue growth factor, and increased apoptosis (Roberti et al., 2020).

Beneficial effects of olive oil or its bioactive compounds have been observed in models of acute liver injury, improving liver function. Oleuropein in rats (Yin et al., 2019) and hydroxytyrosol in RAW 264.7 macrophages and C57BL/6 mice (Yu et al., 2020) decreased the production of pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-2, IL-6, IL-10, IL-4), reduced histological damage, and inhibited apoptosis in injured cells (Yin et al., 2019; Yu et al., 2020). Treatment with hydroxytyrosol attenuated M1 macrophages and increased M2 macrophages after stimulation with LPS and increased expression of antiinflammatory cytokines via the suppression of the ERK pathway (Yu et al., 2020). EVOO has also shown antiinflammatory effects in animal models of nonalcoholic fatty liver disease. Dietary supplementation in docosahexaenoic acid (DHA, C22:6*n*-3) and EVOO on oxidative stress and metabolic disturbances induced by a diet high in fat has been tested in mice (Hernández-Rodas et al., 2017). There were synergistic beneficial effects in preventing hepatic steatosis, with upregulation of PPAR-α and Nrf2 associated with downregulation of SREBP-1c and NF-κB. EVOO could repair liver damage induced by a high-fat diet, possibly via an antiinflammatory effect in adipose tissue and modifications in liver lipid composition and signaling pathways (Jurado-Ruiz et al., 2017). Analysis of the hepatic ingenuity pathway revealed the regulation of proteins involved in lipid metabolism, small molecule biochemistry, gastrointestinal disease, and liver regeneration in EVOO groups. Virgin olive oil rich in polyphenols also reduced insulin resistance, liver inflammation and improved mitochondrial dysfunction in rats fed the high-fat diet (0.290 mg/kg/day for 6 weeks), preventing the progression of nonalcoholic fatty liver disease (Lama et al., 2017).

Several other studies have evaluated the potential effect of EVOO and some of its bioactive components on inflammatory bowel disease. The effect of dietary supplementation with hydroxytyrosol-acetate and 3,4-dihydroxyphenylglycol was tested in the inflammatory response associated with a model of colitis in mice (Sánchez-Fidalgo et al., 2015). The antiinflammatory effect in acute ulcerative colitis has been demonstrated by decreased cyclooxygenase (COX)-2 activity, reduced iNOS protein expression, reduced histological damage, and down-regulated phosphorylation of JNK. Some studies have shown that EVOO and its phenolic compounds, especially oleuropein and hydroxytyrosol acetate, are promising agents in treating intestinal inflammation. The main antiinflammatory effects observed in models of inflammatory bowel disease included decreased production of pro-inflammatory cytokines, reduced histological damage (Cariello et al., 2020; Huguet-Casquero et al., 2020), reduced expression of TNF-α gene, total cholesterol, and increased expression of PPAR-α hepatic gene (Bigagli et al., 2019), reduced infiltration of inflammatory cells, and decreased production of ROS (Huguet-Casquero et al., 2020). The phenolic compounds extracted from olive oil were responsible for lowering the redox imbalance induced by oxysterols and pro-inflammatory response in intestinal cells *in vitro* by decreasing the production of inflammatory mediators (IL-6, IL-8, NO and iNOS) and the modulation of the MAPK—NF—κB pathway (Serra et al., 2018). Likewise, phenolic compounds extracted from olive oil modulated the inflammatory response in a model of the intestinal epithelial cell by regulating IL-8 expression through transcriptional and posttranscriptional mechanisms (Muto et al., 2015).

The impact of EVOO in model mice of rheumatoid arthritis revealed various antiinflammatory effects on chemical mediators and histological damage. Rosillo et al. (2016) verified the reduction of paw edema and histological damage in collagen-induced arthritis in DBA-1/J mice, along with improving the activation of JAK/STAT, MAPKs, and NF-κB pathways (Rosillo et al., 2016). Similar antiinflammatory effects were observed in a human synovial sarcoma cell line mediated by oleuropein (Castejón et al., 2017; Rosillo et al., 2019), in addition to hydroxytyrosol (Silva, Sepodes, et al., 2015) and hydroxytyrosol acetate (Rosillo et al., 2015) *in vivo*.

Bioactive olive oil extracts alleviated chronic subclinical inflammation in LPS-stimulated macrophages and male piglets growth (Liehr et al., 2017). Hydroxytyrosol has also shown an antiinflammatory and antioxidant effect in a mouse model of systemic inflammation (Fuccelli et al., 2018). There is also evidence for the antiinflammatory activity of EVOO, via phenolic compounds, in systemic lupus erythematosus (SLE) in humans and mice. An EVOO diet applied to a pristane-induced SLE mouse model resulted in a significant reduction in renal damage and decreased serum levels of MMP-3 and kidney PGE2, as well as production pro-inflammatory cytokines in splenocytes (Aparicio-Soto et al., 2016). The expression of Nrf-2 and HO-1 proteins was up-regulated in these mice. The activation of JAK/STAT, MAPK and NF-κB pathways considerably improved. Additionally, virgin olive oil and its extracted phenolic compounds could counteract inflammatory pathways in a monocyte-macrophage lineage of mice with pristane-induced SLE and healthy subjects (Aparicio-Soto et al., 2018), thus providing a preventive and a palliative role in the management of this disease. In a mouse model of sepsis, oleic acid had a beneficial antiinflammatory role, reducing rolling and influx of leukocytes, balancing cytokine production and controlling bacterial growth, presumably through a mechanism dependent on the PPARγ expression (Medeiros-de-Moraes et al., 2018).

The effect of daily consumption of EVOO in a mouse model of Alzheimer's disease significantly increased levels of synaptophysin in the brain, a protein marker of synaptic integrity (Lauretti et al., 2017). It also improved behavioral deficits and reactivated cellular autophagy. EVOO rich in oleocanthal restored the blood-brain barrier function *in vivo*, promoting a reduction in neuroinflammation (Al Rihani et al., 2019). This effect was achieved by inhibiting the NLRP3 inflammasome simultaneously with the induction of autophagy, thus providing a beneficial effect in slowing or arresting the progression of Alzheimer's disease. On the other hand, hydroxytyrosol suppressed the inflammatory response by modulating the microglia M1/M2 polarization phenotype and downregulating TLR-4 activation mediated by NF-κB and ERK signaling pathway (Zhang et al., 2020). Therefore, hydroxytyrosol has been suggested as a promising therapeutic candidate for treating neuroinflammation in neurodegenerative conditions or brain injury.

Olive oil and its components have also been shown to have beneficial effects on skin inflammation. Olive oil improved pressure ulcers in mice by reducing oxidative damage and inflammation, promoting skin healing (Donato-Trancoso et al., 2016). Hydroxytyrosol and hydroxytyrosol acetate attenuated skin inflammation in human keratinocytes by interfering with the NF-κB pathway (Aparicio-Soto et al., 2019).

The cardioprotective, antioxidant, and anti-inflammatory properties of olive oil and its main bioactive components, such as oleic acid and phenolic compounds, are now well established. In recent years, many studies have corroborated this evidence with a series of laboratory tests prepared to analyze inflammatory intermediates, genes and proteins expression, by regulation of transcriptomes, and potential cellular signaling pathways. The identification of proteins and the genes encoding these proteins has allowed suggesting hypotheses of cell signaling pathways for bioactive compounds in olive oil, for a better understanding of how they act in the cell, and how they exert their beneficial effects. Some schemes have been proposed to illustrate the cellular mechanisms underlying the protective effects of oleic acid, hydroxytyrosol (Scoditti et al., 2015, 2019), oleuropein (Castejón et al., 2017; Yin et al., 2019), or oleocanthal (Al Rihani et al., 2019). Studies in cell lines or animal models are not sufficient to conclude on effects in humans. Therefore, there are limits to extrapolating the evidence obtained. However, the accumulated evidence supports the hypothesis that consuming olive oil, especially EVOO, has beneficial health effects. The bioactive components of olive oil, especially those mentioned in this chapter, can be used as adjunctive therapy, as part of a healthy diet, and as a basis for developing nutritional strategies to prevent various pathologies.

7. Evidence from clinical trials

Several studies have evaluated the effect of olive oil consumption on cardiovascular risk factors and markers of inflammation in humans. Most of these studies were randomized controlled clinical trials (RCTs), with a crossover design, in

Caucasian populations with a daily intake of 20−60 mL of olive oil, usually for short periods (c. three weeks). Numerous trials have been carried out in southern European countries such as Spain and Italy, where the Mediterranean diet is predominant. Some studies have compared olive oil to other vegetable oils in healthy cohorts. Most approaches compared different types of olive oils like, for example, EVOO versus lower quality olive oils, or comparisons between EVOOs with varying levels of phenolic compounds or triterpenes, to assess the effect of lower or higher concentrations in these compounds. More specific cohorts were used to assess cardiovascular risk factors and biomarkers in the context of a particular disease (Table 4.2).

CVD markers are positively affected by functional virgin olive oils in several RCTs. These functional virgin olive oils are virgin olive oils enriched with their phenolic compounds and with phenolic compounds extracted from thyme (Farràs et al., 2015; Fernández-Castillejo et al., 2017; Martín-Peláez et al., 2016; Pedret et al., 2015; Valls et al., 2017). In hypercholesterolemic patients, functional virgin olive oils enhanced distribution and composition of HDL-C subclasses, as well as metabolic and antioxidant enzymatic activities (Farràs et al., 2015). However, a synergistic effect between phenolic compounds and other olive oil components on HDL-C properties could not be overlooked (Farràs et al., 2015). Also, the HDL-C proteome changed in hypercholesterolemic patients after intervention with different types of virgin olive oil. Functional virgin olive oils containing 500 mg/kg of phenolic compounds had a positive impact on the HDL-C proteome in a cardioprotective manner that could improve HDL functionality (Pedret et al., 2015). Fifteen proteins, involved in the activation of liver X receptor (LXR) and retinoid X receptor (RXR), acute-phase response, and atherosclerotic pathways, were expressed differently (Pedret et al., 2015). Besides, the fatty acids and phenolic compounds of olive oil had a strong effect on HDL-C remodeling (Pedret et al., 2015).

Regarding the LDL-C levels, consumption of high-phenolic virgin olive oil decreased the levels and atherogenicity of plasma LDL-C in healthy young men (Hernáez et al., 2015). This may be due to an improved systemic oxidative status or increased lipoprotein lipase (expression of the *LPL* gene). Phenolic compounds are mostly associated with antioxidant effects. However, neither the effect of phenolic compounds from other food components could be excluded, nor their synergistic effects (Hernáez et al., 2015). An isolated intake of EVOO rich in phenolic compounds (one dose of 25 g), in postprandial time, has been shown to have a positive effect on various CVD outcomes, in young, healthy participants (Table 4.2). One dose intake per day has been shown to reduce cardiovascular risk due to improved lipid profile and plasma antioxidant properties, reduced oxidative stress, protection of LDL-C particles against oxidative damage, and enhanced antiinflammatory properties (Perrone et al., 2019).

The effects of consuming EVOO on cardiovascular risk factors have also been studied in populations where neither olive oil nor the Mediterranean diet is a staple. Blood lipids are affected differently by butter and coconut oil, which are mostly composed of saturated fats, compared to olive oil (50 g/day) (Khaw et al., 2018). However, coconut oil had a similar effect to olive oil in LDL-C and had no significant difference in healthy individuals. The interaction of different profiles in individual fatty acids, processing methods and diets should be further elucidated to understand the effects of various dietary fats on lipid profiles, metabolic markers, and health outcomes (Khaw et al., 2018). In a healthy Australian cohort, high-phenolic olive oil (360 mg/kg phenolic compounds, 60 mL/day) significantly decreased peripheral blood pressure and systolic blood pressure, providing evidence for a potentially widely available dietary intervention to prevent CVD in a multiethnic population (Sarapis et al., 2020). In this short-term RCT, there was no effect on diastolic blood pressure or arterial stiffness (Sarapis et al., 2020). In Brazilian women with excess body fat, a daily intake of 25 mL of EVOO combined with an energy-restricted Western diet, for 9 weeks, significantly reduced body fat (80% higher loss compared to control) and decreased diastolic blood pressure (Galvão-Cândido et al., 2018). EVOO also increased serum creatinine and decreased hepatic alkaline phosphatase and IL-1β levels (Galvão-Cândido et al., 2018). Another study on healthy European men showed that high-phenolic EVOO (366 mg/kg of phenolic compounds, 25 mL/day, for 3 weeks) significantly reduced systolic blood pressure, modulating the expression of genes linked to the renin-angiotensin-aldosterone system (Martín-Peláez et al., 2017). Silva et al. (2015) obtained different results, showing that supplementation with high-phenolic olive oil or low-phenolic olive oil (20 mL/day, for 6 weeks) did not lead to an improvement in cardiovascular health markers, in a healthy UK cohort (Silva, Bronze, et al., 2015). Neither the secondary outcomes (TG, oxLDL-C, LDL-C) nor the proteomic biomarkers of heart and kidney disease and diabetes were significantly affected. According to the authors, this food supplementation was unlikely to be linked to a direct antioxidant effect (Silva, Bronze, et al., 2015). On the contrary, a 10-year prospective study conducted in Greece with 2020 participants found that olive oil consumption, as the unique source of fat in the diet, prevented primary CVD, in adults without a preexisting disease (Kouli et al., 2019). The authors found a significant mediation of olive oil use on CVD risk through fibrinogen plasma levels by employing a multiadjusted statistical model.

Phenolic compounds from olive oil have been shown to affect other CVD-related biomarkers, such as different variables and mechanisms of the paraoxonase (PON) family of enzymes in hypercholesterolemic patients. PONs are lipo-lactonases

TABLE 4.2 Characteristics of clinical trials examining the effect of regular olive oil consumption on selected biomarkers.

Reference	Country	No. of participants (end of study)	Population, health status	Age, y; mean (SD)	Study design, duration (washout period)	Olive oil intervention	Comparison group(s)	Evidence in outcome parameters Olive oil versus comparator(s)
Farràs et al. (2015)	Spain	33	Women & men, hypercholesterolemic	55.21 (10.62)	RCT, crossover, 3 wk (2 wk)	Dose: 25 mL/d FVOO, enriched with olive oil PC (500 mg/kg oil) and FVOOT (500 mg PC/kg oil) combining olive oil PC (50%) and thyme PC (50%).	VOO (80 mg PC/Kg oil)	*FVOOT affected CVD markers* ↑ large HDL particles ↓ small HDL particles ↑HDL EC/FC content ↑HDL monolayer PL/FC ↑HDL antioxidant enzymes
Pedret et al. (2015)	Spain	33	Women & men, hypercholesterolemic	35–80	RCT, crossover, 3 wk (2 wk)			*All interventions impacted the HDL proteome* Upregulated proteins: Chol homeostasis, protection against oxidation and blood coagulation Down-regulated proteins: acute-phase response, lipid transport, and immune response
Fernández-Castillejo et al. (2017)	Spain	33	Women & men, hypercholesterolemic	35–80	RCT, crossover, 3 wk (2 wk)			*VOO* ↑PON-3 protein *FVOO* ↓ lactonase raw *FVOOT* ↑ PON-1 protein ↓ lactonase specific ↓ paraoxonase raw and specific

Continued

TABLE 4.2 Characteristics of clinical trials examining the effect of regular olive oil consumption on selected biomarkers.—cont'd

Reference	Country	No. of participants (end of study)	Population, health status	Age, y; mean (SD)	Study design, duration (washout period)	Olive oil intervention	Comparison group(s)	Evidence in outcome parameters Olive oil versus comparator(s)
Valls et al. (2017)	Spain	33	Women & men, hypercholesterolemic	35–80	RCT, crossover, 3 wk (2 wk)			*FVOOT and FVOO* versus *VOO on CVD markers* ↑ IRH ↑ HDL-C *Fat-soluble vitamins* ↑ β-cryptoxanthin, lutein, α-tocopherol
Martín-Peláez et al. (2016)	Spain	10	Women & men, hypercholesterolemic	35–80	RCT, crossover, 3 wk (2 wk)			*FVOO* ↑ IgA-coated bacteria ↑ CRP
Martín-Peláez et al. (2017)	Germany, Finland, Spain	18	Men, healthy	36.0 (11.1)	RCT, crossover, 3 wk (2 wk)	Dose: 25 mL/d HPOO (366 mg PC/Kg oil)	LPOO (2.7 mg PC/Kg oil)	↓ SBP ↔ DBP ↓ *IL8RA* gene expression
Hernáez et al. (2015)		Lipid-related outcomes (25) Gene expression outcomes (18)		32.3 (11.2) and 36.9 (12.3)				*CVD markers* ↓ apo B-100 ↓ total LDL particles ↓ small LDL particles ↑ lipoprotein lipase gene expression *Oxidative status* ↑ LDL oxidation lag time
Silva et al. (2015)	United Kingdom	63	Women & men, healthy	LPOO: 30.2 (12.1) HPOO: 31.5 (11.9)	RCT, parallel, 6 wk	Dose: 20 mL/d HPOO (286 mg caffeic acid equivalents/Kg oil)	LPOO (18 mg caffeic acid equivalents/Kg oil)	↔ CVD markers

Pacetti et al. (2016)	Colombia	160	Women & men, healthy	EVOO: 62.8 (6.1) HPO: 64.3 (8.5)	RCT, parallel, 3 mo	EVOO (25 mL/d) added to lunch and dinner meals	Hybrid palm oil (HPO, 25 mL/d) added to lunch and dinner meals	*Both HPO and EVOO* ↓ lipophilic index of erythrocyte membranes ↑ PUFA-esterified PChol and PEth species in erythrocytes
Galvão-Cândido et al. (2018)	Brazil	41	Women, excess body fat	27.0 (0.9)	RCT, parallel, 9 wk	EVOO (25 mL/d on energy-restricted diet)	Soybean oil (25 mL/d energy-restricted diet)	c. 80% fat loss ↓ DBP
Khaw et al. (2018)	United Kingdom	Olive oil: 30 Coconut oil: 28 Butter: 33	Women & men, healthy	Olive oil: 59.1 (6.4) Coconut oil: 59.1 (6.1) Butter: 61.5 (5.8)	RCT, parallel, 4 wk	EVOO (50 g/d)	Coconut oil (50 g/d), Butter (50 g/d)	= LDL-C (vs. Coconut oil) = Chol/HDL-C ratio and non-HDL-C (vs. Coconut oil)
Perrone et al. (2019)	Italy	22	Women & men, healthy	31.0 (11.90)	RCT, parallel, postprandial (1 wk)	p-EVOO (25 g containing 9.4 mg hydroxyphenylethanol and derivates per 20 g oil)	C-OO (25 g containing 2.3 mg hydroxyphenylethanol and derivates per 20 g oil)	↓ oxLDL, malondialdehyde, TGs and visceral adiposity index Upregulation of catalase, SOD1 and upstream transcription factor 1
Rezaei et al. (2019)	Iran	Olive oil: 26 Sunflower oil: 28	Women & men, nonalcoholic fatty liver disease	Olive oil: 46.3 (13.6) Sunflower oil: 40.8 (8.7)	RCT, parallel, 12 wk	OO (20 g/d)	Sunflower oil (20 g/d)	↓ AST and TGs ↓ fat mass, fatty liver grade, body fat % ↑ skeletal muscle mass
Daimiel et al. (2020)	Spain	12	Women & men, healthy	22–60 years	RCT, crossover, postprandial	Dose: 30 mL H-EVOO (750 mg total PC/Kg oil)	L-EVOO (250 mg total PC/Kg oil) and M-EVOO (500 mg total PC/Kg oil)	↓ let-7e-5p regardless of EVOO PC content ↑ miR-17-92 cluster by L-EVOO and M-EVOO
Rus et al. (2020)	Spain	30	Women, fibromyalgic	EVOO: 54.10 (5.57) ROO: 49.81 (5.81)	RCT, parallel, 3 wk (2 wk)	EVOO (50 mL/d)	ROO (50 mL/d)	↓ RBC count ↓ ESR ↓ cortisol (*vs.* ROO) ↑ PDW (vs. ROO)

Continued

TABLE 4.2 Characteristics of clinical trials examining the effect of regular olive oil consumption on selected biomarkers.—cont'd

Reference	Country	No. of participants (end of study)	Population, health status	Age, y; mean (SD)	Study design, duration (washout period)	Olive oil intervention	Comparison group(s)	Evidence in outcome parameters Olive oil versus comparator(s)
Sarapis et al. (2020)	Australia	50	Women & men, healthy	38.5 (13.9)	RCT, crossover, 3 wk (2 wk)	Dose: 60 mL/d HPOO (360 mg/kg PC)	LPOO (86 mg/kg PC)	↓ peripheral and central SBP
Santangelo et al. (2016)	Italy	11	Women & men, overweight type-2 diabetic	64.63 (8.52)	Nonrandomized controlled intervention trial, 4 wk (4 wk)	Dose: 25 mL/d HP-EVOO (577 mg of PC/Kg)	ROO (PC not detectable)	↓ fasting plasma glucose ↓ HbA1c ↓ BMI and body weight ↓ AST, ALT and serum visfatin
Sánchez-Rodríguez et al. (2018)	Spain	51	Women & men, healthy	28-32 (2)	RCT, crossover, 3 wk (2 wk)	Dose: 30 mL/d OVOO (490 mg/kg of PC and 86 mg/kg of triterpenes) and FOO (487 mg/kg of PC and 389 mg/kg of triterpenes)	VOO (124 mg/kg of PC and 86 mg/kg of triterpenes)	*OVOO intake* ↑ HDL-C *OVOO, FOO and VOO* ↓ plasma endothelin-1 *VOO (≥124 mg/kg of PC)* ↑ endothelin-1 No effect of triterpenes
Sánchez-Rodríguez et al. (2019)	Spain	51	Women & men, healthy	28-32 (2)	RCT, crossover, 3 wk (2 wk)	Dose: 30 mL/d OVOO (490 mg/kg of PC and 86 mg/kg of triterpenes) and FOO (487 mg/kg of PC and 389 mg/kg of triterpenes)	VOO (124 mg/kg of PC and 86 mg/kg of triterpenes)	*FOO* versus *OVOO* ↓ Urinary 8-OHdG and plasma IL-8 and TNF-a *FOO* versus *VOO* ↓ IL-8

de la Torre et al. (2020)	Spain	Dose-response study: 18 Bioavailability study: 12	Women & men, healthy	29.3 (8.6 years)	RCT, crossover, 3 wk (2 wk)	Dose: 30 mL/d (single dose after 12 h fasting for dose-response study and 3 wk for bioavailability study). OVOO (490 mg/kg of PC and 83.6 mg/kg of triterpenes) and FOO (487 mg/kg of PC and 389 mg/kg triterpenes)	VOO (124 mg/kg of PC and 83.3 mg/kg of triterpenes)	↑ of MA and OA in biological fluids (dose-dependent) Bioavailability: MA > OA *OVOO and FOO* ↑ IRH ↓ DBP
Carnevale et al. (2019)	Italy	30	Women & men, impaired fasting glucose	58.1 (11.4)	RCT, crossover. Plasma biomarkers measured before, 1h and 2h after the intervention.	EVOO (10 g in a meal)	Meal without EVOO	*2h after intervention* ↓ LPS, apo B-48, sNox2-dp and oxLDL LPS up-regulated Nox2-derived oxidative stress via interaction with Toll-like receptor 4 *in vitro*
Morvaridi et al, (2020)	Iran	32	Women & men, ulcerative colitis	EVOO-CO intervention: 37.0 (12.2) CO-EVOO intervention: 35.9 (9.4)	RCT, crossover, 3 wk (2 wk)	EVOO (50 mL/d)	Canola oil (CO, 50 mL/d)	↓ ESR ↓ hs-CRP ↓ bloating, constipation, fecal urgency, incomplete defecation, and final GSRS

Abbreviations: ↔, no significant change; ↓, significant decrease; ↑, significant increase; *8-OHdG*, 8-hydroxydeoxyguanosine; *ALT*, alanine aminotransferase; *Apo*, apolipoprotein; *AST*, aspartate aminotransferase; *BMI*, body mass index; *Chol*, cholesterol; *CO*, canola oil; *CRP*, C-reactive protein; *CVD*, cardiovascular diseases; *DBP*, diastolic blood pressure; *EC*, esterified cholesterol; *ESR*, erythrocyte sedimentation rate; *EVOO*, extra virgin olive oil; *FC*, free cholesterol; *FMD*, flow-mediated dilatation; *FOO*, functional olive oil; *FVOO*, functional virgin olive oil; *FVOOT*, functional virgin olive oil with thyme; *GSRS*, gastrointestinal symptom rating scale; *H-EVOO*, high-polyphenol extra virgin olive oil; *HbA1c*, glycated hemoglobin; *HDL-C*, high-density lipoprotein-cholesterol; *HP-EVOO*, high-polyphenol extra virgin olive oil; *HPOO*, high-polyphenol olive oil; *hs-CRP*, high-sensitivity C-reactive protein; *Ig*, immunoglobulin; *IL*, interleukin; *IL8RA*, chemokine (C-X-C motif) receptor 2; *IRH*, ischemic reactive hyperemia; *L-EVOO*, low-polyphenol extra virgin olive oil; *LDL-C*, low-density lipoprotein-cholesterol, *LPOO*, low-polyphenol olive oil; *LPS*, lipopolysaccharide; *M-EVOO*, medium-polyphenol extra virgin olive oil; *MA*, maslinic acid; *mo*, month; *OA*, oleanolic acid; *OO*, olive oil; *OVOO*, optimized virgin olive oil; *oxLDL*, oxidized low-density lipoprotein; *p-EVOO*, phenols-rich extra virgin olive oil; *PC*, phenolic compound; *PChol*, phosphatidylcholine; *Peth*, phosphatidylethanolamine; *PL*, phospholipids; *PON*, paraoxonase; *PUFA*, polyunsaturated fatty acid; *RBC*, red blood cells; *RCT*, randomized controlled trial; *ROO*, refined olive oil; *SBP*, systolic blood pressure; *SNox2-dp*, soluble Nox2-derived peptide; *SOD*, superoxide dismutase; *TC*, total cholesterol; *TG*, triglyceride; *TNF*, tumor necrosis factor; *VOO*, virgin olive oil; *wk*, week.

with unique substrate specificities associated with several human diseases, like CVD, atherosclerosis, obesity, or cancer (Furlong et al., 2016). PON3, in particular, seems to be susceptible to obesity, gallstone formation, and atherosclerosis (Furlong et al., 2016). Thus, the expression of PON3 protein, which appears to have a protective role in hypercholesterolemic patients, increased significantly after virgin olive oil consumption (80 mg phenolic compounds/kg) (Fernández-Castillejo et al., 2017). Consumption of EVOO also modulates circulating postprandial CVD-related microRNAs, which was suggested as a potential mechanism for its associated cardiovascular benefits (Daimiel et al., 2020). The miR-17-92 cluster, which was involved in fatty acid metabolism and nutrient sensing, increased after consumption of low-phenolic and medium-phenolic EVOO (30 mL) (Daimiel et al., 2020). The consumption of EVOO (25 mL/day, for 3 months), in healthy adults, also improved the membrane fluidity of erythrocytes, thanks to an increase in the degree of membrane lipids unsaturation, which could be associated with a lower risk of developing CVD (Pacetti et al., 2016). In contrast, the lipophilic index of erythrocyte membranes decreased, showing the positive effects on the phospholipid molecular species composition in erythrocytes of treated individuals (Pacetti et al., 2016).

The status of endothelial function may reflect a propensity to develop atherogenicity, oxidative stress and inflammation (Bonetti et al., 2003). Consumption of olive oil pentacyclic triterpenes, along with phenolic compounds, has been associated with an improved endothelial function (de la Torre et al., 2020). The effect of functional virgin olive oils with different doses of phenolic compounds and triterpenes has been evaluated on biomarkers of metabolic syndrome and endothelial function. HDL-C levels were improved in healthy subjects after the intake of virgin olive oil with 490 mg/kg of phenolic compounds and 86 mg/kg of triterpenes (Sánchez-Rodríguez et al., 2018). Besides, virgin olive oils with varying concentrations of triterpenes and phenolic compounds were able to decrease plasma endothelin-1 levels (Sánchez-Rodríguez et al., 2018). A significant effect of triterpenes in olive oils was observed in a similar study, in a healthy Spanish population, on biomarkers of oxidative stress and inflammation (Sánchez-Rodríguez et al., 2018). Olive oil containing 487 mg/kg triterpenes significantly reduced urinary 8-hydroxydeoxyguanosine levels and plasma IL-8 and TNF-α levels compared to low-triterpenic acids olive oil (86 mg/kg). Virgin olive oil with different concentrations of phenolic compounds and triterpenes significantly increased ischemic reactive hyperemia and decreased diastolic blood pressure (de la Torre, 2020). In this study, only when triterpenic acids were added to olive oil rich in phenolic compounds, there was a significant improvement in postprandial endothelial function. There was a direct association between increased concentrations of ischemic reactive hyperemia and nitrite, a surrogate marker for nitric oxide bioactivity. The effect on endothelial function may be due to interactions between phenolic and triterpene compounds from virgin olive oil, or due to other dietary components (de la Torre et al., 2020). Plasma levels of fat-soluble vitamins (β-cryptoxanthin, lutein, α-tocopherol) increased significantly after a prolonged intake of phenol-rich olive oils (25 mL/day, 3 weeks, 500 mg phenolic compounds/kg), in Spanish hypercholesterolemic patients (Valls et al., 2017). The values of ischemic reactive hyperemia and the levels HDL-C also increased after the treatment. The intake of functional virgin olive oil produced an increase in the plasmatic concentrations of fat-soluble vitamins and also an improvement in the endothelial function (Valls et al., 2017). Metabolic control and circulating inflammatory adipokines improved in overweight patients with type-2 diabetes, after daily consumption of EVOO rich in phenolic compounds (25 mL/day) (Santangelo et al., 2016). Consumption of high-phenolic EVOO increased the serum levels of apelin, a protein with hypotensive effects (Bertrand et al., 2015). It also led to reduced fasting glucose, glycated hemoglobin (HbA1c), and visfatin expression, an adipocytokine with potentially significant effects on glucose metabolism and atherosclerosis (Saddi-Rosa et al., 2010). Overall, these changes may to improve metabolic disorder in unhealthy people (Santangelo et al., 2016). In patients with nonalcoholic fatty liver disease, consumption of ordinary olive oil improved the severity of the disease by lowering the fatty liver stage and reducing the percentage of body fat but did not affect liver enzymes or cardiovascular risk factors (Rezaei et al., 2019). EVOO consumption (50 mL/day for 3 weeks) decreased cortisol levels in patients with fibromyalgia and increased the platelet distribution width compared to refined olive oil. EVOO significantly reduced erythrocyte count and erythrocyte sedimentation rate after treatment (Rus et al., 2020).

The phenolic compounds from olive oil can influence microbial populations in the gut and the metabolic output (Martín-Peláez et al., 2016). High amounts of olive oil phenolic compounds (500 mg/kg) increased immunoglobulin A (IgA) coated bacteria in hypercholesterolemic patients, suggesting stimulation of the mucosal immunity of the gut (Martín-Peláez et al., 2016). However, after the treatment, a decrease was observed for C-reactive plasma protein, a marker of systemic inflammation. Despite the conflicting results, high doses of olive oil phenolic compounds were suggested to potentially boost the immune system (Martín-Peláez et al., 2016). The consumption of EVOO (50 mL/day for 3 weeks) decreased inflammatory markers and improved gastrointestinal symptoms in patients with ulcerative colitis (Morvaridi et al., 2020). Daily consumption of EVOO has been suggested as complementary medicine for these patients. Administration of EVOO (10 g in a meal) reduced inflammation related to postprandial oxidative stress triggered by LPS produced in the intestine (Carnevale et al., 2019). A significant association was shown between postprandial LPS and markers of oxidative stress, such as oxLDL-C and sNox2-dp, suggesting that LPS could be an oxidative stress trigger of Nox2. *In vitro* LPS up regulated Nox2-derived oxidative stress via interaction with toll-like receptor 4. Despite this, the antioxidant effect mediated by phenolic compounds in EVOO could not be excluded (Carnevale et al., 2019).

8. Evidence from systematic reviews and meta-analyses

The effect of olive oil consumption on cardiovascular risk factors and markers of inflammation has also been evaluated through systematic reviews and meta-analyses. Meta-analyses have the advantage of controlling for several confounding factors and analyzing the risk of bias in RCTs and observational studies. However, given the strict inclusion and exclusion criteria for this type of analysis, only a small number of studies are eligible (George et al., 2019; Hohmann et al., 2015; Schwingshackl et al., 2019). Besides, the interpretation of results and causal links with significant evidence must be cautious, given the considerable number of limitations in these studies (Fernandes et al., 2020; George et al., 2019; Hohmann et al., 2015; Schwingshackl et al., 2015, 2018, 2019; Schwingshackl & Hoffmann, 2014; Zamora-Zamora et al., 2018).

The existence of a causal relationship between dietary fatty acids, blood lipids and CVD is still controversial (Schwingshackl & Hoffmann, 2014). The consensus is that replacing foods rich in saturated fatty acids with others rich in unsaturated fatty acids reduces the risk of coronary heart disease, as vegetable oils are more effective at lowering LDL-C than butter (Schwingshackl & Hoffmann, 2014). Also, plants rich in omega-3 and omega-6 fatty acids are more effective at lowering LDL-C and total cholesterol than olive oil (Schwingshackl & Hoffmann, 2014). Oils rich in unsaturated fatty acids, such as olive oil, are more effective at lowering LDL-C, compared to foods rich in saturated fatty acids, such as butter or lard, since most of the evidence comes from indirect comparisons and biomarkers of CVD risk (Schwingshackl et al., 2018). MUFAs, in general, have been shown to reduce the overall risk of all-cause mortality (11%), cardiovascular mortality (12%), cardiovascular events (9%), and stroke (17%) (Schwingshackl & Hoffmann, 2014). On the other hand, combined MUFAs of animal and plant origin have no significant effects on these indicators. The MUFAs of the Western diet come mainly from animal sources, and the MUFAs of the Mediterranean diet come mostly from olive oil. As olive oil is associated with a reduced risk of CVD, it is believed that these studies did not consider the different sources of MUFAs, and MUFAs could also come from other vegetable oils (canola, sunflower) or dried fruits (Schwingshackl & Hoffmann, 2014). Further studies with specific sources of MUFAs are needed, namely, RCTs and not observational studies based on nutritional assessment, such as cohort studies (Schwingshackl & Hoffmann, 2014). Thus, different meta-analyses were inconclusive regarding the role of MUFAs in the risk of coronary heart disease.

The health properties associated with consuming olive oil are now recognized to be primarily mediated by phenolic compounds. The effect of high- or low-polyphenol olive oil in cardiovascular risk factors and olive oil as a stand-alone intervention has been evaluated in several controlled clinical trials (George et al., 2019). High-phenolic olive oil confers some CVD-risk reduction benefits provided by phenolic compounds, regardless of the high MUFA content (George et al., 2019). High-phenolic olive oil improved total cholesterol and HDL-C and outcomes related to oxidative stress, by reducing malondialdehyde and oxLDL-C. Olive oil having high phenolic content reduces the risk of CVD, despite the short-term studies on Mediterranean cohorts (George et al., 2019). Consumption of high-phenolic olive oil does not affect TG levels and has little benefit both on systolic blood pressure and on the oxidative state of the serum. However, it exerts a beneficial effect on oxLDL-C in patients with CVD (Hohmann et al., 2015). A meta-analysis from Schwingshackl et al. (2019) concluded that different types of olive oil show distinct effects on cardiovascular risk factors: EVOO with high-phenolic content slightly reduced LDL-C compared to low-phenolic EVOO; high-phenolic and low-phenolic EVOO reduced systolic blood pressure compared to refined olive oil; high-phenolic EVOO may improve oxLDL-C compared to refined olive oil; and EVOO may reduce oxLDL-C compared to refined olive oil (Schwingshackl et al., 2019). Besides, there was a dose−response relationship between higher intakes of phenolic compounds from olive oil and lower systolic blood pressure and lower levels of oxLDL-C. High-phenolic EVOO has been ranked as the best treatment for LDL-C. Finally, it was concluded that high-phenolic EVOO improve certain cardiovascular risk factors (Schwingshackl et al., 2019). From this body of evidence, one can concluded that olive oil having a high phenolic content should be recommended in the Mediterranean diet as a means of the nutritional prevention of CVD (Hohmann et al., 2015) or adjusted to dietary plans with specific medical purposes aiming to hinder or to lower CVD risk.

Regular consumption of olive oil has been shown to have beneficial effects on markers of inflammation and endothelial function (C-reactive plasma protein, IL-6, flow-mediated dilatation, and sE-selectin) (Schwingshackl et al., 2015). Daily consumption of olive oil is an adequate alternative to dietary fat, given its antiinflammatory potential, especially for significantly decreasing IL-6 (Fernandes, 2020). However, data on TNF-α, adiponectin and flow-mediated dilatation were not affected, or the results were inconclusive (Schwingshackl et al., 2015). Also, at a daily dose of 10−50 mg/day, EVOO lowered diastolic blood pressure in healthy subjects or those with cardiovascular risk factors, including people with high blood pressure (Zamora-Zamora et al., 2018). Despite the small number of studies, olive oil represents a key ingredient to the cardiovascular protective effects of the Mediterranean diet (Schwingshackl et al., 2015).

Meta-analyses have highlighted several limitations of controlled clinical trials and cohorts that will need to be overcome to reach more robust conclusions in the future. The main limitations are related to heterogeneous study designs, which include: the nature of interventions with olive oil (e.g., olive oil used as a supplement or as part of a diet such as the Mediterranean diet); variations in the diet of control groups (who may also have consumed olive oil) (Schwingshackl et al., 2015; Zamora-Zamora et al., 2018); daily dosage of olive oil; types of olive oil (uncertainty of its classification on studies (Schwingshackl et al., 2019)); duration of the intervention and periods of follow-up (Fernandes et al., 2020; Schwingshackl et al., 2015); characteristics of the populations (different countries, mainly carried out in the Mediterranean, participants with distinct risk factors, limiting external validation (Zamora-Zamora et al., 2018)); small number of studies and participants (Hohmann et al., 2015); nature of the evidence (most come from indirect comparisons, such as intermediate biomarkers of CVD risk (Schwingshackl et al., 2018, 2019)); low-to-moderate risk of bias in most studies (George et al., 2019); outcome data relevant to patients (such as coronary heart disease or stoke); duration of controlled clinical trials (often they were too short, less than 12 weeks) (Schwingshackl et al., 2019).

More studies on olive oil consumption are needed to confirm the evidence with more well-designed controlled clinical trials. Despite the limitations, the scientific evidence demonstrates the beneficial effects of olive oil on cardiovascular risk factors and markers of inflammation.

9. Functional olive oils

EVOO is considered a functional food in its own right. EVOO should be consumed raw to exert its recognized benefits after ingestion in order to complement a healthy and balanced meal, generally associated with the Mediterranean diet. However, it is difficult to obtain virgin olive oils with a polyphenol content compliant with the EFSA health claim ($\geq$250 mg/kg hydroxytyrosol and its derivatives) (EFSA, 2011). Several varieties and ripening stages of olives naturally rich in phenolic compounds have been studied to produce potential EVOO that comply with these claims without adding phenolic compounds (López-Huertas et al., 2020). Other approaches aim to design virgin olive oils enriched or fortified with bioactive compounds (phenolic compounds, n-3 essential FA, or carotenoids) from natural sources, which increase their nutritional and medicinal value and improve their oxidative stability and their shelf-life, also known as "functional olive oils."

Different natural sources of biologically active substances have been used to enrich virgin olive oils. Mainly herbs and spices (thyme, oregano, rosemary, sage, mint, or basil), but also plant leaves (olive, oleaster and citrus), seed oils (flaxseed, safflower), oily fruits (pistachio, walnut), citrus peels (lemon and orange), among others, as discussed below.

Herbs and spices modify the flavor and increase the content of bioactive compounds in virgin olive oils. In general, extracts of herbs and spices do not significantly increase oxidative stability or the evolution of oxidation rates in enriched virgin olive oils. However, in several cases, its antioxidant capacity increases due to the greater amount and different activities of specific bioactive compounds (reviewed by Reboredo-Rodríguez et al., 2017). As mentioned earlier, several clinical trials have demonstrated the beneficial effects of ingested functional virgin olive oils enriched with different concentrations of phenolic compounds extracted from herbs (e.g., thyme) on CVD markers.

Citrus peels are suitable for improving the phytochemical composition and sensory profile of olive oils due to the content of bioactive compounds such as carotenoids, flavonoids (e.g., naringenin), limonoids, and phenolic compounds in higher concentrations than olive oil (tyrosol and hydroxytyrosol) (Flori et al., 2020). Virgin olive oils with citrus peels (orange and lemon) were produced using an innovative method (cryomaceration with solid carbon dioxide) in which the peels were cold macerated and then pressed with the olives. These citrus oils can also be used as ingredients for pastry and bakery, having peculiar sensory attributes due to their sweet notes (Ascrizzi et al., 2019).

Fortified virgin olive oils with orange or lemon peels (25%, w/w of peels to olives) have been shown to have protective effects in rats with metabolic syndrome and oxidative stress induced by a high-fat diet (Flori et al., 2020). Positive outcomes were observed after ingestion for glucose and serum lipid levels, the metabolic activity of adipocytes, myocardial tissue functionality, oxidative stress markers, and endothelial function at the blood vessel level (Flori et al., 2020). The addition of citrus peels increases the antioxidant activity of olive oils; however, it decreases the oxidative stability and degradation rate of bioactive compounds (Reboredo-Rodríguez et al., 2017). Therefore, it is necessary to determine the ideal conditions for the production of these fortified olive oils.

The nutraceutical properties of EVOO can also be enhanced by adding olive leaves when pressing olives. In a study with very ripe olives and cryomacerated olive, orange, and lemon leaves, the chemical and sensory qualities of these oils were compared. The composition of the antioxidants varied according to the botanical origin of the leaves. Leaf-added olive oils contained more antioxidants than leafless olive oils, and olive oil with olive leaves had 50% more oleuropein than pure olive oil. There was an increase in vitamin E in fortified olive oils, since vitamin E is present in the leaves. Leafy olive oils have improved organoleptic properties compared to pure olive oil in terms of overall pleasantness, complexity and aroma (Sanmartin et al., 2019).

Oleaster (*Elaeagnus angustifolia* L., wild olive tree) leaves have shown great potential to fortify olive oils by improving organoleptic characteristics and oxidative stability (Hannachi & Elfalleh, 2021). After the enrichment process, there was a high concentration of phenolic compounds (354.95 mg/kg), chlorophylls (8.13 mg/kg), and carotenoids (1.51 mg/kg) and also an improvement of the profile of phenolic compounds. The optimal conditions of the olive oil enrichment process with oleaster leaves were determined by the surface response methodology based on a mathematical model: 7.76 g of dry powder from oleaster leaves, 116.44 mL of olive oil, ca. 42 h of maceration (Hannachi & Elfalleh, 2021).

Several works have enriched olive oil with polyphenols from olive fruit and specifically with hydroxytyrosol, tyrosol, and oleuropein aglycones to produce a final product with greater bioactivity and an official health claim. The most efficient methods of producing olive oil enriched with olive polyphenols s are the addition of polyphenolic derivatives encapsulated in liposomes or the extraction of polyphenols from olive oil using an organic solvent and then incorporating them with a cryogenic sublimation into another olive oil (Tsilfoglou et al., 2017). The final product combines nutritional and medicinal benefits in disease prevention and can be used to improve food and pharmaceutical products (Tsilfoglou et al., 2017).

EVOO fortified with genistein, an isoflavone (phytoestrogen) with antioxidant and antiinflammatory activities, was administered to rats with ulcerative colitis (the daily intake of genistein was 100 mg/kg body weight). Fortified olive oil was able to attenuate the high levels of IL-1β and the oxidative damage induced by colitis, to a greater extent compared to pure olive oil or with genistein fortified canola and rice oils. The incorporation of genistein into EVOO improves its bioavailability, given its hydrophobicity, and suggests new ways of treating ulcerative colitis through nutritional supplementation (Tanideh et al., 2020).

Functional olive oils have also been developed using refined olive oil enriched with phenolic extracts of pistachio and nuts. The pistachio kernels (ca. 7 g/kg) and walnuts (ca. 10 g/kg) had a high content of phenolic compound. In the fortified formulations, the concentration of phenolic compounds reached 340−570 mg/kg, demonstrating a high antioxidant activity, mainly with the phenolic extracts of walnuts (Fregapane et al., 2020).

Besides adding natural antioxidants, adding essential FA to olive oil is an important factor since as it is mainly monounsaturated fat. A formulation of mixed oils was developed to obtain a product rich in essential FA and a reduced *n*-6/*n*-3 ratio (Meinhart et al., 2017). The formulation with the best sensory acceptance contained 85% EVOO, 3% flaxseed oil, and 12% rapeseed oil. This formulation had a lower *n*-6/*n*-3 ratio than EVOO, higher oxidative stability, higher levels of phytosterols and tocopherols, and can be used as an ingredient in various food products (Meinhart et al., 2017). Further studies have shown that the transesterification of α-linolenic acid (C18:3*n*-3, ALA) (obtained from flaxseed oil) mediated by commercial lipases in the TG of olive oil is a suitable technique for enriching olive oil in *n*-3 PUFA. *Candida rugosa* has been identified as a good source of lipases for the transesterification of ALA with olive oil and olive oil enriched with 27% ALA (Rupani et al., 2014).

Although pure EVOO is generally the option of traditional consumers, namely those in Mediterranean countries, enriched/fortified virgin olive oils, also called “gourmet olive oils” can represent a market strategy to increase consumption of olive oil by nontraditional consumers or as part of nutritional strategies for a diet richer in specific micronutrients.

10. Conclusion

Olive oil has a unique chemical composition which distinguishes it from other food oils. High-quality olive oil production systems, the development of new production technologies and compliance with high-quality management practices in the olive mill are essential to obtain a product that meets regulated standards, industry requirements and consumer tastes. Olive oil has a health claim linked to its phenolic compounds by its beneficial role against blood lipids oxidation. However, the bioavailability and bioaccessibility of phenolic compounds from olive oil in humans are limited. It is still necessary to understand the underlying mechanisms, within the framework of synergistic effects with other compounds and in the context of a dietary pattern. There is a large body of scientific evidence of the health attributes of olive oil or its components, namely oleic acid and phenolic compounds, in improving risk markers of CVD, atherogenicity, endothelial function, liver disease, diabetes associated with obesity, metabolic syndrome, autoimmune diseases, neuroinflammation, neurodegenerative diseases, sepsis, or skin inflammation. Scientists claim that more controlled clinical studies in humans are needed to demonstrate the positive effects of (extra virgin) olive oil on several pathologies. But there is also a consensus that extra virgin olive oil for its quality and chemical composition is a central ingredient when included in a balanced diet and healthy lifestyle. In addition, future work on "functional olive oils" should assess their stability during storage (shelf-life), during cooking (quality), their sensory characteristics, verify the positive effects of bioactive compounds (medicinal value), determine the biologically active concentrations (nutritional and nutraceutical value), as well as their bioavailability and bioaccessibility.

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Chapter 5

Olive, apple, and grape pomaces with antioxidant and anti-inflammatory bioactivities for functional foods

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1. Introduction

There is now substantial evidence from several epidemiological and clinical studies showing the benefits of plant-based healthy diets, such as the Mediterranean Diet, which has shifted the global trend toward consuming plant-based products, especially from those sources that some of the major components and beverages of the Mediterranean Diet are produced, such as olive oil and alcoholic beverages like wine, with a plethora of reported functional properties and health benefits (Tierney et al., 2019). However, this shift of the consumers has also remarkably increased the need for increased production of these healthy foods, which are usually accompanied by a subsequent coproduction of several food industry byproducts, such as olive pomace, grape pomace, and apple pomace. These pomaces were initially treated as "wastes" of the food industry, due to their environmental impact, and thus classic waste management was followed for their removal, with further increased overall cost and loss of the functional bioactive that are found in these pomaces. Besides representing an economic problem for producers, these byproducts also pose serious environmental concerns, thus their partial reuse, like that of all agronomical production residues, represents a goal to pursue. This aspect is particularly important since such byproducts are rich in bioactive compounds, which, once recovered/extracted, may represent ingredients with remarkable added value for food, cosmetic, and nutraceutical industries (Schieber, 2019; Antónia Nunes et al., 2018). The attempts for the recovery of such bioactive compounds found in these pomaces have been recently increased thoroughly, aiming to their valorization as added ingredients for increasing the functionality of the enriched food products or for developing novel products with enhanced functional properties and health benefits, including supplements and nutraceuticals while reducing also their environmental impact and the economic costs for the food industries.

2. Olive pomace

The Mediterranean diet includes virgin olives and olive oil as the main lipids' sources, which contain an extensive number of bioactive compounds with several functional properties and health benefits (Jimenez-Lopez et al., 2020). Currently, olive oil production represents an important economic income for Mediterranean countries, where roughly 98% of the world's production is located. During the production of olive oil, several byproducts are produced, with the main ones being the olive pomace, and olive mill wastewater, which are also considered as rich sources of valuable biofunctional compounds, such as organic acids, and lipid bioactive, and above all, phenolic compounds (Antónia Nunes et al., 2018). Yet, extraction and recovery of such bioactive components from olive pomace constitute a critical issue for their rational valorization and detailed identification and quantification are mandatory. Nevertheless, several applications now exist for

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the recovery and valorization of these valuable compounds from the olive pomace, including those in the functional foods, nutraceutical, pharmaceutical, and cosmeceutical sectors, and, consequently, to foster the sustainability of the olive-oil chain (Madureira et al., 2022). Of special interest are applications of using the valuable compounds recovered from olive pomace, as functional ingredients in enriched added value food products, without however modifying substantially the organoleptic parameters of the novel products.

2.1 Introduction to olive pomaces

The olive oil industry produces quite a few byproducts together with olive oil, including olive leaves, olive stones, olive pomace (OP), and olive mill wastewaters (OMWW). Extra virgin olive oil (EVOO) is obtained from the olive fruit by mechanical procedures, whereas its production involves one of the following extraction processes: (1) discontinuous (press) extraction, (2) three-phase centrifugal extraction, or (3) two-phase centrifugal extraction (Rahmanian et al., 2014) (Fig. 5.1).

From the three-phase mode, water can be added to the olive paste, which can be next separated into a solid residue of olive pomace, an oily phase, and a water phase. The two-phase method is fundamentally the exact same procedure, only without adding water into the first olive mash. Olive pomace is the principal residue following the two kinds of separation methods and it comprises crushed olive stones, process water, and all the substances coming from the olive drupe except the olive oil. It represents the principal residue of the olive oil extraction procedure by weight and its composition differs based on whether the processing is based on the two or three-phase method.

The total amount of pomaces produced per 100 kg of olives is around 87 kg in a “two-phase” method (wet olive pomace) and 62 kg in a “three-phase” method (olive pomace) (Peri, 2014). Nowadays, more than 90% of the olive mills operate with the two-phase system that generates wet olive pomace instead of OMWW (Kapellakis et al., 2008). The two-phase centrifugal system was introduced during the 1990s in which the olive paste is separated into phases of olive oil and wet pomace (sludge byproduct) results in the reduction of the volume of OMWW (Dermeche et al., 2013).

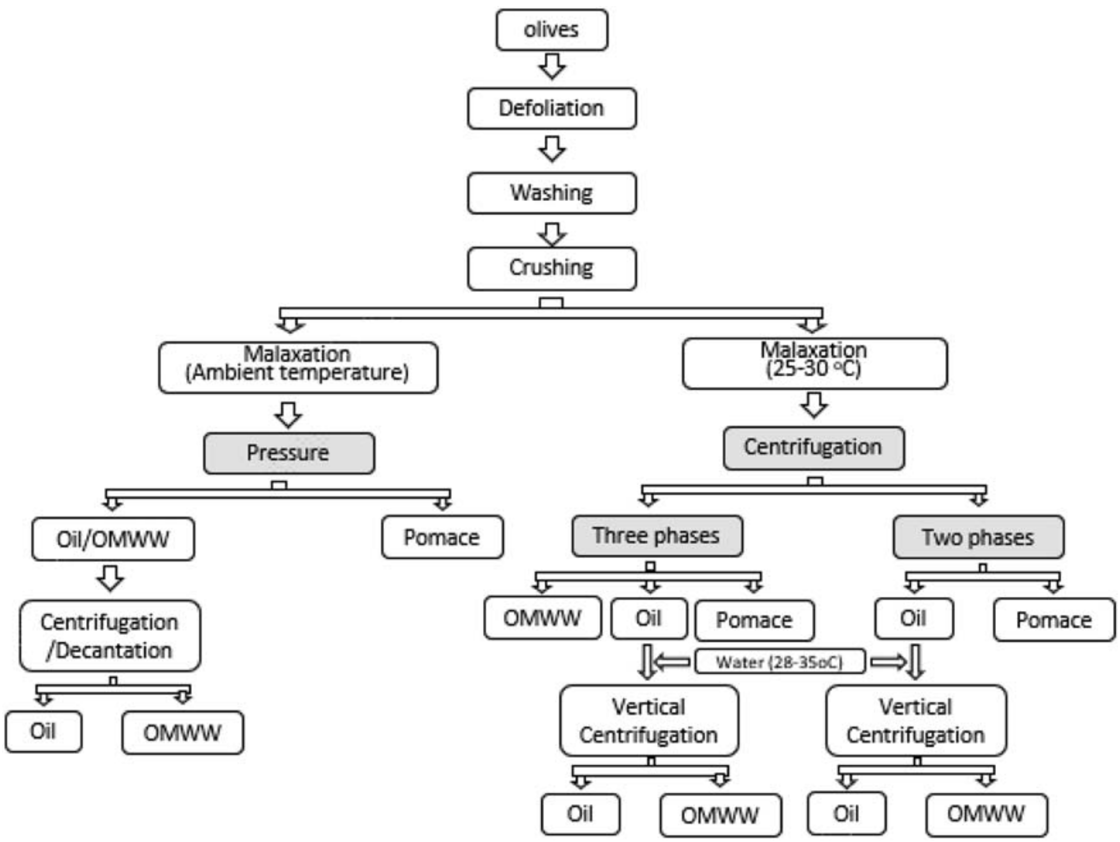

FIGURE 5.1 Extraction processes for producing extra virgin olive oil and acquiring olive pomace.

Olive pomace has a high amount of extractives (Ballesteros et al., 2001). This residue contains, among others, some residual olive oil and polyphenols. It may be processed to extract, usually with n-hexane, the so-called "pomace olive oil" that results in a solid residue called "extracted dry pomace residue". This residue is also characterized by high extractive content and it can be furtherly used as a source of valuable bioactive molecules such as tyrosol, hydroxytyrosol, oleuropein, and others (Rodríguez et al., 2008), or for other applications such as energy production. Pomaces have an acidic pH (around 5.2), a high content of phenolic substances (1%−3% of dry matter), and lipids (8%−14% of dry matter) (Alburquerque et al., 2004).

2.2 Olive pomace bioactive compounds and health effects

Factors that greatly influence the occurrence and concentration of particular bioactive molecules in olive oil industry byproducts are fruit cultivar, maturity, irrigation, storage time, and extraction techniques (Aviani et al., 2012; Lesage-Meessen et al., 2001; Mulinacci et al., 2001; Obied et al., 2008).

Extra virgin olive oil is a high-quality product. Its' production processes include olive tree cultivation, olive harvesting, olive processing, oil storage, oil bottling oil distribution, bottled oil for sale, and culinary usage. Among them olive cultivation, harvesting, and processing affect not only the quality of the extra virgin olive oil but also that of the olive pomace. Olive integrity during cultivation and harvesting is very important for the quality of olive pomace. Climatic conditions or pest attacks that damage olives result in an irreversible loss of quality. Having undamaged and healthy olives the quality is affected by the climate, the soil, the ripeness of olives at harvesting, and the oil processing conditions. Olive cleaning, olive milling, olive pitting, and olive paste malaxation are also steps in olive processing that affect olive pomace quality. Among them, olive paste malaxation is a processing step where molecular changes take place (Catania et al., 2016, 2017; Miho et al., 2020; Sánchez-Ortiz et al., 2016; Servili et al., 2015).

Olive pomace is a good source of bioactive compounds. Bioactive compounds found in olive pomace belong mainly to the classes of phenolic compounds and lipids. Table 5.1 presents the most important molecules that have been found in those classes (Dermeche et al., 2013; Nunes et al., 2018; Seçmeler et al., 2018; Stavroulias & Panayiotou, 2005; Pérez-Serradilla et al., 2008; Gallardo-Guerrero et al., 2002).

Phenols are today among the most debated groups of natural antioxidants, widely dispersed in the plant kingdom and available in our diet (Boskou, 2006). They contain one or more classes of hydroxyls (the polar part) directly bound to the aromatic ring (the nonpolar part) and are mostly found as esters or glycosides rather than free molecules in plants. The inverse correlation between cancer risk, coronary disorders, diabetes, multi-age chronic diseases, and dietary consumption rich in phenols and antioxidants has been shown in recent epidemiological studies (Karadag et al., 2009; Obied et al., 2009; Visioli et al., 1999; Yang et al., 2008).

Phenols may occur naturally in the olive fruit or are produced during the processing of olive oil (Obied et al., 2005). Only 2% of the olive phenols migrate into olive oil (Rahmanian et al., 2014), thus 98% of the phenols contained in olive fruit remain in olive pomace (Rodis et al., 2002).

TABLE 5.1 Indicative important bioactive compounds found in olive pomace.

Phenolic acids	Secoiridoids	Flavonoids	Lipids
Cinnamic acid	Oleuropein	Luteolin	α-Tocopherol
p-coumaric acid	Demethyloleuropein	Luteolin 7-O-glucoside	α-Tocotrienol
Caffeic acid	Verbascoside	Hesperidin	β-Tocopherol
Ferulic acid	Ligstroside	Quercetin	γ-Tocopherol
Vanillic acid	Tyrosol	Apigenin	β-carotene
Gallic acid	Hydroxytyrosol	Apigenin 7-O-glucoside	β-sitosterol
Syringic acid		Cyanidin 3-O-rutinoside	Squalene
Ainapic acid		Cyanidin 3-O-glucoside	Oleic acid
Homovanillic acid			Linoleic acid

Phenolic acids and alcohols, secoiridoids, and flavonoids are present in olive fruit (Boskou, 2008; Obied et al., 2007) and most of them remain in olive pomace. Phenolic compounds such as o- and p-coumaric, cinnamic, caffeic, ferulic, gallic, sinapic, chlorogenic, protocatechuic, syringic, vanillic, and elenolic acids are found in phenolic acids (D'Alessandro et al., 2005; Mulinacci et al., 2001). The most typical and abundant phenolic alcohols in olive oil and pomace are tyrosol and hydroxytyrosol (DellaGreca et al., 2004).

Other phenolic compounds contained in olive pomace are oleuropein, dimethyl-oleuropein, verbascoside, catechol, 4-methyl catechol, p-cresol, resorcinol, and high amounts of secoiridoid derivatives like hydroxytyrosyl acyclodihydroelenolate, comselogoside, and dialdehyde 3,4-dihydroxyphenyl-ethanol-elenolic ether linked to hydroxytyrosol, that is generated during olive fruit malaxation by the hydrolysis of oleuropein and dimethyl oleuropein (Damak et al., 2012; Japón-Luján & De Castro, 2007; Lo Scalzo & Scarpati, 1993; Obied et al., 2009; Servili et al., 1999). Numerous flavonoids such as apigenin, hesperidin, cyanidin flavone, anthocyanin, and quercetin may also be present in olive pomace (Obied et al., 2005; Visioli et al., 1999).

Olive pomace phenols are well known for their healthy strong antioxidant properties that further suggest their reuse as antioxidants in foods (Galanakis, 2012). Indeed, their ability to scavenge free radicals, both *in vitro* and *in vivo*, is one of their most known bioactivities. Several radical-generator compounds such as $DPPH^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl), $ABTS^{+}$ (2,2′-asinobis (3-ethylbenz-thiazoline-6-sulfonic) diammonium salt) or hyperoxide anion salt have been tested for this capacity (De Marco et al., 2007; Visioli et al., 1999). In addition, some studies have documented the antimicrobial effects of olive polyphenols against *Staphylococcus aureus*, *Bacillus subtilis, Bacillus cinerea*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Obied et al., 2007).

Among olive pomace phenolics, hydroxytyrosol is known, to exert advanced antiradical properties similar to vitamins E and C (Fernández-Bolaños et al., 2006). The antioxidant potential of hydroxytyrosol has been demonstrated in the plasma and liver of rats (Visioli et al., 2001) and its cardioprotective role on human cells (Leger et al., 2000). It is known to exert a defensive effect against low-density lipoprotein (LDL) oxidation *in vitro* along with other o-phenolics (e.g., oleuropein and caffeic acid) and it appears that it can be most effective at low concentrations to shield human erythrocytes and DNA from oxidative harm (Covas et al., 2006).

The beneficial effect of hydroxy tyrosol as an important hypoglycemic and antioxidant agent has also been shown by reducing oxidative stress and free radicals, as well as by strengthening enzymatic protective activities in diabetic rats. The European Food Safety Authority (EFSA) has licensed hydroxytyrosol-rich olive oils for their potential to sustain stable amounts of LDL cholesterol and lipid antioxidants (EFSA, 2012).

Numerous patents for the production of target phenolic compounds (e.g., oleuropein, hydroxytyrosol) from byproducts of olive oil manufacturing have now been filed. Olive pulp extracts with generally recognized as safe (GRAS) status (GRN No. 459) to be used as antioxidants in various foods (e.g., baked goods, drinks, and dressings) up to a maximum of 3 g/kg (0.3% w/w) in the final food have been commonly licensed by the Food and Drug Administration (FDA) in the United States (Galanakis et al., 2015).

Some studies, although limited in number, have shown a health beneficial role of the olive pomace and especially its polar lipids against the risk for cardiovascular diseases and the development of atherosclerosis, as well as against renal disorders such as glomerulosclerosis, through the inhibition of platelet-activating factor activity and regulation of its metabolism (Table 5.2).

TABLE 5.2 Health effects of biofunctional olive pomace lipid extracts.

Sample	Effect	Experimental model	References
Olive pomace	1. *In vitro* platelet antiaggregatory activity.	Washed rabbit platelets.	(Karantonis et al., 2008)
Olive pomace polar lipids	1. *In vitro* antiglomerulosclerotic activity. 2. *Ex vivo* platelet antiaggregatory activity. 3. *In vivo* antiatherogenic activity. 4. *In vivo* atherogenic regression. 5. No significant effect on coagulation markers (e.g., *ex vivo* human platelet aggregation and circulating plasminogen activator inhibitor-1 (PAI-1) and P-selectin (sP-selectin) concentrations) in adults.	Human mesangial cells. Rabbit platelet-rich plasma. Male New Zealand rabbits. Male New Zealand rabbits. Double-blind randomized cross-over trial in humans.	(Tsoupras et al., 2007, 2011) (Tsantila et al., 2007) (Tsantila et al., 2010) (Tsantila et al., 2010) (Ntzouvani et al., 2021)

2.3 Application for functional food production

Recently, staple foods have been fortified with olive pomace to produce functional foods that influence one or more target functions of the body beneficially, beyond sufficient nutritional impact, in a way that is important to either enhanced health or well-being condition and/or reduction of risk of disease. Indeed olive pomace has been shown to exert antioxidant (Radić et al., 2020; Ribeiro et al., 2021; Vergani et al., 2016) and anti-inflammatory (Carito et al., 2015; Herrero-Encinas et al., 2020; Tsoupras et al., 2007, 2011) properties. Since oxidation and inflammation are key mechanisms underlying the onset of chronic diseases such as cardiovascular diseases, type II diabetes, and various types of cancer (Demopoulos et al., 2003; Nasopoulou, Karantonis, et al., 2014a), utilization of olive pomace could lead to functional foods whose consumption may reduce the incidence of chronic diseases.

2.3.1 Edible oils

Edible oils high in unsaturated fatty acids are more prone to oxidation and antioxidants may be applied to improve their shelf life. Natural antioxidants derived from byproducts and waste are preferred to synthetic ones such as butylated hydroxyanisole (BHA, E-320), butylated hydroxytoluene (BHT, E-321), *tert*-butylhydroquinone (TBHQ, E-319), and propyl gallate (PG, E-310), due to their possible harmful impact on human health (EFSA, 2011, 2012, 2014, 2016; Araújo et al., 2015; Sánchez De Medina et al., 2011). Polyphenols derived from olive pomace have been added to test the consistency and efficiency of various processed oils such as maize, soy, high oleic sunflower, sunflower, olive, and rapeseed oil. The result was that all enriched oils were improved in terms of oxidation stability (Orozco-Solano et al., 2011; Sánchez De Medina et al., 2011).

2.3.2 Pasta

Pasta as a staple food has been the subject of many studies for nutritional improvement (Oliviero & Fogliano, 2016). Among other approaches, the fortification of pasta has been performed by replacing durum wheat semolina with 5% and 10% olive pomace powder resulting in increased total phenol content and antioxidant efficiency (Simonato et al., 2019). To obtain a food capable of having a beneficial effect on the health of customers, functional spaghetti from durum wheat semolina enriched with olive paste powder (10−15%) was produced. In enriched spaghetti, the levels of overall free phenolics and flavonoids were almost 50 times and 15 times higher than in control spaghetti, respectively. In addition, the new product with olive pomace was richer in fiber, carotenoids, and tocopherols (Padalino et al., 2018). Increased antioxidant activity in olive pomace-enriched pasta was also observed in a similar study (Cecchi et al., 2019; Cedola et al., 2020).

Besides increased nutritional value in enriched pasta and the findings of the market test showing that customers liked and were able to pay more for fortified pasta than the regular one, the overall output was lower in enriched pasta products compared to regular products in terms of organoleptic characteristics (Cedola et al., 2020), highlighting the need for further research in enrichment methodologies.

2.3.3 Bread

Bread fortified with 10% of olive pomace had better antioxidant efficacy, leading to a higher prevalence of phenolic acids and flavonoids, and a better glycemic response due to higher fiber content (Cedola et al., 2020).

2.3.4 Biscuits

Biscuits enriched with 6%−7% (w/w) olive pomace were richer in dietary fiber than controls, showing significantly lower glycemic index and energy value. Consumption of biscuits enriched with olive pomace powder increased homovanillic and 3,4-dihydroxyphenyl acetic acid amounts, which are believed to be involved in lowering oxidative LDL cholesterol. The intake of enriched biscuits also raised the number of phenolic acids in the urine, suggesting an upregulation of polyphenol biotransformation in the intestine and hydroxytyrosol derivatives in the blood (Conterno et al., 2019; Lin et al., 2017).

2.3.5 Snacks

An amount of 20% fermented olive pomace in an Italian savory snack called "taralli" resulted in a new product higher in hydroxytyrosol, triterpenic acid, lutein, and β-carotene, and lower saturated fatty acid content compared to the control. Oxidation in enriched snacks was significantly lower compared to the original snack resulting in a longer shelf life (Durante et al., 2019).

2.3.6 Fish

Replacing an amount of fish oil with olive pomace in the diet of gilthead sea bream exhibited no significant difference in the growth performance factors of fish fed with OP diet compared with the control group. Both eicosapentaenoic acid (EPA) (C20:5n-3) and docosahexaenoic acid (DHA) (C22:6n-3) content in gilthead sea bream muscle of fish fed with OP enriched diet were found to be statistically decreased compared to control group (Nasopoulou et al., 2011). Moreover, bioactive lipid fractions of OP with antiaggregatory effects on platelets stimulation-aggregation have effectively enriched gilthead sea bream feed and monitored in aquacultured fish (Nasopoulou et al., 2013), while structural elucidation of OP fed gilthead sea bream phospholipids with antiaggregatory activity against platelets aggregation underline the fact that OP inclusion in fish feed reinforces fish cardioprotective properties (Sioriki et al., 2016).

Sea bass treated with an experimental diet enriched with 4% OP showed no significant difference in fish feed conversion ratio and specific growth rate when compared to the control group, suggesting that sea bass fed with the experimental OP diet exhibited satisfactory growth performance, similar to that of the control group (Nasopoulou, Smith, et al., 2014b). Structural elucidation of phospholipids, present in OP-fed sea bream, that exert potent inhibitory activity against platelet activating factor (PAF) action, a known lipid mediator of inflammation and thrombosis, present in OP-fed sea bream, highlights the cardioprotective properties of fish nourished with OP enriched fish feed (Nasopoulou, Smith, et al., 2014b).

Partial substitution of wheat flour and corn meal with olive pomace in the diet of rainbow trout exhibited no significant difference in growth performance and feed utilization between the fish fed with the OP-treated diets when compared to the control group. Regarding the fatty acid profile alpha-linoleic acid (C18:3n-3) amount increased in the muscle of fish of almost all experimental groups received a diet enriched with OP compared to control, whereas arachidonic acid (C20:4n-6) content remained unchanged in the muscle. Eicosapentaenoic acid (C20:5n-3) exhibited significantly decreased levels in the muscle of the fish treated with an experimental diet enriched with more than 4% OP (Khoshkholgh et al., 2020).

Regarding the hematological characteristics hematocrit and hemoglobin exhibited an OP dose-dependent reduction, with the lowest decrease to be observed in the experimental group treated with the highest OP levels (Khoshkholgh et al., 2020). Increased content of tannin in the diet containing 100 g/kg OP could be the reason for hemoglobin reduction since tannin is a polyphenolic compound acting as an immunostimulant and antioxidant factor, interfering in the minerals binding, thus iron absorption (Banavreh et al., 2019). Additionally, serum LDL exhibited decreased levels with increased OP content within the experimental diets, while serum HDL levels increased. These findings are an indication that an increase in cellular lipid and metabolism improved the growth performance (Khoshkholgh et al., 2020). Experimental diets containing increased OP content reinforced serum lysozyme activity an important hydrolytic enzyme of the fish's innate immune system (Khoshkholgh et al., 2020).

Wheat flour replacement with OP up to 100 g/kg diet showed no considerable difference in the final weight, feed conversion ratio, feed intake and specific growth rate between the fish treated with OP enriched experimental diets and the control group. Digestibility of crude protein and crude lipid showed no significant difference between the control diet and experimental diets. Regarding the fatty acid profile, both docosahexaenoic acid (C22:6n3) and the ratio of n-3/n-6 fatty acid exhibited significantly increased levels in fish treated with OP-enriched diets compared to the control diet (Banavreh et al., 2019).

2.3.7 Meat

Rabbits fed with feeds enriched with olive pomace led to meat higher in monounsaturated and lower in polyunsaturated fatty acids with higher oxidative stability due to polyphenols and other antioxidants in olive pomace. These effects were proportional to the content of antioxidants in the olive pomace sample (Dal Bosco et al., 2012). A study in chickens resulted in a final product with increased nutritional value since chicken meat exerted higher oxidative stability compared to control due to tyrosol, hydroxytirosol, and verbascoside that were found in chicken meat (Branciari et al., 2017). Broilers were also researched for their beneficial properties in their breast and thigh meet after being fed olive pomace (Nasopoulou et al., 2018). This study displayed very promising results with not only increased antithrombotic characteristics but also beneficial sensory properties. Similar studies in lambs fed with a mixture of olive pomace and linseed resulted in meat with increased oxidative stability and higher levels of polyunsaturated fatty acids and vitamin E (Luciano et al., 2013).

2.3.8 Milk, cheese, and yogurt

Ewes fed with olive pomace resulted in an increased ratio of unsaturated/saturated fatty acids and decreased atherogenic and thrombogenic index (Chiofalo et al., 2004; Vargas-Bello-Pérez et al., 2013). In addition, buffalo fed with olive pomace led to milk with higher content of total tocopherols and hydroxytyrosol, and a fatty acid composition increased in monounsaturated and polyunsaturated fatty acids and decreases in saturated fatty acids resulting in a decreased ratio of saturated/unsaturated fatty acids (Terramoccia et al., 2013).

Similar results concerning improvement in the lipid profile were observed in cheese from ewe and cow milk, where monounsaturated and some polyunsaturated fatty acids increased by supplementing the feed with olive pomace resulting in healthier cheese products (Castellani et al., 2017; Vargas-Bello-Pérez et al., 2013).

The addition of aqueous phenolic extracts from olive pomace to fermented milk using *Streptococcus thermophilus* and *Lactobacillus acidophilus* cultures gave an enriched yogurt with higher total phenolics and antioxidant activity compared to control (Aliakbarian et al., 2015).

Based on the fact that lipid bioactives from apple pomace were found to modulate *in vitro* PAF-metabolism toward favorably reducing the levels and activities of this inflammatory mediator (Tsoupras et al., 2007, 2011), a randomized, double-blind, three-arm trial was recently performed in 92 apparently healthy, but mainly overweight volunteers (35−65 years), based on a new functional yogurt enriched with such PAF inhibitors of natural origin from the olive pomace, in order to evaluate the putative beneficial effects of this fermented product on PAF metabolism (Detopoulou et al., 2021). The intake of the enriched yogurt resulted in reduced activities of the main regulatory enzymes of PAF-biosynthesis, while no difference was observed in the activities of the two isoforms of the main catabolic enzyme of PAF. Thus, intake of yogurt enriched in olive pomace-derived PAF inhibitors can favorably modulate PAF biosynthetic and catabolic pathways (Detopoulou et al., 2021).

2.3.9 Eggs

Laying hens fed with olive pomace-supplemented feed resulted in eggs with lower cholesterol levels in egg yolk compared to control animals. A whole bleed transcriptomic study on the laying hens found that gene expression was impaired by the supplementation and many downregulated genes in the biosynthetic cholesterol pathway were identified showing positive effects of dried olive pomace-supplemented diet on inflammation and cholesterol (Iannaccone et al., 2019).

3. Apple pomace

3.1 Introduction

The modern apple industry produces a massive 70 million tons of apples worldwide each year (Perussello et al., 2017) and with a vast volume of these apples going to the juice, cider, and vinegar processing plants comes large amounts of apple pomace waste. Apple pomace is the waste product after juicing stage where the apples are crushed and squeezed to remove all the juice that will be later manufactured into juice, cider, or vinegar (Fig. 5.2). Annual processing of up to 10−15 million tons of apples worldwide for the production of juice and cider generates a huge volume of relative products, byproducts, and wastes. The disposal of these wastes leads to additional waste treatment costs for farms/companies/industries, while if not treated correctly, this waste may cause environmental issues.

Apples are widely consumed worldwide due to their nutritional values and their basic availability to the public all year round (Wojdyło, 2008). The apple is primarily composed of water, vitamins, minerals, and carbohydrates such as fructose and fiber.

3.2 Apple pomace composition, nutritional value, and associated health benefits

Apple pomace consists of approximately 95% flesh and skin with the remaining 5% consisting of seeds (2%−4%) and stems (1%) (Bhushan et al., 2008; Grigelmo-Miguel et al., 1999). The constituents of the pomace have been thoroughly researched throughout the literature displaying the variances due to the processing involved in juice production and the types of apples used (Vendruscolo et al., 2008). The variances of apple pomace's physical-chemical composition do not alter the fact that apple pomace contains an abundance of healthy compounds and nutrients such as proteins, minerals, vitamins, and phytochemicals (Bhushan et al., 2008). The pomaces carbohydrates are primarily composed of simple sugars in the form of fructose (23.6%), glucose (22.7%), and galactose (6%−15%) (Ktenioudaki et al., 2013). The apple pomace minerals consisted of Calcium (0.06%−0.1%), Magnesium (0.02%−0.36%), Phosphorus (0.07%), and Iron (31.8 mg/kg DW) (Lyu et al., 2020). Apple pomace contains an abundance of polyphenols such as flavanols, cinnamate esters, and dihydrochalcones (Will et al., 2006). Notably, apple pomace has been displayed throughout the research to contain natural antioxidants especially quercetin glycosides, phloridzin, and other phenolic, antioxidant compounds (Lu et al., 2006; Schieber et al., 2002). Throughout the extensive research, it is clear that apple pomace possesses health beneficial

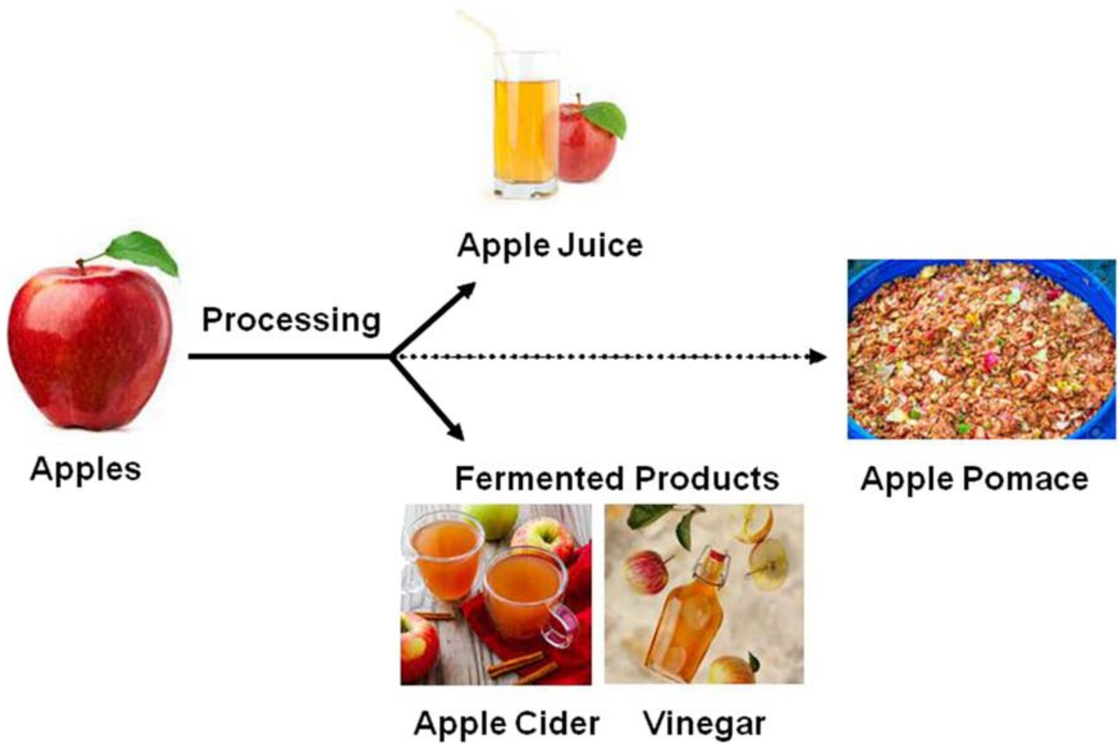

FIGURE 5.2 Apple pomace is produced as a byproduct of apple processing for making apple juice, cider, and vinegar.

compounds, which may be underutilized by classifying it as a waste product and not characterizing pomace as a cheap, alternative, health ingredient within the food industry.

Apple waste such as pomace is produced by the juice, cider, and vinegar industry during the pressing stage. Both apple products and the remaining apple pomace have also been found to contain several other bioactive compounds, like phytochemicals and polar lipids (PL), the composition of which vary according to the apple variety, the methods applied to produce apple products, and several other parameters (Blidi et al., 2015; Boyer & Liu, 2004; Tsoupras, Moran, Pleskach, et al., 2021; Tsoupras, Moran, Byrne, et al., 2021).

More specifically, during the picking, it is important to note the apple variety, which season it is, and the weather conditions. These factors can influence the composition of the apples and further affect the quality and shelf life of the products. The apples must be inspected for imperfections they may have such as pest damage, bruising, and any imperfections they may have to ensure the product is not affected later in processing.

Apple juice and apple cider manufacturing/processing can vary throughout the industry leading to the alterations in the apple pomace produced. The most common juicing process is a continuous press however in the smaller production lines or homebrew systems, a discontinuous vertical hydraulic may be in use (Canteri et al., 2012). Another difference in pomace composition is the additional step that occurs before pressing where the stalks, skin, and pips are removed from the pressing stage leaving fewer components within the waste pomace (Rabetafika et al., 2014).

As with the variety of apple juice products comes an array of apple varieties that are used. This variation brings a variety of composition to the pomace too. Tannins are a well-known natural phenolic compound utilized for their astringency and bitter taste (Ma et al., 2014). As for this, they are primarily used in fermented cider and other alcoholic fruit beverages such as grape fermentation to wine (Ashok & Upadhyaya, 2012). Apple cider production utilizes these types of compounds to bring a unique taste and aroma of apple cider.

Apples have always displayed promising health-beneficial properties and are well renowned for being healthy fruit. This has been mainly attributed to the many healthy apple phytochemicals, but also to some sugars, dietary fibers, and bioactive lipids (Tsoupras, Moran, Pleskach, et al., 2021; Tsoupras, Moran, Byrne, et al., 2021). Thus, the apple pomace produced as byproducts of cider breweries and apple juice production units can also serve as another source of bio-functional compounds with several health benefits that can be utilized for the production of several other functional foods and nutritional supplements.

There have been several studies on apples, apple products, and their components regarding their health benefits and their biofunctionality (Cory, 2018; Tsoupras, Moran, Pleskach, et al., 2021; Tsoupras, Moran, Byrne, et al., 2021). For example, apples and subsequently apple wastes like apple pomace, have been shown to be a rich source of lipid bioactive and bioactive phytochemicals, such as several phenolic compounds (Tsoupras, Moran, Pleskach, et al., 2021; Tsoupras, Moran, Byrne, et al., 2021). Apples and in particular the peels of apples have significantly higher polyphenols, phenolic acids, and total radical-trapping antioxidative potential (TRAP) values than other fruits such as pears and peaches (Leontowicz et al., 2002). It is widely accepted that the main health benefits of phenolic compounds compose of their antioxidant properties and decrease/prevent oxidation of LDL-cholesterol (Frankel, 1993.). Fruits, specifically apples, supplemented with rats have shown to have a hypocholesterolemic effect when fed cholesterol and even positively affect the plasma antioxidant potential (Leontowicz et al., 2002). Phenolic compounds such as tannins and flavan-3-ols found in apples and apple pomace, but also in grapes and peaches have been central to many research studies regarding antioxidant activities, free radical scavenging, and metal chelating activities (Lee, 2003; Woodside, 2013).

Apple pomace is also rich in fiber, carbohydrates, proteins, amino acids, fatty acids, minerals, vitamins, and several other compounds (Perussello et al., 2017). The literature describes numerous possible extraction and application methods that display the range of potential use of the byproduct as a functional ingredient, and substrate. The extraction method varies depending on the compounds of interest and the advantages/disadvantages that come with the process. Several reviews and articles throughout the literature have shown the extraction and applications display very positive outcomes with regard to health benefits however the sensory evaluation is often affected (Perussello et al., 2017).

3.2.1 Apple pomace bioactive lipid compounds and health benefits

Lipids are a large, complex group of biological compounds composed of several structures to allow them to possess beneficial, bioactive properties and functions. This complex group of macronutrients can be separated into two subgroups known as NL and PL. NL are mainly highly hydrophobic molecules such as waxes, triacylglycerols, cholesterol esters, and terpenes whereas PL like phospholipids and glycolipids are less lipophilic molecules with amphiphilic properties, which contain hydrophilic groups (two fatty acid moieties) and a hydrophobic head residue. Plant lipids and fatty acids (FA) are keys to the structure and the metabolic constituents of the plant and fruit cells. The plant membranes and orderly functions are dependent on these compounds to continue physical/chemical reactions within the fruit cells. The apple lipid composition has been shown to possess bioactive activity against inflammatory pathways and platelet aggregation induced either by the inflammatory and thrombotic mediator PAF or by classic platelet agonists like ADP (Tsoupras, Moran, Pleskach, et al., 2021; Tsoupras, Moran, Byrne, et al., 2021). Such lipid bioactive in apple products and byproducts seem to be putative candidates to be used as ingredients for the production of novel functional foods and nutritional supplements with improved anti-inflammatory health benefits, as well textural characteristics like aroma and taste.

More specifically, apples and apple pomace are composed of numerous lipid classes that possess bioactive properties and functions (Tsoupras, Moran, Pleskach, et al., 2021; Tsoupras, Moran, Byrne, et al., 2021). Apples are a diverse group of fruit that changes in composition depending on what variety/breed it is, how ripe the fruit may be, and any other complications during the growth/packaging/processing (Marangoni et al., 1996; Tsoupras, Moran, Pleskach, et al., 2021; Tsoupras, Moran, Byrne, et al., 2021) These factors can influence not only the total lipid (TL) content within the apple product but also the ratio between PL and NL. The general apple pomace was found to contain small amounts of TL (less than 1 g/100g of lipids) compared to other plant-derived food sources and byproducts. However, the amounts of TL in apple pomace were found to be one order of magnitude higher than the TL found in the actual apple products of apple cider breweries (apple juice, apple cider, and apple vinegar), while in the apple pomace TL the majority (more than 80%) were PL with potent bioactivities (Tsoupras, Moran, Pleskach, et al., 2021; Tsoupras, Moran, Byrne, et al., 2021). Thus, apple pomace contains comparable to other plant sources amounts of bioactive PL that can be more easily extracted since they almost represent the whole amount of apple pomace TL. This outcome further supports the notion of valorizing apple pomace as sustainable source of biofunctional PL for functional foods and nutraceuticals.

It should also be highlighted that the most bioactive PL from apple and apple pomace were those of the phosphatidylcholine (PC) family, while the apple pomace PL and especially their PC molecules were also found to contain biofunctional monounsaturated fatty acids (MUFA) like the oleic acid, as well as omega-3 (n-3) polyunsaturated fatty acids (PUFA), mainly the alpha-linolenic acid (ALA) and in much lower but considerable amounts the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), providing a rational for their strong antiinflammatory and antithrombotic bioactivities (Tsoupras, Moran, Pleskach, et al., 2021; Tsoupras, Moran, Byrne, et al., 2021). More specifically, Tsoupras, Moran, Byrne, et al. (2021) also found that within the PUFA content of apple pomace the omega-6 (n-6) linoleic acid (LA; 18:2 n6) and the n-3 ALA (18:3, n-3) were the most abundant PUFA, while palmitic acid (16:0) and stearic acid (18:0) were the most abundant within the saturated fatty acids (SFA) of apple pomace PL SFA content and the omega-9 (n-9) oleic acid (18:1c9) along with n-9 eicosaenoic acid (20:1 c11) being the most abundant monounsaturated fatty acids (MUFA) in apple pomace PL. The results found within this study are in accordance with previous publications in regard to plant-derived beverages such as tea (Tsoupras, Lordan, Harrington, et al., 2020) and Beer (Lordan, O'Keef, Tsoupras, et al., 2019; Lordan, O'Keef, Heffernan, et al., 2019; Tsoupras, Lordan, O'Keefe, et al., 2020).

The n-3 PUFA such as those found in apple pomace PL is defined as "essential" as they cannot be synthesized within the body and are needed in homeostasis maintenance. Therefore, must be consumed regularly through healthy dietary sources, such as oily marine sources and plant-derived sources (Lordan et al., 2020; Simopoulos, 2008; Tsoupras, Moran, Pleskach, et al., 2021; Tsoupras, Moran, Byrne, et al., 2021). Recent studies have also found that apple pomace seems to be one of such sustainable plant food sources mostly for the essential n-3 PUFA ALA, as well as small amounts of EPA and DHA, but also for the essential n-6 PUFA LA (Tsoupras, Moran, Byrne, et al., 2021). A plethora of studies has also shown the key roles of n-3 PUFAs in their influence on gene expression, maintenance of membrane homeostasis, and the overall importance of balance of the inflammatory effects/pathways of the n-6 PUFA (Simopoulos, 2008). EPA is widely accepted to have antiplatelet effects and DHA is needed more in the nervous system (Lordan et al., 2020). In addition, it is also proposed that diets and foods rich in n-3 PUFA such as ALA and especially those with beneficial low values of their n-6/n-3 PUFA have been found to exhibit several health benefits against associated with antiplatelet and anti-inflammatory properties against inflammation-related disorders such as atherosclerosis and CVD (de Lorgeril et al., 1994; Simopoulos, 2008; Tsoupras et al., 2018).

Nevertheless, it has also been shown that the polar forms of the n-3 PUFA when bound in PL are much more bioactive and such PL has superior bioavailability and efficacy rather than the neutral forms of the n-3 PUFA (as esters or esterified in triglycerides) (Lordan et al., 2020). Thus, only high amounts of the neutral forms of EPA and DHA, such as those of 4 g/day found only in supplements and not in foods have shown some anti-inflammatory and cardioprotective properties (Tsoupras & Zabetakis, 2020). In contrast, PL rich in n-3 PUFA seems to be more bioactive in fewer amounts, such as those that can be achieved by dietary sources and functional foods.

Interestingly, it has been previously proposed that plant sources do not contain the long chain n-3 PUFA EPA, DPA, and DHA due to lack of appropriate enzyme machinery for producing them from ALA and LA, yet Guil et al. (1996) have reported the presence of low amounts of both EPA and DHA in several natural plants (Guil et al., 1996), which was also recently observed in tea PL bioactives (Tsoupras, Lordan, Harrington, et al., 2020) but also in the ACBP-derived PL bioactives assessed in the present study and in the PL bioactives of apple products (apple juice and cider) from the same apple varieties (Jonagold, Dabinett, and Aston Bitter) (Tsoupras, Moran, Pleskach, et al., 2021; Tsoupras, Moran, Byrne, et al., 2021), while apple-derived rhamnogalacturonan II, the most structurally complex segment of the 10% of apple pectin, and its unusual sugar-based compounds have also been reported to contain DHA as determined by GC−MS of their trimethylsilyl-esters O-methyl glycosides (Giovanetti et al., 2012).

In addition, the presence of such essential n-3 PUFA (mostly ALA, but also much lower but considerable amounts of EPA, DPA, and DHA) bound in the bioactive PL extracts of apple pomace, and especially in their most potent phosphatidylcholine (PC) bioactive, further support their anti-inflammatory potency and provide a rationale for their strong anti-PAF effects as previously described in several other bioactive dietary PLs and PCs of natural origin, and especially in PL bioactives from healthy food sources (Tsoupras et al., 2018). On this accord, food sources and especially sustainable ones, such as the apple pomace that was found to be rich in bioactive PL with high content in oleic acid and the n-3 PUFA ALA, along with beneficially low levels of n-6/n-3 PUFA ratio, may be an alternative approach for producing functional foods containing bioavailable and bioactive PL molecules rich in essential n-3 PUFA and with potent antithrombotic and anti-inflammatory cardioprotective properties (Tsoupras, Moran, Byrne, et al., 2021). Nevertheless, appropriate efficient, and eco-friendly extraction methodology deserves further investigation, in order to acquire and valorize such bioactive PL from apple pomace, based on innovative technologies according to food grade approaches, and especially as far as scaling-up is concerned.

3.2.2 Apple phenolics and health benefits

As mentioned previously, apple pomace contains also an abundance of phenolic compounds, which display antioxidant properties such as phloretin, epicatechin, quercetin, caffeic acid, chlorogenic acid, and protocatechuic acid (Lohani & Muthukumarappan, 2016; Lu & Foo, 2000). These polyphenols also contribute to the sensory properties of foods and act as oxidative stability components of foods by preventing damage to the cells by reactive oxygen species (ROS) and other free radicals (Perussello et al., 2017). These molecules covert free radicals to a neutral form during cell's processing. The literature on polyphenols and human consumption display a protective properties of the compounds against human infections, chronic diseases, aging, and brain dysfunction. Phloridzin is a cotransporter of inhibitors of sodium and glucose located in the kidneys and intestines. Phenolics target these in order to reduce glucose serum levels, which can improve the health of diabetic patients (Masumoto et al., 2009).

Apples contain more than 60 phenolic compounds including the four key groups, hydroxycinnamic acids dihydrochalcone derivatives (especially phloridzin), benzoic acids, flavan-3-ols, and flavanols (Franquin-Trinquier et al., 2014; Grigoras et al., 2013). Notably, the largest subclass of phenols is flavonoids, flavanols, and flavanones, respectively.

Such phenolic compounds have been shown to affect the central phases of colorectal carcinogenesis, namely DNA damage (Comet assay), colonic barrier function (TER assay), cell cycle progression (DNA content assay), and invasion (Matrigel assay) (McCann et al., 2007). These studies have shown that the phenolic content within the apple waste products can act as protection against damage to DNA, increase the function of barriers, prevent invasion, and other uses in pharmaceuticals, cosmetic gelling agents, and stabilizers (Kalinowska et al., 2014; McCann et al., 2007).

The health benefits that can be derived from apple and apple pomace phenolics can also be supported by the fact that a specific lipophilic fraction of apple pomace extracts rich in phenolic compounds (HPLC-fraction 1) was observed to possess considerable anti-inflammatory and antiplatelet potency against both the PAF and ADP pathways of platelet aggregation (Tsoupras et al., 2021b), while several other plant-derived and fruits-derived phenolics like resveratrol from grapes have also shown strong anti-platelet and anti-inflammatory properties against the PAF-associated inflammatory pathways and PAF-synthesis (Fragopoulou et al., 2004; Tsoupras et al., 2007, 2009), but mainly in *in vitro* studies. Since limited evidence exists that phenolics can actually inhibit oxidative damage *in vivo*, since phenolic compounds have poor bioavailability and their antioxidant activities are affected and altered by their metabolic transformation, suggesting that the favorable effects of plant-derived phenolics against several chronic disorders, including CVD, seem to have little to do with their antioxidant properties rather than with other health-related mechanisms, including favorable effects on endothelial function, inflammation, and the risk of thrombosis and platelet activation.

Nevertheless, more studies are needed to evaluate the valorization of apple pomace polyphenolics as functional compounds with *in vivo* health benefits. On the other hand, the antioxidant activity of apple pomace phenolics can also be valorized when utilizing such phenolic compounds for the preservation of other susceptible to oxidation oily foods and products. Again, more studies are also needed for such an application of apple pomace phenolics in the food industry.

Antioxidants are defined widely as hydrophobic or hydrophilic. The solubility of the antioxidants will determine the role of the compounds within the body. Hydrophilic antioxidants interact with the cell, cytosol, and plasma, whereas lipophilic compounds prevent lipid peroxidation to the cell membranes (Sharma et al., 2015). Extraction using organic and water-soluble solvents such as acetone and methanol can be done to extract phenolic compounds in apple pomace (Reis et al., 2012). This displays that apple pomace is abundant in both antioxidant types. Numerous studies have found high concentrations of antioxidants, although these concentrations are heavily influenced by the extraction method and solvents used (Rana et al., 2015; Perussello et al., 2017). The polar paradox is a theory of how compounds act within certain emulsions and surroundings that should be noted in this research. This theory states the paradoxical nature of antioxidants in alternate environments/solutions can be defined by polar compounds are more effective in lipid dominant media (bulk oils), whereas the nonpolar compounds tend to display higher efficiency in polar dominant media (oil-water emulsions) (Shahidi et al., 2011).

Usually, the majority of the bioactive fruits-derived phenolics are water soluble and apple and apple pomace are not exceptions to that. Thus, apple pomace phenolics are usually extracted by applying hot water as an easily implementable food grade process for polyphenols extraction (Fernardez et al., 2019). The amount of the main native phenolic compounds extracted from apple pomace like the flavan-3-ols decrease 50% after hot water extraction, while the other classes remain usually unchanged. However, dihydrochalcones and hydroxycinnamic acid oxidation products can also be observed, alongside nonextractable oxidized procyanidins that represent more than 4-fold the amount of native apple polyphenols in the pomace. Microwave superheated-water extraction of the insoluble cell wall material in water/acetone solutions and the high amounts of polyphenols that were insoluble in water/ethanol solutions suggested that oxidized procyanidins could be covalently linked to polysaccharides. These complexes represented up to 40% of the available polyphenols from apple pomace, potentially relevant for food waste valorization.

The high amounts of antioxidants, hypolipidemic properties, fiber, and phenolic compounds make apples and apple products the ideal fruit supplement for dietary prevention/treatment of chronic inflammatory diseases such as CVD (Leontowicz et al., 2002).

Apple Pomace phenolic compounds generally consist of phloridzin, epicatechin, chlorogenic acid, and quercetin glycosides (Lohani & Muthukumarappan, 2016; Schieber et al., 2003). The addition of polyphenols alters the organoleptic characteristics including flavor, odor, and color while also improving the health properties of foods making phenolic compounds an ideal supplement in functional foods (Perussello et al., 2017; Rana et al., 2015). Common extraction methods include maceration and Soxhelt extraction (Alberti et al., 2014; Wijngaard & Brunton, 2010), microwave extraction (Bai et al., 2010), and ultrasound extraction (Ajila et al., 2011), and enzymatic extraction method (Oszmiański et al., 2011). Benvenutti and others (2019) observed the solid-liquid extraction method of phenols to incorporate them into cider production. They observed the increase in antioxidant activity while producing a darker, more bitter, and astringent cider product. Although the phenols affected the sensory characteristics of the cider, the overall product came in with acceptable levels in sensory evaluation to other ciders. Overall, the research on the extraction and incorporation of apple pomace phenolic compounds is relatively low and further evaluation of these extracts is required in order to fully understand and utilize the compounds to their full health beneficial capacity.

Nevertheless, the water-soluble content of apples has shown little bioactivities against platelet aggregation and subsequent inflammatory manifestations (Tsoupras, Moran, Pleskach, et al., 2021; Tsoupras, Moran, Byrne, et al., 2021). However, this is not the case for the lipid content of apple pomace. Thus, another kind of apple pomace bioactive compound with potent anti-inflammatory and antiplatelet bioactivities were also shown to be some biofunctional apple lipid compounds. As it was found in apple products, in apple pomace also the bioactive properties are more abundant within their PL while their neutral lipids (NL) are considerably less bioactive (Tsoupras, Moran, Pleskach, et al., 2021; Tsoupras, Moran, Byrne, et al., 2021). More specifically, apple pomace PL extracts rich in PL bioactive and phenolics exhibited potent exhibited anti-inflammatory properties against PAF pathways of human platelet aggregation, but also potent antiplatelet effects against a classic platelet agonist, adenosine diphosphate (ADP) (Tsoupras, Moran, Byrne, et al., 2021).

3.2.3 Other components of apple pomace with functional health benefits

Polysaccharides such as cellulose and xyloglucan are considered valuable components of the cell walls in apple pomace. Xyloglucan can be converted and reformed into thickening agents and texture modifiers in the replacement of cellulose in food products. Xyloglucan also contains bioactive properties against tumors due to their oligosaccharides (Fu et al., 2006). This was observed using ultrasound technology to extract the xyloglucan from the pomace. The potassium hydroxide concentration along with sonication extraction time was evaluated to optimize the extraction using a central-composite rotatory design.

Apples are well known for their aromatic and sensory properties, which allows them to be used in numerous applications as a food ingredient. The aromatic compounds vary in composition due to variables such as benzoic acid, terpendiol, 2-phenylatic acid, methyl syringate benzyl alcohol, and several others (Kuś et al., 2013).

3.3 Apple pomace as an ingredient and substrate in functional food production

Millions of tons of apple pomace are produced each year during the processing of apple products such as juice, cider, and vinegar. This pomace is generally disposed of however the nutritional value of the waste is highly undervalued. The apple pomace that goes to waste causes an environmental impact and public health hazards. This byproduct is mostly composed of the apple flesh and skin that remains after juicing, which contains valuable macro and micronutrients such as sugars, lipid, and phenolic bioactive compounds, minerals, and dietary fiber. These valuable components can be extracted through a variety of processing techniques and utilized in the production of functional foods. If utilized correctly, apple pomace can be used as an ingredient in the processing of functional foods with a wide range of health benefits. It is important to note that the addition of apple pomace and apple pomace extracts can influence products in the negative sensory analysis if the pomace is added in large volumes. Therefore, it is necessary to observe a variety of apple pomace types and volumes added in order to produce products that are both sensory and health beneficial (Fig. 5.3).

3.3.1 Applications in bread and other bakery products

Bakery products are widely consumed around the world for their low cost and general ease of production, which makes them the ideal product to include apple pomace extracts. The addition of apple pomace to baked goods generally focuses on the implementation of health benefits and increased dietary fiber (Wang & Thomas, 1989). The addition of apple pomace into bakery products comes with changes to its sensory characteristics with minimal addition of the pomace extracts. The volume, texture, crust hardness, and taste are all sensory characteristics that are altered during the addition of the extracts. The optimal addition to bread ranges less than 3% of pomace extract ingredient to optimize the sensory characteristics while also increasing the beneficial health properties of the bread (Wang & Thomas, 1989).

However, in other sweet bakery products, the addition of pomace increased the sensory properties in abundance. The addition of up to 30% in cakes and 50% in muffins of apple pomace increased flavor, aroma, and texture while increasing

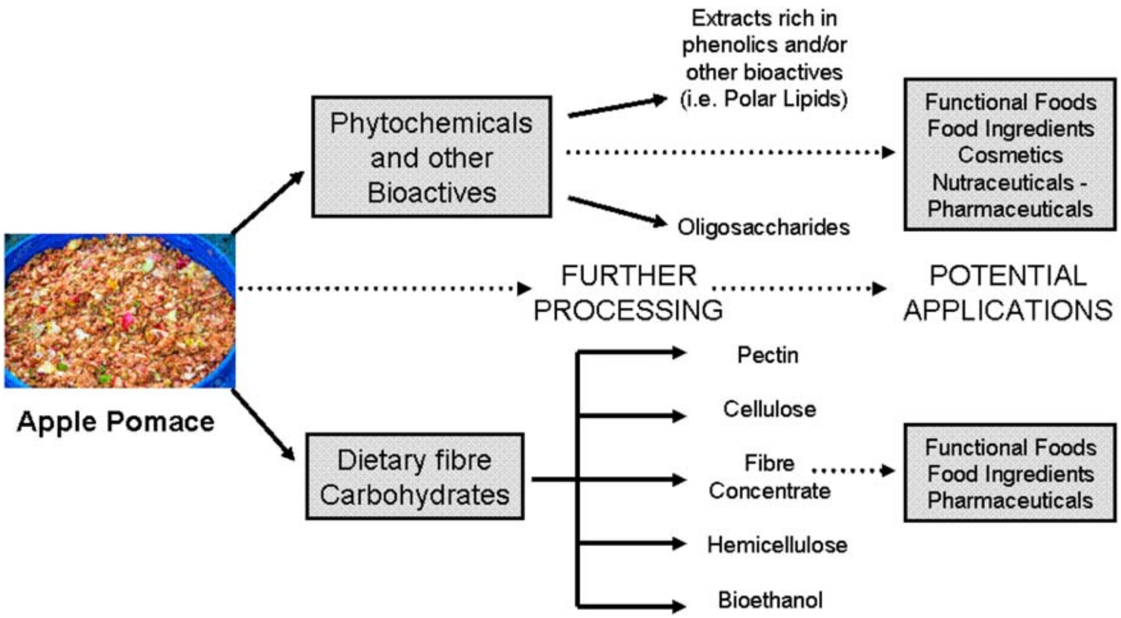

FIGURE 5.3 Apple pomace bioactive and potential applications.

the dietary fiber, antioxidant properties, and phenolic content. Sudha et al. (2007) analyzed the use of apple pomaceinfused wheat flour with alternating amounts of finely ground apple pomace (5%−30% added pomace). Water absorption increased with the mixing tolerance however there was a decrease in dough stability and an overall decrease in the strength of the dough. The volume of the cakes postbaking decreased from 850 to 620cc with the concentrations of apple pomace 0% and 30% respectively. With this decrease in volume came an increase in functional properties such as an improved dietary fiber and polyphenol content of the final cake product (Sudha et al., 2007).

3.3.2 Applications in apple pomace flour

Apple pomace is infused in products as an ingredient however it can also be infused into the ingredients prior to food production. Apple pomace flour is produced industrial through a range of techniques by grinding down the pomace and dehydrating it to remove the moisture content and produce a flour-like an ingredient. Gorjanović and others (2020) observed the effects of the process and the possible benefits of the use of the nutrient-dense, apple pomace flour. They produced flour that was rich in polyphenols, and dietary fiber with properties such as high AO activity, WHC, and OHC. This research displays the wide range of applications of apple pomace flour in a vast number of diets with the incorporation of health-beneficial compounds. The antioxidant, antidiabetic, and anti-obesity possibilities along with the availability in most diets including gluten-free displays the use of it as a dietary supplement to prevent oxidative stress and inflammatory disease (Gorjanović et al., 2020).

3.3.3 Applications in alcoholic beverages

Apples are well known for their alcoholic cider products however the pomace produced from this industry can also be fermented. Apple pomace is the ideal substrate to be used in fermentation due to its low cost, nutritional value, and minimum land requirement (Hang et al., 1981; Nogueira et al., 2005). The most common method of fermentation of pomace is solid-state fermentation (Ngadi et al., 1992) or even a substrate mix of molasses and pomace (Kaur, 1989). The apple pomace fermentation creates a mildly alcoholic beverage with enhanced flavors (Li et al., 2015).

3.3.4 Applications in confectionary products

Pomace is a byproduct rich in sugars, flavors, aromas, and health-beneficial compounds making it a low-cost ingredient that can be used in the production of jelly. The product was developed using a puree of the pomace and the fruit, quince (Royer et al., 2006). It was found that the apple pomace and quince puree did not affect most characteristics however the overall acceptability of the jelly was found to be the sample with the lowest sugar concentration and the highest amount of quince.

Apple pomace was also studied in an apple-pomace-based jam (Hussein et al., 2015). It displayed relatively high concentrations of phosphorus, and flavonoids while also having fruity flavors and a satisfying appearance due to the pomace.

3.3.5 Applications in dairy products

Apple pomace was researched as a stabilizer and texturizer in yogurt products (Wang et al., 2019). Various concentrations of additional apple pomace were shown to decrease the gelation time, raise the onset of pH, and improved the consistency of the yogurt products during storage.

Freeze-dried pomace powder was also analyzed as a possible dairy ingredient in yogurts exhibiting beneficial properties in lowering fermentation periods and increasing gelation while producing a firmer and more consistent yogurt gel (Wang et al., 2020). This research also displayed the potential health benefits of the addition of apple pomace as a food ingredient by increasing the polyphenol content and dietary fibers within dairy products.

3.3.6 Applications in meat

Within the meat production industry, apple pomace is primarily utilized to increase dietary fiber. Meats such as chicken (Verma et al., 2010; Yadav et al., 2016), and mutton (Huda et al., 2014; Rather et al., 2015) products such as sausages and nuggets have been subject to these studies.

Apple pomace-infused buffalo meat patties (Younis & Ahmad, 2018) have been investigated for their moisture, fat, and fiber levels. The addition of the pomace displayed a very positive correlation between the addition of the biproduct and the investigated properties however there was a decline in texture and structure of the patty.

3.3.7 Applications in mushrooms

As apple pomace is rich in nutrients and is easily broken down, it makes for the ideal substrate in mushroom cultivation. Mushrooms thrive on polymers (Lignin) and minerals (Nitrogen) that apple pomace brings to the soil (Lyu et al., 2020). Worrall and Yang (1992) examined the effects of apple pomace and sawdust on specific mushroom breeds such as oyster and shiitake mushrooms. This found the mushroom yield to be significantly higher in pomace alone than in combination with both and sawdust alone. Park and others (2012) observed the effects with similar parameters (Park et al., 2012). It was observed that with the addition of 2.5% pomace came a rise in mycelial growth values in liquid, solid, and solid-state fermentation. Park and others (2012) also found 2.5% additional apple pomace to be the ideal quantity to induce an increase in mycelial growth with anything over 5% having a negative effect. Further parameters are required to determine the possibilities for pomace to be used as a substrate in mushroom cultivation.

3.4 Health hazards associated with the apple pomace

There is no significant evidence of health hazards following consumption of apple pomace or apple pomace products. There are, however, concerns about the consumption of natural plant toxins and pesticide residue (Skinner et al., 2018). Apple seeds contain amygdalin, which can cause acute cyanide poisoning however research shows for this to become a risk a person would need to consume roughly 800g of apple pomace (Skinner et al., 2018). This seems highly unlikely but to increase the safety of products seeds may need to be monitored or if possible, removed prior to production.

Pesticide residues have been detected in apple pomace, but the quantities are deemed insufficient to be removed by extraction methods (Chen et al., 2014). Neonicotinoid residues were analyzed in eight apple types and found that most of the neonicotinoids displayed negligible content within the pomace (Chen et al., 2014). Acetamiprid was found in two varieties of apples however the content has been determined to have a low risk of toxicity (Skinner et al., 2018). The increase in fungicide and pesticide use throughout agriculture, it causes questions about the safety of the use of products such as apple pomace (Lyu et al., 2020). Fungicides that are detected in apples and their bi-products are primarily carbendazim, thiophanate, and pyrimethanil (Liu et al., 2016). The EPA has deemed these and similar chemicals to have a low acute toxicity value (US EPA—Pesticides).

Other residue chemicals of concern included naphthaleneacetic acid (Maiti et al., 2018) and diphenylamine (Lozowicka, 2015), plant growth regulators. These compounds were researched in numerous papers however they were also described as having little no risk of toxicity (Lozowicka, 2015; Reregistration Eligibility Decision) (EPA—Pesticides—Fact Sheet). Therefore, apple pomace can be deemed safe for human consumption as of now however further research and studies are required to investigate the overall safety of the product as a food ingredient.

4. Grape pomace

4.1 Introduction

Grapes are amongst the most common fruit consumed worldwide through simple grape, raisins, and beverage products such as juice and wine. The grape is well renowned for its utility in the food industry however the grape is also a source of many health-beneficial compounds such as vitamins, minerals, fibers, phenolic compounds, antioxidants, and many bioactive compounds that have been linked to health benefits such as anti-inflammatory effects.

Since the evidenced cardioprotective benefits of moderate consumption of wine (de Lorgeril, 1992; de Lorgeril et al., 1994), there has been an influx of wine production, which has inevitably increased the production of byproducts in this industry. The primary organic waste produced from squeezing/juicing grapes to produce wine or grape juice is known as grape pomace (Abarghuei et al., 2010; Christ & Burritt, 2013; Cuccia, 2015) (Fig. 5.4). This byproduct consists of the skin, seeds, and stems of the grapes and until recently it was disposed of as waste or for animal feed. The waste management of grape pomace is an important environmental issue since if disposed of in landfills its compounds like polyphenolics can have negative effects on biodegradation and land microbiota.

On the other hand, there is an increasing demand for healthy and natural food ingredients that can replace synthetic antioxidants and food preservation substances Due to a lack of some essential nutrients the use of grape pomace as a composting material is not economically viable, while on the other hand, grape pomace contains significant amounts of substances that can be considered beneficial to health, such as fiber and polyphenolics (Antonić et al., 2020). Thus, apple pomace has been widely researched for its biofunctional components, with potential health benefits, which may be utilized in the food industry as a functional food ingredient (Antonić et al., 2020; Chamorro et al., 2012; Duba et al., 2015; Beres et al., 2016; Bail et al., 2008; Fernandes et al., 2013; Fiori et al., 2014) (Fig. 5.4).

Further research is required to fully understand the possibilities of this byproduct however if the food industry could utilize this pomace the wine industry would not only become economically richer, but it could also lower its reasonably high greenhouse gas emissions (Amienyo et al., 2014; Castillo-Vergara et al., 2015; Cuccia, 2015).

4.2 Grape pomace composition, nutritional value, and associated health benefits

This grape pomace is primarily composed of the skin and seed of the grapes, which in themselves hold very beneficial compounds. The primary health beneficial compounds researched have been the presence of dietary fiber and oils within the pomace and specifically their seeds. These compounds have not only been researched for their nutritional value but also for the extraction methods available to utilize the potential use of these components as a functional food ingredient. This byproduct full of potentially bioactive lipids and phenolic compounds specifically proanthocyanidins. These are polyphenols that have the ability to be utilized as a functional food ingredient specifically used to naturally increase the biological pathways and processes within the body. Other phenolic compounds include anthocyanins and flavanols, which have been heavily researched for their bioavailability and health-beneficial properties (García-Lomillo & González-SanJosé, 2017).

The grapes' weight consists of 2%−5% seeds and roughly 38%−52% of the solid waste matter produced by the manufacturing of wine (Brenes et al., 2016). These seeds are made up of approximately 40% fiber, 10% protein, 10%−20% lipids, and a further 30%−40% sugars, complex phenolics, vitamins, and minerals (Rockenbach et al., 2012).

These grape seeds values are mainly sought after for their oil as they contain an abundance of unsaturated fatty acids such as oleic and linoleic acids along with being a rich source of phenolic compounds (Bail et al., 2008; Hanganu et al., 2012). A rich-polyphenolic residue remains postextraction of the oil as a dry fraction of the seeds roughly 77% of the original weight (Luque-Rodríguez et al., 2005). This fraction has been less studied than the pomace or the seeds however it has been shown to contain 46% lignin, 11% protein, and 6.5% moisture (Prado et al., 2014).

4.2.1 Grape pomace phenolic compounds

Phenolic compounds are phytochemicals possessing a vast array of biological properties with health beneficial properties, including cardioprotective, antioxidant, anti-inflammatory, antithrombotic, antimicrobial, and the prevention of chronic diseases and cancer (Averilla et al., 2019; Choleva et al., 2019; Tsoupras et al., 2009). These properties have been associated with the consumption of fruit and vegetables, which have been heavily studied in the food industry (Haminiuk et al., 2012). This is why they are always considered in the conversation of health beneficial compounds and especially considered during the utility of byproducts such as grape pomace to be used as a functional food ingredient.

Red wine and grape pomace byproducts contain an abundance of these phenolic compounds and have also been heavily researched in the industry for their health beneficial properties (Antonić et al., 2020; Boussetta et al., 2012; de Lorgeril, 1992). Having noted this, the wine extraction does not extrude all of the polyphenols within the fermentation. The extraction only averages about 30%–40% of the phenolic compounds during the vinification process (location, and technical parameters considered) (Ky et al., 2014). This leaves a large portion of unutilized polyphenols with highly beneficial properties behind in a residue as a byproduct of the wine production industry that may be used as a bioactive food ingredient. These phenolics generally contain an aromatic ring and contain at least one hydroxyl substituent (Beres et al., 2017).

There has been an abundance of phenolic compounds founds and identified within the grape pomace composition including a rich source of hydroxybenzoic, hydroxycinnamic, and anthocyanins acids, flavanols, flavan-3-ols, and stilbenes (Kammerer et al., 2004; Pinelo et al., 2006; Soto et al., 2015). The polyphenolic compounds, colorants, and anthocyanins of grape pomace are the main carriers of its antioxidant potential, with several health benefits and uses in food preservation, due also to the inhibition of lipid oxidation and antibacterial effect (Antonić et al., 2020; Peixoto et al., 2018).

The anthocyanins are what give the grapes their reddish/wine color, which is produced by the fruit during the ripening process (Castañeda-Ovando et al., 2009; Xia et al., 2010). These phenols are very sensitive to a number of factors such as temperature, oxygen, light, pH, and solvents, which may cause chemical transformations. Therefore, the main priority of the research into these compounds is their chemical structure and formations stability, for their use as a natural food dye. 3-O-glycosides of malvidin, petunidine, cyanidin, peonidin, and delphinidine are the primary anthocyanins found in the skin (Souza et al., 2014; Xu et al., 2015).

The phenolic acids contain carboxylic acid functional groups, which are segregated into hydroxybenzoic acids and hydroxycinnamic acids. Fatty acids such as caffeic, gallic, ferulic, chlorogenic acids, and those aromatic compounds including side chains of three carbons are known as hydroxycinnamic. Whereas the hydroxybenzoic acids include the protocatechuic, p-hydroxybenzoic acid, syringic, and vanillic acids (Ignat et al., 2011; Yu & Ahmedna, 2013).

Proanthocyanidins and catechins are flavan-3-ols, which are the typical flavonoids within the human diet. These flavonoids are commonly found in fruits and vegetables. These flavonoids are some of the primary compounds in the sensory properties of wine. However, not all of these flavonoids are extracted during the vinification process and a large portion is left unused in the byproduct residue left after extraction. The seeds contain a large quantity of these compounds as well as the skin. Notably, the skins and seeds differ in the flavan-3-ol composition (González-Manzano et al., 2004).

A phytoalexin known as stilbenes is that occur fruit bearing plants, and this is especially the case when it comes to the grape. Resveratrol is the primary stilbene found in grapes and grape products such as wine and pomace. The amount of this phytoalexin depends on factors such as the fruit variety and the stage of maturation of the fruit. More specifically, the most health-beneficial stilbenes with the grape is resveratrol-3-O- β-glucopyranoside, piceatannol, cis, and trans-resveratrol and resveratrol dimers (Flamini et al., 2013). Resveratrol has been proposed as the most bioactive component of wine and its grape pomace byproduct, with well-established antioxidant, antiinflammatory, antithrombotic and antiatherogenic properties (Choleva et al., 2019; Fragopoulou et al., 2000, 2004; Tsoupras et al., 2007; Vlachogianni, Fragopoulou, et al., 2015; Vlachogianni, Fragopoulou, et al., 2015). Apart from the antioxidant capacity of grape pomace resveratrol, its anti-inflammatory potency has also been attributed to its high specificity against the PAF-related inflammatory pathways, as well as against its biosynthesis and thus promoting the reduction of the levels of this inflammatory mediator, with several anti-inflammatory and antiatherogenic benefits (Fragopoulou et al., 2004; Tsoupras et al., 2007, 2009; Vlachogianni, Fragopoulou, et al., 2015).

The phenolic compounds in grapes are dispersed amongst all components of the grape. The phenolic compounds' types and amounts depend on what component of the grape they are extracted from (i.e., whole grape, pomace, seeds, or skin). The seeds contain the highest extractable phenolics in the whole grape with 60%–70% while other components such as skin (28%–35%) and the pulp (10%) (Shi et al., 2003). With this being said, the actual weight of the phenolic composition ranges from 5% to 8% of the weight of the seed (Baiano & Terracone, 2011). These grape seed extracts have been studied for their abundance in proanthocyanidins commonly polymers and oligomers of polyhydroxy flavan-3-ols such as (+)-catechin and (−)-epicatechin (Brannan, 2008).

4.2.2 Other functional compounds

The oily part of the grape pomace is rich in unsaturated fatty acids, colorants, and minerals. Another class of biofunctional compounds found in this oily part of grape pomace are several polar lipid bioactives with strong antithrombotic and anti-inflammatory potency against the PAF and thrombin pathways (Choleva et al., 2019; Fragopoulou et al., 2004). In addition, ethanolic extracts of grape pomace rich in such bioactive polar lipids were found to contain also the essential n-6

PUFA linoleic acid (C18:2n6) and the n-3 PUFA alphalinolenic acid (C18:3n3) (Choleva et al., 2019). Thus, the presence of such antiplatelet lipid bioactive and essential fatty acids in ethanolic extracts of grape pomace, along with the phenolics being coextracted in such extracts, further suggest that ethanol extracts of grape pomace winery byproducts exert a potent antiplatelet effect and its valorization could lead to the production of functional foods with cardioprotective properties (Choleva et al., 2019).

Grape pomaces contain biofunctional dietary fibers, comprising mostly of insoluble fibers from grape skin like lignin, cellulose, and hemicelluloses, which have been proposed to improve the efficiency of the digestive tract (Antonić et al., 2020; Bender et al., 2017). Moreover, some grape pomace phenolic compounds bond chemically to its fiber substances, creating thus antioxidant dietary fibers and giving the pomace stronger radical scavenging potential, which makes them have a higher nutritive value in comparison to dietary fiber present in other plant sources like cereals, with several proposed health benefits (Antonić et al., 2020; Mildner-Szkudlarz et al., 2011).

Grape pomace also contains essential minerals and trace elements, like potassium, iron, and zinc. Potassium has been proposed to lower blood pressure and decrease the risk of osteoporosis due to reduced urinary calcium excretion, while iron and zing have a large impact on the antioxidant potential of grape pomace (Sousa et al., 2014).

4.3 Grape pomace as an ingredient and substrate in functional food production

Fruit pomace, as mentioned earlier, has a wide variety of applications in many industries and grape pomace is no different. Previous research has shown not only the food industry benefits from the byproduct but also the pharmaceutical, and cosmetic sectors too (Prodanov et al., 2005). In the food industry there have been many applications for the grape pomace extracts such as a preserver in fish products as it increases the prevention of lipid oxidation (Pazos et al., 2005) and also increases their antibacterial capacity from a wide spectrum of bacteria (Katalinić et al., 2010; Xu et al., 2016). Pomace flour has also taken interest in the food industry with grape pomace flour displaying a lipid oxidation suspension effect and very high antioxidant levels. The seedless grape pomace is showing possibilities in functional food products as a promising applicant for its bactericidal effects on the aerobic mesophilic bacteria, lactic acid, and Enterobacteriacea (García-Lomillo et al., 2014).

The extract from grape pomace can be used as a functional food ingredient in the food manufacturing process to improve the overall nutritional value of the products or to be used as a supplement to osmotic solution to obtain the dehydrated fruit incremented in the phenolic content (Rózek et al., 2010). Chitosan edible film was infused with the extract also to encourage antioxidant characteristics and to extend the expiration date (Ferreira et al., 2014).

The grape pomace extract could be used as a replacement for the synthesized antioxidants in current food products today. Pork burgers infused with red grape pomace extract were studied to observe the increase in natural antioxidants (Garrido et al., 2011). It was found that the extract had the possibility to be used as a preservative in meat products from observing the overall acceptability and also color stability, and lipid oxidation. Similar effects were also found in lamb meat, where the extract was infused with a vitamin E supplement to improve the overall expiration of the product (Guerra-Rivas et al., 2016).

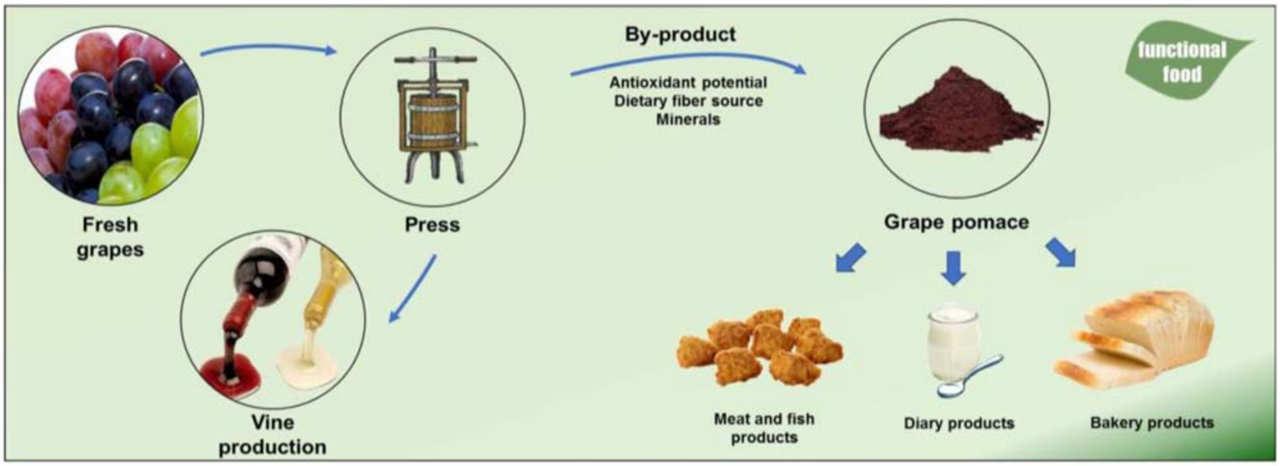

FIGURE 5.4 Grape Pomace production and valorization. *Reproduced with permission from Antonić, B., Jančíková, S., Dordević, D., & Tremlová, B. (2020). Grape pomace valorization: A systematic review and meta-analysis.* Foods, 9*, 1627.* *https://doi.org/10.3390/foods9111627*

It was found that the extract supplemented with vitamin E displayed an approximate 20% reduction in lipid oxidation over the first 7 days of storage.

Phenolic extracts from grape seeds were studied for their use in plants and potato products to observe their effect on the formation of acrylamide formation during the Maillard reaction. In plants, it was found that the production of acrylamide was lowered by 54% (Zhu et al., 2009) whereas potato products showed drastically higher reduction rates with up to 90% less production of acrylamide (Xu et al., 2015).

The grape pomace extract exhibited beneficial health effects when given to hamsters consistently over the period of 3 months (Auger et al., 2004), with plasmatic cholesterol lowering of 25%. It was also found that the supplement showed an effect on cancer cells in several cancer types (i.e., prostate, breast, skin, etc.) (Kaur et al., 2009).

Resveratrol seems to contain strong antioxidant and anti-inflammatory potency (Tsoupras et al., 2007, 2009; Vlachogianni, Fragopoulou, et al., 2015; Vlachogianni, Fragopoulou, et al., 2015), with potent possibilities of health beneficial characteristics. However, within the functional food and functional food ingredient production process, there are very few applicable options due to its low water solubility along with problems in its bioavailability and chemical stability. With the use of emulsions as an enveloped system for delivering the lipid, there may be promising outcomes by preventing oxidation and degradation by protecting it with a lipid casing. An option of using grape seed oil and the extract from the grape skin displayed a promising emulsion system that would allow resveratrol to be consumed and be delivered with minimal damage against UV-light and oxidation (Davidov-Pardo & McClements, 2015).

Another grape pomace-based product that is rich in both phenolics like resveratrol and polar lipid bioactives is the ethanolic extracts of grape pomace, which have been proposed as candidates for food supplements and nutraceuticals, since they have shown potent antiplatelet properties, due to the high specificity of both resveratrol and polar lipid bioactives found in these extracts against the PAF-related inflammatory pathways, as well as against its biosynthesis and thus promoting the reduction of the levels of this inflammatory mediator, with several proposed health benefits (Fragopoulou et al., 2004; Tsoupras et al., 2007, 2009; Vlachogianni, Fragopoulou, et al., 2015).

Overall, many studies have shown the successful incorporation of grape pomace and its biofunctional compounds as fortifying agents in several plant-derived and animalderived foodstuffs, including plant-based muffins, cookies, biscuits, bread, extruded cereals, noodles, pancakes, pasta, and tomato puree, as well as the dairy yogurt, cheese, fermented milk, and ice cream products and other animal-based meat and fish products, such as pork burgers, beef frankfurters, pork sausages, pork loin marinade, chicken meat, salmon burgers, and minced fish muscles, which have thoroughly been reviewed by Antonić and others (2020). The addition of grape pomace and/or its biofunctional compounds in these foodstuffs resulted mainly in increased levels of total polyphenolic contents and thus oxidative stability and prolonged shelf life in all fortified final products (Antonić et al., 2020). However, it should be stressed that a higher fortification degree with higher grape pomace concentration mainly adversely affected textural and sensory characteristics, including color changes (darker, reddish, and bluish) (Antonić et al., 2020). Thus, apart from the undeniable positive impact and health benefits of grape pomace on all types of food commodities, its effects on sensory properties suggest that individual foodstuffs have to be tested separately for possible grape pomace fortification.

5. Conclusions and future perspectives

Agriculture has a severe impact on the environment in today's growing society that must be addressed in order to produce sustainable foods, oils, and beverages' manufacturing industries. Today, there are numerous countries that rely heavily on the agricultural industry to produce products however this industry has control policy's that must be adhered to in order to create a sustainable production process.

With respect to olive oil production and its waste byproduct, olive pomace, solvent extraction has long been used to recover oil from olive pomace for the need of human nutrition. For many decades, olive pomace along with OMWW have only been considered as materials that are an environmental problem that needs to be solved. Nowadays, researchers and manufacturers of olive oil are focused on the recovery of olive pomace polyphenols and industrial application is already a reality since companies manufacture phenol-rich extracts from these byproducts and trade them in foods as natural preservatives or bioactive additives. The well-known antioxidant and anti-inflammatory properties of the main phenolic compounds of olive pomace have been utilized in the production of various functional foods with promising results.

In addition, during the production of apple products the most common waste obtained is apple pomace, with bioactive compounds of nutritional value and health benefits. The literature shows the value of these compounds found in apple waste and its potential to be used as a functional food ingredient to increase health benefits, lower economic costs, and improve the sustainability of common foods. Research has shown that the use of solvent extraction methods can be used to extract compounds such as phenols and pectin to be used as a functional food ingredient with the addition of not affecting and even improving sensory analysis. The apple pomace, as an ingredient or substrate, can improve the aroma, taste, and texture of functional foods. The health benefits of these extracted compounds from apple pomace are linked to a variety of beneficial properties such as inhibition of inflammatory pathways, lowering of LDL-cholesterol, and antioxidants. The literature and research show the beneficial use of these extractants and the possibilities of utilizing apple pomace as a functional food ingredient. The use of apple byproducts such as apple pomace is an area that requires further research in order to fully understand and perfect the applications, therefore, utilizing its properties to the highest standards. As of today, the use of apple pomace as a food ingredient and/or substrate is extremely promising in improving the efficiency and sustainability of the apple juice, cider, and vinegar production industry.

Concerning the grape and wine industry byproducts, there is a massive issue with the overproduction of byproduct waste in the form of grape pomace that must be addressed in order to create a sustainable production process that can meet the demands of an ever-increasing and aging worldwide population. These industries such as winemaking create byproduct residues containing an abundance of bioactive and bioavailable compounds that may be utilized through extraction methods to be used in many different areas in the food industry. This utilization might not only improve the sustainability of food production industries such as wine but also improve the economic value and lower the environmental effect that the agricultural industry has on the world today. The extraction and incorporation of the byproduct, grape pomace looks to be a very interesting and possible area in the food industry as an improvement on the system in place today however further research is needed in order to fully utilize the compounds and promote a “greener” process.

Overall, today’s human diet has been subject to constant improvements in both nutritional value and sensory characteristics. For consumers along with new nutritional value laws, there is a demand for natural health beneficial compounds to replace the synthetic additives that have been subject to ridicule in recent years. This demand became promising to fill as researchers have looked at byproducts such as olive, apple, and grape pomaces, as a source of biofunctional compounds with health benefits that can be valorized as fortifying ingredients for the production of novel functional foods. It is important to note that any effort for the recovery of bioactive molecules should analyze and define food-specific applications for functional food products to make sense and be successful. In this effort, studies show that enrichment methodology is an open field of research that requires improvements so that the enrichment of foods with olive pomace, apple pomace, grape pomace, or other similar valuable byproducts leads to end products not only healthier but also with high acceptance by consumers in terms of their organoleptic characteristics.

6. Conflicts of interest

The authors declare no conflict of interest.

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Chapter 6

Berries

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1. Introduction

It is well recognized that regular consumption of fruits and vegetables in the diet yields health benefits and reduces risks of lifestyle-associated health conditions (Del Río-Celestino & Font, 2020; Yahia et al., 2019). Fruits, especially berries, contain natural antioxidants that are essential components of healthy diets. Numerous studies have shown that bioactive components including vitamins, carotenoids, terpenes, and phenolic compounds in berries are strongly associated with antioxidant properties derived from berry intake. Antioxidants in berries work by scavenging reactive oxygen species that can cause oxidative damage to cellular macromolecules leading to formation of tumors and carcinogenesis (Sosa et al., 2013). The literature is replete with health benefits derived from berry consumption (Afzal et al., 2019; Baby et al., 2018; Lavefve et al., 2020). Although the *in vivo* bioactivity and bioavailability of berry bioactives are not fully understood, the antioxidant and antiinflammatory actions of these bioactives may decrease the risk of cardiovascular diseases (CVD), metabolic syndrome, inflammations, and neurodegenerative diseases (Bhaswant et al., 2017; King & Bolling, 2020). It has also been well documented that flavonoids present in blackcurrants protect retinal cell types from death due to oxidative stress (Cortez & Gonzalez de Mejia, 2019). Furthermore, anthocyanin rich berries including blueberries, blackberries, and black raspberries showed antioxidant, antiinflammatory, and antimicrobial activities and also played an essential role in preventing diabetes, cancer, and CVD when consumed regularly (Peña-Sanhueza et al., 2017). In addition to these health promoting bioactives, berries contain significant quantities of minerals, sugars, and dietary fibers with well-established health benefits (Castro & Teodoro, 2015). In view of these numerous health benefits, berries are marketed as "superfoods" and they are considered ideal candidates for functional food applications. Berries are normally consumed fresh or in frozen/processed forms (Nile & Park, 2014). Some widely consumed berries include strawberry, raspberry, blueberry, blackberry, Indian gooseberry, bilberry, cranberry, lingonberries, chokeberry, and blackcurrants among others.

Within the farm-to-fork framework for berries, preharvest production factors and postharvest processing methods can influence the composition of bioactives in berries and ultimately the known health benefits derived from berry consumption (Di Vittori et al., 2018). Firstly, preharvest considerations including genotype, environmental, and agronomic interventions can alter the accumulation of berry bioactives (Evdokimenko et al., 2021; Gündüz & Özbay, 2018). For instance, it has been shown that genotype exerted an influence on quality of berries, including blackberries (Vagiri et al., 2014), blueberries (Balducci et al., 2014), and strawberries (Diamanti et al., 2012). Additionally, environmental factors have an impact on important traits of berry quality. In fact, the composition of phenolic compounds in blueberries were shown by Karppinen et al. (2016) to be strongly affected by environmental factors such as light conditions and temperature (Karppinen et al., 2016). Other authors also underlined the importance of climatic factors in influencing the overall berry quality (Uleberg et al., 2014; Vagiri et al., 2016). Furthermore, Valentinuzzi et al. (2015) demonstrated that agronomic factors including fertilization, irrigation, and cultivation systems are essential inputs of berry nutritional quality (Valentinuzzi et al., 2015). Interestingly, while less significant, harvesting factors such age, maturity stage, and harvest time have been reported to influence accumulation of bioactive compounds in berries (Syukri & Chamel, 2021). However, berries, despite their health benefits, have limited shelf lives. To ensure their availability throughout the year and promote their consumption, postharvest processing technologies are employed to reduce fruit senescence, microbial growth, and fruit decay (Arfaoui, 2021; Sun et al., 2019). Among these, fresh pack processing technologies including cold temperature, irradiation, fumigation (1-MCP, SO_2, ozone), chlorine sanitization, edible films/coatings, blanching, controlled

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atmosphere, and modified atmosphere packaging (MAP) among others have been demonstrated to effectively extend the shelf lives of berries (Dermesonlouoglou et al., 2018; Nayak et al., 2015; Nilsen-Nygaard et al., 2021). Furthermore, industrial and domestic processing technologies including drying, freezing, pureeing, juicing, and jamming have been used to preserve berries (Li, Chen, et al., 2017). However, these technologies have been reported in some applications to alter the stability of bioactive compounds in processed berry products following storage (Aaby et al., 2018; Martín-Gómez et al., 2020; Weber & Larsen, 2017). Frequently, in industry, one of the above processing methods is combined with another, so that the knowledge about the changes that occur at the level of bioactive compounds is essential for the assessment of the final berry product quality.

In this chapter, the different aspects of berry production, starting from the raw material to harvested fruits, on berry bioactives as well as loss or accumulation of bioactive compounds during postharvest processing is reviewed. To accomplish this, the nutritional composition of berries will be evaluated and the impact of each production stage on the nutritional quality discussed. This information could be applied to predict fate of bioactive compounds with documented health-related properties in processed berry products, which would be useful in functional food formulations.

2. Botanical classification of berries

Botanically, a berry is a fruit with seeds and pulp produced from the ovary of a single flower with a fleshy pericarp (Joseph et al., 2014). However, the term "berry" has also been used to refer generally to a small, pulpy and often edible fruit (Olas, 2018; Venskutonis, 2020, pp. 95−125). Olas (2018) noted that while several families of berries exist, the Rosaceae, including black chokeberry (*Aronia melanocarpa*), strawberry (*Fragaria ananassa*), red raspberry (*Rubus ideaus*), black raspberry (*Rubus occidentalis*), blackberry (*Rubus fruticosus*), cloudberry (*Rubus chamaemorus*), and the Ericaceae, including cranberry (*Vaccinium macrocarpon*), bilberry (*Vaccinium myritillus*), lowbush blueberry (*Vaccinium angustifolium*), and highbush blueberry (*Vaccinium corymbosum*) are the most common (Olas, 2018). Examples of berries from other families include blackcurrants (*Ribes nigrum*; family: Grossulariaceae), sea buckthorn (*Elaaagnus rhamnoides* (L.); family: Elaeagnaceae) and grapes (*Vitis*; family: Vitaceae), as shown in Table 6.1.

TABLE 6.1 Botanical designations of commonly consumed edible berry fruits[a].

Berry	Family	Genus
Cranberry	Ericaceae	*Vaccinium oxycoccos*
Lingonberry	Ericaceae	*Vaccinium vitis-idaea*
Blueberry "Northblue"	Ericaceae	*Vaccinium corymbosum* "Northblue"
Blueberry "Northcountry"	Ericaceae	*Vaccinium corymbosum* "Northcountry"
Rabbiteye blueberry	Ericaceae	*Vaccinium virgatum*
Lowbush blueberry	Ericaceae	*Vaccinium angustifolium*
Bilberry	Ericaceae	*Vaccinium myrtillus*
Gooseberry (green)	Grossulariaceae	*Ribes uva-crispa*
Black currant	Grossulariaceae	*Ribes nigrum* "Ojebyn"
Red currant	Grossulariaceae	*Ribes_pallidum* "Red Dutch"
White currant	Grossulariaceae	*Ribes_pallidum* "White Dutch"
Green currant	Grossulariaceae	*Ribes nigrum* "Vertti"
Jostaberry	Grossulariaceae	*Ribes nidigrolaria*
Chokeberry	Rosaceae	*Aronia mitschurinii* "Viking"
Rowanberry	Rosaceae	*Sorbus aucuparia*
Sweet rowan	Rosaceae	*Grataegosorbus mitschurinii* "Granatnaja"
Strawberry	Rosaceae	*Fragaria_ananassa* "Senga Sengana"

TABLE 6.1 Botanical designations of commonly consumed edible berry fruits[a].—cont'd

Berry	Family	Genus
Cloudberry	Rosaceae	*Rubus chamaemorus*
Red raspberry	Rosaceae	*Rubus idaeus*
Arctic bramble	Rosaceae	*Rubus arcticus* "Pima' and "Mespi"
Dog rose	Rosaceae	*Rosa canina*
Eastern Shadbush	Rosaceae	*Amelanchier canadensis*
Red raspberry	Rosaceae	*Rubus idaeus*
Deerberry	Rosaceae	*Rubus occidentalis*
Blackberry	Rosaceae	*Rubus allegheniensis*
Sea buckthorn berry	Elaeagnaceae	*Hippophae rhamnoides*
Crowberry	Elaeagnaceae	*Empetrum nigrum*
Goji berry	Solanaceae	*Lycium barbarum*
Elderberry	Adoxaceae	*Sambucus nigra*
White mulberry	Moraceae	*Morus alba*
Hardy kiwifruit	Actinidiaceae	*Actinidia arguta*
Grape	Vitaceae	*Vitis vinifera*

[a]*Table was originally adapted from (Sater et al., 2020).*

3. Nutrient composition of berries

Berries are a source of nutritive compounds which serve as macronutrients (carbohydrates, lipids, proteins) and micronutrients (vitamins and minerals) in the human diet (Olas, 2018). Additionally, berries contain significant amounts of non-nutrients including phenolic compounds, carotenoids, and isoprenoids (terpenes and terpenoids) among others (Jimenez-Garcia et al., 2018). Therefore, it suffices that the nutritional quality of berries as well as associated health benefits may be related to the presence of these nutritive and non-nutritive compounds (Foito et al., 2018). An extensive body of work has been undertaken to assay the composition of some of the widely consumed berries to characterize phytochemicals possessing bioactive properties (Bilawal et al., 2021; Gündeşli et al., 2019; Schulz & Chim, 2019). Thus, the phytochemical and nutritional composition of berries are reviewed along with their associated health benefits in the following sections.

3.1 Health benefits of nutritive bioactives in berries

Berries, like most fruits contain carbohydrates which occur mainly as soluble sugars and dietary fibers. In particular, berries have been shown to contain *ca.* 15% soluble solids which consist mainly of soluble sugars and minor electrolytes (Yadav, 2021). Among these sugars, sucrose, glucose, and fructose abound in berries (Česonienė et al., 2021). Generally, when fully ripened, berries contain higher fructose and glucose contents in relation to sucrose and as such the levels of these sugars tend to vary with maturity of fruits. Furthermore, other studies have identified sorbitol, maltose, lactose, and xylose in berries including sea buckthorn berries, grapes, rowanberries, and chokeberries but these were at much lower concentrations compared to sugar, glucose, and fructose (Jaśniewska & Diowksz, 2021; Lodaya & Gotmare, 2018; Sidor & Gramza-Michałowska, 2019). Berry sugars provide a source of dietary calories and influence consumer taste and acceptance of fresh and processed berry products (Souza Gonzaga et al., 2021; Vilela & Cosme, 2016). Basile et al. (2020) showed that soluble sugars and organic acids influenced berry taste and were major consumer preference driving factors (Basile et al., 2020). It is worth noting that the enrichment of fructose makes fully ripened berries valuable caloric sources for managing diabetes and diabetes-related complications (Hameed et al., 2020a). This was demonstrated in a clinical study involving type 2 diabetic patients. Here, it was reported that increased intake of low glycemic index fruits including blueberries reduced blood pressure, coronary heart disease risks and type 2 diabetes (Jenkins et al., 2011). These results appear to corroborate the reduction of risk factors of diseases associated with sugar rich diets and regular berry consumption (Mursu et al., 2014). However, these reports may not be wholly acceptable on face value: systematic reviews and meta-analysis of controlled trials have not shown that fructose-containing sugars behave any differently from other forms of digestible carbohydrates when they are consumed in excess (Khan & Sievenpiper, 2016). Nonetheless, a recent systematic and updated meta-analysis of prospective cohort studies reaffirmed that diets with low glycemic index were strongly associated with type 2 diabetes reduction (Livesey et al., 2019). It appears that the benefits from berry consumption on diabetes management could be best realized if berries are consumed in moderation since excessive sugar intake could be associated with dental and caloric harm. In addition to soluble sugars, berries are rich in health promoting dietary fibers. The major dietary fibers in berries consist mainly of carbohydrates (cellulose, hemicellulose and pectin) and lignin (Reboredo-Rodríguez, 2018). These compounds are resistant to digestion and absorption in the intestines where they undergo fermentation (Devries, 2003). Typically, dietary fiber content in berries ranges from 1%—7% based on total fresh weight (Aura et al., 2015; Niro et al., 2017). Nutritionally, fruit fibers act as an intestinal regulator by influencing metabolism and absorption of nutrients including carbohydrates, fats, and sterols (Ramadan et al., 2008). Furthermore, it is well known that a daily consumption of whole berries or as part of the normal diet provide sufficient soluble dietary fibers to ensure regular stool production and bowl movements (Căpriţă et al., 2010). Another noteworthy benefit from dietary fiber intake through berry consumption is the effect dietary fibers have on reducing risks of type 2 diabetes and related health conditions as demonstrated in a number of human clinical studies (Camerotto et al., 2019; Davison & Temple, 2018). For instance, in a previous study involving 50 type 2 diabetes patients with mild hypertension, it was reported that a higher fiber, high carbohydrate dietary regimen produced substantial reduction in serum lipids and lipoproteins along with improvements in cardiovascular risk (Pacy et al., 1984). These results were in good agreement with those reported in recent clinical studies on the impact dietary fiber consumption on insulin resistance and prevention of type 2 diabetes (Harding et al., 2008; Lattimer & Haub, 2010; Liu et al., 2003). However, other reports have revealed some contradictory results with positive effects being noted for cereal based fibers whereas fruit derived fibers had little protection against type 2 diabetes (Davison & Temple, 2018). These were corroborated by clinical and meta-analysis studies, wherein it was shown that consuming high amounts of dietary fibers, especially cereal fibers including β-glucan or psyllium produced substantial reduction in blood glucose concentrations and glycosylated hemoglobin percentages, which are known to reduce the incidence of type 2 diabetes and diabetic kidney disease (Mcrae, 2018; Quesada-Morúa et al., 2020; Weickert & Pfeiffer, 2018). In contrast, in a meta-analysis study of clinical cohort studies published from 1966 to 2014, Wang et al. (2016) concluded that a higher intake of fruits, especially berries were strongly associated with lower risk of type 2 diabetes (Wang et al., 2016). When taken together, these reports support the recommendation on increasing consumption of berries as a dietary fiber source for the prevention of many chronic ailments, including type 2 diabetes. However, additional studies appear to be needed to explore the mechanisms that underpin the associations.

Berries are a significant source of vitamins in the human diet (Česonienė et al., 2021; Rajakaruna et al., 2021). Among these, ascorbic acid (the reduced form of vitamin C), an essential water soluble vitamin has received particular interest due to its reducing properties, well known by its antioxidant activity due to the neutralization of physiologically relevant free radicals, oxygen and nitrogen reactive oxygen species, formed via cell metabolism, which are associated with several forms of tissue damage and diseases (Hemilä, 2017; Klimant et al., 2018). Additionally, vitamin C can reduce non-radical species and regenerate circulatory antioxidant molecules such as vitamin E (Carr & Frei, 1999). However, vitamin C can exhibit pro-oxidant properties under conditions including high oxygen tension and in the presence of metal ions (Padayatty & Levine, 2016). Under such conditions it can catalyze the reduction of Fe^{3+} to Fe^{2+} (Griffiths & Lunec, 2001), which results in the production of highly reactive radicals capable of oxidative damage to cellular biomolecules (Duarte & Lunec, 2005). In comparison to other dietary sources including citrus fruits, berries are among the richest sources of vitamin C with strawberries higher in vitamin C compared to oranges based on edible flesh weight basis (Richardson et al., 2018). Among berry species, strawberries have similar content to raspberries, but four-times more vitamin C than blueberries (Skrovankova et al., 2015). Nutritionally, sustained deficiency of dietary vitamin C is known to result in scurvy, a condition characterized by hypochondriasis and depression; perifollicular hyperkeratosis with coiled hair; anemia, erythema, and purpura; breakdown of old wounds; bleeding into the skin, subcutaneous tissues, muscles, and joints; fever; infections; and confusion (Padayatty & Levine, 2016). When untreated, scurvy can be fatal. Furthermore, *in vitro* and *in vivo* animal studies appear the support the antioxidant role vitamin C in disease prevention. Vitamin C was reported to inhibit atherosclerosis induced by cigarette smoke *in vivo* by preventing leukocyte aggregation and adhesion to the endothelium (Lehr et al., 1994). It has also been reported to prevent lipid peroxidation related atherosclerosis by inhibiting oxidation of LDL and absorption of oxidized LDL (Frei, 1991; Padayatty et al., 2003). These results were in line with antioxidant effects of vitamin C in mitigating the effects of x-irradiation on nucleated bone marrow cells in mice (Harapanhalli et al., 1996).

As a potent antioxidant, vitamin C has been postulated in clinical trials to mitigate the earliest stages of atherosclerosis (Aguirre & May 2008) and reduce the risk of CVD by contributing to the maintenance of the total pool of nitric oxide (NO) within cells (Ashor et al., 2019; Förstermann, 2010). Also, the benefits of administration of high dose vitamin C for targeting cancer vulnerabilities including leukemia, pancreatic, lung, colorectal, prostrate, and gastric cancers in clinical trials have been well reviewed and shown to be promising for patient populations who may benefit from high-dose vitamin C therapy for various types of cancers (Cimmino et al., 2018; Ngo et al., 2019). A number of epidemiological studies have reported that regular consumption of antioxidants, including vitamin C is beneficial for reduction of risk of CVD, that is, coronary heart disease, stroke, or peripheral arterial diseases (Ellingsen et al., 2009; Ye & Song, 2008). For example, meta-analysis review of double-blind randomized control trials comparing vitamin C use to placebo in children with upper respiratory tract infections (URTI) revealed that vitamin C supplementation reduced the duration of URTI (Vorilhon et al., 2019). As upper respiratory tract infections (URTI) is a viral respiratory infection, the authors postulated that vitamin C possesses anti-viral properties and could be a suitable supplementation in place of prescribed antibiotics for children and those at a higher risk of URTI. Furthermore, the anti-viral properties of vitamin C in clinical trials as adjunctive treatment for reducing the risks of sepsis, common colds, influenza, herpes infections, acute herpetic pain, and postherpetic neuralgia has been recently reviewed (Colunga Biancatelli et al., 2020; Kuhn et al., 2018). In another systematic review and meta-analysis of randomized control trials, Mason et al. (2021) showed that vitamin C supplementation improved glycemic control and blood pressure in participants with type 2 diabetes (Mason et al., 2021). These findings were in agreement with a related meta-analysis study of the effects of vitamin C supplementation on essential hypertension, which showed significant reduction of blood pressure in patients with hypertension following vitamin C supplementation (Guan et al., 2020). However, as with many clinical trials on the nutritional benefits of dietary antioxidants, meta-analysis conducted by different groups often reached different conclusions and have raised questions on the health claims associated with vitamin C consumption (Ashor et al., 2014, 2015; Montero et al., 2014). While a study involving over 20,000 participants by the European prospective investigation into cancer and nutrition (EPIC) Norfolk determined that increased plasma vitamin C concentration through consumption of fruits and vegetable rich in vitamin C was inversely associated with the incidence of cardiovascular related deaths, the results of majority of meta-analysis studies based on vitamin C supplementation concluded that vitamin C has no effect on this end point (Goszcz et al., 2015). Furthermore, a report from another study showed that regular intake of vitamin C rich diet lowered the incidence of stroke in patients over 65 years, but no significant effect was observed so far as coronary heart dieses was concern (Gale et al., 1995). In summary, it appears that consumption of fruits and vegetables rich in vitamin C has a significant protective effect on plasma vitamin C and cardiovascular health, and the effects may be more related to other nutrients present in these foods and not necessarily to vitamin C itself.

In addition to vitamin C, berries contain the water-soluble B vitamins (B1, B2, B3, B6, B9, and B12), but at lower concentrations compared to vitamin C. With regard to physiological functions, Joshi et al. (2001) demonstrated the hydroxyl and lipid peroxyl radical scavenging properties of folate (vitamin B9) in rat liver microsomes (Joshi et al., 2001). Deficiency of folate (vitamin B9) was reported to promote depression, anxiety, dementia, Alzheimer's disease, and CVD in a recent meta-analysis study (Al Mansoori et al., 2021). Perhaps, contributing to the protective effects in CVD, folate (vitamin B9) supplementation *in vivo* decreased LDL peroxidation and number of atherosclerotic plaques in Apo-E deficient mice (Carnicer et al., 2007). B vitamins including folate (vitamin B9) are also involved in metabolism of the essential amino acid, methionine, which impacts both homocysteine and the cellular antioxidant glutathione (Goszcz et al., 2015). Animal studies have shown involvement of methionine in the synthesis and maintenance of cellular glutathione pools which supress cellular oxidative damage by neutralizing radicals, and reactive nitrogen and oxygen species. In contrast, a review by Goszcz et al. (2015) implicated homocysteine in endothelial dysfunction and vascular inflammation which are risk factors for CVD (Goszcz et al., 2015). These reports appear to be in agreement with the results from clinical studies involving 155 patients that demonstrated the influence of high homocysteine and low folate plasmatic levels in acute coronary syndromes (García-Pinilla et al., 2007). Other clinical studies and trials showed that supplementation of folate (vitamin B9), vitamin B12, and vitamin B6 in participants reduced serum homocysteine and risk factors for stroke, peripheral vascular disease, CVD, and coronary artery disease (Antoniades et al., 2007; Heijer et al., 1998; Naurath et al., 1995; Robinson et al., 1998; Till et al., 2005). However, other contrasting reports from the vitamin intervention for stroke prevention (VISP) randomized control trials found no effect on the incidence of cerebral infarction, coronary diseases or cardiovascular mortality following folate (vitamin B9), vitamin B6, and vitamin B12 supplementation (Toole et al., 2004). Furthermore, Cochrane systematic review of vitamin B6, B9, and B12 revealed that there is no evidence in support of the use of B vitamins to reduce cardiovascular conditions, which could be due to the mis-match between the benefits of vitamin B supplementation observed *in vivo* and those reported by cohort, prospective, and retrospective clinical studies, and randomized control trials (Debreceni & Debreceni, 2012).

Vitamin A (retinol), carotenoids, and E are among the lipid soluble vitamins present in berries. These compounds generally possess bright colors which confer photo-protection in plants as well as attracting pollinators. Endogenously, vitamin A (retinol) may been synthesized from β-carotene by gut microbes (Norum & Blomhoff, 1992). Importantly, carotenoids including lycopene, lutein, α-carotene, and β-carotene represent the abundant carotenoids in berries. The antioxidant properties of vitamin A (retinol) and the carotenoids have been well researched (Goszcz et al., 2015). For example, *in vitro* studies based on plasma from human subjects showed that β-carotene, lycopene, and lutein inhibited LDL oxidation caused by Cu^{2+} ions (Romanchik et al., 1995). The radical scavenging properties of lycopene, the precursor of β-carotene is about 10 times more potent than α-tocopherol, and has been ascribed to the conjugated pi system in its chemical structure (Di Mascio et al., 1989). β-carotene and lycopene have also been demonstrated to be effective *in vivo* at reducing plasma cholesterol levels and circulating LDL, which are known to collectively reduce the risk factors for cardiovascular diseases (Fuhrman et al., 1997). These reports have been corroborated by clinical studies and trials which have demonstrated cardioprotective benefits from intake of carotenoid rich fruits and vegetables including berries. Furthermore, carotenoids supplementation including β-/α-carotene and were found in human clinical studies to reduce the incidence of coronary artery disease (Liu et al., 2001), atherosclerosis (D'Odorico et al., 2000), myocardial infarction (Karppi et al., 2011) as well as inhibiting inflammations, oxidative stress, and endothelial dysfunction (Hozawa et al., 2007). Vitamin E, on the other hand, is a term referring to eight isomeric compounds known as tocopherols and tocotrienols (Goszcz et al., 2015). While α-, β-, γ-, and δ-isoforms have been identified in berries, it appears that α-tocopherols are the most abundant isomer in berries (Bastías-Montes et al., 2020; Dienaitė et al., 2021). Functionally, *in vitro* studies have demonstrated that α-tocopherol is capable of exerting a cardioprotective effects, protecting against oxidative damage, inflammations, and endothelial dysfunction, which are all risk factors for atherosclerosis (Ranard & Erdman, 2017). For instance, vitamin E (α-tocopherol) inhibited monocyte adhesion to the endothelium (Cominacini et al., 1997; Devaraj et al., 1996; Islam et al., 1998), while suppressing the proliferation of vascular smooth muscle through the inhibition of protein kinase C activity (Tasinato et al., 1995). Furthermore, *in vivo* animal studies showed that vitamin E supplementation reduced the development of atherosclerosis, intravascular fatty deposits, and ischemia reperfusion by protecting against LDL peroxidation (Dröge, 2002; Otero et al., 2005; Reaven et al., 1993; Sagach et al., 2002). With regard to clinical trials and studies, while some reports exist appearing to suggest that vitamin E supplementation had no effect on cardiovascular outcomes in patients susceptible to cardiovascular events and diabetes (Lonn et al., 2002; Yusuf et al., 2000), the overwhelming evidence along with the antioxidant Cochrane review showed that vitamin E supplementation had a promising cardioprotective effect (Bjelakovic et al., 2014; Stephens et al., 1996).

Berries are also a good source of dietary minerals including phosphorus, selenium, potassium, calcium, magnesium, iron, manganese, copper, sodium, and aluminum (Di Vittori et al., 2018). In fact, it was reported that 100 g of edible portion of raspberries could provide more than 50% of Recommended Dietary Allowance (RDA) for manganese (Kowalenko, 2005). A similar conclusion was obtained for blackberries and blueberries (Nile & Park, 2014). In addition to their nutritional roles in the development of bones and teeth, a number of minerals including copper, zinc, manganese, and selenium are essential cofactors required for cellular antioxidant functions (Goszcz et al., 2015). As a result, their deficiencies have been suggested to result in lowered cellular antioxidant capacity leading to increased incidence of cardiovascular events (Fairweather-Tait et al., 2011). Regarding lipid composition of berries, while some berries have been shown to contain free fatty acids, they are universally not considered significant dietary source of lipids. Berry lipids include triglycerides, fatty acids, fatty alcohols, alkanes as well as sterols (Schulz & Chim, 2019). Dietary lipids including palmitic acid, stearic acid, linoleic acid, elaidic acid, and linoelaidic acid have been deteccted in blueberries, lingonberries, bilberries, and cranberries (Klavins et al., 2019, pp. 198–203). Furthermore, in golgi berries, the most abundant fatty acids were linoleic, oleic, palmitic, and stearic acids, accounting for about 95% of total fatty acids in these berries (Ilić et al., 2020). Apart from their primary function as energy sources, berry lipids including the essential omega-6 polyunsaturated fatty acids (ω6-PUFA) possess anti-inflammatory properties (Kapoor & Huang, 2006). In addition, other clinical studies have shown that infants from mothers whose diets were supplemented with essential fatty acids had higher mental processing scores, psychomotor development, hand-eye coordination and stereo acuity (Singh, 2005). The effect of ω6-PUFA supplementation through berry consumption is of particular interest from a health perspective due to their effect on CVD. Various meta-analysis of randomized control trials showed that the increased intake of ω6-PUFA may reduce myocardial infarction, total serum cholesterol (Calder, 2009; Farvid et al., 2014; Marklund et al., 2019). However, an extensive assessment of the effects of ω6-PUFA supplementation on cardiovascular based on the antioxidant Cochrane systematic review found no evidence in support of increasing ω6-PUFA supplementation and reduction in risks of cardiovascular events (Hooper et al., 2018).

3.2 Health benefits of non-nutritive bioactives in berries

The major non-nutritive bioactives in berries are the phenolic compounds which have been implicated in the defense mechanisms of plants against pathogens and ultraviolet radiation (Rocha et al., 2012). Phenolic compounds are phenol group containing compounds that are grouped into several subclasses: flavonoids, phenolic acids, tannins, lignans, and stilbenes as shown in Fig. 6.1 (Rodríguez-García et al., 2019). Phenolic compounds are well known for their antioxidant action in view of their interaction with reactive oxygen species (Galleano et al., 2010; Rodrigo et al., 2011). Additionally, they are able to block the action of enzymes involved in the formation of superoxide radicals, such as xanthine oxidase and protein kinase C, as well as activating antioxidant enzymes (Procházková et al., 2011; Quideau et al., 2011). Nutritionally, intake of fruits and vegetables rich in phenolic compounds has been demonstrated to exert a cardioprotective effect by reducing the risk of CVD (Nicholson et al., 2010; Varì et al., 2011). However, it is worth noting that pro-oxidant properties of phenolic compounds have been reported especially in the presence of transition metal ions (Babich et al., 2011). Under these conditions, phenolic compounds oxidize leading to the generation of superoxide radicals, quinones, semiquinones, and hydrogen peroxide, which could result in cellular oxidative damage (Gil-Longo & González-Vázquez, 2010; Halliwell, 2008).

Among phenolic compounds, flavonoids and phenolic acids represent the most abundant phenolic classes present in berries (Liu et al., 2020). Berry flavonoids include flavonols (quercetin, kaempferol, myricetin), flavanols (catechins and epicatechin), and anthocyanins (cyanidin glucosides and pelargonidin glucosides). Anthocyanins represent the majority of flavonoid compounds present in berries (Skrovankova et al., 2015). Anthocyanins are among the major colored pigments in berries especially those with red, blue or purple pigments. As natural antioxidants, their anti-cancer, cardioprotective, neuroprotective, anti-diabetic, anti-inflammatory, and anti-aging properties have been well investigated and reviewed (Kalt et al., 2020; Krga & Milenkovic, 2019; Sandoval-Ramírez et al., 2022). The principal anthocyanins found in berries are cyanidin glucosides and pelargonidin glucosides (Yousefi et al., 2021). Furthermore, among flavonoids, catechins are known for their anti-cancer, anti-obesity, anti-diabetic, hepatoprotective, and neuroprotective effects (Bernatoniene & Kopustinskiene, 2018; Ide et al., 2018; Shirakami & Shimizu, 2018). Similar antioxidant and cardioprotective effects have been reported for quercetin (Kumar et al., 2017; Patel et al., 2018). Additionally, evidence from several epidemiological studies has shown a positive association between flavonoid supplementation and reduced risk of CVD in certain high-risk population groups including postmenopausal women (Dower et al., 2016; Mink et al., 2007; Rees et al., 2018).

Compared to flavonoids, phenolic acids including hydroxybenzoic acids and hydroxycinnamic acids constitute about a third of phenolic compound compositions in berries (Kim, 2018; Di Vittori et al., 2018). Phenolic acids may occur as free acids or esterified with organic acids and sugars (Subbiah et al., 2020). Among these, chlorogenic acid derivatives (esters of caffeic and quinic acids) are the common esters of hydroxycinnamic acids (Paredes-López et al., 2010). Furthermore, gallic and vanillic acids represent the most abundant hydroxybenzoic acids in berries (Golovinskaia & Wang, 2021; Shabani et al., 2020). Phenolic acids have gained widespread recognition owing to their antioxidant, anti-inflammatory,

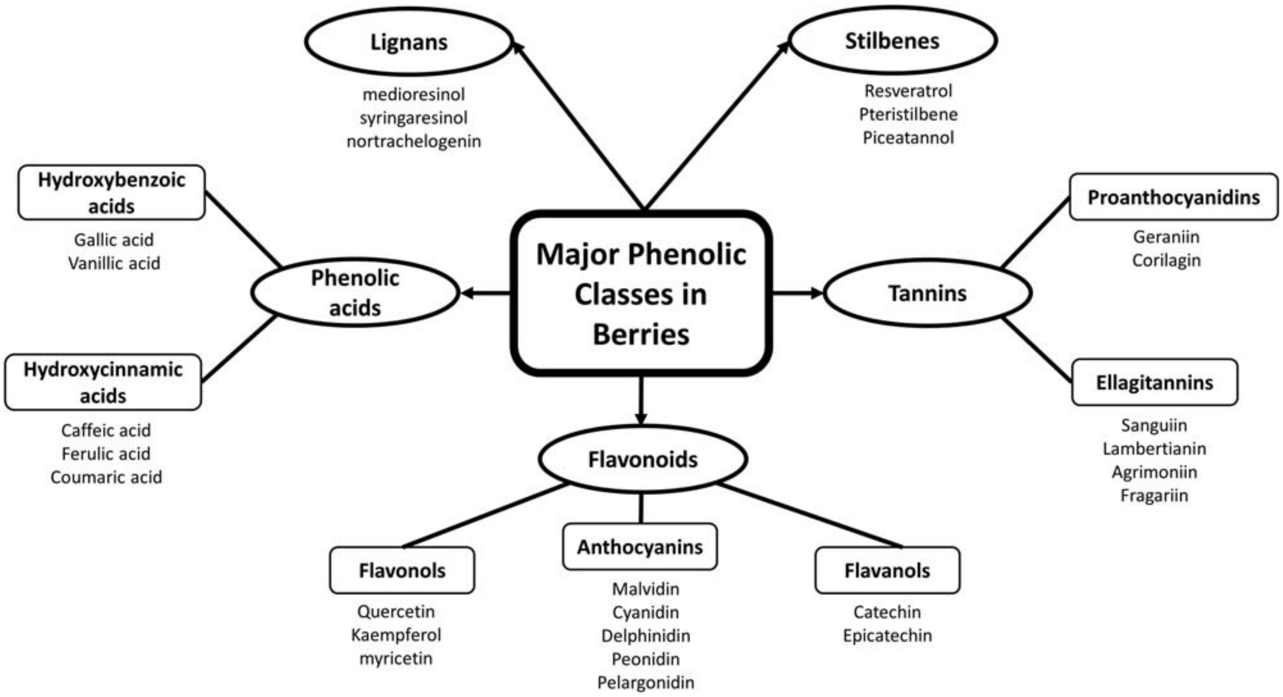

FIGURE 6.1 Phenolic classes in berry fruits. *Adapted from (Di Vittori et al., 2018).*

immunoregulatory, anti-allergenic, anti-atherogenic, anti-microbial, anti-thrombotic, cardioprotective, anti-cancer, and anti-diabetic properties which confer health benefits (Anantharaju et al., 2016; Kumar & Goel, 2019). The reader is directed to a recent review by Kiokias et al. (2020) wherein *in vitro* and *in vivo* antioxidant activity of dietary phenolic acids were well discussed (Kiokias et al., 2020). In a related study, Rosa et al. (2016) demonstrated that phenolic acids were efficacious for the treatment of various forms of cancer, with focus on colon cancer in human colon adenocarcinoma cells (Rosa et al., 2016). Furthermore, a comprehensive review by Vinayagam et al. (2016) noted the anti-diabetic effects of simple phenolic acids (Vinayagam et al., 2016), while the neuroprotective effects of phenolic acids for the treatment of Alzheimer's disease have been reported (Shahidi & Yeo, 2018).

Tannins occur either as condensed (proanthocyanidins) or hydrolysable (ellagitannins) tannins in berries (Bernjak & Kristl, 2020). However, it is been found that condensed tannins are more widely distributed in berries compared to hydrolysable tannins (Seeram et al., 2001; Shahidi & Naczk, 2003). Tannins play an essential role in shaping the sensory properties of berries (Nile & Park, 2014). Puupponen-Pimia at al. (2005) reported that tannins impart a tarty taste to berries and berry juices (Puupponen-Pimiä et al., 2005). Furthermore, when tannins bind to anthocyanins to form copolymers, they help to stabilize the colors of berries and berry juices (Lorenzo et al., 2005). Berries are known to contain a significant source of dietary tannins. It has been reported the level of ellagitannins is three times higher in berries compared to other established dietary sources including walnuts and pecans (Beekwilder et al., 2005; Paredes-López et al., 2010). In a recent review, the cardioprotective, antioxidant, anticancer, anti-allergic, anti-inflammatory, antihelminthic, and antimicrobial activities of tannins were investigated (Sharma et al., 2021). For instance, *in vivo* tests screening cancer-preventive agents, Okuda (2005) showed that the hydrolysable tannins: geraniin, and corilagin exhibited anticancer activities (Okuda, 2005). The author also reported antitumor activities in studies using the ellagitannins, sanguiin H6, and lambertianin. Furthermore, clinical studies using grape seed supplementation rich in tannins revealed an improvement in cardiovascular health of patients in the test group by lowering plasma triacylglycerols and blood pressure (Sivaprakasapillai et al., 2009; Zern et al., 2005).

Stilbenes are one of the classes of phenolic compounds derived from phenylpropanoid pathway (Chong et al., 2009). However, compared to flavonoids, phenolic acids and tannins, they are not widely distributed in berries and occur only in plants which have the gene encoding the enzyme stilbene synthase (Błaszczyk et al., 2019). Among berries, grapes, blueberry, bilberry, partridgeberry, cowberry, lingonberry, and deerberry provide dietary sources of stilbenes (El Khawand et al., 2018). Stilbenes have attracted interest due to the health benefits associated stilbenoid compounds such as resveratrol, pterostilbene, piceatannol, 3′-hydroxypterostilbene, and piceid (Rimando et al., 2004). In systematic reviews, the anticarcinogenic, anti-allergenic, algicidal, antidiabetic, anti-obesity, anti-aging, and antimutagenic effects of these stilbenoid compounds were discussed (Paredes-López et al., 2010; Shakibaei et al., 2009; Tsai et al., 2017). For example, Cui et al. (2010) demonstrated in vivo that resveratrol reduced colon tumor incidence, tumor multiplicity, and tumor volume in mice (Cui et al., 2010). These results were in line with those from other in vivo animal studies based on resveratrol (Miki et al., 2012; Nutakul et al., 2011). Other related mice based in vivo studies have also demonstrated the efficacy of resveratrol formulations for treating inflammations (Lee et al., 2015; Sánchez-Fidalgo et al., 2010), breast cancer (He et al., 2011; Scarlatti et al., 2008), prostrate cancer (Sheth et al., 2012), diabetes (Do et al., 2012; Mohammadshahi et al., 2014), obesity (Chang et al., 2016; Kim et al., 2011), and aging (Baur et al., 2006; Monserrat Hernández-Hernández et al., 2016; Pearson et al., 2008). In addition to these animal studies, randomized control trial and clinical studies have demonstrated resveratrol formulations to be effective for reducing type II diabetes mellitus (Bhatt et al., 2012) and obesity (Konings et al., 2014) in human volunteers.

Lignans are phenolic compounds formed by the union of two cinnamic acid residues or their biogenetic equivalents (Ayres & Loike, 1990). Smeds et al. (2012) reported that medioresinol, nortrachelogenin, syringaresinol, lariciresinol, pinoresinol, and secoisolariciresinol are among the most abundant lignans in berries (Smeds et al., 2012). Lignans exert a range of effects including anticancer (Su & Wink, 2015), anti-aging (Corrêa et al., 2018; Su & Wink, 2015), antioxidant (Huang et al., 2015), anti-inflammatory and cardioprotective (Chen et al., 2013), antimicrobial (Álvarez-Martínez et al., 2020), antitumorigenic (Saarinen et al., 2007; Webb & Mccullough, 2005), antidiabetic, and anti-obesity (Bhathena & Velasquez, 2002; Vanharanta et al., 1999). For example, regarding antimicrobial effects, Azman et al. (2018) demonstrated that pinoresinol exhibited in vivo antiviral activity against influenza virus (IC_{50} of 30.1 ± 11 μM) and Coxsackie virus B3 (IC_{50} of 7.1 ± 3 μM) (Azman et al., 2018). Concerning anti-inflammatory and cardioprotective effects, Rodriguez-Garcia et al. (2019) reported that lignans reduced the risks of inflammatory cell infiltration (Rodríguez-García et al., 2019) and CVD (Chun et al., 2014). Furthermore, a study based on the United States Cancer Center Support Grant found that higher lignan consumption was inversely correlated with the risk of breast cancer among pre- and menopausal women (McCann et al., 2011). The results were supported by other meta-analysis studies that demonstrated that high levels of plant lignan consumption was strongly correlated with a reduction in postmenopausal breast cancer risk (Buck et al., 2010; Velentzis et al., 2009). Dietary lignans have also been implicated in the reduction of CVD risks in postmenopausal women

(Sammartino et al., 2006). Here, it was demonstrated that an inverse association existed between high lignan consumption and development of hypertension and CVD in postmenopausal women and elderly men (Pellegrini et al., 2010; Witkowska et al., 2018).

Several studies have been undertaken to investigate the mechanism of action of phenolic compounds *in vivo* (Goszcz et al., 2015). However, *in vitro* studies that focused on the antioxidant properties of berry-derived phenolic compounds were based on micromolar concentrations of phenolic extracts which were higher than actual plasma concentrations (0.1−1 μM) of phenolic compounds (Nicholson et al., 2010; Rodrigo et al., 2011). The low bioavailability of phenolic compounds in the blood following consumption of phenolic-rich foods appears to stem from the poor absorption of phenolic compounds and appreciable metabolism they undergo in the gut, which have led to the postulation that they will not have any significant impact on plasma antioxidant potential in view of their low physiological concentrations (Goszcz et al., 2017a, 2017b). To account for the *in vivo* physiological effects of phenolic compounds, it has been suggested that phenolic compounds or their metabolites may act to stimulate endogenous antioxidant defenses through activation of the NRF-2/ARE-1 signaling pathway (Grossini et al., 2015; Muggeridge et al., 2019; Tang et al., 2018). Furthermore, low molecular weight phenolic metabolites appear to be more stable in physiological environments than their intact forms, which may better account for the physiological effects of phenolic intake (Wallace, 2011).

In addition to phenolic compounds, berries contain several non-nutritive compounds whose structures do not incorporate phenolic ring (s). Notables are terpenes, amino acids, and organic acids. These phytochemicals, though less widely distributed in berries compared to phenolic compounds, have received considerable interest due to nutritional and organoleptic properties. Regarding isoprenoids (terpenes and terpenoids), these compounds are derived from a common biosynthetic pathway based on mevalonate and are composed of isoprene units (Gupta & Prakash, 2014). They occur mainly as essential oils which impact berry aroma and flavor, and ultimately consumer sensorial preference (Carbone et al., 2008). Terpenes, especially monoterpenes, such as phellandrene, sabinene, and γ-terpinene, have been reported only in goji berry (Chang et al., 2019). Kupska et al. (2014) identified 44 terpenes including eucalyptol (−)-β-pinene and (−)-terpinen-4-ol, (−)-camphor and (+)-α-terpineol in blue honeysuckle berries and blueberries (Kupska et al., 2014). Other notable berries rich in terpenes include golgi berries and acai berries (Gao et al., 2015). In addition, terpenes play significant roles in human health owing to their apophlegmatic, anti-bacterial, anti-virial, cholagogic, spasmolytic, pain-releasing, and anti-carcinogenic properties (Bakkali et al., 2008; Dillard & German, 2000).

4. Factors affecting berry nutritional quality

Whether consumed fresh or processed, berries are among the richest sources of nutrients and health-promoting bioactive compounds in the human diet. However, the distribution of these metabolites is not the same for all berry types, being dictated by preharvest factors (Battino et al., 2009). Furthermore, even for the same species of berries, variations in metabolite composition have been identified based on cultivar differences (Routray & Orsat, 2011). In addition, there is amble literature that shows that environmental and agronomic interventions during plant growth are capable of altering metabolite composition in berries (Labanca et al., 2017). On top of these, metabolite compositions of non-climacteric berries including grapes, strawberries, and raspberries among others are impacted by harvesting practices (Ali et al., 2011). Lastly, postharvest processing and storage conditions can potentially alter the nutritional composition of raw and processed berries (Kumar et al., 2018). Importantly, many pre- and postharvest factors are closely interrelated and often difficult to isolate experimentally (Downey et al., 2006). Fig. 6.2 outlines the factors influencing the metabolite and nutritional quality of berry species.

4.1 Genetic factors

Genetics is the first factor that determines the metabolite composition of a plant since the biosynthesis of metabolites is strictly controlled by genes (Castellarin & DI Gaspero, 2007). As macro- and micronutrients in berries are mainly primary metabolites, they occur in all berry species, albeit in varying quantities. Furthermore, bioactive non-nutritive compounds including phenolic compounds constitute secondary metabolites in berries whose distribution, though under genetic control, may also be altered by biotic and abiotic (agro-environmental) stressors. Thus, whether consumed fresh or processed, the influence of genetic differences, either interspecies (varietal) or intraspecies (cultivaral) on metabolite composition of berries cannot be overemphasized, as has been extensively researched and reviewed (Alvarez-Suarez et al., 2014; Di Vittori et al., 2018; Baghdady et al., 2020).

Regarding interspecies differences, for example, Hegedűs et al. (2008) observed variations in antioxidant and phenolic compositions of three berry species including strawberry, raspberry, and redcurrant (Hegedűs et al., 2008). Here, total

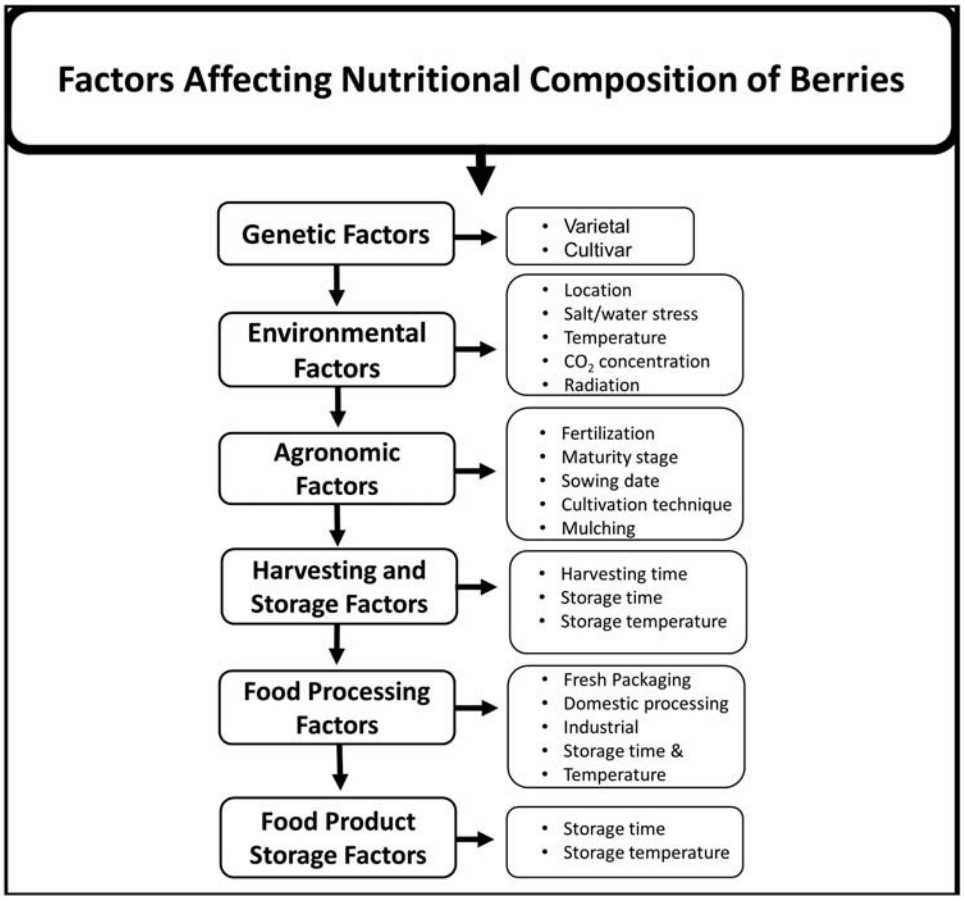

FIGURE 6.2 Factors affecting nutritional composition of berries. *Adapted from (Tiwari & Cummins, 2013).*

phenolic content (TPC) varied in the relative order of raspberry = red currant < strawberry whereas ferric reducing antioxidant power (FRAP) radical scavenging activities varied in the relative order of strawberry < raspberry = redcurrant. In a related study, interspecies differences accounted for the presence of catechin in strawberries, which is absent in raspberries (Nile & Park, 2014). These results corroborate the variations in anthocyanin contents observed among lowbush blueberry (*Vaccinium angustifolium*), highbush blueberry (*Vaccinium corymbosum*), cranberry (*Vaccinium oxycoccos*), raspberry (*Rubus idaeus*), strawberry (*Fragaria x ananassa*), blackcurrant (*Ribes nigrum*), redcurrant (*Ribes sativum*), blackberry (*Rubus mesogaeus*), bilberry (*Vaccinium myrtillus*), and cowberry (*Vaccinium vitis-idaea*) species (Katsube et al., 2003). In another study investigating the phenolic profile of 18 species of Scandinavian berries, Määttä-Riihinen et al. (2004) observed distinct differences in the anthocyanin content and profiles of berry species belonging to Grossulariaceae, Ericaceae, Rosaceae, Empetraceae, Elaeagnaceae, and Caprifoliaceae families (Määttä-Riihinen et al., 2004). Similar interspecies diversity has been reported in *Vaccinium* berries. For instance, Prior et al. (1998) studied 23 genotypes of four species (*V. corymbosum* L., *V. ashei Reade*, *V. angustifolium* Aiton, and *V. myrtillus*), Ehlenfeldt and Prior (2001) examined 87 *Vaccinium corymbosum* L. and hybrid genotypes, and Moyer et al. (2002) evaluated 30 genotypes of nine species (*V. angustifolium*, *V. constablaei* **x** *V. ashei*, *V. corymbosum*, *V. membranaceum*, *V. myrtilloides Michaux*, *V. ovalifolium*, *V. ovatum Pursh*, and *V. parvifolium Smith*) and found marked interspecies variability in total phenoic contents, total anthocyanin contents, and antioxidant capacities between berry groups (Lee et al., 2004). These were in line with the results obtained when Lee and Wrolstad (2004) studied 48 genotypes of three species (*V. membranaceum*, *V. o valifolium*, and *V. deliciosum*) (Lee et al., 2004); Taruscio et al. (2004) evaluated nine species (*V. angustifolium*, *V. corymbosum*, *V. deliciosum*, *V. membranaceum*, *V. ovalifolium*, *V. ovatum*, *V. oxycoccus*, *V. parvifolium*, and *V. uliginosum*) (Taruscio et al., 2004), and Kalt et al. (1999a, 1999b) evaluated 17 genotypes of four species (*V. myrtillus* L., *V. myrtilloides* L., *V. corymbosum* L., and *V. angustifolium*) (Kalt et al., 1999b).

Intraspecies variations in metabolite compositions have also been reported in berries. For instance, Hakala et al. (2003) observed differences in vitamin C and mineral contents between strawberry cultivars "Senga Sengana," "Jonsok," "Korona," "Polka," "Honeoye," and "Bounty" cultivated under identical growth conditions (Hakala et al., 2003). In another study, a marked difference in TPC was observed between blackcurrant and redcurrant cultivars (Hegedűs et al., 2008). Here, the authors reported that TPC was considerably higher in all blackcurrant cultivars ("Fertődi 1," "Otelo," and "Titania") compared to redcurrant cultivars ("Detvan," "Jonkheer van Tets," and "Rondom"). The variability in the phenolic and antioxidant activities of redcurrant and blackcurrant cultivars was in line with the higher anthocyanin content of blackcurrant cultivars which were roughly 37-fold higher than redcurrant cultivars (Wu et al., 2006). Similar cultivaral differences in sugars, vitamins, phenolic compounds, and antioxidant activities have been reported in blueberry (Gao & Mazza, 1994; Kalt et al., 1999b; Prior et al., 1998), strawberry (Caracciolo et al., 2013; Diamanti et al., 2012; Doumett et al., 2011), raspberry (Pantelidis et al., 2007, de Ancos et al., 1999), cranberry (Pappas & Schaich, 2009; Wang, Catana, et al., 2002), currants, and blackberry (Milivojević et al., 2011; Siriwoharn et al., 2004) among other berries. Table 6.2 summarizes the nutritional composition of different species of berries. For further reading, readers are directed to these reviews that have extensively evaluated the effects of genetics on berry nutritional quality (Alvarez-Suarez et al., 2014; Di Vittori et al., 2018; Wang, 2007).

4.2 Environmental factors

With regards to environmental factors, the accumulation of nutrients and bioactive compounds in berries is influenced by environmental conditions as illustrated in Fig. 6.2. Significantly, while the genetic background is the first factor determining the nutritional quality of berries, the full capacity is determined by interaction with environmental conditions, as has been reported and reviewed (Dal Santo et al., 2018; Karppinen et al., 2016). In addition to primary metabolites such as proteins, fats, and carbohydrates, berries produce secondary metabolites which constitute key components in plant defense and adaptation to both biotic and abiotic stress conditions (Wang, 2007; Wink, 2008). In fact, secondary metabolite pools in plants are known to respond to environmental and biotic stresses (Harborne, 1999). Taken together, environmental conditions may trigger mechanisms in plants that could result in accumulation or depletion of secondary metabolite pools to enable plants survive adverse environmental stresses including temperature, water, salt, altitude, geographic location, carbon dioxide (CO_2) concentration, and radiation (Yang et al., 2018).

4.2.1 Geographic location

Geographic location incorporates components such as altitude, latitude, and soil profile which influence berry phytochemical composition and nutritional quality (Karppinen et al., 2016; Koundouras et al., 2006). In particular, light intensity generally increases as latitude decreases, which in turn impacts photosynthetic processes in plants (Jackson & Lombard, 1993). Furthermore, there is also a decrease in air and soil temperature at higher latitudes and altitudes, which in turn could impact the rates of biochemical processes including those leading to the formation of metabolites in plants. For example, De Silva and Rupasinghe (2021) had shown that the total sugar content (TSC), total anthocyanin content (TAC), TPC, and total antioxidant activity (TAA) of haskap berries (*Lonicera caerulea* L.) cultivated at different locations across Canada were significantly impacted by the growing location (De Silva & Rupasinghe, 2021). The TAC, TPC, and TAA of the berries varied from 88 to 273 mg C3GE/100 g of fresh weight (FW), 256−442 mg GAE/100g of FW, and 27−52 μmol TE/g of FW, respectively. In another study, Krüger et al. (2012a, 2012b) reported that sugars and organic acids in strawberries were influenced by latitude with northern European sites generally possessing higher values of these metabolites compared to central and southern European sites (Krüger, Josuttis, et al., 2012). Moreover, the authors observed that fruits grown at the southern sites were redder compared to those of the northern sites. These results were corroborated by Cocco et al. (2015) for strawberries grown in northern and southern Italy. Here, the TSC, total ascorbic acid, and TAC were found to be significantly higher in fruits cultivated in southern Italy compared to Northern Italy (Cocco et al., 2015). Similarly, Mikulic-Petkovsek et al. (2015) showed that the growing location influenced the quantity of sugars, organic acids, and phenolic compounds in wild bilberry (Mikulic-Petkovsek et al., 2015). In grape (*Vitis vinifera*) cultivars Touriga Nacional and Touriga Francesca, anthocyanin content was observed to increase with increasing altitude from 150 to >250 m above sea level (Mateus et al., 2002). In contrast, in a related study by the same author, flavan-3-ol monomer and total proanthocyanidin content in both cultivars decreased with increasing altitude (Mateus et al., 2001). Similarly, blackcurrant cultivars grown at higher latitude (66 degrees 34′ N) had lower contents of total flavonols, total anthocyanins, and total phenolic compounds than those grown at lower latitude (60 degrees 23′ N) in Finland (Zheng, Yang, Ruusunen, et al., 2012). These results were in good agreement with those reported for wild Finnish bilberries (Lätti et al., 2008). Here, a lower content of the total anthocyanins was observed in the berries of the southern region compared to those in the central and northern regions of Finland. However, it is unlikely that these results are strictly effects of altitude but rather the effects of different climatic conditions at each site, with the higher latitude sites cooler than the lower sites. The diversity in

TABLE 6.2 Nutritive composition of berries per 100 g[a].

Group	Nutrient	Straw berry*	Rasp berry*	Black berry*	Blue berry**	Goose berry***	Cran berry**	Black currant***	Cloud Berry*	Elder berry†	Mul berry††	Green kiwifruit†*	Grapes*‡
Proximates	Energy (kcal)	32	52	43	157	44	46	63	51	73	43	61	57
	Protein (g)	0.67	1.2	1.39	0.74	0.88	0.46	1.4	2.4	0.66	1.44	1.14	0.81
	Lipid (g)	0.3	0.65	0.49	0.33	0.58	0.13	0.41	0.8	0.5	0.39	0.52	0.47
	Carbohydrates (g)	7.68	11.9	9.61	14.5	10.2	12	15.4	8.6	18.4	9.8	14.7	13.9
	Dietary fiber (g)	2	6.5	5.3	2.4	4.3	3.6	–	–	7	1.7	3	3.9
	Sugar (g)	4.89	4.42	4.88	9.96	–	4.27	–	–	–	8.1	8.99	–
	Sucrose	0.47	0.2	0.07	0.11	–	0.16	–	–	–	–	0.15	0.57
	Glucose	1.99	1.86	2.31	4.88	–	3.44	–	–	–	–	4.11	3.67
	Fructose	2.44	2.35	2.4	4.97	–	0.67	–	–	–	–	4.35	3.92
	Starch	0.04	0	0	0.03	–	0	–	–	–	–	0	–
Minerals	Calcium (mg)	16	25	29	6	25	8	55	18	38	39	34	37
	Copper (mg)	0.048	0.09	0.165	0.057	0.07	0.056	0.086	–	0.061	0.06	0.13	0.119
	Iron (mg)	0.41	0.69	0.62	0.28	0.31	0.23	1.54	0.7	1.6	1.85	0.31	0.26
	Magnesium (mg)	13	22	20	6	10	6	24	–	5	18	17	14
	Manganese (mg)	0.386	0.67	0.646	0.336	0.144	0.267	0.256	–	–	–	0.098	1.97
	Phosphorus (mg)	24	29	22	12	27	11	59	35	39	38	34	24
	Potassium (mg)	153	151	162	77	198	80	322	–	280	194	312	203
	Selenium (μg)	0.4	0.2	0.4	0.1	0.6	0.1	–	–	0.6	0.6	0.2	–
	Sodium (mg)	1	1	1	1	1	2	2	–	6	10	3	1
	Zinc (mg)	0.14	0.42	0.53	0.16	0.12	0.09	0.27	–	0.11	0.12	0.14	0.11
Vitamins	Thiamin (mg)	0.024	0.032	0.02	0.037	0.04	0.012	0.05	0.05	0.07	0.029	0.027	–
	Riboflavin (mg)	0.022	0.038	0.026	0.041	0.03	0.02	0.05	0.07	0.06	0.101	0.025	1.5
	Niacin (mg)	0.386	0.598	0.646	0.418	0.3	0.101	0.3	0.9	0.5	0.62	0.341	–

	Pantothenic acid (mg)	0.125	0.329	0.276	0.124	0.286	0.295	0.398	–	0.14	–	0.183	–
	Pyridoxine (mg)	0.047	0.055	0.03	0.052	0.08	0.057	0.066	–	0.23	0.05	0.063	–
	Folate (mg)	24	21	25	6	6	1μg	–	–	6	6	25	2
	Choline	5.7	12.3	8.5	6	–	5.5	–	–	–	12.3	7.8	–
	Ascorbic acid (mg)	58.8	26.2	21	9.7	27.7	14	181	158	36	36.4	92.7	6.5
	α-tocopherol (mg)	0.29	0.87	1.17	0.57	0.37	1.32	1	–	–	0.87	1.46	–
	Vitamin K (μg)	2.2	7.8	19.8	19.3	–	5	–	–	–	7.8	40.3	–
	B-carotene (μg)	7	12	128	32	–	38	–	–	–	9	52	39

* = Rosaceae; ** = Ericaceae; *** = Grossulariaceae; † = Adoxaceae; †† = Moraceae; †* = Actinidiaceae; *‡ = Muscadine grapes.
[a]*USDA National Nutrient Database (http://ndb.na.usda.gov/nda/, US Department of Agriculture, 2018).*

metabolite composition and antioxidant activities of berries as a result of geographic locations has been noted in grape (Mateus et al., 2001, 2002), strawberry (Hårdh & Hårdh, 1977; Josuttis et al., 2012), currant (Zheng et al., 2009), bilberry (Lätti et al., 2010; Rieger et al., 2008; Vänninen et al., 1988; Åkerström et al., 2010), blackberry (Johansson et al., 1997; Reyes-Carmona et al., 2005; Zheng et al., 2009), cloudberry (Johansson et al., 1997), blueberry (Connor, Luby, Tong, et al., 2002), and sea buckthorn (Zheng, Yang, TRÉpanier, & Kallio, 2012). For comprehensive evaluation of the effects of geographic location on berry metabolite composition, readers are directed to the following reviews (Downey et al., 2006; Jackson & Lombard, 1993; Mansour et al., 2022; Teixeira et al., 2013).

4.2.2 Temperature

As with growing location, temperature is one of the climate factors which has received a lot of attention owing to the effect it has on plant metabolic processes (Bonada & Sadras, 2015; De Orduna, 2010). There is also recognition that rising global temperatures associated with climate change will impact many of the world's berry-growing regions as average air and soil temperatures rise (Gouot et al., 2019). As such, preharvest temperature stresses are expected to result in accumulation or degradation of metabolites in berry fruits, and consequently postharvest nutritional quality (Rienth et al., 2021). Studies focusing on the sole effect of temperature separated from CO_2, light, and water found that temperature extremes during fruit development affected a wide range of berry metabolites, in particular sugars, organic acids, and phenolic compounds (Gouot et al., 2019). However, results are often ambiguous, which makes it difficult to draw general conclusions about temperature effects. Furthermore, study results are affected by genetic factors (inter- and intraspecies), temperature ranges, as well as variations in the duration of stress application from a couple of hours to several months. Nonetheless, with few exceptions, it appears cooler preharvest temperatures favor accumulation of organic acids, phenolic compounds, and vitamin C in berries (Koshita, 2015; Lee & Kader, 2000), whereas higher temperatures have been shown to lead to higher sugar contents (Karppinen et al., 2016). In *Vaccinium* berries, for example, northern and southern clones of bilberry (*Vaccinium myrtillus* L.) fruits showed higher levels of anthocyanins, flavanols, hydroxycinnamic acids, quinic acid, and carbohydrates (*myo*-inositol, fructose, glucose, and sucrose) when plants where raised at 12°C compared to those cultivated at 18°C (Uleberg et al., 2012). In another study, Zoratti et al. (2015a, 2015b) compared the effect of light-temperature combinations on bilberry and highbush blueberry nutritional composition (Zoratti, Jaakola, Häggman, & Giongo, 2015). Here, the authors noted that lower temperatures favored the accumulation of anthocyanins in the berries. In bilberry, an increase in the levels of hydroxylated anthocyanins of fruits was recorded when growth temperature decreased from 25°C to 10°C, whereas in blueberry, anthocyanins were accumulated in plants raised at 25°C compared to plants grown at 30°C. A similar accumulation of anthocyanins in cranberry was reported by Hall and Stark (1972), when fruits were maintained under cooler night temperatures (Hall & Stark, 1972). Martinussen et al., 2010 also reported the accumulation of TPC and TAC in cloudberry when cultivated at 9 and 12°C compared to fruits grown at higher temperatures (Martinussen et al., 2010). This contrasted with the results obtained by Van Heuvel and Autio (2008) in cranberry (*Vaccinium macrocarpon* Ait.) fruits (Heuvel & Autio, 2008). The authors noted that warmer preharvest temperatures increased TAC, TPC and total flavonol content of the fruits. These results were generally in good agreement with those reported in blackcurrants where TAC, TPC, and vitamin C content were higher in fruits grown at 12°C compared to those cultivated at 24°C (Woznicki et al., 2016). The effects of temperature on nutritional composition of berries have been reported in grape (Mori et al., 2007; Ortega-Regules et al., 2006; VAN Leeuwen & DESTRAC-Irvine, 2017; Yamane et al., 2006), currant (Krüger, Dietrich, et al., 2012; Zheng et al., 2009), strawberry (Davik et al., 2006; Tulipani et al., 2011; Wang & Zheng, 2001), sea buckthorn (Zheng, Yang, TRÉpanier, & Kallio, 2012), and cloudberry (McDougall et al., 2011). Other reviews have also evaluated the effect of temperature on the accumulation of non-nutritive bioactive metabolites in berries (Karppinen et al., 2016; Remberg et al., 2010; Zoratti, Jaakola, HÄ Ggman, & Giongo, 2015).

4.2.3 Photo-radiation

The light conditions under which plants grow have been found to have a significant role in the metabolism of flavonoids in berries (Karppinen et al., 2016). However, the effect of light on the accumulation of flavonoids in berries seems to be regulated in a species-specific manner (Winkel-Shirley, 2002). For instance, it was reported that bilberries accumulated higher levels of anthocyanins, flavonols, hydroxycinnamic acids, and total phenolics when grown under higher photosynthetic active radiation (Mikulic-Petkovsek et al., 2015). Although blueberries are also shade-adapted species they seem to require higher solar exposure for normal ripening and anthocyanin accumulation (Zoratti, Jaakola, Häggman, & Giongo, 2015). In other pre- and postharvest studies, light had also a positive effect on the accumulation of phenolic acids and anthocyanins in cranberry (Zhou & Singh, 2004), raspberry (Wang et al., 2009b), bayberry (Niu et al., 2010), bilberry (Uleberg et al., 2012), and strawberry (Anttonen et al., 2006). The positive effect of high light exposure on flavonoid biosynthesis has been reported to be due to increased expression of flavonoid biosynthetic genes in berry skins, leading to elevated content of flavonoids (Cortell & Kennedy, 2006; Koyama et al., 2012).

It is important to point out that the effect of light on berry bioactive compounds can be transmitted through perception of other attributes such as light quality and day length (Zoratti, Karppinen, et al., 2014). Longer days are associated with more intense flavonoid production than shorter days (Jaakola & Hohtola, 2010; Mazur, Sønsteby, et al., 2014). In bilberry, the effect of photoperiod appears to be one reasons for more rapid accumulation and higher concentrations of anthocyanins at northern latitudes compared to southern growth conditions (Uleberg et al., 2012). In addition to light intensity, the quality of light is an important factor influencing the biosynthesis of flavonoids in berries. For example, Karppinen et al. (2016) reported that exposure of bilberry fruits to blue, red, and far-red light during ripening enhanced the total anthocyanin content in ripened fruits (Karppinen et al., 2016). Similar results were obtained by Zoratti et al. (2014a, 2014b) where exposure of bilberries to blue wavelengths resulted in accumulation hydroxylated anthocyanins; delphinidins, petunidins, and malvidins, but not cyanidins and peonidins (Zoratti, Sarala, et al., 2014). In other related studies, post-harvest exposure of blueberries to ultraviolet-B (UV−B) and ultraviolet-C (UV−C) light-induced anthocyanin accumulation (Nguyen et al., 2014; Perkins-Veazie et al., 2008; Wang et al., 2009a). Furthermore, longer light exposure has been implicated in the accumulation of flavonoids in berries (Jaakola & Hohtola, 2010; Mazur, Sønsteby, et al., 2014). In bilberry, the effect of photoperiod appears to be one reason for more rapid accumulation and higher concentrations of anthocyanins at northern latitudes compared to southern growth conditions (Uleberg et al., 2012). The variation in berry metabolite composition with photo-irradiation has been demonstrated in grape (Bergqvist et al., 2001; Pereira et al., 2006; Ristic et al., 2007; Spayd et al., 2002; Van Leeuwen & Destrac- Irvine, 2017) and bilberry (Jaakola et al., 2004; Uleberg et al., 2012). Along with temperature and photo-radiation, atmospheric CO_2 concentration has been shown to also impact metabolite composition in grape (Salazar Parra et al., 2010; Wohlfahrt et al., 2020), blueberry (Kuusela et al., 2006; Tjosvold, 1994), and strawberry (Itani et al., 1998; Sun et al., 2012; Wang et al., 2007).

4.3 Agronomic factors

Agronomic management practices are among the interventions most commonly used to influence the nutritional quality of berries before they are harvested (Alem et al., 2019; Hasanaliyeva et al., 2021; Stojanov et al., 2019; Tomás-Barberán & Espín, 2001). Agronomic factors may include water management, mineral nutrition, grafting, application of elicitors, stimulating agents, plant activators, as well as agricultural practices such as organic versus conventional agricultural practices (Anjos et al., 2020). Organic agricultural practices include the addition of nutrient amendments that enhance soil fertility and health, and the exclusion of mineral inorganic fertilisers. In contrast, conventional agricultural practices have a potential to impact moderate increment of most nutrients, this being dependent on the levels and forms of the fertilization applied to berry crops, the balance of macro- and micronutrients in the soil and soil quality (Alem et al., 2019). Mineral nutrition plays an important role in the accumulation of phenolic compounds in berries. Furthermore, the effect of water stress, salt stress, and soil cultural practices may trigger a response in plants leading to the accumulation or depletion of metabolite pools (Di Vittori et al., 2018).

4.3.1 Cultivation technique

Cultivation techniques including organic and conventional farming have been demonstrated to impact the nutritional quality of berries as recently reviewed (Di Vittori et al., 2018). In particular, Conti et al. (2014) showed that organically grown strawberries possessed higher levels of sucrose, vitamin C, β-carotene, malic acid, and mineral elements than conventionally cultivated strawberries (Conti et al., 2014). These results were in agreement with those reported by Olsson et al. (2006) where total phenolics, ellagic acid, and flavonol contents were significantly higher in organic strawberries compared to conventional strawberries (Olsson et al., 2006). Furthermore, Ochmian et al. (2015) reported similar results on the effects of organic farming on the nutritional quality of blueberries; in this study, the cultivars from organic plantations were characterized by a higher content of phenolic compounds. In particular, the level of delphinidin-3-galactoside was higher in organic berries compared to their conventional counterparts (Ochmian et al., 2015). In another study, Ponder and Hallmann (2019) reported higher phenolic acids and flavonoids, including myricetin, quercetin, luteolin, and quercetin-3-O-rutinoside in organic raspberries than conventional ones (Ponder & Hallmann, 2019). These results appear to support the higher levels of phytochemicals produced by wild berries compared to cultivated berries. This is related to the increased content of protective phytochemicals accumulated in berry fruits when cultivated under the harsher conditions posed by organic farming. For example, Veberic et al. (2015) reported higher levels of anthocyanins in wild compared to cultivated raspberries, blackberries, and strawberries (Veberic et al., 2015). However, contrasting results on the effects of organic farming on accumulation of bioactive compounds in berries have been reported. In particular, Häkkinen and Törrönen

(2000) reported no significant enhancement of bioactive compounds and antioxidant levels between organic and conventional strawberries (Häkkinen & Törrönen, 2000), as was also reported by Ochmian et al. (2015) where lower sugar and organic acids contents were found in organic blueberries and strawberries compared to conventional fruits (Ochmian et al., 2015). These results contrasted with the decreased phenolic content in strawberries following foliar application of titanium dioxide solution (Choi et al., 2015).

4.3.2 Plant nutrition and fertilization

Concerning bioactive compounds in berries, there is ample evidence that support the significant role of fertilization schemes in modulating the nutritional composition of berry crops. For instance, with regard to fertilization schemes, application of nitrogen fertilization decreased total anthocyanin and sugar contents in cranberry (Jamaly et al., 2021). This was in agreement with the results obtained by Cocco et al. (2021) who showed that nitrogen fertilization decreased the anthocyanin content, organic acid content, and sugar content of grapes (Cocco et al., 2021). Similarly, Rupp and Tränkle (2000) reported decreased amount of vitamin C and sugars in redcurrant following excess nitrogen fertilization (Rupp & Tränkle, 2000), while nitrogen, phosphorus, and potassium fertilizations did not produce significant changes in sugar, organic acids, nitrogen, magnesium, and zinc levels in blueberries (Milošević et al., 2018). However, contradictory results exist regarding the effect of fertilization regimes on berry nutritional quality. For instance, Abd El-Razek et al. (2011) reported an increase in sugar content in grapes following potassium fertilization (Abd El-Razek et al., 2011). Similarly, fertilization with phosphorus, magnesium, and potassium caused an accumulation of sugars and anthocyanins in grapes (El-Badawy, 2019; Topalović et al., 2011). Furthermore, Castellanos-Morales et al. (2010) reported an accumulation of phenolic compounds in strawberries following nitrogen fertilization (Castellanos-Morales et al., 2010). Regarding plant nutrition, it was reported that iron deficiency produced a significant increase in total phenolic and total anthocyanin contents of strawberries (Valentinuzzi et al., 2015). Additionally, Pestana et al. (2012) showed that zinc and copper were accumulated in strawberries cultivated under iron deficient conditions (Pestana et al., 2012), whereas Valentinuzzi et al. (2015) showed that potassium deficiency resulted in increased accumulation of calcium in fruits (Valentinuzzi et al., 2015).

4.3.3 Mulching

Mulching is an agronomic management practice widely employed in berry cultivation to preserve soil moisture, maintain soil temperature, provide nutrients, and control weed growth (Di Vittori et al., 2018; Hegazi, 2000; Burkhard et al., 2009). In addition to these benefits, mulching is able to impact the accumulation of bioactive compounds in berries and ultimately the nutritional composition of fruits (Daugaard, 2008; Kumar et al., 2012; Strik & Davis, 2021). For instance, when investigating the effect of production systems on strawberry quality, Fan et al. (2011) demonstrated that plastic mulch with row covers improved postharvest nutritional value by increasing total phenolic and antioxidant levels (Fan et al., 2011). Other studies have demonstrated that the application of black polythene mulching alone (Bakshi et al., 2014; Singh et al., 2007) and in combination with *azotobacter* bio-fertilization improved total sugar, ascorbic acid, and protein content in strawberries (Tripathi et al., 2017). Moor et al. (2004) reported similar increase in vitamin C content with plastic and straw mulch (Moor et al., 2004), while Wang et al. (1998) demonstrated accumulation of ascorbic acid, ellagic acid, and carbohydrates in strawberry plants grown using black polyethylene, red polyethylene and straw-vetch mulches (Wang et al., 1998). The application of mulching to improve berry nutritional quality has not been limited to only strawberries. Todic et al. (2008) showed that application of reflective plastic foils increased total phenolic and anthocyanin content of Cabernet Sauvignon (*Vitis vinifera* L.) grapes (Todic et al., 2008). Furthermore, in maritime growing environments subjected to low light conditions, placement of reflective mulches alongside blueberry plants enhanced the total anthocyanin content and antioxidant activity of fruits (Petridis et al., 2021). Similar results were reported by Magee and Spiers (1996) who showed that mulching preserved the sugar and anthocyanin content of blueberries (Magee & Spiers, 1996).

4.3.4 Water and salt stress

Water stress is common abiotic constraint on fruit biochemical quality (Mirás-Avalos & Intrigliolo, 2017). It is generally accepted that water stress resulting from severe water shortages or prolong drought can be detrimental to the growth and development of plant species including berries. In contrast, some reports exist in the literature which appear to suggest an accumulation of phytochemicals when plants are subjected to water stresses. For instance, the sugar content, phenolic content, and antioxidant capacity of strawberry increased following mild water stress occasioned by deficit irrigation (Terry et al., 2007; Valentinuzzi et al., 2015). The beneficial effects of mild water stress on grapevine bioactive composition were reaffirmed by other reports including increased total soluble solids (Dos Santos et al., 2003, Degaris et al., 2015), and greater accumulation of phenolics, anthocyanins, and resveratrol (Caruso et al., 2022; Conesa et al., 2016). In a related study, Lobos et al. (2016) showed that applying mild water stress corresponding to 25% less water to highbush blueberries did not reduce fruit quality or levels of antioxidants (Lobos et al., 2016). In a recent review of the effects of water stress on grape composition (Mirás-Avalos & Intrigliolo, 2017), it was suggested that grapevine responded to drought by modulating several secondary metabolic pathways involved in phenyl propanoid, isoprenoid, carotenoid, amino acid, and fatty acid metabolism which may account for the accumulation of phenolic and antioxidant compounds (Deluc et al., 2009; Savoi et al., 2016). As alluded to previously, water deficit does not always result in enhancement of bioactive composition in berries as seen especially under severe water stress conditions. Usually, higher levels of water stress are reported to reduce anthocyanin and sugar contents in berries (Mirás-Avalos & Intrigliolo, 2017). However, these responses depend on other factors such as crop load, vineyard age, fertilization, soil type, berry maturation stage at harvest, and canopy development, among others. For instance, total phenolic content, total anthocyanin content, and sugar content decreased linearly in grapevines with increasing water stress (Girona et al., 2009).

Salt stress may result from prolonged drought conditions as well as from the use of recycled water for irrigation purposes (Hamilton et al., 2007; Mirás-Avalos & Intrigliolo, 2017; Mosse et al., 2011). Generally, minimum soil salinity can induce a stress response mechanism that promotes the production and accumulation of phytochemicals, in particular polyphenols, as reported under water stress conditions (Walker et al., 2000). For instance, increased antioxidant capacity, antioxidants pools (ascorbic acid, anthocyanins, superoxide dismutase) and minerals including sodium, potassium, phosphrus, and zinc were reported in strawberry under long term sodium chloride stress conditions (Keutgen & Pawelzik, 2008b). Similarly, salt stress caused an accumulation of sugars and organic acids in Chilean strawberry (*F. chiloensis*) (Garriga et al., 2015). Other studies have shown that the effect of salt stress may be cultivar dependent. For instance, moderate salinity resulted in an increase in antioxidant capacity, phenolic content, and anthocyanin content of strawberry but the increase was higher in cv. Elsanta cultivar compared to cv. Korona (Keutgen & Pawelzik, 2007). However, the ascorbic acid content decreased in both cultivars under the salt stress conditions investigated. Similar results were reported by Crizel et al. (2020) who showed that salt stress induced by increased soil sodium chloride content triggered the production of proline, pelargonidin-3-O-glucoside, ascorbic acid, and phenolics in strawberries (Crizel et al., 2020). These results were in good agreement with those reported in other studies for strawberry (Cardeñosa et al., 2015; Garriga et al., 2015) and raspberry (Neocleous & Vasilakakis, 2008). As with water deficits, extreme salt stress can lead to reduction in the accumulation of bioactive compounds in berries. This was demonstrated in the reduction of ascorbic acid in strawberry under extreme and prolong salt stresses (Celikli et al., 2017; Keutgen & Pawelzik, 2007).

4.3.5 Soil culture systems

Soil management systems including raised bed, hill plasticulture, and closed soilless systems such hydroponics have been immensely profitable to crop production owing to the flexibility in nutrient management these systems provide (Poling, 2016; Wang, Zheng, & Galletta, 2002). Raised bed and hill plasticulture systems have advantages of enhanced weed control, advanced harvest, increased yield and fruit size, prevention of bed erosion, and increased fruit cleanliness (Baumann et al., 1995; Himelrick, 1982). In addition to these benefits, application of soil cultural systems for berry cultivation can impact the postharvest nutritional composition of berry fruits. For example, in a study to investigate the effect of different cultural systems on strawberry quality, Wang and Millner (2009) reported that berries grown in a compost sock system had significantly higher total sugar (fructose, glucose, and sucrose), total organic acid (malic acid, citric acid) and total flavonoid content (Wang & Millner, 2009). In a related study by the same group, Wang et al. (2002a, 2002b) showed that cultural systems and genotype significantly affected strawberry fruit quality (Wang, Zheng, & Galletta, 2002). The authors reported that strawberries grown using hill plasticulture system had higher total sugar content, organic acid content, flavonoid content, and antioxidant capacities than fruits grown in a matted row system. Similar results were reported by Hakala et al. (2003) who showed that the soluble solids, total sugars, ascorbic acid, and titratable acidity was increased in strawberries grown under hill plasticulture system compared to matted row cultivation system (Akhatou et al., 2016).

Soilless cultivation systems have been reported to produce higher quality berry fruits as this system excludes soil-borne disease pathogens (Asaduzzaman & Asao, 2020). For instance, Akhatou and Fernández Recamales (2014) reported that strawberries grown under soilless conditions showed higher sugar and organic acid content compared to conventionally grown fruits (Akhatou & Fernández Recamales, 2014). In another study to produce low potassium containing strawberry fruits for consumption owing to the association of high potassium consumption and increased risks of chronic kidney disease, Mondal et al. (2017) cultivated strawberries using potassium nitrate (KNO_3) fertilizer in nutrient solution from anthesis to the harvest period (Mondal et al., 2017). The authors reported that fruits exhibited a potassium reduction of about 64% when plants were grown in nutrient solution with KNO_3 at 1/16 of the normal level. Furthermore, citric acid and ascorbic acid contents of the fruits were reduced with decreasing KNO_3 concentrations in the nutrient solution. These results were supported by those reported by Rhee et al. (2006) who demonstrated that hydroponically produced strawberry contained higher soluble solids (sugars) and organic acid content compared to conventionally grown berries (Rhee et al., 2006). However, it has been pointed out that the practice of recycling hydroponic solutions to make hydroponic systems cost-effective, sustainable, and environmentally friendly can lead to an accumulation of plant root exudates including phenolic acids which could inhibit plant growth and development when allowed to accumulate (Asaduzzaman & Asao, 2020; Kitazawa et al., 2005). Several methods have been developed to reduce autotoxicity arising from the build-up of allelopathic phenolics acids in hydroponic systems including activated charcoal, electrodegradation, and microbial strain (Asao et al., 1999, 2004, 2008). For instance, Asao et al. (2008) demonstrated that electrodegradation treatment at 10.0 and 2.0 V to a benzoic acid nutrient solution could mitigate the autotoxicity of strawberry plants grown by closed hydroponic culture (Asao et al., 2008).

5. Postharvest technological processing factors affecting berry nutritional quality

From the moment that berries are harvested, the fruits no longer receive nutrients from the mother plant, provoking changes at structural, chemical, nutritional and biochemical level, until it is no longer edible. Furthermore, berries are susceptible to microbial contamination and mechanical damage, which results in a short life span and loss of commercial value during postharvest storage (Zhou et al., 2019). To prolong the shelf life of fresh berries, several approaches have been developed to reduce postharvest decay. However, these approaches can impact the nutritional quality of fresh and processed berries. These approaches include fresh pack processing, technological processing (juicing, pureeing, canning, winning, drying), and domestically processed products including jams and jellies (Horvitz, 2017; Kårlund et al., 2014). The next sections review the recent literature on the effect of processing approaches on the nutritional quality of berries.

5.1 Fresh pack processing

The commonly used technologies used for fresh packaging of whole berries include low-temperature storage (Ambaw et al., 2016), edible coatings (Mannozzi et al., 2017), irradiation (Horvitz, 2017; Zhao et al., 2020), 1-methylcyclopropane fumigation (Wang et al., 2019), atmospheric cold plasma (Dong & Yang, 2019; Hu et al., 2021), controlled atmosphere storage, and modified atmosphere processing (Pinto et al., 2020; Saito et al., 2020). Here, the effect of some of these technologies on berry nutritional quality will be reviewed.

5.1.1 Low-temperature processing

Short-term low-temperature postharvest storage strongly influences nutritional quality and phytochemical profiles of berries, and storage temperature is one of the key factors affecting the stability of phenolic and vitamin antioxidants in fruits, during postharvest storage (Kumar et al., 2018; Rodionova et al., 2021). Short-term storage of berry fruits under refrigerated conditions (temperature of $-1°C$ to $+2°C$) allows preservation of their quality by slowing down the natural metabolic processes—respiration and transpiration (Dziedzic et al., 2020). Moreover, low temperatures could be expected to stabilize the antioxidants present in fruits, such as phenolic compounds and vitamin C. The literature is replete with several applications of low temperature either alone or in combination with other fresh pack technologies to reduce spoilage and extend berry shelf lives (Handojo et al., 2022; Rosenbloom et al., 2020). For example, one study found that the most evident effect of low-temperature storage (storage for 2 days at 4°C and 1 day at room temperature, in the dark) on strawberry cultivars was on folate content. The authors reported that the levels of folate increased in all the cultivars studied when stored for 2 days at 4°C (Tulipani et al., 2009). Other authors also reported that the total flavonoid concentration of strawberries was higher in fruits after short-term low-temperature storage compared to fruits stored at room temperature, which appears to support cold temperature as a suitable technique to preserve the nutritional quality of berries (Gil et al., 2006; Olsson et al., 2004; Tulipani et al., 2009). Significantly, in all these reports, it was observed that the total phenolic, anthocyanin content, and vitamin C content were not affected by cold temperature storage, which suggests the successful retention of these bioactives during low-temperature storage. The increase in the levels of health-promoting folate and flavonoids in the berries could be due to the fact that during short-term low-temperature storage, there is an increase in the content of early eluting polar phenolic antioxidants as fruit cell walls disintegrates as a function of time (Tulipani et al., 2009). This appears to be the case underlying the increase in total phenolic and total anthocyanin contents of strawberries subjected to extended low-temperature storage conditions. It was also suggested that the elution of phenolic compounds increased if storage temperature and period were extended. For instance, Kalt et al. (1999a, 1999b) investigated the composition of strawberries stored at 0°, 10°C, 20°C, and 30°C for up to 8 days (Kalt et al., 1999a). The authors found that anthocyanin content increased an average of 4.3-fold after 8 days, and the magnitude of increase was related to temperature. When strawberries were stored at 0°C for 8 days, anthocyanins increased 1.7-fold, while for the same period at 30°C, the apparent increase was 6.8-fold. Other authors obtained similar results with higher levels of total phenolic and anthocyanin content present in fruits stored at 5°C compared to those stores at 0°C over a 7 days of period (Ayala-Zavala et al., 2004; Jin et al., 2011). Short-term low-temperature storage positively affects the antioxidant capacity of berry fruits, since the complex reactions taking place within the fruits during postharvest storage period may facilitate formation of compounds with enhanced antioxidant capacity, even when fruit attributes such as taste and smell have deteriorated (Piljac Zegarac & Samec, 2013). Similar results have been reported concerning the effects of short-term low-temperature on berry nutritional quality In blueberries, for instance, Kalt et al. (1999a, 1999b) reported that *V. corymbosum* L. cultivar Bluecrop demonstrated a 1.2-fold increase in anthocyanin content over an 8-day period when stored at 20°C, which was accompanied by a 1.2-fold increase in the oxygen radical antioxidant capacity (ORAC) (Kalt et al., 1999a). Storage for the same period at 0°C or 10°C did not result in significant changes in anthocyanins, vitamin C, total phenolics, or ORAC antioxidant activity in this cultivar. However, the authors noted a 27% loss in vitamin C in berries stored at 20 and 30°C. Similarly, Kalt and Mcdonald (1996) also reported 18% increase in anthocyanin content in three lowbush (*V. angustifolium* Ait.) blueberry cultivars when stored at 1°C for 2 weeks (Kalt & Mcdonald, 1996). These results appear to corroborate those obtained by Connor et al. (2002a, 2002b) where the total antioxidant activity, total phenolic content, and anthocyanin content were determined for blueberry (Vaccinium L. sp.) cultivars stored at various time periods at 5°C (Connor, Luby, Hancock, et al., 2002). The authors showed that only the cultivar-MSU-58 demonstrated a 29% increase in antioxidant activity. Furthermore, no significant decrease in antioxidant activity, total phenolic content, and anthocyanin content of the other berry cultivars were observed during cold temperature storage. Thus, it appears short terms low-temperature storage (>0°C but less than room temperature) positively affected phenolic metabolism in berries, thus enhancing, in most cases, phenolic content and total antioxidant activity of berries.

Freezing is a beneficial method to store berries and maintain quality before processing into juice and allows production to continue after harvest seasons are over. How long berries are kept in freezing temperatures affects the quality of the berry and the subsequent berry juices (Mcanalley et al., 2003). Freezing is an important storage method used to retain fruit quality during long-term storage and enables the year round processing of seasonal fruit products. A storage temperature of −18°C is typically used to reduce the chemical and biological spoilage of fruits and to extend their shelf life. However, freezing causes cell breakage, allowing enzymatic reactions to occur (Bonat Celli et al., 2016). Therefore, anthocyanins and other phenolic compounds can degrade during freezing and more extensively during thawing, due to their interaction with oxidative enzymes. However, the effects of cold temperature may depend among other factors on the freezing rate and genotype of berry under investigation (Bonat Celli et al., 2016). For example, the effect of freezing rate on vitamin C content was assessed in Iranian strawberries (var."Kordestan"). Two conditions were evaluated: slow freezing at −20°C for 24 h (in a domestic freezer) and quick freezing in liquid nitrogen at −50 to −100°C for 11 min (Sahari et al., 2004). The authors also evaluated the following storage conditions: at −12°C, −18°C, and −24°C in a domestic freezer for 3 months. The authors noted major losses of vitamin C during the first 15 days of storage and the percentages were 64.5%, 10.7% and 8.9% at −12°C, −18°C and −24°C, respectively. However, no significant differences were observed between the −18°C and −24°C freezing methods in vitamin C losses. In a related study involving blueberries, there was a 59% reduction in total anthocyanin content when fruits were subjected to cold temperature storage (Celli et al., 2011). In red raspberries frozen at −20°C for 1 year, an 18% decrease in anthocyanins was also observed (De Ancos et al., 2000). The decrease in anthocyanin and antioxidant compounds were attributed to the fact that while anthocyanin degrading endogenous enzymes found in berries are slowed down by freezing temperatures, enzymatic activities are not completely stopped (Bello & Sule, 2012; Chisari et al., 2007). Thus, prolonged cold temperature tended to lead to slow degradation of these bioactive compounds compared with short-term cold temperature storage.

In contrast, some reports exist that indicate that freezing may not be detrimental to the nutritional composition of berries. These discrepancies have been attributed to a number of factors including fruit maturity (Skrede, 1996), cultivar differences (Sapers & Philiips, 1985), and improved extraction following freezing-thawing cycles (Ścibisz & Mitek, 2007). For instance, freezing temperatures have been proven to have minimal effect on anthocyanins in red and black raspberries over cold storage period of 6 months (De Ancos et al., 2000; Hager et al., 2008). In another study done by De Ancos et al. (2000), ellagic acid, total phenolic, and vitamin C contents were quantified by HPLC in fresh, just frozen, and frozen stored (−20°C for 1 year) raspberry fruits. The results showed that after 12 months, there was no change in total phenolic content, whereas a significant decrease in ellagic acid and vitamin C contents was observed (De Ancos et al., 2000). These reports were reaffirmed by other studies which showed that total antioxidant capacity of blueberries was preserved during 6-month cold storage even though anthocyanin content decreased during this period (Brownmiller et al., 2008; Reque et al., 2014).

Furthermore, no changes in anthocyanin content and antioxidant activity of blueberries were observed following storage for 3 months at −20°C (Lohachoompol et al., 2004). These results were confirmed by Ścibisz and Mitek (2007) using highbush blueberries stored at −18°C and −35°C for 6 months (Ścibisz & Mitek, 2007).

5.1.2 Controlled atmosphere (CA) and modified atmosphere (MA) storage

Controlled or modified atmosphere storage can be used as a complement to proper temperature and relative humidity management to extend the shelf lives of berries (Kitinoja & Kader, 2002). Beneficial effecs of controlled atmosphere and modified atmosphere atmosphere storage on berry quality rely on their fungistatic properties, whereas inappropriate gas concentrations during fruit storage trigger physiological damage (Ehlenfeldt & Martin, 2002). Controlled atmosphere (CA) storage refers to the maintenance of continuously controlled gas atmosphere during storage whereas modified atmosphere (MA) storage refers to a gas composition that is initially modified at the start of storage period. Significantly, the gas composition within a modified atmosphere storage unit will change over time due to the respiration rate of fruits and the permeability of the packaging (if any) surrounding the fruit products (Jayas & Jeyamkondan, 2002). The widely used gas composition used for storage under controlled or modified atmospheres consists of oxygen (O_2) and carbon dioxide (CO_2). The primary benefit of lowering O_2 and increasing CO_2 surrounding the produce is the effect it has on lowering the respiration/metabolic rate of the fruits, leading to slowing down of natural senescence processes during storage (Dumont et al., 2016). On the other hand, it has been shown that increased CO_2 concentration in the storage atmosphere can also be a factor inducing abiotic stress in berry fruits, while increasing in the concentration of phenolic compounds (Khorshidi et al., 2011). However, internal gas concentrations in berries are affected by temperature, which modifies skin resistance, gas solubility and metabolic rates (Beaudry et al., 1992). These internal changes could influence composition of bioactive compounds leading to changes in nutritional quality of berries. The literature is replete with several applications of controlled atmosphere storage to preserve the nutritional quality of berries which is reviewed further.

For example, in one study, the effects of CA storage on the total phenolics content, flavonoids content, and total antioxidant activities of Pilgrim and Stevens cranberry cultivars (*Vaccinium macrocarpon* Aiton) were studied during storage in atmospheres of 2%, 21%, and 70% O_2 with 0%, 15%, and 30% CO_2 (balance N_2); and 100% N_2 at 3°C (Gunes et al., 2002). Here, it was demonstrated that the controlled storage atmospheres did not significantly affect the content of total phenolics or flavonoids during the sampling period, which suggest a retention of these bioactive compounds during storage. In another study by Schotsmans et al. (2007), two rabbiteye blueberry cultivars ("Centurion" and "Maru") were stored at 1.5°C in either regular air or controlled atmosphere (2.5 kPa O_2 + 15 kPa CO_2) for up to 6 weeks to assess the effect of low-temperature CA storage on berry nutritional quality (Schotsmans et al., 2007). The authors reported that "Centurion" fruits had higher antioxidant activities and total phenolics content than those from "Maru" fruit at harvest and after storage in controlled atmosphere than regular air, which reaffirmed the utility of CA for preservation of the nutritional quality of berries when combined with low-temperature storage. Another report of the beneficial effect of CA storage was shown for Muscadine grapes (*Vitis rotundifolia* Michx) (Shahkoomahally et al., 2021). Here, fruits of two muscadine grape cultivars were stored at 4°C with 95% relative humidity in either regular air (AIR), regular CA (RCA; 6% O_2 + 10% CO_2), or CA with extreme CO_2 level (ECA; 4% O_2 + 30% CO_2) for up to 42 days. In particular, the authors reported application of regular and extreme controlled atmospheres (RCA and ECA) for storage led to higher total antioxidant activities and total phenolic contents in both grape cultivars compared to storage in regular air (AIR). These results demonstrated that maintaining CA storage conditions during postharvest storage led to improved preservation of nutritional quality of harvested muscadine grapes compared with regaular air storage. Other reports of the beneficial effects of CA storage on maintaining grape nutritional quality have been reported (Basiouny, 1996; Himelrick, 2003; Mercer & Smittle, 1990; Romero et al., 2019; Sanchez-Ballesta et al., 2006, 2020).

However, there exist contrasting reports in the literature that appear to suggest that controlled atmosphere (CA) storage could have detrimental effects on berry nutritional quality. Nonetheless, it is important to point out that the effects of controlled atmosphere storage depend on the berry cultivar, temperature, and gas mixture, which may differ between reported studies. This was particularly the case during storage under controlled ozone conditions. For example, in one study using controlled atmosphere by Tabakoglu and Karaca (2018), changes in quality parameters of mulberries were investigated during storage in rooms with ambient air and ozone atmospheres (0.64 and 5.14 mgm^{-3}) for 6 days at 2°C and 95% relative humidity (Tabakoglu & Karaca, 2018). The authors noted that while there was an overall decrease in vitamin C and anthocyanin content over the storage period for all berry samples, the losses observed for samples stored in controlled atmospheres were significantly lower than those stored in air. A similar decrease in vitamin C content was observed in strawberries following storage in ozone atmosphere (Pérez et al., 1999). However, anthocyanins in other berry fruits including blackberry (Barth et al., 1995) and strawberry (Keutgen & Pawelzik, 2008a) were not adversely affected by ozone treatments. In another study by Perkins Veazie and Collins (2002), "Navaho" and "Arapaho" blackberries (*Rubus* sp.) were stored at 2°C in ambient atmosphere (0.03 kPa CO_2, 21 kPa O_2) and in controlled atmosphere (15 kPa CO_2, 10 kPa O_2) with the following treatments: 3 days of controlled atmosphere storage plus 11 days in air; 7 days of controlled atmosphere storage plus 7 days with air; 14 days of constant controlled atmosphere storage (Perkins Veazie & Collins, 2002). The authors noted that both blackberry cultivars had less decay when stored for 7 days under the controlled atmosphere conditions with no adverse on flavour, compared with fruits stored in ambient atmosphere. Furthermore, the anthocyanin content decreased by 20% after 7 days controlled atmosphere storage but the loss was lower in controlled atmosphere compared with ambient atmosphere storage for both blackberry cultivars. Similar results were reported following storage of strawberries in carbon dioxide enriched controlled atmospheres at 5°C (Holcroft and Kader, 1999a, 1999b).

Concerning the application of modified atmosphere (MA) storage for preservation berries, Dziedzi et al., 2020 reported that modified atmosphere packaging (MAP) using Xtend bags preserved bioactive compounds in honeysuckle berries (Dziedzic et al., 2020). Here, the authors noted that short-term storage of blue honeysuckle berries in Xtend bags containing a modified atmosphere composed of 20% carbon dioxide and 5% oxygen preserved the total anthocyanins content and antioxidant activity of fruits compared with fruits maintained under ambient atmosphere. Furthermore, the authors noted that higher oxygen concentrations ($\sim$21% CO_2) in normal air packaging compared to modified atmosphere packaging (MAP) had a deteriorating effect on phenolic compounds including anthocyanins present in the blue honey suckle berries. These results confirmed the advantages of modified atmosphere packaging over ambient atmosphere packaging for preserving the postharvest nutritional quality of berries as demonstrated for retaining phenolic compounds in strawberry (Oliveira et al., 2015) and blueberry (Koort et al., 2018). In another study by Pinto et al. (2020) to evaluate the combined effect of modified atmosphere packaging (MAP) and a gaseous ozone pretreatment on the physico-chemical parameters of strawberries, raspberries, and blueberries during cold storage (Pinto et al., 2020), fruits were exposed to 13 mg m^{-3} of gaseous ozone (about 6 ppm) for 16 h at 1°C (±0.5°C) and then stored for 15 days under modified atmosphere conditions (10 kPa O_2 and 40 kPa CO_2) or in air at 4°C. The authors showed that compared to storage in air, the combined effects of gaseous ozone pretreatment and modified atmosphere packaging (MAP) led to a pretention of anthocyanins in all three berries during cold temperature storage with blueberries showing the highest increase in anthocyanin content during the storage period. Taken together, these reports were in agreement with those obtained when blackberries were refrigerated at 5°C in ambient air compared with refrigerated storage in oxygen and carbon dioxide enriched atmospheres for 15 days (Van De Velde et al., 2020). Here, the authors demonstrated that refrigerated storage of blackberries in modified atmosphere packaging (70 kPa O_2 + 20 kPa CO_2) provided afforded greater retention of vitamin C and phenolic compounds in blackberries with concomitant increase in antioxidant capacity compared to berries stored in ambient air atmosphere. Additional data in support of the benefits of modified atmosphere (MA) storage were provided by Krupa and Tomala (2007) and Zhang et al. (2003) who observed a lower content of phenolics and anthocyanins in blueberry, blackberry, and strawberry stored in an ambient air atmosphere compared to fruits stored in modified atmospheres (Krupa & Tomala, 2007; Zhang et al., 2003).

It is important to recognize that underling preservation of bioactive compounds during controlled atmosphere (CA) and modified atmosphere/modified atmosphere packaging (MA/MAP) storage are reports which indicate that after harvest, biochemical changes still occur in the fruits including synthesis of phenolic compounds such as anthocyanins (Horvitz, 2017). In addition, postharvest changes in berry storage conditions, including low temperature and gas compositions, cause abiotic stress, as a result of which berry fruits may respond by accumulating secondary metabolites including phenolic compounds (Cisneros-Zevallos, 2003; Senica et al., 2018). Furthermore, it is also well known that the postharvest phenolic content of berry fruits are strongly affected by the composition of the storage atmosphere, in particular the carbon dioxide (CO_2) concentration in the atmospheres in which the fruits are stored. In this regard, hight carbon dioxide (CO_2) and low oxygen (O_2) enriched atmospheres are known to enhance the accumulation of phenolic compounds in berry fruits (Khorshidi et al., 2011). On the one hand, it has been noted that higher carbon dioxide (CO_2) levels may be detrimental for preserving the postharvest berry nutritional quality. For instance, storage of 'Duke' blueberries under 18 kPa CO_2, coupled with 3 kPa O_2 resulted in accelerated softening og berries and lower levels of anthocyanins (Harb et al., 2014; Rahman, 2000). The decrease was attributed to supression of key enzymes including chalcone synthase (*VcCHS*) invovled in polyphenol biosynthesis, which are severely affected by storage in a high carbon dioxide atmosphere (18 kPa CO_2). Notwithstanding, there appears to be overwhelming evidence in the literature in support of high CO_2 coupled with low O_2 concentrations in controlled or modified atmospheres for storage of berries and retention or accumulation of health-promoting bioactives including phenolic compounds.

5.1.3 Plasma processing

Over the past decade, plasma processing techniques including cold plasma (CP) or atmospheric plasma (AP) have gained significant interest for berry storage applications as a non-thermal technology for fresh packaging of berries (Pankaj et al., 2018). The application of ionizing gas, referred to as plasma jets, in cold plasma or atmospheric plasma for food processing has been demonstrated to be effective for food decontamination (Misra et al., 2011), enzyme inactivation (Misra et al., 2016), and toxin removal from diferrent food matrices including berries (Misra, 2015). In particular, when used in complementary to fresh pack storage techniques, plasma processing has been shown to be effective against major food-borne pathogenic microorganisms such as *Escherichia coli* (Bermúdez-Aguirre et al., 2013), *Salmonella typhimurium* (Fernandez et al., 2013), *Staphylococcus aureus* (Kim et al., 2014), and *Listeria monocytogenes* (Grunert, 2005). The literature is replete with applications of plasma processing techniques for preserving berry postharvest nutritional quality. For instance, in a study involving the use of plasma-activated water (PAW), Cong et al. (2022) demonstrated that PAW treated goji berries possessed higher total phenolic content, flavonoid content, vitamin C content, and β-carotene content compared with controls treated with distilled water. Thus, PAW treatment of goji berries was effective for maintaining higher quality attributes and nutritive properties of fruits compared with the controls which were treated with only distilled water (Cong et al., 2022). A similar result was reported by Li et al. (2019) for strawberries subjected to dielectric barrier discharge (DBD) cold plasma treatment where the total phenolic content, flavonoid content, and antioxidant activity of fruits increased following 1 min of plasma treatment and 1-week cold storage at 4°C compared to untreated control fruits (Li et al., 2019). In another example using dielectric barrier discharge (DBD) for cold plasma generation, blueberries were subjected to cold plasma treatment for 0−5 min by Sarangapani et al. (2017). The authors reported that total phenolics and flavonoids contents of blueberries increased significantly following treatment for 1 min for all applied voltages of cold plasma (Sarangapani et al., 2017). However, despite the benefits plasma processing techniques confer on postharvest nutritional quality and shelf lives of berries, the ionizing nature of plasma can potentially degrade bioactive compounds, especially when applied at high voltages and for extended periods thereby compromising the postharvest nutritional quality of berries. For instance, in the same study by Sarangapani et al. (2017), the authors noted that DBD cold plasma treatment at higher voltages and longer exposure times significantly decreased the total phenolic, total flavonoid, ascorbic acid, and anthocyanin contents of the blueberries. These results were in good agreement with those obtained in a study by Lacombe et al. (2015) to assess the potential use of cold plasma in food processing. Here blueberries were treated with atmospheric cold plasma for 0, 15, 30, 45, 60, 90, or 120 s at a working distance of 7.5 cm with a mixture of four cubic feet/minute (cfm) of cold plasma jet and 7 cfm of ambient air (Lacombe et al., 2015). The authors observed significant reduction in anthocyanin content of the berries after 90 s of cold plasma treatment even though significant suppression of microbial growth occurred after only 30s. Taken together, these results along with other reports in the literature suggest that non-thermal cold plasma processing is beneficial for preserving the nutritional quality of berries, but the exposure time has to be carefully monitored to ensure bioactive compounds are not destroyed by ionizing gases (Bovi et al., 2019; Ji et al., 2020; Pan et al., 2019; Park et al., 2021; Zhou et al., 2020).

5.1.4 Fumigation

Ethylene is one of several plant growth regulators that affects growth and developmental processes including ripening and senescence (Iqbal et al., 2017). It is a simple hydrocarbon that can diffuse into and out of plant tissues from both endogenous and exogenous (non-biological and biological) sources (Sholberg, 2009) and has been the subject of extensive research on its biosynthesis and actions (Ma et al., 2021; Wang, Dai, et al., 2021; Yamazaki et al., 2021). Ethylene exposure can profoundly affect quality of harvested berry fruits. These effects can be beneficial or deleterious depending on the fruit product, its ripening stage, and its desired use (Yamazaki et al., 2021). Endogenous ethylene production is an essential part of ripening of climacteric fruits and probably acts as rheostat for ethylene-dependent processes (Watkins, 2006). Fumigants are thought to interact with ethylene receptors and thereby to prevent ethylene-dependent responses (Sholberg, 2009; Sisler & Serek, 2003). During fumigation, berry fruits are subjected to gas atmospheres that supress endogenous production of ethylene. Typically, such ethylene supressing atmospheres are produced by fumigants including sulfur dioxide, ozone, 1-methylenecyclopropene (1-MCP), volatile acetic acid, acetaldehyde, ally-Isothiocyanates, chlorine, chlorine dioxide, hexane, 2-trans-hexenal, nitrous oxide, and thymol (Sholberg, 2009). However, modifications to internal gas composition of fruits may influence the nutritional quality of berries by modifying the activity of enzymes associated with phenolic compound synthesis. For example, in one study, Aleatico grapes (*Vitis vinifera* L. cv. Aleatico) were treated with air containing 500 mg/L ethylene for 15 h, or 1 mg/L 1-MCP (1-methylcyclopropene) for 15 h, or 1 mg/L 1-MCP for 15 h + 500 mg/L ethylene for further 15 h, or air (control), to investigate the effects of postharvest ethylene and 1-MCP treatments on the nutritional and sensory quality of the grapes (Bellincontro et al., 2006). TPC was enhanced by ethylene treatment from 520 mg/L up to 710, while total anthocyanin content was lost in air-treated samples (from 175 mg/L to 120) and remained constant in ethylene and 1-MCP-treated grapes. In another study by Xu et al. (2020), the effect of 1-methylcyclopropene (1-MCP) with and without ethylene on postharvest functional components and antioxidant activity of blueberries were evaluated (Xu et al., 2020). The authors found that ethylene treatment accelerated ethylene production and respiration rate, resulting in decreased antioxidant activity and functional component contents of blueberries in comparison with the untreated samples. Significantly, the degradation effect of ethylene was effectively neutralized by 1-MCP. Treatment of 1-MCP with or without ethylene enhanced the phenolic content and antioxidant activity of berries whilst suppressing ethylene production compared with the control. Similar application of fumigation for shelf life extension and retention of functional components including phenolic and antioxidant compounds in berries has been reported for Red Globe and Niagara grapes (Chen, Zhang, et al., 2019; Liu et al., 2022), for Misty and Berkeley blueberry cultivars (Totad et al., 2019a; Xu et al., 2020), for blackberries (Li et al., 2018), for kiwi fruits (Xia et al., 2021), and strawberry (Taş et al., 2021). Other reports demonstrating application of 1-MCP based fumigants to improve self-life and nutritional quality of berries exist for golden berry (*Physalis peruviana*), Xinjiang Munake grape, Red Globe 'Campbell' table grape (Cuaspud Cuaical et al., 2019; Deng et al., 2018; Jia et al., 2020; Kim et al., 2019).

5.1.5 Edible coatings

In recent years, edible films and coatings have been considered as one of the technologies with greater potential to improve microbiological safety of fruits and protect them from the influence of external environmental factors, thus increasing shelf-life of berry fruits (Panahirad et al., 2021). One of the major potentials in the development of coatings/films is the use of materials derived from renewable sources such as hydrocolloids of biological origin, and incorporation of functional ingredients that can be used, for example, as antioxidants and/or antimicrobial agents to improve the final quality of fruit products.

For instance, in a study by Fan et al. (2019) to evaluate the effect of composite coatings with lotus leaf extract (LLE) on the quality of fresh goji fruits during postharvest storage at ambient temperature (Fan et al., 2019), the fruits were coated with LLE incorporated composite coatings. It was found that the LLE treatment effectively maintained ascorbic acid, organic acids, and health-promoting antioxidant enzymes including superoxide dismutase, catalase, peroxidase activities at higher levels in the fruits compared to the controls. Using LLE incorporated coatings also extended the shelf life of the berries for about 4 days. In a related study by Jatoi et al. (2017) to enhance storage life and poststorage quality of fresh goji berries, three treatments with lecithin coatings (1, 5, 10 $g\,L^{-1}$) and two storage times (8, 16 days) were evaluated (Jatoi et al., 2017). The authors demonstrated that the antioxidant activity of berries increased after 16 days of storage, as did the total phenolic content and total flavonoid content when coatings contained lecithin at 1 $g\,L^{-1}$. However, it was noted that higher doses of lecithin (>1 $g\,L^{-1}$) in the coatings were not beneficial for fruit quality. Furthermore, in another study to evaluate the effect chitosan based coatings enriched with procyanidin by-product on quality of fresh blueberries during 14 days of storage at 4°C (Mannozzi et al., 2018), the authors reported that chitosan coating and chitosan-procyanidins based coatings increased the anti-radical activity of the berries with the highest values obtained for berries coated with chitosan - procyanidins films. In another study, flame seedless grapes (*Vitis vinifera* L.) cv. were dipped in different concentrations of spermine (0.0—control, 0.5, 1.0, and 1.5 mmol/L) for 5 min, and thereafter stored in a cold room (3−4°C, 90%−95% RH) for 30, 45, 60−75 days (Harindra Champa et al., 2015). Here, the authors reported that spermine coatings at a dose of 1.0 mmol/L effectively stabilized anthocyanins, suppressed the activity of pectin methyl esterase, and reduced membrane electrolyte leakage in the grapes during storage. Furthermore, treatment of berries with 1.0 mmol/L spermine extended the postharvest shelf life and nutritional quality of grapes for up to 45 days compared with untreated berries. In fact, the literature is replete with several applications of edible coatings to successfully preserve the nutritional quality and shelf lives of berries. This was seen with the use of methylcellulose based edible coatings to preserve the nutritional and sensory quality of "Misty" blueberries (Totad et al., 2019b), strawberries (Nadim et al., 2014), and Jamun fruits (Gol et al., 2015). Similar edible coatings based on chitosan, pectin, pullulan, and alginate proved effective for preseving bioactives in table grapes (Chen, Wu, et al., 2019), raspberries (Guerreiro et al., 2015), strawberries (Li, Sun, et al., 2017), blueberries (Chiabrando & Giacalone, 2015), and blackberries (Tumbarski et al., 2020). However, there are other reports which showed that edible coatings adversely impacted the nutritional quality of blackberries as was shown with starch-beeswax coatings (Pérez-Gallardo et al., 2015) and cassava starch coatings (Oliveira et al., 2014). These reports were collaborated by Vargas et al. (2006) when strawberries were dipped in chitosan-based coatings resulting in decreased total phenolic content (Vargas et al., 2006). A number of factors have been postulated to rationalize these lossess. For instance, it has been reported that edible coatings may cause decreased oxygen atmosphere around fruits leading to reduced anthocyanins synthesis (Romero et al., 2008) which may account for the decrease in total phenolic content reported in other studies. A decrease in available oxygen may also restrict phenolic compounds accumulation (Bodelón et al., 2010). Moreover, carbon dioxide accumulation which results from edible coatings may damage internal tissues by promoting oxidation of phenolic compounds by enzymatic reactions involving polyphenol oxidase and peroxidase as reported by Duan et al. (2011) for blueberries following treatment with edible coatings (Duan et al., 2011). Furthermore, modification of fruit internal atmosphere that accompany treatment of fruits with edible coatings may slow down the biochemical reactions leading to anthocyanins synthesis with concomitant reduction in postharvest anthocyanin levels (Tzoumaki et al., 2009).

5.2 Domestic processing

Berries are generally consumed as fresh fruits, even if many products such as juices and purees are available on the market. However, berries may be domestically processed into jams and jellies. Several studies have shown that such processed berry products possess decreased nutritional quality compared to fresh fruits, and that the degree of reduction is strictly related to production time and processing steps, such as heat treatment (Alvarez-Suarez et al., 2014). For example, Bursać Kovačević et al. (2009) reported that during thermal processing of fresh fruits of strawberry cultivars into jams, the total phenolic content decreased in all jam samples by 45%−63%, flavonoids content by 10%−36% and nonflavonoids by 7%−40% compared to processed fruits (Bursać Kovačević et al., 2009). These results were in overall agreement with those reported by Häkkinen et al. (2000) where the loss was about 15%−20% for flavonoid and 20% for total ellagic contents (Häkkinen et al., 2000). In a related study, strawberry (cv. Senga Sengana) and raspberry (cv. Veten) were processed into jams at 60, 85 or 93°C and stored at 4 or 23°C for 8 and 16 weeks (Martinsen et al., 2020). Here, it was shown that high processing temperatures significantly reduced ascorbic acid, total anthocyanins, and total phenolic contents in strawberries but not in raspberries. This discrepancy may be due to the greater stability of phenolic compounds present in raspberries (Boyles & Wrolstad, 1993). Processing of berries into jams, jellies, purees, and juices mostly involves heating to inactivate microorganisms and endogenous enzymes like polyphenol oxidase which degrade the shelf lives and bioactive compounds in berries (Holzwarth et al., 2012). For instance, thermal treatment degraded total vitamin C, anthocyanin, and total phenolic contents by non-enzymatic reactions during processing of jams from strawberries (Klopotek et al., 2005; Ngo et al., 2007; Patras et al., 2009) and raspberries (García-Viguera et al., 1999; Mazur, Nes, et al., 2014). Furthermore, the loss of bioactive compounds has been found to increase when thermal processing is performed at higher temperatures (Holzwarth et al., 2013). Taken together, it appears that thermal processing of berries to extend shelf life has a negative impact on nutritional quality of the processed products.

6. Berries in cardiovascular diseases (CVD) management and functional food development

CVD are a group of disorders of the heart and blood vessels and include coronary heart disease (CHD), cerebrovascular disease (CBD), rheumatic heart disease and other conditions. CVD are considered the major cause of morbidities and mortalities globally, and it is estimated that CVD are taking nearly 17.9 million lives each year, 32% of all annual deaths in the world. It is further projected that CVD-rendered mortality score may rise to almost 23.6 million deaths annually by the year 2030 (WHO, 2022). Numerous risk factors of CVD have been discussed in literature; however, hyperlipidemia, hypertension, unhealthy diets, physical inactivity, smoking, and alcoholism are the key factors for this combined pathology. Hyperlipidemia is associated with obesity/overweight and it is stated that hyperlipidemia can increase the incidence rate of CVD by 3-times whereas reduction in cholesterol level (by 1%) can reduce the CVD risk by threefold (Huang et al., 2016). Similarly, in a meta-analysis of nine studies showed that gestational hypertension associated with a 67% higher risk of CVD and 75% higher risk of subsequent CVD-related mortality (Virani et al., 2021). Regarding nutrition and diets, diets/meals having high-fat/high-carbohydrate/high-calorie as major constituents (normally >45%) are considered main risk factor for CVD occurrence. On the other hand, American Heart Association (AHA) reported that intake of balanced and calorie-restricted Mediterranean-style diets resulted in almost 30% reduction of stroke, myocardial infarction, in large primary prevention trial among patients with CVD risk factors (Virani et al., 2021).

In light of the above facts, various approaches have been adopted in order to cope with the risk factors of CVD and hence lessen the epidemic of CVD worldwide. Among these, two approaches i.e., pharmacological and nutritional approaches have been largely in practice. However, lately, the former approach has been found to be associated with high economic cost and possible side effects. As such nutritional approaches are gaining attention as both a preventive, and treatment strategies to circumvent the risks of developing CVD. For this purpose, the concept of precision nutrition or individualised nutrition has been introduced recently. Precision nutrition is the provision of individualized dietary and nutritional recommendations to vulnerable group of people (Hameed et al., 2020a). Numerous functional foods and nutraceuticals have been suggested for the individualized nutrition. Diets high in fruits and vegetables including berries are highly recommended as part of an individualized nutrition or diet to reduce and manage CVD. Berries are marketed as superfunctional foods and recent experimental and epidemiological evidence have shown that berries have therapeutic and preventive effects for reducing the risk factors of CVD, as well as cancer and diabetes (Yang et al., 2019; Huntley). Most of these studies used blueberry, bilberry, mulberry, raspberry, cranberry, currants, blackberry, maqui berry, and acai berry in their interventions.

These berries are rich source of bioactive secondary metabolites, vitamins, polyunsaturated fatty acids, folate, fiber, and micronutrients whereas flavonoids, in particular anthocyanin, are the most abundant phenolic classes accounting for their cardiovascular health-promoting properties. In fact, a growing number of studies have shown that these phenolics may prevent CVD through various mechanisms (Table 6.3).

In summary, CVD is multifaceted disease with different stages of development, and which usually starts with sedentary lifestyle and unhealthy diets in addition to genetic background making some group of people more vulnerable. Sedentary lifestyle and unhealthy diets result in disturbance of redox-balance (balance of GSH/GSSG, NAD^+/NADH and NADP/NADPH) in biological system, alterations in lipids metabolism, and progression toward (acute) inflammatory diseases. These conditions may then aggregate toward weight-gain, obesity, insulin insensitivity, hyperglycaemia, hypoinsulinemia, (pre)diabetes, arthrosclerosis, hypertension, stroke, myocardial infarction etc. The disturbance of redox-balance increases oxidative stress which in-turns promoted the production and transport of LDL cholesterol (LDL-C) subjecting to oxidation to generate oxidized LDL (oxLDL). The oxLDL then infiltrates the cells due to disturbed flow dynamics of endothelium which increased gene expression of adhesion molecules (such as intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin) and chemotactic factors. Up-regulation of ICAM-1 and VCAM-1 enhances the attachment of immune cells (e.g., monocyte and T-lymphocyte) on the endothelial wall after which monocytes attached to artery walls mediated by monocyte chemoattractant protein 1 (MCP-1). These artery-resident-monocytes differentiate into macrophages upon exposure toward comitogenic mediators. These macrophages have the capacity to take up and degrade oxLDL through overexpressed scavenger receptors but under oxidative stress the classical LDL receptor (LDLR), which is downregulated by increasing cellular cholesterol levels, the ability of scavenger receptors to take up modified LDL is not inhibited by increasing cellular cholesterol. This leads to the appearance of macrophage-derived foam cells, whose cytoplasm is swollen with lipid droplets. Lipid-laden foam cell formation is considered the most significant early hallmark of atherosclerosis, and hence CVD. Similarly, oxidative stress also causes defects in insulin-dependent substrate proteins (e insulin receptor substrate (IRS) proteins one and 2) and their mediated signaling pathways leading to metabolic syndromes and diabetes. These pathways mediate the cellular response to insulin and involves a large array of insulin-stimulated protein kinases including the serine/threonine kinase AKT and protein kinase C (PKC) that phosphorylate a large number of Ser/Thr residues in the IRS proteins involved in the metabolic response to insulin. Additionally, AMP-activated protein kinase, G protein-coupled receptor kinase 2, and c-Jun N-terminal protein kinase (noninsulin-dependent kinases) phosphorylate the two insulin-responsive substrates and any oxidative-stress led disruption in the AKT and PKC kinases is central to the development of diabetes and is associated with all major features of the disease including hyperinsulinemia, dyslipidemia and insulin resistance (Kharroubi & Darwish, 2015; Sagesaka et al., 2018). However, the inclusion of antioxidant-rich berries in diets can help to reverse all these above-mentioned biological process by rebalancing the redox-balance and hence mitigate the risk factors of CVD. The addition of berries to the diet can positively affect risk factors to cardiovascular health by free radical scavenging, modulation of eicosanoid metabolism, inhibiting inflammation, improving endothelial function, normalizing the glucose/lipid metabolism, reducing blood pressure, inhibiting platelet aggregation/activation, increasing resistance of LDL to oxidation and increasing circulatory high-density lipoproteins (HDL-C). Many clinical studies have been completed both at *in vitro* and *in vivo* levels validating the health-claims of berries in this regard. A list of these clinical studies comprising only human subjects is presented in the Table 6.3.

7. Berry-based therapeutic functional foods for CVD

Over the last 2 decades, there has been seen an enormous spike in the demand of berry-based functional foods and/or berry-based functional ingredients having both health promoting and disease preventive properties. As mentioned-above, berries are rich source of phytonutrients, dietary fiber, minerals, vitamins, carotenoids, lycopenes, terpenoids, and fatty acids. There are multiple ways of using berries as functional foods which can be broadly categorize into three i.e., (**a**) usage of whole freshly harvested berries themselves and/or incorporating them into various food items and traditional food recipes;

TABLE 6.3 A comprehensive list of human clinical studies demonstrating the intake of different berries and their subsequent health-promoting effects in managing, treating, or reducing CVD risk factors.

No.	References	Study design	Study subject	Duration	Berry intervention	Intervention diet	Effect on risk-factors of CVD
1.	(Cassidy et al.)	Prospective cohort study	Nurse's Health Study, (Women, n = 93,600)	18 years	Intake of >3 portions (1 cup per portion) of strawberries and blueberries per week, calculated from assessment of intake of the different flavonoid subclasses and phenol-explorer database	Nil	Combined intake of two anthocyanin-rich foods, blueberries and strawberries, tended to be associated with a decreased risk of myocardial infarction by 34% over an 18-year follow
2.	(Rodriguez-Mateos et al.)	Two randomized, controlled, double-blind, crossover human-intervention trials	Healthy men (n = 21)	Single day	Intake of macronutrient and micronutrient matched blueberry drink that contained 0 (control), 766, 1278, and 1791 mg total blueberry polyphenols (equivalent to 240, 400, and 560 g fresh blueberries, respectively)		Time-dependent increase in flow-mediated dilation (FMD) (vascular function), with significant increases at 1—2 and 6 h after consumption of blueberry polyphenols
3.	(Rodriguez-Mateos et al.)	Randomized controlled trial	Healthy men (n = 10)	Single-day	Blueberry powder rich baked products or juice (containing a total of 34 g freeze-dried blueberry powder, equivalent to 240 g of fresh blueberry. Control products were without blueberry		FMD increased after 1, 2, and 6 h consumption of the baked products or juice
4.	(Rodribguez-Mateos et al.)	A double-blinded, 5-armed randomized controlled crossover study	Volunteers (n = 5, n = 10, n = 5, n = 40, n = 22)	4 week	Pure anthocyanin content of 160 mg, or wild blueberry drink, made of 11 g of freeze-dried wild blueberry powder supplying equivalent anthocyanin		Acute and chronic flow-mediated dilation improvements, and 13-fold increase in peripheral blood mononuclear cells
5.	(Del Bo' et al., 2017)	A randomized controlled crossover pilot study	Healthy males (smokers = 12, nonsmokers = 12)	Single-day	Blueberry treatment (300 g of blueberry); control treatment (300 mL of water with sugar) with/without smoking		Improvements in blood pressure, peripheral arterial function (reactive hyperemia index, reactive hyperemia index (RHI), a marker of endothelial function) and arterial stiffness while mitigating the effect of smoking

6.	(Nyberg et al.)	A randomized cross-over trial	Healthy men and women (n = 15 + 17 = 32)	4 week	Intake of 150 g of blueberries, or not (control), and exercise by running/jogging 5 km five times/week and 4 weeks of minimal physical activity		Increased levels of both fasting glucose and HDL-cholesterol, insulin, and triglyceride levels were reduced after intake of blueberry
7.	(Anchalee et al., 2016)	Randomized controlled trial	Hypercholesterolemic subjects (n = 60)	6 week	45 g of freeze-dried mulberry (equivalent 160 g fresh mulberries, 325 mg anthocyanins) per day		Consumption of mulberry fruits significantly lowered total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations in hypercholesterolemic subjects
8.	(Anchalee et al., 2016)	Open prospective, and single-blinded design	Middle-aged subjects (n = 26)	6-week	Mulberry fruit extracts (MFEs) 100 mL/day		Levels of C-reactive protein (CRP), serum triglyceride (TG) and LDL-cholesterol had significantly decreased, whereas serum levels of HDL-cholesterol significantly increased
9.	(On-Nom et al., 2020)	Open label trial	Dyslipidemia volunteers (total cholesterol >200 mg/dL; LDL cholesterol >130 mg/dL; HDL cholesterol 150 mg/Dl; triglycerides >150 mg/dL) (n = 15 + 7)	7-day	Mulberry fruit powder (MFP) jelly containing 14 g MFP (191 mg anthocyanin) per serving size (170 g). one serving of MFP jelly every day for 7 days	A high-fat meal with 652 kcal contains 32% of fat distribution for energy per day	Fasting blood cholesterol, low-density lipoprotein (LDL), and inflammatory markers (interleukin-6 levels) of the subjects were significantly lower with increased insulin sensitivity
10.	(Wang, Yu, et al., 2021)	Randomized controlled trial	Dyslipidemic patients with myocardial infarction or angina pectoris (n = 160)	1 year	Treatment group orally administrated with 150 mg of Mulberry leaves Deoxynojirimycin (MLD) per day whereas The control group was orally administrated continuously with starch placebo		Improved antioxidant, anti-inflammatory, and serum lipid profile in CVD patients. The carotid intima-media thickness (IMTs) values were lower in treated group than controlled group
11.	(Arevström et al., 2019)	A prospective, open-label, randomized, controlled clinical trial	Patients after acute myocardial infarction (AMI) (n = 25 + 25 = 50)	8 week	Taking total of 40 g of bilberry powder per day with meals 3 times a day, equaling approximately 480 g of fresh berries	Standard medical diet post-AMI	No obvious effect to standard therapy post-AMI on the primary biochemical end point i.e., high-sensitivity C-reactive protein (hs-CRP) but significant improvement in exercise capacity, i.e., Six-minute walk test (6 MWT) was seen

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TABLE 6.3 A comprehensive list of human clinical studies demonstrating the intake of different berries and their subsequent health-promoting effects in managing, treating, or reducing CVD risk factors.—cont'd

No.	References	Study design	Study subject	Duration	Berry intervention	Intervention diet	Effect on risk-factors of CVD
12.	(Habanova et al., 2016)	Randomized controlled trial	Random volunteers (n = 36)	6 week	150 g of frozen stored bilberries 3 times a week		Bilberries intake contributed to beneficial effects on CVD risk reduction, such as decreasing TG, glucose, γ-glutamyltransferase, LDL-C and TG and increasing HDL-C
13.	(Bergh et al., 2021)	A prospective, randomized, double-blind, placebo-controlled multi-center trial	Patients post-AMI (n = 900)	12 week	Isocaloric beverages with/without bilberry (+ oat fiber) powder (active) for consumption twice daily (160 kcal/day)		Helpful in lower LDL cholesterol and inflammation markers than standard therapy alone
14.	(Bryl-Górecka et al., 2020)	Open-label study	Patients post-AMI (n = 900)	8 week	Bilberry-supplemented group ingested three doses of 13.3 g of bilberry each day, totalling 40 g daily that corresponded to 480 g fresh bilberries. The diet contained 2250 mg of anthocyanins per 100 g; thus, the patients consumed 300 mg anthocyanins in a single dose		Bilberry reduced both endothelial extracellular vesicle (EMVs) and platelet-derived vesicles (PMVs) in the plasma of MI patients. It also reduced endothelial vesiculation through several molecular mechanisms (i.e., reduced expression of Rab27b and Rab27a and prevented bzATP-induced increase of Rab27b, Rab27a, and SMPD1 gene expression)
15.	(Karlsen et al., 2010)	Randomized controlled trial	Volunteers with increased risk of CVD (n = 62)	4 week	330 mL/day bilberry juice (Corona Safteri, Rotvoll, Norway) or water for 4 weeks		Modulate NF-κB-related inflammatory markers
16.	(Duffey & Sutherland, 2015)	Randomized controlled trial	Regular consumer (n = 330)	Single-day	Cranberry juice cocktail (CJC) consumption 404 mL (14 fL oz) for 2 nonconsecutive 24-hour dietary recalls		Significantly lower levels of CRP, anti-inflammatory markers, and body mass index in treated group as compared to controls
17.	(Rodriguez-Mateos et al., 2016)	A double-blind randomized controlled crossover trial	Healthy males (n = 10)	2 day	Consumption of cranberry juices containing 409, 787, 1238, 1534, and 1910 mg of total cranberry (poly) phenols(TP), and a control drink		Improve vascular function in healthy males which is related plasma metabolite of cranberry (i.e., ferulic and caffeic acid sulfates, quercetin-3-O-ß-D-glucuronide and a-valerolactone sulfate)

18.	(Novotny et al., 2015)	A double-blind, placebo-controlled, parallel-arm study	Women and men (n = 30 + 26 = 56)	8 week	240 mL of low-calorie cranberry juice (LCCJ) (containing 173 or 62 mg of phenolic compounds) or the placebo beverage		Fasting plasma glucose, triglycerides (TGs), serum C-reactive protein (CRP), and diastolic blood pressure (BP) were lower after consuming LCCJ as compared placebo
19.	(Hsia et al., 2020)	A randomized, double-blind, placebo-controlled, parallel-designed	Individuals with obesity and with elevated fasting glucose or impaired glucose tolerance (n = 35)	8 week	450 mL of low-energy cranberry (LCCJ) beverage or placebo daily		Consumption OF LCCJ can lead to a reduction in TAG concentrations and a reduction in oxidative stress levels in people with elevated CRP concentrations
20.	(Richter et al., 2021)	A randomized, placebo-controlled, crossover trial	Middle-aged adults with overweight/obesity and elevated brachial blood pressure (n = 40)	Single-day	Consumption of 500 mL/d of cranberry juice (∼16 fL oz; 27% cranberry juice) or a matched placebo juice in a randomized order		Reduced 24-h diastolic ambulatory BP by ∼2 mm Hg compared to the placebo, modest effects on 24-h diastolic ambulatory BP and the lipoprotein profile
21.	(Xiao et al., 2019)	A controlled, three-arm, single-blinded, crossover trial	Prediabetic subjects (n = 32)	3 day	125-to-250g of frozen red raspberries	Challenge breakfast meal (high carbohydrate/moderate fat) on three separate days	Raspberry intake relieved the individuals from prediabetic state after taking high carbohydrate/moderate fat meal as glucose level dropped from 8.1 ± 0.2 mmol/L (prediabetic state) to 7.2 ± 0.2 mmol/L (normal state)
22.	(Park et al., 2015)	Randomized, placebo-controlled clinical trial	Healthy male smokers (n = 39)	4-week	Smokers received either 30 g of freeze-dried black-raspberry or placebo consumed equivalent amounts of calorie-matched drink		Raspberry intake significantly increased the activity of the antioxidant enzymes, glutathione peroxidase and catalase, and reduced plasma lipid peroxidation
23.	(Istas et al., 2018)	Three-arm, double blind, crossover randomized controlled trial	Volunteers (n = 10)	Single-day	Consumption of 200 and 400 g of red raspberries containing 201 or 403 mg of total (poly)phenols, or a micro- or macronutrient matched control drink		It leads to improvements in endothelial function for at least 24 h after consume which corresponds to 10%–15% less chances of CVD
24.	(Franck et al., 2020)	Two-arm parallel-group, randomized, controlled trial		8-week	Consume 280 g of frozen Raspberry daily (roughly 2 cups)		The intervention did not significantly affect plasma insulin, glucose, inflammatory marker concentrations, nor

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TABLE 6.3 A comprehensive list of human clinical studies demonstrating the intake of different berries and their subsequent health-promoting effects in managing, treating, or reducing CVD risk factors.—cont'd

No.	References	Study design	Study subject	Duration	Berry intervention	Intervention diet	Effect on risk-factors of CVD
							blood pressure. But it does enriched several functional pathways and differently expressed the 43 genes leading to improvement in metabolism phospholipids
25.	(Jeong et al., 2016)	A prospectively randomized trial	Patients with prehypertension (n = 45)	8 week	Moderate-to-high dose of black raspberry dose (1500−2500 mg/day) daily		Significantly reduced 24-h systolic blood pressure
26.	(Aghababaee et al., 2015)	Randomized clinical trial	Dyslipidemic patients (n = 72)	8 week	Intake of 300 mL/day blackberry juice with pulp and for control group:usual diets)		Blackberry consumption exerted beneficial effects by improving apolipoproteins (lipid transporters in blood) and HDL-C concentration, blood pressure, and reducing inflammatory markers hs-CRP which ultimately reduced the CVD risk factors
27.	(Quesada-Morúa et al., 2020)	Randomized cross-over design	Healthy individuals (n = 13)	2 week	Intake of 250 mL of either blackberry beverage or water with every meal, three times a day	High-fat, high-carbohydrate diet challenge for breakfast, lunch, and dinner (4000 Kcal/day)	Blackberry nullified the effect of high-fat/high-carbohydrate diet and improved the lipid profile of participants
28.	(Solverson et al., 2018)	Randomized, placebo controlled, cross-over with two treatments	Overweight/obese men (BMI >25 kg/m2)	3 week	Intake of 600 g of whole blackberries (BBs) (∼1476 mg of flavonoids and ∼361 mg of total anthocyanin daily) or a calorically matched amount of artificially flavored gelatine (GEL) daily		Improved insulin sensitivity and glucose tolerance was observed in treated groups as blackberry intervention improved homeostasis model assessment of insulin resistance (HOMA-IR), respiratory quotient (RQ) (an indicator of fat burning and oxidation) area under the curve (AUC) of insulin availability and sensitivity
29.	(Hassimotto et al.)	Open, single-center, Randomized clinical trial	Healthy human subjects (n = 6)	4h	Intake of 200 mL of blackberry juice (BBJ) equivalent to 400 mg of cyanidin equivalent/50 kg of body weight		Improved plasma and urine antioxidant system

30.	(Aghababaee et al.)	Randomized clinical trial	Dyslipidemic patients (n = 72)	8 week	Intake of 300 mL of BBJ (equivalent to 316 mg/100 g polyphenols) of blackberry		Increased apo A-1 and HDL-C along reduction in apo B and hsCRP
31.	(Okamoto et al., 2020)	A randomized, double-blind, placebo-controlled, crossover design study	Older adults (n = 14)	7-day	Intake of two capsules of black currant extract (each 300 mg capsule contains 35% blackcurrant extract		Carotid-femoral pulse-wave velocity, central blood pressure, carotid femoral pulse-wave velocity, central arterial stiffness, and central blood pressure was reduced significantly in black-currant recipients
32.	(Okamoto et al., 2020)	Double-blinded and randomly assigned study	Healthy males *(n = 10)*	Single day	Intake of blackcurrant extract (1.87 mg total anthocyanins/kg bodyweight) or placebo powder		Black-currant intervention maintained the e forearm blood flow (FBF) and forearm vascular resistance (FVR) during an extended period of sitting, without influencing exercise performance
33.	(Cook et al., 2017)	Counter-balanced Latin square design	Endurance-trained male cyclists (n = 15)		Intake of black-currant extracts (300, 600 or 900 mg day) or placebo (control)		Black-currant extracts helped to keep unchanged the systolic and diastolic blood pressure, heart rate and ejection time. Cardiac output and stroke volume increased dose-dependently in treated versus placebo-controlled cyclists
34.	(Castro-Acosta et al., 2016)	A randomized, double-blind, cross-over design	Healthy subjects (n = 22)		Intake of low sugar beverages containing blackcurrant extract providing 150 to 600-mg total anthocyanins or no blackcurrant extract (Control) were administered immediately before a high-carbohydrate meal	High-carbohydrate meal	Lower postprandial glycemia and inhibited the secretion of insulin and incretins which may reduce the vascular burden of glucose-induced oxidative stress and endothelial dysfunction involved in the progression of atherosclerosis
35.	(Lappi et al., 2021)	A randomized, controlled crossover postprandial study	Healthy subjects (n = 26)		Consumed four test products contained 31 g of available carbohydrates and had similar composition of sugar components:		Blackcurrant intervention resulted in reduced sugar-induced hypoglycaemic response at the late postprandial phases

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TABLE 6.3 A comprehensive list of human clinical studies demonstrating the intake of different berries and their subsequent health-promoting effects in managing, treating, or reducing CVD risk factors.—cont'd

No.	References	Study design	Study subject	Duration	Berry intervention	Intervention diet	Effect on risk-factors of CVD
					300 mL water with sucrose, glucose and fructose (SW; reference), blackcurrant purée with added sugars (BCs), a product consisting of the blackcurrant purée and a product base with fermented quinoa (BCP) and the product base without blackcurrant (PB)		
36.	(Jurado, 2017)	Randomized clinical trial	Female participants (n = 20)	15 day	Participants received 40 g of dehydrated goji berry, consumed in two daily servings of 20 g, one at breakfast and the other after lunch		Goji berry intake decreased body weight, abdominal circumference, glycaemia, LDL, systolic, and diastolic blood pressure and increased serotonin, and may be indicated as a complementary treatment for diabetes, hypertension, and depression
37.	(Van den Driessche et al., 2019)	Randomized clinical trial	Healthy, overweight men (n = 17)	Single-day	A meal containing 25 g of dried *Lycium barbarum* fruit or a control meal matched for caloric content and macronutrient composition		Goji berry intake significantly increased the energy expenditure and hence prohibiting the weight gain
38.	(de Souza Zanchet et al., 2017)	Randomized clinical trial	Patients with metabolic syndrome (n = 50)	12-week	Intake 14 g of the natural form of goji berry in the diet		An increase in serum antioxidant capacity and Reduced glutathione (GSH) and a decrease in lipid peroxidation, LDL cholesterol, and waist circumference after long-term ingestion of goji berry
39.	(Erkel et al., 2017)	Randomized clinical trial	Elderly participants (n = 18)	Single-day	Intake of capsules of goji berry extract (14 g) per day		Supplementation of goji berry extract reduces waist circumference and the percentage of body fat percentage

40.	(Van den Driessche et al., 2019)	A double-blind, randomized, controlled, cross-over study	Healthy overweight men (n = NS)	Single meal challenge	Meal containing 25 g of dried goji berry fruit	Ready-made meal with a fixed macronutrient composition (30%–40% fat, 40%–50% carbohydrates, and 13%–16% proteins)	No-single-dose effect on substrate oxidation and postprandial-energy-expenditure
41.	(Sadowska-Krepa et al., 2015)	Randomized clinical trial	Elite junior hurdlers (n = 7)	6-week	100 mL of the Acai berry MonaVie Active juice blend		Significantly improved the antioxidant capacity of plasma, lipid profile, and exercise induced muscles damage
42.	(Do-Jin Kim, 2018)	Randomized clinical trial	Middle-aged women in their 40 and 50s (n = 30)	8-week	Participants received 5 g of acai berry powder before breakfast and dinner, and ingested it by melting them in water. The intervention was combined with exercise held three times a week for 8 weeks, each for 60 min including warming up and cooling down		Mild or nonsignificant effect of acai berry intake on insulin and HOMA-IR, glycated hemoglobin (HA1bc)
43.	(Nam & Kang, 2014)	Randomized clinical trial	Middle-aged women in their 40 and 50s (n = 30)	8-week	5 g acaiberry dilutes with 100 mg water to inhale before breakfast and dinner along walking exercise 3 times per week for 8 weeks		Acaiberry intake has positive effect in inflammatory markers in middle age women
44.	(Udani et al., 2011)	open label pilot study	Overweight adults (n = 10)	30 days	Intake of 100 g AB pulp twice daily		Postprandial increase in the AUC of plasma glucose with reduced TC, LDL-C, and LDL-C/HDL-C
45.	(Alqurashi et al., 2017)	A double-blind, randomized, controlled, cross-over study	Overweight healthy males (n = 23)	Single day Meal challenge	Frozen AB pulp (150 g) was prepared in a smoothie with 50 g banana	50 g banana and matched for fat with 1.5 g hexadecanoic acid [palmitic acid (16:0)] and 8.5 g sunflower oil [30% (9Z)-Octadec-9-enoic acid (oleic acid [18:1]), 60% (9Z,12Z)-9,12-Octadecadienoic acid (linoleic acid [18:2]), and 10% palmitic acid (16:0)]	Lower incremental area under the curve (iAUC) for total peroxide oxidative status after açai and increased the iAUC for insulin

Continued

TABLE 6.3 A comprehensive list of human clinical studies demonstrating the intake of different berries and their subsequent health-promoting effects in managing, treating, or reducing CVD risk factors.—cont'd

No.	References	Study design	Study subject	Duration	Berry intervention	Intervention diet	Effect on risk-factors of CVD
46.	(Alvarado et al., 2016)		36 prediabetic subjects		A single dose of delphinidin-rich extract from maqui berry (Delphinol) (nil, 60, 120, or 180 mg) on different test days, with minimum of 1-week intermission between tests		Delphinidin-rich extract from maqui berry significantly (dose-dependently) reduced the basal and postprandial glycemia and insulinemia by either improving insulin sensitivity, incretin-mediated effect, and inhibition of intestinal glucose transporters
47.	(Ávila et al., 2019)	A randomized type I clinical trial with a crossover design	Healthy male subjects (n = 11)		250 mL of the drink containing maqui berry-derived polyphenols(approximately 1000 μmol equivalents of gallic acid) before each test meal	Test meals containing food-grade glucose and rice, containing 50 g of carbohydrates by each meal	Inclusion of maqui berry derived polyphenols reduced the glycemic index in participants after consuming the high-carbohydrate meals
48.	(Davinelli et al., 2015)	A double-blind, placebo-controlled design	42 participants	4-week	Intake of standardized extract of maqui berry (162 mg anthocyanins) or a matched placebo		Reduced levels of oxidized cholesterol in treated groups without affecting the anthropometric characteristics, ambulatory blood pressure, and lipid profile
49.	(Alvarado et al.)	an open prospective design	Prediabetic subjects (n = 31)	12 weeks	One capsule containing 180 mg Delphinol in the morning with approximately 200 mL water before breakfast		Fasting glucose and insulin level was found to be decrease without affecting the oral Glucose Tolerance Test (OGTT). An LDL-C level was significantly reduced after 3 months without any impact on total cholesterol or triglycerides
50.	(Corsi et al., 2018)	Prospective observational study	Middle-aged participants (n = 21)	8 week	Two tablets per day of an MCN (Eonlipid) (containing maqui, 300 mg in each tablet)		Improvement of most atherogenesis and oxidative stress biomarkers

(**b**) bioactive ingredients (fiber, natural colorants, essential aromatic oils etc.) obtained from berries for subsequent use in further food/beverage processing and preparation; and (**c**) isolation of (specific) phytochemical(s) or class of phytochemical (enriched) extracts/powders. The first one is the most commonly used approach in which, generally, freshly-harvested whole berries can be incorporated into high-glycemic numerous bakery items (bread, biscuits/cookies, cakes, bagels, buns, muffins etc.), dairy products (yoghurt, smoothies, ice cream, (sour) cream etc.), high-calorie beverages (juices, sports-drinks, alcoholic drinks, energy-drink, fizzy drinks, cocktails, nectars etc.), where they can serve as to nullify the effect of high-glycemic/high-calorie foods/beverages as confirmed in clinical studies. Similarly, fresh berries can also be syruped, pickled, fermented, freeze-dried into powders and can be used in various food/beverage recipes at home. The freeze-dried powders show up to 4.3-times higher antioxidant potency as compared to fresh produce (Miller et al., 2000). Likewise, berries can also be source of bioactive ingredients such as natural colorants (lycopene, carotenoids, lutein), dietary fiber (pectin) antioxidants (ascorbic acid, inulin), fruit-fillings, natural fruit sweeteners with low glycemic index and high soluble solid content which can be used in processing/preparation of numerous food/beverages (Wallace et al., 2020). Moreover, berry-derived anthocyanin and flavonoids can be extracted using, supercritical fluid extraction, accelerated solvent extraction, followed by concentration using methods like RapidVap concentration, ultrafiltration, CentriVap or concentration and/or freeze drying (Kennedy & Panesar, 2005; Shi et al., 2005, Sun-Waterhouse, 2011).

The applications of berry-derived polyphenolic extracts can be mainly used to fortify or enhance foods or beverages. There are many examples where fruit-based polyphenolic extracts are already available in the market for both research and commercial purposes. Some of the commercial suppliers include the companies Just the Berries New Zealand, Berryfruit New Zealand, Penglai Marine BioTech China, GNT International The Netherlands, Herbstreith & Fox Germany etc. (Sun-Waterhouse, 2011). Furthermore, previous studies found these extracts were pH-sensitive and thermo-labile (Hameed et al., 2020b) putting forward an issue for their stability and bio-accessibility/bioavailability at intestinal level. However, this issue can be overcomed by (micro/nano)-encapsulating these polyphenolic extracts to form fine-flowing and readily dissolvable powders (Hameed et al., 2020a). Encapsulation is not only a good measure for targeted delivery of these bioactive compounds but also improve stability and bioavailability (Wattanathorn et al., 2019). Berry polyphenols can also be transformed into natural health products where a single phytochemical or otherwise a group of polyphenols can be commercially available in the form of capsules. An example of this is delphinidin-rich Maqui berry extract (Delphinol) (Alvarado et al., 2016). Moving forward, whatever health-promoting products are developed from berries, one should keep in mind the consumer perception/well-being, food safety issues, (inter)national food regulations, target population, target (group of) bioactive compounds, target site, target dose, and health-claims.

8. Conclusions

There is a diverse array of berries found wild in tropical, temperate and arid ecosystems or cultivated in both field and control environments across the globe. It is evident berry genetics, species, growth environment, cultivation techniques, postharvest management practices, packaging and processing affect the nutritional and functional properties of berries. The level and composition of functional and nutritional compounds in berries are primarily responsible for their health promotive properties. In particular, anthocyanins and flavonoids are shown to be very effective in managing, treating and reducing CVD risks in humans; and the effects are even more pronounced when combined with personalized nutrition or diets and physical activities. Globally, there is a steady increase in CVD incidences and associated deaths. There is a need for interventive strategies to reduce these CVD incidences and associated deaths. Personalized nutrition and diets containing increase levels or consumption of fresh berries, berry-based functional foods, nutritional products, or nutraceuticals could be an effective long-term strategy to reduce CVD disease risks, as well as improve population health globally.

Author contributions

All authors have participated actively in the design and conception of this chapter. All authors have assessed the present form of the review and have approved it for publication.

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Part II

Dairy foods

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Chapter 7

Yogurt and health

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1. Introduction

Yogurt, the major type of fermented milk, is a tremendously popular product consumed around the world. Yogurt is thought to have first appeared around 5000 BCE in Mesopotamia. For centuries, yogurt making was a known safe method for preserving milk, other than drying it (Fisberg & Machado, 2015). Indian Ayurvedic scripts, dating from about 6000 BCE, refer to the health benefits of consuming fermented milk products (Brothwel, 1997). Yogurt was well known in ancient Greece and the Roman empire, and the Greeks were the first to mention it in written references in 100 BCE, noting the use of yogurt by barbarous nations (McGee, 2004). A type of yogurt known to Greeks since classical times was Pyriate or Oxygala ('Οξύγαλα'; 'Oxy' means "sour" and gala "milk"), − a sort of thickened sour milk, which nowadays consists one of the major fermented milk in Greece (Dalby, 1996, p. 66). The ancient Greek philosopher and physician Galen of Pergamon says that 'Οξύγαλα' was eaten alone with honey, just as thick Greek yogurt is today (Hoffman, 2004, p. 471). To this type of yogurt Galen devoted a few sections of his treatise (Galen, AD 12 9-c.200/c.216) discussing the influence of 'Οξυγαλα' consumption on human health. It is believed that the word "yogurt" has been first used by the Turks in the eighth century and comes from the Turkish word "yoğurmak", which means to thicken, coagulate, or curdle (Kashgari et al., 1984).

According to the Food and Agriculture Organization of the United Nations (FAO, 2015), world milk production derives from cows (85%), buffalos (11%), goats (2.3%), sheep (1.4%), and camels (0.2%). Milk is processed into 5000 different fermented foods worldwide (Tamang et al., 2016). Yogurt, the main fermented dairy product, is manufactured by lactic acid fermentation enabled by the symbiotic yogurt cultures *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* (*S. thermophilus*). The Codex Alimentarius (2003) and the US Code of Federal Regulations in 21CFR131.200 (CFR, 2017a) specify that yogurt should contain a minimum of 2.7% (w/w) milk proteins, at least 3.5% (w/w) milk fat, a minimum of 0.6% titratable acidity (expressed as % w/w of lactic acid) and a minimum of 1×10^7 cfu/g of the starters as a whole, to the date of shelf life. If the product is heat-treated after fermentation the requirement for viable microorganisms does not apply.

Yogurt has a wide acceptance worldwide, and its nutritional and health benefits are well known for centuries (Cormier et al., 2016). The yogurt market has existed and developed for centuries. In 2019, the global yogurt production reached approximately 85.54 billion $ US, and EU-28 production of acidified milk (yogurt and other) for the year 2019 was estimated at 8.12 million tons (Eurostat, 2019). The highest overall consumption of yogurt can be found in France and Germany, whereas the highest per capita consumption of yogurt is recorded in Sweden (33.4 kg), the Netherlands (30.5 kg), and Finland (23.8 kg) (Eucolait, 2017). With increasing numbers of product launches and preferences for yogurt among consumers, professionals believe that the yogurt market is projected to register a compound annual growth rate (CAGR) of nearly 5% by 2023 (FAO, 2020).

The European, North American, Australian, and New Zealand market is dominated by three types of yogurt. The set yogurt, which is packed immediately after inoculation with the starters and incubated in the package; the stirred yogurt, which is inoculated and incubated in a tank, and after incubation is cooled, stirred, and packaged; and the last one, and maybe the most popular, known as Greek-style yogurt, with a high level of milk total solids (TS) (∼20% w/w TS, with ∼10% w/w fat), with higher consistency, viscosity, and nutritional value (Kilara & Chandan, 2013).

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2. Yogurt manufacturing practice and its impact on nutritional value

Irrespective of the milk type used, the technology of yogurt manufacturing is more or less standardized and includes the following main steps, also shown in Fig. 7.1.

2.1 Milk standardization and yogurt ingredients

The main ingredients in yogurt production are milk and the starter culture. Cow milk is the most common milk used for producing yogurt in the Western world; however, yogurt from sheep, goat, and buffalo milk is used as well (Korhonen & Pihlanto, 2007). Milk consists of a complex mixture of components. The major components are lactose, lipids, proteins, minerals, and water. The composition of milk varies and depends on different factors, such as the breed, the individual animal, the stage of lactation, the feed, etc (Walstra et al., 2005). Concerning the yogurt variety or type, the milk may be either full fat, or partially and fully skimmed. The milk cream is used to adjust the fat content, while when modification of the total solids is needed, skim milk powder or milk whey powder is added. Stabilizers, sweeteners, and other complementary ingredients may be used to improve the consistency and avoid whey separation, but these must appear on the product's label.

Milk standardization is performed to comply with the legal requirements for milk/dairy products (Codex Alimentarius, 2003), provide the consumer with a uniform product, and ensure an end-product with certain physical properties and flavor. In yogurt production, milk solids content may vary from 14% to 15% (w/w) and minimum nonfat solids from 8.2% to 8.6% (w/w), to meet the minimum legal requirements of national food regulatory authorities (FDA, 2020). Reduced milk nonfat solids (SNF) content results in lumpy and watery texture, while increased content leads to a "full" taste, higher viscosity, and a stable gel, with no whey separation during cold storage (Shiby & Mishra, 2013). However, given that an acceptable end-product should have around 4%−5% (w/w) protein with at least 8.25% (w/w) milk SNF according to the US Code of Federal Regulations (CFR, 2017a), the level of protein in cow milk is not adequate for a desirable texture in a high-quality yogurt. Traditionally, the increase of solids content of milk was achieved by boiling the milk for about 10 min and condensing it at the desired level of two-thirds of its original volume. Nowadays, many alternative procedures are in use, such as (i) addition of skim milk powder, caseinates (sodium, calcium, or sodium-calcium), milk whey powder, and dehydrated milk whey proteins to liquid milk; (ii) milk condensation by vacuum evaporation, ultrafiltration, or reverse osmosis systems. Milk standardization is often used for enhancing the nutritional value of the end-product by increasing the final concentration of several functional ingredients. Fortification of yogurt with vitamins A and C, fiber, iron, fruits, and vegetables leads to quality improvement and consequently, increases its acceptability by, among others, preventing diseases associated with nutritional deficiencies, such as osteoporosis, iron deficiency anemia, gastrointestinal disorders, etc (Gomez-Gallego et al., 2018).

2.2 Homogenization

The purpose of milk homogenization is to reduce the average fat globules diameter (d < 2 μm) and prevent the formation of a fat layer on the yogurt surface. Additionally, casein micelles are scattered; thus, fat-protein complexes are formed, which improve the consistency of the final product (Kneifel & Seiler, 1993). The disruption of fat globules results in an

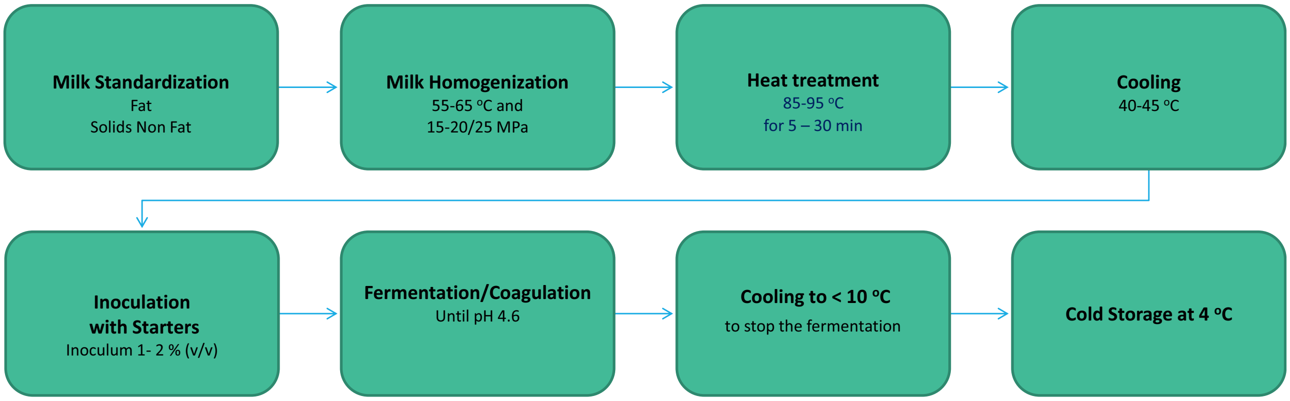

FIGURE 7.1 Main steps of yogurt manufacturing.

increased amount of milk fat globule membrane (MFGM) material, while phospholipids and proteins of the MFGM, whey proteins of the skim phase, and some casein micelles bind with the newly formed fat globules and stabilize them. According to Sieber et al. (1997), milk homogenization seems to improve milk digestibility in people suffering from gastrointestinal tract (GIT) diseases by facilitating the bioavailability of the nutritional yogurt components, such as fat and protein. Besides, smaller lipid droplet sizes would conceivably favor lipolysis, increasing the bioavailability of the fatty acids (Claeys et al., 2013). In an *in vitro* gastrointestinal digestion study using a fasting model, the free fatty acids release rate was higher in yogurt prepared from homogenized milk, while palmitic, stearic, and oleic acids degraded faster than myristic acid and other fatty acids (Tunick et al., 2016).

2.3 Heat treatment

Milk heat treatment is carried out to ensure product safety and exploit the effects on the yogurt microstructure and physical properties. In commercial practice, the most commonly used heat treatment of milk in the yogurt manufacturing process is performed at 85°C for 30 min (Tamime & Robinsons, 2007, pp. 11−118; Walstra et al., 2005), which is then cooled to 42°C where starter cultures (2%) are used to inoculate the milk. There are also other heat treatments, such as at 90−95°C for 5−10 min (Chandan & 2006), but ultimately they all have the same purpose.

The thermal processing of milk has the following effects: ensures milk safety as it destroys the most vegetative forms of microorganisms, among them *Coxiella burnetii,* which is the most heat-resistant pathogen currently recognized as the cause of Q-fever in humans. Additionally, it inactivates lipases and proteinases from milk psychrotrophic bacteria, denatures most whey proteins, develops a "cooked" flavor in yogurt, and improves viscosity and firmness. Static *in vitro* experiments have shown that heating milk at high temperatures increases the formation of peptides within the first 30 min of gastric digestion (Lamothe et al., 2017), thus, improving milk protein digestion. In general, homogenization and thermal processing in combination change the size, the zeta-potential, the microstructure, the phospholipid layer, and the glycosylated molecules at the surface of milk fat globules and modify the profile of the released fatty acids. These compositional and microstructural milk fat globules changes contribute to better digestion of milk fat globules (Zhao et al., 2019). Moreover, heat treatment decreases the redox potential of milk and favors the growth of yogurt bacteria resulting in a higher acidification rate, which contributes to higher flavor compound production during lactic acid fermentation (Cheng, 2010).

2.4 Fermentation

After heat treatment, milk cooled down to 40−45°C, a favorable temperature for the thermophilic yogurt starter microorganisms, which inoculated at a level of about 2% (v/v). The typical yogurt culture consists of *S. thermophilus* and *L. bulgaricus* in a 1:1 ratio. Although the two microorganisms can grow individually in milk, they have a symbiotic interaction called "proto- in mixed cultures, which means that they are mutually beneficial during fermentation, by performing many biochemical conversions of milk components like as: (i) conversion of lactose into lactic acid or other metabolites (glycolysis) (ii) hydrolysis of caseins into peptides and free amino acids (proteolysis) and (iii) breakdown of milk fat into free fatty acids (lipolysis) (Hatti-Kaul et al., 2018).

Lactic acid fermentation is the most essential part of yogurt preparation, which mainly defines the process of coagulation, the organoleptic characteristics, and the functionality of the end product (Tamime & Robinson, 2007, pp. 11−118). Lactic acid contributes to the acidic taste of yogurt and the gel formation, while the by-products (predominantly acetaldehyde) contribute to its characteristic aroma and flavor. Acidification in a pH 4.6 and below tends to increase the hydration of proteins contributing to yogurt consistency improvement.

Due to proteolysis caused by the starter cultures, amino acids (mainly proline and glycine) are released into yogurt, even during cold storage at 4°C (Vedamuthu, 2006). Regarding lipids, several free fatty acids, mostly stearic and oleic acid, are released due to the lipase activity of the starters (Santo et al., 2012). Additionally, an increase in vitamins B, such as B2 (riboflavin), B3 (niacin), and B12 (cobalamin), during fermentation has been reported (Gille et al., 2015).

2.5 Cooling

Cooling is the final step in yogurt manufacture, which signals the end of the fermentation and the start of yogurt preservation. After reaching pH 4.6, yogurt is cooled at around 4−6°C, aiming at suspending bacterial activity and biochemical reactions. Survival and metabolic activity of *S. thermophilus* and *L. bulgaricus* at cooling temperature create excessive acidity and it is called postacidification (Tamime & Robinson, 2007, pp. 11−118). High acidity causes leakage of whey proteins out of the yogurt gel, called syneresis, which reduces both yogurt quality and shelf-life (Lucey, 1999). Post-acidification manipulation can be achieved by changing the microbial ratio or selecting suitable bacterial strains as starters. Yogurt cooling can be performed by either rapid or gradual decrease of the temperature to less than 10°C, leading to yogurt with increased viscosity and limited syneresis. Moreover, during the first 24−48 h of cold storage, improvement of the physical characteristics of the coagulum is observed, mainly due to the hydration and stabilization of the casein micelles (Rasic & Kurmann, 1978). Recent studies (Khan et al., 2020) indicated that milk type and postfermentation cooling patterns have a pronounced effect on the antioxidant characteristics, fatty acid profile, lipid oxidation, and textural characteristics of yogurt.

3. Yogurt nutritional and bioactive components

Yogurt is the main dairy fermented product consumed globally due to its nutritional and physiological function, and health-promoting properties. The nutritional components of yogurt include the macronutrients lactose, proteins, fat, and micronutrients, e.g., calcium, phosphorus, magnesium, zinc, vitamins, and live microbes (Souza et al., 2018). During fermentation, due to the metabolic activity of the starters, many components are transformed, synthesized, or released, such as among them bioactive peptides, conjugated linoleic acid (CLA), exopolysaccharides (Marsh et al., 2014). Yogurt is a food matrix with viscous and elastic properties, component interactions, increased nutrient bioavailability, and synergistic properties (Marette & Picard-Deland, 2014). These may generate interactions among the food matrix components and alter the bioactive properties of nutrients in a different way that is not predictable from the nutrition-label information. Due to these synergistic phenomena, the addition of ingredients, such as skim milk powder (SMP) or whey protein concentrate (WPC) during yogurt manufacture, increases the buffer capacity of the yogurt matrix and this modifies both the composition of the formed organic acids and the lactic acid content (Perotti et al., 2019). During fermentation, lactic acid and low pH have a preservative role by inhibiting the growth of spoilage bacteria, yeasts, and molds, while they may influence the absorption of minerals (Flach et al., 2018). Moreover, lactic acid affects the physical properties of caseins inducing a better rearrangement and thus promoting digestibility (Corzo-Martínez, 2012).

The beneficial effect of the remaining lactose is well established with regards to calcium absorption, GIT activity stimulation, and enhancement of phosphorus utilization (Adolfsson et al., 2004). Additionally, calcium absorbability and bioavailability may also depend on different physiological factors, such as age, menopause state, intestinal villus width, obesity, etc., and interactions between nutrients (Gueguen & Pointillart, 2000). The viscoelastic form of yogurt might also help mineral absorption, by increasing the orocecal transit time (Oak et al., 2019). Interestingly, Ünal et al. (2005), using an *in vitro* simulation of gastrointestinal digestion, showed that calcium in yogurt had the highest bioavailability compared to other dairy products (e.g., milk, infant formulas).

Today, milk proteins are considered the most important source of bioactive peptides (Muro Urista et al., 2011). Bioactive peptides are largely found in milk, fermented kinds of milk, and cheeses. Proteolytic enzymes naturally occurring in milk, and enzymes from the LAB or exogenous sources contribute to the generation of bioactive peptides. Bioactive peptides are released via the proteolytic breakdown of milk proteins, either through digestion in the human GIT or by microorganisms during milk fermentation (Park, 2015). The quantity of active peptides released from milk caseins and whey proteins is estimated as 25−130 mg/g of protein in fermented milk and cheeses (Bos et al., 2000). The major biological activities assigned to bioactive peptides are antimicrobial, antihypertensive, antioxidative, opioid, and immune-modulatory (Jayathilakan et al., 2018; Mohanty et al., 2016). Despite the short yogurt fermentation time, the production of bioactive peptides has been reported. In particular, the production of the antihypertensive peptides, known as ACE-I (angiotensin-converting enzyme inhibitor), by either the yogurt starters when grown as single cultures in milk or during yogurt production has been shown by Ashar and Chand (2004), Donkor et al. (2007) and Georgalaki et al. (2017). Moreover, several commercial products containing bioactive peptides have been developed, such as the “AmealPeptide”, containing casein-deriving peptides (lactotripeptides), the Japanese lactic acid drink “Amino-CALPIS” and the Swedish fermented milk “Evolus” (Valio Oy, Helsinki, Finland), with health benefits having been reported (Lavikka et al., 2008; Yamamura et al., 2009). Interestingly, interactions in the yogurt matrix among bioactive peptides and nonprotein milk components, such as oligosaccharides, fats, and glycolipids, have been reported with health benefits as well (Bos et al., 2000; Marette & Picard-Deland, 2014).

During the fermentation process, the milk fatty fraction also goes through biochemical changes releasing free fatty acids, among them the conjugated linoleic acids (CLA) (Adolfsson et al., 2004). CLA occurs mainly in products of animal origin (Shingfield et al., 2013) and it is considered to exhibit anti-inflammatory, antiatherogenic, and antioxidant activities (Chinnadurai et al., 2013; Penedo et al., 2013). CLA formation in fermented milk products can be affected by several factors, such as bacterial species involved, bacterial cell number, growth substrate composition, and incubation time

(Kim & Liu, 2002). Various studies have shown that LAB can bioconvert linoleic or linolenic acid to CLA during milk fermentation (Balakrishnan & Agrawal, 2014). According to Aneja and Murthi (1990), the fermented yogurt-like Indian product, named "Dahi", contains more CLA (26.5 mg/g fat) than the unfermented milk (5.5 mg/g fat), while Shantha et al. (1995) observed that in a low-fat yogurt (fat 0.05% w/w) CLA content increased from 4.40 mg/g to 5.25 mg/g during cold storage. Moreover, yogurt fermentation alters the composition and antiplatelet properties of milk polar lipids (Lordan et al., 2020).

Although yogurt is a good source of proteins, calcium, bioactive compounds, and occasionally probiotics, its content of vitamin D is low, ranging between 2 and 3 μg per 100 g serving (USDA, 2014), which is below the recommended dietary allowance of 15 μg (600 IU) per day up to the age of 70 years (Ross et al., 2011). In addition, the consumers' preference for low-fat yogurt consumption limits the dietary intake of this fat-soluble vitamin. Therefore, yogurt is considered a good choice for vitamin D fortification to improve the positive health outcomes associated with its consumption (Gasparri et al., 2019). Several studies have indicated the stability of vitamin D in milk and other dairy products during processing and storage (Kazmi et al., 2007; Upreti et al., 2002; Wagner et al., 2008). Moreover, studies on yogurt fortified with vitamin D indicated no loss of vitamin D bioavailability during heat treatment, homogenization, and fermentation (Jafari et al., 2016; Hanson & Metzger, 2010).

4. Yogurt and probiotics

According to FAO/WHO (2002), probiotics are defined as "live microorganisms, which when administered in adequate amounts confer a health benefit on the host". The majority of probiotic bacteria are either LAB, mainly of the genus *Lactobacillus*, or bifidobacteria. These microorganisms, to be effective, should maintain viability throughout their transit through the GIT, and expose specific beneficial effects to the host, while not altering the sensory characteristics of the final product (Champagne et al., 2005). Probiotics can normalize the intestinal microbiota composition by excluding pathogens through competition for nutrients and binding sites, and by the *in situ* production of antimicrobials. Strains have also been shown to prevent antibiotic-associated diarrhea, improve the mucosal barrier function, modulate immune responses in the host, reduce lactose intolerance, serum cholesterol, and constipation, or even produce vitamins (Zoumpopoulou et al., 2017).

Fermented milk, and in particular yogurt, are the best-known vehicles of probiotic strains. According to Future Market Insight (FMI) analysis, probiotic yogurt sales have grown at 5.1% CAGR (Compound Annual Growth Rate) between 2016 and 2020, while the global probiotic yogurt market is expected to grow at 6.3% CAGR through 2031 (https://www.futuremarketinsights.com/reports/probiotic-yogurt-market). Probiotic yogurt products play a significant role in human health by providing both the natural milk nutrient components and at the same time exposing a specific impact on human health that goes beyond that of basic nutrition. Probiotics are incorporated either in combination with the starter cultures, added in yogurt after fermentation, or even used as sole starters (Soccol et al., 2010). It has been suggested that a probiotic product should contain at least 10^6–10^7 CFU/g of the probiotic bacteria or a total of 10^8–10^9 CFU to exert a therapeutic effect, based on the minimum daily consumption of 100 g or 100 mL of food (Jayamanne & Adams, 2006). The viability of probiotics in fermented milk appears to be stable for up to 4 weeks at 5°C. Viable counts above 10^8 CFU/g have been reported (Rutella et al., 2016), while the addition of skimmed milk powder (10%–15% w/w) can further increase the viability of probiotics (Maganha et al., 2014). The clinical efficacy of probiotics in milk and yogurt matrices is well documented for a variety of conditions including constipation, antibiotic-associated diarrhea (AAD), necrotizing enterocolitis; *Helicobacter pylori* infection, high blood pressure, and lactose intolerance (Barclay et al., 2007; Koebnick et al., 2003; Sachdeva & Nagpal, 2009; Walstra et al., 2005; Wenus et al., 2008; Zhou et al., 2019).

5. Yogurt and human health

The role of yogurt consumption on human health has been the subject of extensive research, including epidemiological, observational, and clinical studies. Table 7.1 presents several representative recent studies mainly focusing on yogurt consumption and its impact on human health.

5.1 Lactose intolerance

Lactose intolerance is a common condition caused by a decreased ability to digest lactose, the main carbohydrate of milk, and is due to the lack or decreased expression of the enzyme lactase in the small intestine, which breaks lactose down into glucose and galactose. Symptoms may include abdominal pain, bloating, diarrhea, gas, and nausea. The symptoms'

TABLE 7.1 Selected studies on the health impact of yogurt consumption.

Study design	Country	Number of participants	Results	Authors (year)
Animal study	India	6 *Rattus norvegicus* rats	Excess and continued consumption of yogurt leads to the higher development of advanced glycation end products (AGEs), oxidative stress, and inflammatory markers (prediabetic-like state)	Patil et al. (2021)
Randomized controlled trial	China	100 obese women 36–66 y	Yogurt consumption (220 g yogurt for 24 weeks) was associated with a lower risk of MetS	Chen et al. (2019)
A parallel randomized controlled trial	Canada	30 males 20.6 ± 2.2 y	Consumption of Greek yogurt during a training program resulted in improved strength, muscle thickness, and body composition over a carbohydrate-based placebo	Bridge et al. (2019)
Observational	Canada	198 children and young adults	Consuming yogurt may protect against insulin resistance more specifically among youth at risk of obesity	Panahi et al. (2019)
Longitudinal study	USA	1371 adults 30–64 y	Yogurt and cheese consumption was associated with an increased risk of central obesity and metabolic syndrome (MetS)	Beyduoun et al. (2018)
Meta-analysis of 40 observational studies	Korea	N/A	The risk of MetS was reduced by 18% with 100 g of yogurt added to the daily diet	Jin et al. (2018)
Crossover study	Spain	N/A	No alteration to plasma lipoprotein cholesterol levels was observed for yogurt from Ewe's and cow's milk consumption in apparently healthy individuals.	Olmedilla-Alonso et al. (2017)
A randomized, double-blind, placebo-controlled study	Brazil	50 35–60 y	Probiotic consumption improved the glycemic control in T2D individuals and decrease the inflammatory cytokines (TNF-α and resistin)	Tonucci et al. (2017)
Observational	Spain	N/A	Yogurt consumption, in the context of a healthy dietary pattern, may reduce the risk of type 2 diabetes in healthy and older adults at high cardiovascular risk	Salvado et al. (2017)
Cross-sectional	Iran	1009 male and female	High-fat yogurt consumption was associated with a lower risk of high triglyceride concentration. Low-fat yogurt consumption was associated with a lower risk of abdominal adiposity and fasting plasma glucose	Falahi et al. (2016)
Observational	Canada	664 18–55 y	Yogurt consumption may be associated with lower anthropometric indicators and a more beneficial cardio-metabolic risk profile in overweight/obese individuals	Cormier et al. (2016)
FHS cohort study	USA	2636 male and female	Yogurt consumption for >15 years was associated with a lower risk of hypertension	Wang et al. (2015)
Observational	USA	N/A	Yogurt consumption as a part of a healthy diet may be beneficial in the prevention of CVD	Astrup et al. (2014)
Cohort study	USA	6526 Male and female	Yogurt consumption was inversely associated with all of the MetS components	Wang et al. (2013)
Meta-analysis of 6 prospective cohort studies	China	264,268 male and female	Dietary calcium intake with yogurt consumption was not independently associated with the risk of type 2 diabetes.	Dong and Qin (2012)
Birth cohort study (PASTURE)	5 European countries	856 children	The introduction of yogurt in the first year of life significantly reduced the risk of atopic dermatitis	Roduit et al. (2012)
Cross-sectional	USA	4,519male and female	Each serving of yogurt was associated with a 2–2.5 -fold reduction in the prevalence odds of obesity, central obesity, and MetS	Beydoun et al. (2008)
Epidemiological study	Japan	134 Juniors	Lactic acid bacteria and bifidobacteria intake involve in the regulation of nasal allergy development	Enomoto et al. (2006)

CVD, Cardiovascular diseases; *FHS*, Framingham Heart Study; *MetS*, Metabolic Syndrome; *N/A*, Not Available; *PASTURE*, Protection Against Allergy-Study in Rural Environments.

severity typically depends on the amount of lactose a person consumes (Walstra et al., 2005). There is strong clinical evidence that by consuming yogurt, there is a marked improvement in lactose utilization (Savaiano, 2014). During yogurt fermentation, the lactose content of milk is reduced by about 30% due to the breakdown of lactose by the beta-galactosidase activity of *S. thermophilus* and *L. bulgaricus* and the catabolism of glucose (and usually galactose as well) to lactic acid. The lactose content also varies with the duration of yogurt's cold storage and the postacidification activity of the starter cultures. Thoreux et al. (1998) have suggested that lactic acid bacteria (LAB) may induce lactase activity in the intestinal endothelial cells. Moreover, yogurt is tolerated better by people who are lactose-intolerant because of the presence of bacterial beta-galactosidase (Savaiano, 2014). The FAO, the World Health Organization (WHO), and the European Food Safety Authority (EFSA) recognize this effect at the regulatory level (EFSA, 2018; FAO/WHO, 2018).

5.2 Obesity control

Obesity is nowadays an increasing public health problem all over the world and one of the main factors of metabolic syndrome (MetS), responsible for heart disease, stroke, and type 2 diabetes (Zimmet & Shaw, 2005). The metabolic syndrome refers to the cooccurrence of several known cardiovascular risk factors, including insulin resistance, obesity, atherogenic dyslipidemia, and hypertension, and it is clear that diet and lifestyle are very significant factors in the prevention and treatment of this disease (Lee et al., 2020). Concerns about obesity are often the reason for limiting the consumption of milk products especially those made with full-fat milk. However, numerous observational studies in adults have shown that yogurt consumption has beneficial effects on obesity (Bridge et al., 2019; Panahi et al., 2019) and lipid metabolism (Cormier et al., 2016). Evidence from randomized controlled trials on obese individuals has indicated that calcium sourced from dairy products can promote weight and fat loss (Zemel et al., 2004). A cohort study conducted by Beydoun et al. (2008) showed that consumption of 100 g of yogurt per day was associated with a 2−2.5-fold decrease in the risk of obesity, waist circumference, and MetS prevalence. Recently, a meta-analysis study indicated that the risk of MetS was reduced by 18% with 100 g of yogurt added to the daily diet (Jin et al., 2018). In a cross-sectional study conducted on 973 adults in Iran by Falahi et al. (2016), the plasma high triglyceride level decreased by consumption of full-fat yogurt, while the risk of abdominal obesity and fasting blood glucose levels decreased by consumption of low-fat yogurt. Panahi et al. (2019) have shown that calcium in yogurt increases the inhibition of lipogenesis, lipolysis, lipid oxidation, and thermogenesis and thus affects adiposity. Moreover, calcium absorption is increased with the high acidity of yogurt, thus contributing to the decrease in obesity risk (Jeon et al., 2019).

5.3 Impact on cardiovascular diseases

Globally, cardiovascular diseases (CVD) is the foremost cause of mortality. It includes heart and blood vessel disorders and can arise from many risk factors, such as diabetes, hypertension, hyperlipidemia, etc. It is generally accepted that a healthy diet is substantial to preventing health problems. Almost 20 years ago, consumption of milk and dairy products was negatively correlated with CVD. Interestingly, according to WHO, consumption of dairy products should have been limited to minimize the intake of cholesterol and saturated fatty acids (SFA) to reduce the incidence of CVD (WHO, 2002). Nowadays, the point of view has changed. Research data obtained from clinical studies suggest that yogurt consumption as a part of a healthy diet may be beneficial in the prevention of CVD (Astrup, 2014a, 2014b). Because of the inherent calcium and potassium bioavailability, yogurt prevents high blood pressure, which is a high-risk factor for CVD. Results from a cohort study on 2636 men and women aged 28−62 years (part of the Framingham Heart Study) showed that yogurt consumption for >15 years was associated with a 6% lower risk of developing incident hypertension (Wang et al., 2015). Moreover, another study by Buendia et al. (2018) on a large cohort of 173,229 men and women aged 30−75 years with prevalent High Blood Pressure (HBP) has shown that yogurt intake was inversely associated with CVD risk (myocardial infarction and stroke). Furthermore, several clinical studies suggest that the consumption of yogurt has beneficial effects on cardiometabolic diseases and metabolic syndrome-related to the protein composition, and the emergence of bioactive peptides during the metabolic activity of LAB (Lanchais et al., 2020). According to Salvado et al. (2017), the bioactive peptides in yogurt are responsible for the stimulation of the gastrointestinal hormone insulin and incretin (GLP-1) release and consequently reduced energy intake. Also, probiotics used as adjunct cultures in yogurt help to partially reduce cholesterol levels circulating in the blood. Several studies have indicated a modest lowering of serum cholesterol in subjects consuming milk fermented with probiotic strains of *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus,* and yogurt cultures (Nami et al., 2019; Park et al., 2018; Yadav et al., 2019). Beltran-Barrientos et al. (2016) reported that milk fermented with *L. helveticus* contributes to blood pressure and heart rate lowering, which is ascribed to the bioactive peptides generated by this culture. Moreover, according to a crossover study by Olmedilla-Alonso et al. (2017), who studied the lipid profile of subjects consuming yogurt made from semiskimmed and whole sheep and cow milk, no alteration to plasma lipoprotein cholesterol levels was observed for all yogurt types in apparently healthy individuals.

5.4 Impact on type 2 diabetes (T2D)

Type 2 diabetes (T2D) is a chronic metabolic disease characterized by hyperglycemia resulting in increased blood glucose levels. According to the American Diabetes Association, yogurt is recommended as part of healthy nutrition in essentially managing T2D and T2D complications (American Diabetes Association, 2019). It is well known that yogurt modulates the host intestinal microbiota, resulting in the reduction of low-grade gut inflammation, weight gain, and risk of insulin resistance (Chen et al., 2014). It has been also suggested that calcium is essential in insulin secretion and action (Dong & Qin, 2012). On the other hand, there have been several randomized controlled trials evaluating the effects of probiotic yogurt on the glycemic index in patients with T2D; however, these trials have produced contradictory results (Ostadrahimi et al., 2015; Tonucci et al., 2017). At the same time, several reports have shown that yogurt consumption enhances the risk of insulin resistance in T2D owing to its fatty acid composition (Salvado et al., 2017). A recent animal study by Patil et al. (2021) demonstrated a positive correlation between consumption of yogurt and glycation of proteins leading to the higher production of Advanced Glycation End Products (AGEs), oxidative stress, and inflammatory markers, concluding that excess and continuous consumption of yogurt can lead to a prediabetic like a state. Therefore, the nutritional benefit of yogurt is offset by its role in protein glycation.

5.5 Atopic diseases

The immune system protects the body against environmental challenges and is divided into innate and adaptive immune systems. Yogurt consumption may enhance immune response and reduce infectious disease risk. The components of yogurt that may be involved in enhancing immunity include zinc, vitamin B_6, protein, and bacteria, as evidenced by human and animal studies (Fox et al., 2015).

Atopic diseases (e.g., dermatitis, rhinitis, asthma) arise from abnormal immune responses to allergens. Recent studies have shown a correlation between AD on the one hand and cardiovascular risk factors, CVD, and metabolic abnormalities on the other. This suggests that systemic inflammation in AD may contribute to the development of these comorbidities over time (Brunner et al., 2017).

According to Van de Water et al. (1999), the consumption of 200 g of yogurt/day was associated with reduced nasal allergies. Similar results were obtained from an epidemiological study in Japanese students having nasal allergies (Enomoto et al., 2006). The birth cohort study “Protection Against Allergy—Study in Rural Environments” (PASTURE) in five European countries (Austria, Finland, France, Germany, and Switzerland) showed that the introduction of yogurt in the first year of life significantly reduced the risk for atopic dermatitis (Roduit et al., 2012).

6. Functional applications of yogurt

Besides the potential health benefits of conventional yogurt, consumers’ preferences for functional foods are driving the dairy industry to produce fortified dairy products with various phytochemicals, and dietary fibers, providing specific health benefits, such as antioxidant, anti-inflammatory, and antimicrobial activities (Fardet et al., 2017; Kaczmarczyk et al., 2012). Yogurt is the prominent dairy product for the development of functional foods due to its nutritional and physicochemical properties that make it the most suitable carrier for probiotics and bioactive compounds.

Phytochemicals, recognized for their health potential, include phenolic compounds (i.e., flavonoids, phenolic, phytoestrogens), carotenoids, phytosterols, phytostanols, and plant organosulfur compounds (Rodriguez et al., 2006; Saxena et al., 2013). Phytochemicals can be introduced into yogurt in the form of essential oils (tea oil, lemongrass oil, cinnamon oil, peppermint oil, etc.), plant extracts, and fruits. Adding basil or peppermint essential oil to probiotic yogurt formulation can improve its functionality and provide an inhibitory effect against *Listeria monocytogenes* and *Escherichia coli* (Azizkhani & Tooryan, 2016). Besides, fruits are a potential source of phenolic compounds, vitamins, bioactive compounds, and minerals, contributing also to enhanced probiotic cultures viability in yogurt (Bara & Ozcan, 2017; Nualkaekul & Charalampopoulos, 2011). In recent years, growing demand for fermented milk enriched with fruits may be an alternative way to deliver probiotic bacteria (Bara & Ozcan, 2017).

Other plant compounds recognized for their beneficial effects on consumers’ health are phytosterols. Yogurt fortified with phytosterols (free and esterified sterols and stanols) has been reported to decrease total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-cholesterol) in hyperlipidemic subjects due to their ability to reduce intestinal cholesterol absorption (Ferguson et al., 2016; Rocha et al., 2016; Soto-Méndez et al., 2019). One of the most important essential nutrients in dietary patterns is omega-3 fatty acids (omega-3FA) because their regular intake prevents the prevalence of a large number of diseases (e.g., CVD, hypertension, diabetes, arthritis, and some cancer types) or inflammatory and autoimmune disorders (Asbell et al., 2018; Dawczynski et al., 2013; Gutierrez-Delgado et al., 2019; Gutierrez- Miura et al., 2019), in a randomized placebo-controlled intervention with n-3 long-chain polyunsaturated fatty acids-supplemented yogurt, concluded that omega-3 FA fortification of yogurt is a simple method of increasing omega-3 FA content of plasma lipids, which can help to reduce inflammation and may improve cardiovascular health in people with hypertriglyceridemia. Moreover, dairy products fortified with omega-3 FAs have been reported to improve cardiovascular risk factors in adults (Astrup, 2014a, 2014b; Eilat-Adar et al., 2013; Soto-Méndez et al., 2019).

Fortifying yogurt or dairy products with dietary fibers is of increasing interest toward the development of functional foods with health benefits and increased functionality. Moreover, fibers from various plant sources are added to yogurt because of their water-holding capacity, their ability to increase the yogurt production yield, and improve its textural properties and structure (Larrauri, 1999; Luana et al., 2014). Consumption of high-fiber yogurt may prevent or reduce hypercholesterolemia, hyperlipidemia, hypertension, coronary artery disease, diabetes, cancer, gastrointestinal disorders, constipation, and ulcerative colitis, but also promote the intestinal microbiota balance and gastrointestinal immunity (Dello Staffolo et al., 2017; Tomic et al., 2017).

Finally, yogurt is considered the most suitable food to be fortified with vitamin D, given that its usual vitamin D content is low, ranging between 2 and 3 μg per 100 g (USDA, 2014) providing about 5% of the recommended dietary allowance (RDA), respectively, per 250 g (FAO, Food Balance Sheets, 2010). Vitamin D is necessary for proper skeletal development and plays a fundamental role in regulating serum calcium and phosphorus concentrations in the body (Dawson-Hughes et al., 2010). In particular, vitamin D-fortified yogurt would be beneficial to populations with limited exposure to sunlight that reduces vitamin D content, especially in the winter months. Yogurt fortification with vitamin D could be a suitable strategy to reduce the prevalence of vitamin D deficiency and consequently improve the serum lipid profile in hyperlipidemic individuals (Mousavi et al., 2011). Gasparri et al. (2019) in a systematic review and meta-analysis of nine randomized trials showed that blood levels of vitamin D increased in people consuming vitamin D-fortified yogurt. Compared with the "unfortified yogurt" group, people who consumed vitamin D-fortified yogurt lost more weight and had healthier blood fat levels. They also appeared to have better blood glucose control, suggesting that vitamin D fortified yogurt might be associated with reducing the risk of diabetes.

7. Conclusions

Yogurt is one of the most popular fermented milk products worldwide and has gained widespread consumer acceptance as a healthy food. It provides an array of nutrients in significant amounts, concerning its energy, protein, and fat content, making it a nutrient-dense food. Yogurt provides bioavailable proteins of high quality and contains all nine essential amino acids necessary to maintain good health. Besides the standard nutritional components originating from milk, yogurt confers a variety of bioactive components, through the protein and fat degradation by the starters during the fermentation process. The minerals, in particular calcium, and vitamins found in yogurt are highly bioavailable. Furthermore, yogurt may confer health benefits beyond the basic nutrition it provides, such as improved lactose tolerance, regulation of body weight and fat loss, attenuation of CVD and MetS as well as T2D and its complications. Moreover, yogurt prepared with probiotic bacteria balances the host GIT microbiota and facilitates further probiotic actions. Finally, the relationship between yogurt and well-being is further enhanced with the incorporation of bioactive compounds in yogurt varieties to act as functional components for health maintenance. Yogurt fortification, with several micronutrients, is an important tool to improve nutrient intake, especially for young children and the elderly, thus preventing nutrition-related diseases.

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Chapter 8

Cheese and cardiovascular diseases

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1. Cardiovascular diseases

Cardiovascular diseases (CVD) is a group of disorders of the heart and blood vessels that include; Coronary Heart Disease, which refers to disorders of the blood vessels supplying the heart muscle, Cerebrovascular Disease, which refers to the blood vessels that supply the brain, and Peripheral Arterial Disease, which involves the blood vessels supplying the arms and legs, and Deep Vein Thrombosis, which involves blood clots, often in the leg veins dislodging and moving to the heart. Some of the main risk factors for developing CVD include tobacco use, unhealthy diet, obesity, physical inactivity, elevated blood pressure, high level and poor ratio of cholesterol (High-Density Lipoprotein-cholesterol (HDL-C)/ Low-Density Lipoprotein-cholesterol (LDL-C)) and diabetes; "Life's Simple 7" as described by the American Heart Association (Virani et al., 2021).

The prevalence of chronic diseases, which include CVD is increasing globally and CVD is now the number one cause of death worldwide (Alwan, 2011). World Health Organization (WHO) data indicate that up to 17.9 million people died from CVD in 2019, representing 32% of all global deaths of which 85% were due to heart attack or stroke (WHO, 2021). Heart attacks and strokes are acute events and are mainly caused by a blockage that prevents blood from flowing to the heart or brain. The most common reason for this is a build-up of fatty deposits on the inner walls of the blood vessels that supply the heart or brain.

Due to the role of diet and nutrition in the development and control of CVD, there is increasing interest in the identification of functional foods that support cardiovascular health. Much interest is directed toward fermented foods and fermented dairy foods in particular as there is much conflicting information on both their advantages and disadvantages from a general health and CVD perspective.

2. Cheese, a fermented food of ancient origin

Fermented foods have a long history of consumption as demonstrated by the archaeological record dating to 7000 BCE (McGovern et al., 2004) but it is likely that inadvertent production and consumption of fermented foods significantly predate this whereby foods underwent spontaneous fermentation during storage (Tamang et al., 2020). There is increasing interest in the potential health benefits associated with fermented food consumption (Marco et al., 2017). Some researchers have argued that as a consequence of their long history as part of the human diet, and their established and emerging health benefits that they should be included in food consumption guidelines (Chilton et al., 2015). Cheese is a fermented food made from milk of a number of mammal species including cows, sheep, goats, and buffalo through the action of microorganisms and/or enzymes.

Rudimentary processes to convert milk to cheese by fermentation had emerged by 6500 BCE, the key benefit of which was extending the shelf life of milk and thus, providing a mechanism to save food from a time of plenty to one of relative scarcity. Cheese making had the additional advantages of (i) concentrating the fat, protein, and some minerals and vitamins of the milk thus; producing an energy-dense, nutritious food, and (ii) removal of lactose making the cheese accessible to adults who at that time were mostly lactose intolerant and could not consume milk post infancy (Kindstedt, 2012). While there is a vast variety of cheeses available today, all are made using combinations of the same four ingredients; milk, microbes, and sometimes the enzyme chymosin (rennet) or other protease and/or salt. These are processed through a number of common steps including acidification, gel formation, whey expulsion, salt

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addition, and a period of ripening (Fox et al., 2017a). Variations in ingredient blends and process parameters including variations in temperature, curd handling techniques, salting methodology, and ripening conditions have led to the evolution of the variety of cheeses available today. Commonly used cheese classification systems identify up to 18 primary cheese types; however, it is generally accepted that there are well in excess of 1000 cheese varieties available globally arising from changes to the making procedure (Beresford et al., 2001). This review will focus on natural casein-based cheeses (as opposed to whey-based or processed cheeses).

3. Basics of cheese manufacture

Milk is a complex mixture of proteins, fats, carbohydrates, minerals, and vitamins dispersed in water. The composition of this mixture varies depending on the species from which it is derived and will also vary within a species based on the stage of lactation, diet, and genetics of the individual animal (Roy et al., 2020). Cheese manufacture, at its simplest, could be considered a methodology to concentrate the dry matter, primarily protein and fat of milk through the removal of water. The total solids in milk, much of which is concentrated in cheese, range from 11 to 20 g 100 mL^{-1} among milk from cow, sheep, goat, and buffalo, with the protein content ranging from 3.0 to 7.0 g 100 mL^{-1} and fat ranging from 3.0 to 9.0 g 100 mL^{-1}. Milk from sheep tends to have the highest concentration of total solids (Roy et al., 2020).

A typical generic process flow diagram outlining the procedure for cheese manufacture is described in Fig. 8.1. Initially, milk for cheese manufacture is assembled, following which it may be heat treated and its composition manipulated in particular with respect to its fat content. The milk is then delivered into a cheese vat where for most cheese varieties starter cultures are added. The addition of starter culture initiates acidification, which continues throughout the manufacturing process and for some cheese varieties into the early stages of ripening. The liquid milk is then converted into a solid coagulum or gel either as a consequence of acidification, addition of protease enzymes (most typically chymosin), heat, or a combination of these. The gel is then cut and through the action of stirring and heating the process of syneresis is initiated whereby the curd particles contract and expel whey. The curds and whey are then separated and the curd particles are allowed to coalesce. The curd mass may then undergo different processes such as cheddaring or stretching, which facilitate continued acid production and expulsion of whey. The curds are formed into the typical shape of the particular cheese variety and salt may be added either directly to the curd particles, by immersion in brine, or by the addition of salt to the surface of the final cheese structure. The cheese may then be consumed fresh or ripened for a period of up to 1 year or more under controlled temperature and humidity conditions.

The extent of concentration of milk solids varies based on the making procedure and thus, differs for each cheese variety. However, most cheese types have moisture content in the range 35–50 g 100 g^{-1}, but with some outliers such as the fresh cheeses cottage cheese and fromage frais, which have moisture contents of 79 and 77 g 100 g^{-1} respectively while cheeses such as Parmesan, which is designed to have a long ripening period to facilitate the development of a rich variety of aroma compounds, has a moisture level of 18 g 100 g^{-1} (Table 8.1; O'Brien & O'Connor, 2017). The fat and to a lesser extent the protein content of cheese varies inversely with moisture content. The protein content is generally in the range 19–28 g 100 g^{-1}, while fat levels are in the range 23–34 g 100 g^{-1}. Protein levels in cottage cheese and fromage frais are generally in the region of 13.8 and 6.8 g 100 g^{-1} respectively, while fat levels are 3.9 and 7.1 g 100 g^{-1} respectively. Good examples of how the process impacts the composition are provided by cream cheese and reduced fat cheddar. In both of these cases, the milk composition is manipulated as part of the milk pretreatment process. In the case of cream cheese, the cheese milk is fortified with fat, while fat is removed for reduced fat cheddar cheese manufacture. The resulting cheese composition measurements reflect these changes with the fat content of cream cheese being in the region of 47.4 g 100 g^{-1} while that of reduced fat cheddar is 15.0 g 100 g^{-1}.

4. Manufacturing processes that impact the nutritional properties of cheese

Changes to the basic manufacturing process have led to >1000 cheese types manufactured globally today. Manipulation of these technological leavers results in cheeses of different compositions that follow different and diverse ripening pathways, that not only result in cheeses with very diverse techno-functional and sensory attributes but also offer the potential to impact the nutritional value and bio-functionality of the final product (Fig. 8.1). Indeed, this diversity is likely complicating the development of a consistent view with regard to the nutritional and health attributes of cheese.

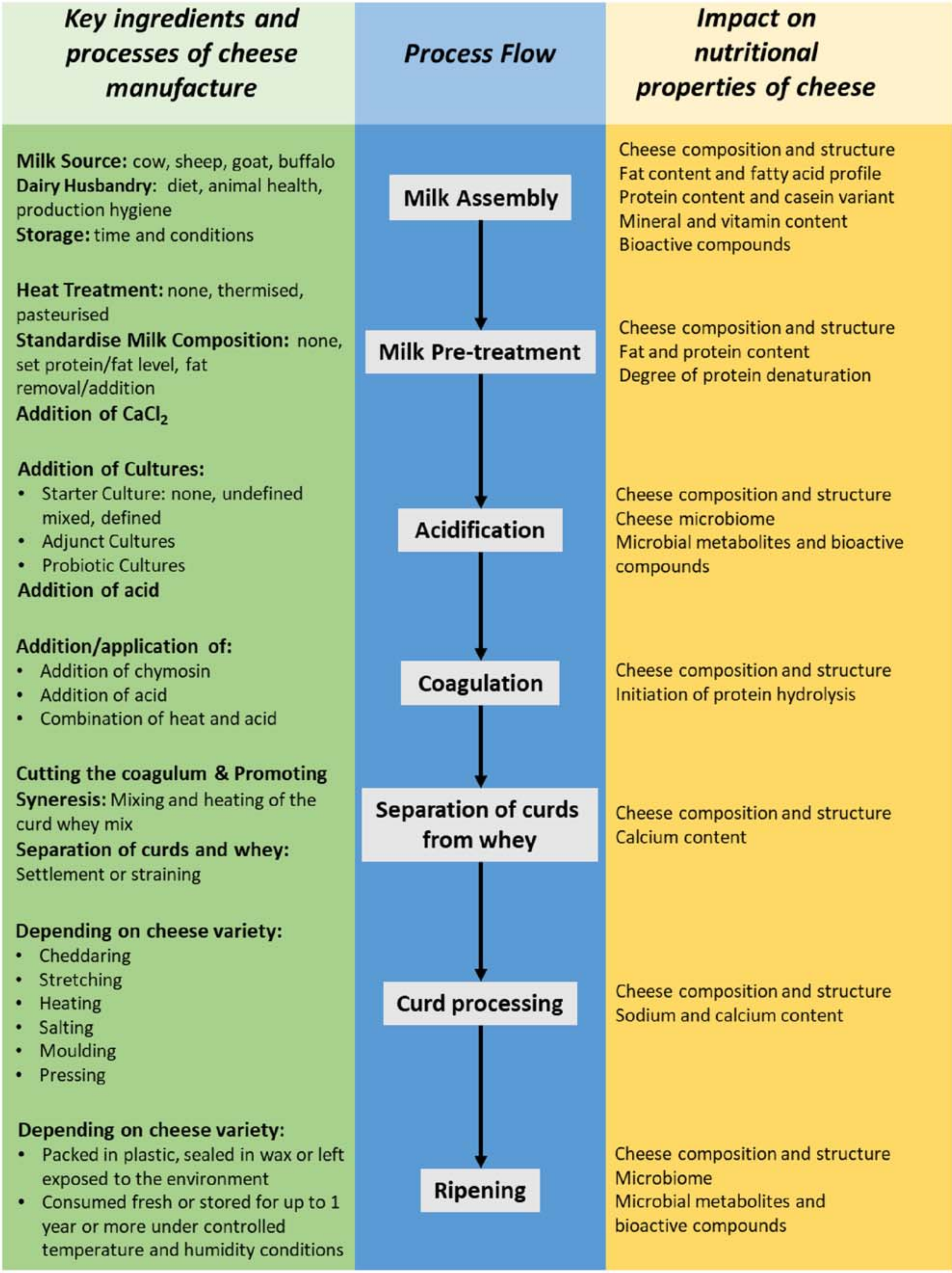

FIGURE 8.1 Generic process for the manufacture of natural casein-based cheeses, the key ingredients and processes involved in their manufacture, and impact of the various components of the cheese-making process on the nutritional properties of cheese.

TABLE 8.1 Composition and energy content of a selection of natural casein based cheeses, per 100g.

Cheese	Water (g)	Protein (g)	Fat (g)	Cholesterol (mg)	Energy (kcal)
Brie	48.6	19.3	26.9	100	319
Caerphilly	41.8	23.2	31.3	90	375
Camembert	50.7	20.9	23.7	75	297
Cheddar (normal)	36.0	25.5	34.4	100	412
Cheddar (reduced fat)	47.1	31.5	15.0	43	261
Cheshire	40.6	24.0	31.4	90	379
Cottage cheese	79.1	13.8	3.9	13	98
Cream cheese	45.5	3.1	47.4	95	439
Danish blue	45.3	20.1	29.6	75	347
Edam	43.8	26.0	25.4	80	333
Emmental	35.7	28.7	29.7	90	382
Feta	56.5	15.6	20.2	70	250
Fromage frais	77.9	6.8	7.1	25	113
Gouda	40.1	24.0	31.0	100	375
Gruyere	35.0	27.2	33.3	100	409
Mozzarella	49.8	25.1	21.0	65	289
Parmesan	18.4	39.4	32.7	100	452
Roquefort	41.3	19.7	32.9	90	375
Stilton	38.6	22.7	35.5	105	411

(Adapted from O'Brien, N. M., & O'Connor, T. P. (2017). Nutritional aspects of cheese. In P. L. H. McSweeney, P. F. Fox, P. D. Cotter & D. W. Everett (Eds.), Cheese; chemistry, physics and microbiology (4th ed.). Academic Press. ISBN: 9780124170179).

4.1 Milk assembly

Many factors from the farm to the processing plant affect milk quality. Animal health will impact the somatic cell count in the milk and the potential for contamination with antibiotics, hygienic practices during milk harvesting and transport, milk storage temperature, and duration of storage, will impact milk quality as it relates to cheese manufacture (Guinee & O'Brien, 2010); however, how most of these relate to the nutritional quality and associated health implications are not well studied. However, husbandry practices and species from which the milk is sourced as discussed above will significantly influence the milk composition (Roy et al., 2020). In addition to total solids; protein, fat, and micronutrient concentrations and diversity will vary between milk from different sources; which in turn leads to changes in its structure and physiochemical properties that will contribute to the techno- and bio-functional properties of the resulting cheese. While the protein content of cow, sheep, goat, and buffalo milk can range from 3.0 to 7.0 g 100 mL^{-1}, the composition of the protein can also vary. The casein content can range from 2.33 g 100 g^{-1} in goat milk to 5.26 g 100 g^{-1} in sheep milk, while the whey protein content ranges from 0.37 g 100 g^{-1} for goat milk to 1.6 g 100 g^{-1} in sheep milk. This has consequences for cheese manufacture as most whey protein is not incorporated into cheese unless it is in a denatured format usually arising as a consequence of heat treatment. Casein, which is the main protein in cheese is composed of α_{s1}-, α_{s2}-, β-, and κ-caseins. Again, the relative abundance of each of these molecules varies between milk from different species (Roy et al., 2020). The casein content and composition will not only influence the techno-functional characteristics of the cheese but will also impact the accumulation of peptides during cheese ripening, some of which have associated bioactivity that may be relevant for CVD as discussed below.

Similarly, the total fat content of milk used for cheese manufacture can range from 3.0 g 100 g^{-1} for goat milk to 9.0 g 100 g^{-1} for sheep and buffalo milk (Roy et al., 2020) and a recent review has highlighted very significant differences not only in overall fat content within a species but also the composition of the fat including different fatty acid profiles (Mollica et al., 2021). During cheese manufacture, most of the fat present in the milk is trapped within the cheese curd particles and thus, partitions with the cheese. Milk fat is primarily composed of triacylglycerols (~98%) with the remainder comprising of di- and mono-acylglycerols, phospholipids, cholesterol, and free fatty acids and is comprised of >400 fatty acids (Alothman et al., 2019; Devle et al., 2012). The fatty acids in cheese are generally ~66% saturated (SFA), 30% monounsaturated (MUFA), and 4% polyunsaturated (PUFA) (O'Brien & O'Connor, 2017). However, milk fat content and composition vary between species with cow milk containing up to 69.7% SFA compared to sheep and goat milk, which contain ~57.5% and 59.9%, respectively, while sheep milk contains up to 39.1% MUFA (Devle et al., 2012). Within a species, diet greatly influences the fat content and composition (O'Callaghan et al., 2016). It was demonstrated that cows fed on pasture, not only had significantly higher concentrations of fat in their milk but also that the fat content was changed with significantly higher concentrations of (i) the SFAs C11:0, C13:0, C15:0, C17:0, and C23:0, (ii) the unsaturated fatty acids C18:2n-6 trans, C18:3n-3, C20:1, and C20:4n-6 and (iii) a >2-fold increase in the conjugated linoleic acid C18:2 cis-9 trans-11 when compared to milk produced from cows fed indoors on mixed rations, while milk produced from cows on mixed ration had increased levels of C16:0, C18:2n-6 cis, C18:3n-6 cis, C22:0 C22:1n-9, and C18:2 cis-10 trans-12. Research from the same group demonstrated that cheese made from milk from pasture-based feeding systems had (i) significantly lower thrombogenicity index scores, which is a measure of the tendency of blood to clot as defined by the ratio of prothrombogenic (SFAs C14:0, C16:0, and C18:0) to antithrombogenetic fatty acids (MUFA n3 and PUFA n6), (ii) >2-fold increase in the concentration of vaccenic acid and the bioactive conjugated linoleic acid C18:2 cis-9 trans-11, while cheeses made from milk produced from a mixed ration feeding system had significantly higher palmitic acid content (O'Callaghan et al., 2017). A study using goat milk demonstrated that milk pasteurization and cheese processing technology resulting in the manufacture of four different cheese types using the same milk had a minimal impact on the polar lipid fatty acid composition of the resulting cheese. However, the fermentation pathways and development of a different microbiome in the four kinds of cheese over ripening lead to changes in the polar lipid fatty acid composition and improvements on antithrombotic properties with a soft cheese displaying the strongest antithrombotic effect (Lordan, Walsh, et al., 2019).

4.2 Milk pre-treatment

The main treatments that milk may receive before being converted to cheese are standardization of the fat to protein ratio and heat treatment such as pasteurization. In some situations, $CaCl_2$ may be added to aid coagulation (Panthi et al., 2017) or homogenization of the cheese milk in the case of cream cheese, which results in smaller milk fat globules. Milk standardization is undertaken to enhance the efficiency of manufacture and to influence the final properties of the cheese as in the case of reduced fat variants. The conventional methodology used is to remove a portion of the fat by centrifugation or to add cream to achieve the desired fat to protein ratio. In some situations, the protein content of the milk may also be standardized by ultrafiltration. The most common heat treatment for cheese milk is pasteurization, which occurs at 72−74 °C for 15s. This kills pathogenic bacteria that may be present in raw milk but it also leads to significant killing of other bacteria in the milk that many consider important for ripening and flavor development. This heat treatment was established with a view to killing pathogenic bacteria but it also transpired that this level of treatment had minimal impact on other cheese-making properties of the milk. If heat treatments in excess of this are applied to the milk it leads to denaturation of whey proteins, which then attach to the casein and get incorporated into the cheese (Rynne et al., 2004). Higher heat treatments are used in the preparation of the milk for some cheese types, for example, 85 °C for 5 min for Queso Blanco and other heat-acid coagulated cheeses as the high heat treatment helps to destabilize casein micelles, which promote coagulation. Higher heat treatments may also be used in the manufacture of low-fat cheese with the objective of retaining more moisture within the cheese matrix leading to improved texture attributes (Rynne et al., 2004). While there is an argument that reduced-fat cheeses may be more beneficial from a CVD perspective, there are few studies linking the cheese microbiome to CVD at this time; however, there is an ongoing debate about the pros and cons of pasteurization and its impact on the microbiome of cheese most of these arguments focus on cheese sensory quality attributes.

4.3 Acidification

For most cheese varieties, acidification results from the growth of lactic acid bacteria that are either naturally present in the cheese milk or are added as starter cultures at the beginning of the manufacturing process (Parente et al., 2017). These bacteria metabolize lactose present in milk and produce lactic acid as an end product. For such cheeses, acidification is ongoing throughout the cheese manufacturing process and in some cases also during the early days of ripening and only terminates when bacterial metabolism is inhibited due to low pH arising from the accumulation of lactic acid or high salt in moisture levels as a result of NaCl addition toward the end of the manufacturing process. For some cheeses such as Paneer and Queso Blanco acidification is brought about by adding acid (citric, acetic, or lactic) directly to the milk (Farkye, 2017, chap. 44). The method of acidification will impact cheese composition and the nature of the cheese matrix, both of which will influence the nutritional value and digestibility of the cheese during gastrointestinal transit (Feeney et al., 2021).

4.4 Coagulation

Casein, which comprises ~80% of the protein in milk is found in colloidal aggregates known as casein micelles. The surface of these micelles is partly composed of κ-casein, which stabilizes the individual micelles and prevents them from aggregating and precipitating out of solution. During cheese making the micelles are destabilized which allows them to aggregate to form a protein network/gel within which are embedded milk fat droplets and water (Dalgleish & Corredig, 2012). Destabilization of the micelle is achieved either by chymosin addition, acidification, heat, or a combination of acidification and heat. The approach used depends on the type of cheese being manufactured and it will impact the structure and techno-functional characteristics of the gel formed. This will in turn impact the cheese composition and influence its digestion characteristics during gastrointestinal transit. In addition, for cheeses manufactured using chymosin, this enzyme initiates casein hydrolysis, which is subsequently complimented by bacterial protease and peptidase enzymes that continue throughout ripening leading to the release of a multitude of peptides some of which have been shown to have associated bioactivity that could impact on CVD (see below).

4.5 Separation of curds from whey

A key feature of all cheese types is that they involve a concentration of milk solids and thus, removal of water. This is achieved subsequent to coagulation either by (i) mechanical separation by filtering or centrifugation as in the case of acid/heat coagulated cheese varieties such as cream cheese, cottage cheese, and quarg or (ii) by exploiting syneresis in the case of chymosin coagulated cheeses. Syneresis is a natural process by which cymosin-coagulated milk gels expel moisture when the gel is destabilized. Destabilization is brought about by initially cutting the gel, followed by stirring the developing curd (gel particles) and whey (liquid) mixture. Contraction of the curd particles is boosted by increasing the temperature (often referred to as cooking) of the curd whey mix and by acid production due to the activity of the starter bacteria (Fox et al., 2017b). Once the desired level of acid production is achieved the curd and whey mix is separated mechanically by filtration or settlement and the individual curd particles are allowed to fuse to form a semisolid structure. The degree to which the gel is cut, the rate of stirring, the extent of cooking, and the level of acid production will all influence the rate and degree of syneresis and thus, impact cheese composition and microstructure.

4.6 Curd processing

While acid/heat coagulated cheese curds undergo little additional manipulation once separated from the whey, significant additional manipulations take place with most chymosin coagulated cheeses and this processing is often very influential in determining the final structure and techno-functional characteristics of the cheese. During the manufacture of some varieties, the curd particles are "washed" via the addition of warm water, which helps to remove residual lactose in the curd particles and thus, decrease acid production by the starter bacteria. Cheddar undergoes the "cheddering" process while pasta-filata-type cheeses undergo hot-water stretching. Salt is added toward the end of the manufacturing process either as dry salt added directly to the curd, by immersing the molded cheeses in brine, or by smearing dry salt directly to the surface of the molded cheese (Fox et al., 2017a). These steps influence the final composition of the cheese but also help to control the amount of calcium remaining in the cheese and the level of sodium added in the form of NaCl, which has obvious health implications.

4.7 Ripening

While some cheese varieties are consumed fresh in the days post-manufacture, most undergo a period of ripening that can vary from as little as 2 weeks for cheeses such as mozzarella to 2 years or more for Parmigiano Reggiano, and extra mature varieties of cheddar, Swiss- and Dutch-type cheeses (McSweeney, 2017). During this time the cheeses are maintained under controlled temperature and for some varieties humidity conditions. Significant changes to the cheese microbiome occur that involve the starter bacteria entering stationary phase growth and in some cases autolyzing and releasing their intercellular enzymes, growth of secondary microbes including propionic acid bacteria (PAB) in Swiss-type cheeses, development of the complex populations of molds, bacteria and yeast that develop in mold and surface smear-ripened cheeses and growth of nonstarter lactic acid bacteria (NSLAB), which grow internally within the cheese matrix during ripening (Cotter & Beresford, 2017). The combination of microbial metabolic activity and enzyme activities from the milk and chymosin retained in the curd or released from autolyzing bacteria result in the accumulation of a wide variety of metabolites that are responsible for the development of the sensory and techno-functional characteristics of the different cheese types. This metabolic activity also provides an opportunity for the development of an array of molecules including vitamins, bioactive peptides, and products of amino acid metabolism that may have bio-functional properties including the potential to positively impact CVD. Indeed, studies on the cheese metabolome and its relationship with the microbiome and cheese quality are an area of ongoing development and likely to reveal new insights into the nutritional and health benefits of cheese over the coming years (Afshati et al., 2020).

There is also a growing interest in the potential of the cheese microbiome to directly impact the health of the consumer by either modulating the gut microbiome or by their metabolic activity within the gastrointestinal tract. To achieve this they need to be able to survive gastric transit. In this regard, there is particular interest in the NSLAB population as they contain species, members of which are often described as probiotic. Studies have demonstrated that the cheese matrix provides protection to probiotic strains deliberately added to cheese during manufacture when they encounter gastrointestinal conditions (Gomes da Cruz et al., 2009). A recent study has indicated that up to 10^7 CFU/g of cheese of endogenous NSLAB could be isolated from cheddar cheeses exposed to simulated gastric conditions and that ~40% of the strains investigated expressed bile salts hydrolase activity, which has been associated with cholesterol-lowering capabilities (Leeuwendaal et al., 2021), suggesting that this may be a mechanism by which cheese could positively impact on CVD.

5. Nutritional value of cheese in relation to cardiovascular diseases

5.1 Energy

In general, dietary guidelines recommend a daily energy intake of 2000 and 2600 kcal for women and men respectively (EFSA 2013). The energy available in the cheeses described in Table 8.1 in general ranges from 300 to 400 kcal 100 g^{-1}, indicating that consuming a typical 50g portion will provide 7.5%−10% of the daily energy requirements for women and 5.7%−7.7% of the requirements for men. While cheese is a rich source of nutrients, minerals, and potential bioactive molecules, its overall contribution to energy intake needs to be taken into consideration when including it as part of a well-balanced nutritional diet. As would be expected, there is a direct correlation between energy value and fat content, while an inverse correlation is observed with water content (Fig. 8.2). It is well established that high energy intake, in particular in the absence of equivalent energy expenditure increases the incidences of obesity (Hall, 2018) and that consumption of energy-dense foods is associated with higher incidences of obesity and CVD (Teo et al., 2021). Thus, the consumer that wants to include cheese in their diet to satisfy nutritional needs in addition to enjoying its eating and culinary characteristics but wishing to limit calorie intake could opt for some of the lower fat higher moisture varieties or include the higher energy cheeses in balance with the overall energy value of their diet.

It is interesting to note that Parmesan has the highest energy value, which is consistent with it having the lowest water content and consequently the highest combined fat plus protein content, while cottage cheese has the lowest energy value while having the lowest fat content and highest water content of the cheeses listed in Table 8.1. Partly in an effort to address the energy density of conventional cheeses, much research has focused on producing reduced fat variants. However, fat plays a significant role in the flavor, texture, and rheological characteristics of cheese, and thus, developing reduced fat variants that fully satisfy consumer demands has proven difficult (Costa et al., 2010; Mistry, 2001). Nevertheless, reducing the fat content of cheddar from 34.4 to 15 g 100 g^{-1} decreases the energy content from 412 to 261 kcal 100 g^{-1} representing a 37% reduction in energy intake.

5.2 Protein

Cheese is a relatively high protein food as can be seen from Table 8.1 with most cheeses having protein contents between 19 and 27 g 100 g^{-1} with the fresh cheeses such as cottage and fromage frais having lower contents with Parmesan being higher. During cheese manufacture, micellar casein is incorporated into the cheese matrix while most of the water-soluble whey proteins partition with the whey. Hydrolysis of the casein commences during cheese manufacture and continues during ripening (McSweeney, 2004). Recent research has demonstrated that the cheese protein matrix is degraded during the intestinal stage of digestion (Žolnere et al., 2019) where the resulting amino acids are absorbed and that casein is highly digestible with a protein digestibility corrected amino acid score (PDCAAS) of 1 (Schaafsma, 2000). In addition the amino acid profile of caseins from species used to make cheese demonstrates that cheese is a good source of amino acids

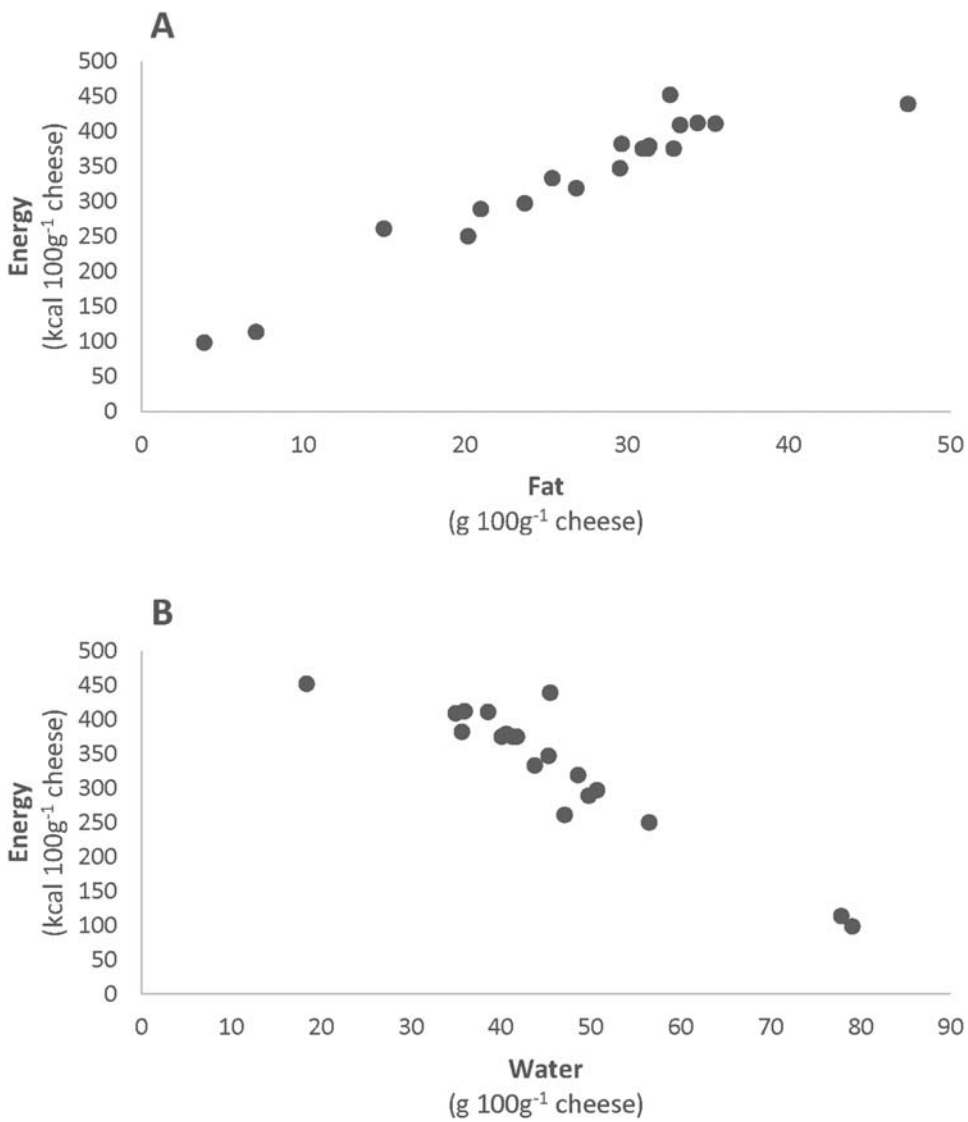

FIGURE 8.2 Relationships between (A) energy and fat and (B) energy and water contents of cheeses from Table 8.1.

including essential amino acids (Rafiq et al., 2016). These observations demonstrate that cheese is a good source of protein. For adults of both sexes, the average requirement (AR) for protein (the amount of protein required to maintain a nitrogen balance) is 0.66 g protein/kg body weight per day (EFSA 2012), which equates to approximately 50 and 40g for men and women respectively, suggesting that a 50g portion of cheddar cheeses would supply 25% and 32% of the protein needs for men and women respectively. However, the average protein intake in absolute amounts within the EU range from approximately 67 to 114 g/d in men and from 59 to 102 g/d in women indicating that a portion of cheddar cheese could provide between 11% and 22% of this daily intake.

There are many studies in the literature linking diet and amount and source of protein to CVD; however, studies on direct relationships between protein and CVD are difficult to find. Nevertheless, many epidemiological studies indicate that increased protein intake is associated with lower blood pressure and an attenuated increase in blood pressure over time (Appel, 2003). A mechanism by which protein could exert such beneficial effects may be through biologically active peptides.

5.3 Fat

Fat has an important role in determining the texture and flavor of cheese, either directly or as a consequence of fat metabolism during manufacture and ripening (McSweeney, 2017). The fat content for most cheese varieties ranges from

$\sim$20 g 100 g^{-1} for Feta to $\sim$35 g 100 g^{-1} for Stilton (Table 8.1). The fat content of the fresh cheeses, fromage frais, and cottage cheese are considerably lower at $\sim$7.1 and 3.9 g 100 g^{-1} respectively, while that of cream cheese is higher at $\sim$47 g 100 g^{-1}. As discussed above, there is a direct correlation between energy and fat content and this needs to be considered by consumers wishing to control their energy intake. Fat contains 9 kcal g^{-1} while carbohydrate and protein contain 4 kcal g^{-1}, making fat more than twice as energy dense. Thus, the fat in a typical 50g portion of cheese will provide from $\sim$90 to 160 kcal. A portion of cheddar contains 17.2 g fat, which supplies 154.8 kcal, which equates to 7.7% and 6.0% of the daily energy requirements of women and men respectively.

The lipid hypothesis (also known as the cholesterol hypothesis) postulates a link between blood serum cholesterol levels and the occurrence of CVD, and as a consequence actions (including medical interventions and diet) to reduce blood serum cholesterol levels, in particular, LDL-C has become the foundation for CVD prevention guidelines (Lordan, Tsoupras, & Zabetakis, 2019). However, cholesterol is a critical component in cellular membranes, is a precursor for all steroids in the body including corticosteroids, sex hormones, bile acids, and vitamin D and plays a role in a number of cell-to-cell signaling pathways (Brown & Sharpe, 2016). Cholesterol can be obtained from the diet but *de novo* synthesis from acetyl-CoA in many tissues throughout the body is a major source (Gu & Yin, 2020; Mayes & Botham, 2003). Cholesterol synthesis is a high-energy consuming process that is highly regulated within individual cells. Thus, the synthesis and metabolism of cholesterol are tightly controlled by a number of genetic and environmental factors including an individual's age, sex, and efficiency of cholesterol absorption and control of cholesterol syntheses (Lecerf & De Lorgeril, 2011).

Much research has indicated that SFAs have the potential to increase cholesterol synthesis leading to increased levels of blood serum cholesterol (Gu & Yin, 2020). The exact mechanism by which SFA promotes cholesterol synthesis has not been fully determined; however, the intracellular cholesterol level is the main factor that modulates the cholesterol synthesis pathway. It is proposed that SFAs suppress endocytosis/transport of cholesterol (LDL-C) into the cell leading to a reduction in intracellular cholesterol and blocking cholesterol movement within the cell from the plasma membrane to the endoplasmic reticulum (where sterol sensors involved in regulating cholesterol metabolism are located); which combine to convince the cell that cholesterol levels are low and thus, promoting new *de novo* synthesis. However, not all SFAs have been implicated in increasing cholesterol and it is thought that short-chain SFAs with chain lengths of 4−10 have little effect the cholesterol synthesis, while in contrast, long-chain SFAs such as C12:0 (lauric acid), C14:0 (myristic acid) and C16:0 (palmitic acid) promote cholesterol synthesis and thus, elevate blood serum cholesterol (Gu & Yin, 2020). While each of C12:0, C14:0, and C16:0 lead to increases in both total and LDL-C blood cholesterol, C18:0 (stearic acid) does not. In addition, SFA also increases the levels of blood HDL-C (which is often referred to as "good cholesterol") and the level of increase is greater as the fatty acid chain length decreases. The consequences of this, are that C12:0 changes the LDL-C/HDL-C ratio in favor of HDL-C implying that C12:0 may in fact be beneficial from an overall cholesterol perspective (Mensink et al., 2003). While no statistical differences were observed in the levels of C12:0 and C14:0 in cheeses made from milk from cows on either total mixed rations, grass, or grass/clover diets, the level of C16:0 was significantly higher in cheeses made from milk from the total mixed rations diets. Whether this increase in C16:0 may be sufficient to impact blood serum cholesterol levels was not determined (O'Callaghan et al., 2017). While there continues to be much discussion on the potential of SFAs to influence the development of CVD, current dietary guidelines recommend that daily intake should be as low as possible, not exceeding more than 10% of the total energy intake and that SFA be replaced with poly- and mono-unsaturated fatty acids (McGuire, 2016; WHO, 2018). However, the energy intake in the form of SFA in most Western diets exceeds this, with $\sim$14% of energy coming from SFA in the Irish diet and $\sim$12% in the U.K (Bates et al., 2014; Tierney et al., 2011). As indicated above, with 66% of the fatty acids in cheese being in the SFA form, cheese is potentially a significant contributor to SFA intake in most western diets, where dairy, in general, can provide up to 20% of the daily SFA intake with cheese specifically accounting for 8.3% of the SFA intake in the Irish diet (Feeney et al., 2016). However, emerging information suggests that the relationship between SFA and CVD is quite complex and that the source of the SFA is of particular importance. A number of recent meta-analyses indicate that while SFAs from meat are associated with detrimental health effects, SFAs from dairy are associated with neutral or even positive health effects (Timon et al., 2020). There is an emerging view that SFAs are not a homogenous group and that different SFAs can exert different health effects. To further complicate the situation, while the dietary source and makeup of the SFA diversity may lead to changes in LDL-C levels, the actual particle size of the LDL-C is a much greater indicator of CVD risk, with small LDL-C particles being much more atherogenic than large LDL-C particles (Astrup et al., 2020). The authors noted that dietary restriction does not result in a decrease in small LDL-C particles in most individuals and could lead to disproportionately large reductions of large LDL-C, which are much less associated with CVD risk.

The basis of the lipid hypothesis is that elevated levels of cholesterol in the blood lead to atherosclerosis, which is manifested by the formation of abnormalities or lesions on the walls of arteries. Atheromatous plaque, made up of fat, cholesterol, calcium, and other substances from blood accumulates in these lesions resulting in a narrowing of the diameter of the artery; thus, impeding blood flow, leading to depletion of oxygen in the parts of the body being serviced by the artery concerned, including the heart. While the exact cause of atherosclerosis is unknown, elevated blood cholesterol, in particular, LDL-C; obesity, diabetes, inflammation, elevated blood pressure, smoking, diet, and family history are all recognized risk factors (Mensah et al., 2017). As a consequence, fatty foods that contain high levels of cholesterol and/or SFAs have been the focus of much research and discussion, with many commentators arguing that such foods should be avoided or limited as part of a healthy diet. However, despite much research, the contribution of dietary cholesterol is difficult to define, with a recent systematic review and meta-analysis not being able to demonstrate a statistically significant association to CVD; while acknowledging that many of the studies included in the analysis were heterogeneous and lacked methodological rigor; thus making definitive conclusion difficult (Berger et al., 2015), with other studies concluding that not all of the evidence supports the hypothesis linking dietary cholesterol to CVD and that it should be reappraised (Dehghan et al., 2018; DuBroff, 2018; Lordan, Tsoupras, & Zabetakis, 2019). Indeed, a number of recently issued guidelines for reduced risk of CVD from the American Heart Association (AHA) (Eckel et al. 2013), the American College of Cardiology (Grundy et al., 2018) and the 2015−20 Dietary Guidelines for America (US Department of Health and Human Services, 2015) have not included explicit guidance for dietary cholesterol and the AHA has recently issued an advisory that dietary guidance should instead focus on healthy dietary patterns that emphasize fruit, vegetables, whole grains, low-fat or fat-free dairy products and lean protein sources including nuts, seeds and liquid vegetable oils (Carson et al., 2019).

The cholesterol content of cheese is directly correlated with its fat content (Fig. 8.3) and ranges from ~65 g 100 g^{-1} in mozzarella cheese to ~105 g 100 g^{-1} in Stilton (Table 8.1). The levels in fromage frais and cottage cheese are lower at ~25 and ~13 g 100 g^{-1} respectively and cholesterol levels in reduced fat cheddar cheese are also lower at ~43 g 100 g^{-1} as a consequence of milk fat removal during the preparation stage of the cheese milk. While, as discussed above there is a move toward advising consumers on an overall healthy diet rather than focusing directly on cholesterol levels, previous advice was to maintain cholesterol consumption below 300 mg per day (Krauss et al., 2001). In this scenario, consuming a 50g portion of cheese would supply between 11% and 18% of the proposed daily dietary intake of cholesterol, with a portion of cheddar providing 16.7% of the recommended amount.

In addition to the direct implications of cholesterol, oxysterols produced by enzymatic and/or chemical oxidation of cholesterol may be present in foods. During processing and storage, in particular following exposure to high temperature and/or air, oxysterols can be formed in foods, including milk containing cholesterol by chemical oxidation (Risso et al., 2021). Oxysterols have been implicated in the formation of fatty deposits in arteries leading to atherosclerosis. However, there is little evidence of significant accumulation of oxysterols in most cheese varieties, probably due to the relatively low temperatures and mild processing conditions used during manufacture (Cilliers et al., 2014).

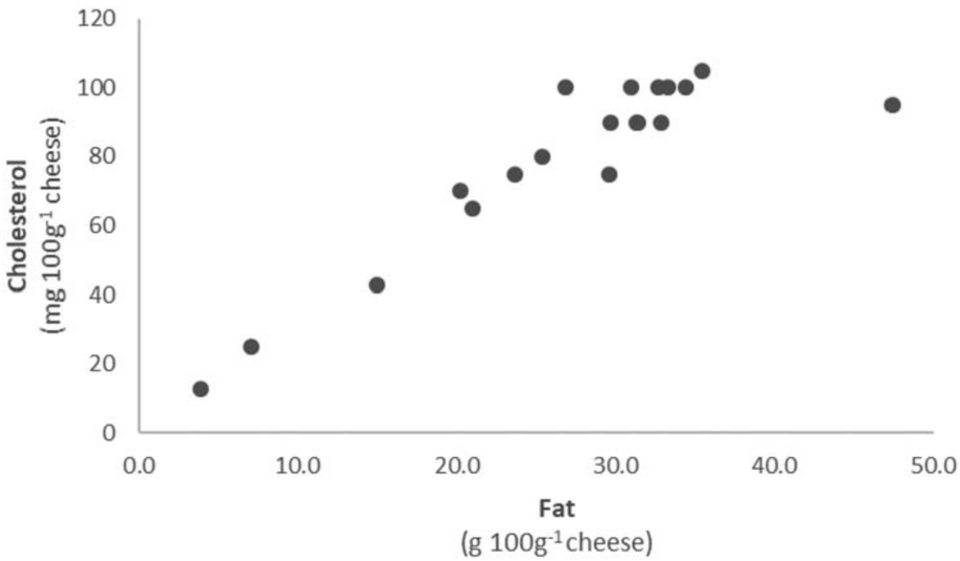

FIGURE 8.3 Relationships between cholesterol and fat contents of cheeses from Table 8.1.

5.4 Carbohydrate

Most of the milk sugar, lactose, partitions with the whey during cheese manufacture, and what remains is metabolized by the starter and NSLAB during the early days of ripening resulting in most cheeses having no or only trace amounts of lactose (O'Brien & O'Connor, 2017). This is significant from a nutrition and health perspective as cheese can thus be consumed by lactose intolerant individuals, which comprise approximately 70% of the global population (Facioni et al., 2020). Diets high in carbohydrates tend to promote obesity and thus increase the risk of CVD; therefore, the very low levels found in cheese are a positive attribute from this perspective.

5.5 Minerals

Cheese contributes a number of important minerals to the diet including iron, potassium, magnesium, zinc, phosphorus, calcium, and sodium (O'Brien & O'Connor, 2017). One of the chief functions of iron is to act as an oxygen carrier in hemoglobin and as such has an indirect role in CVD; however, the recommended daily allowance (RDA) for iron is 7−10 mg, so a typical 50g portion of cheddar cheese would only supply ~2% of daily needs. Similarly, potassium has a role to play in reducing the risk of stroke and protecting blood vessels from oxidative damage, but with an RDA of 4,700 mg a portion of cheddar would only supply ~1.5% of needs. A portion of cheddar cheese could provide up to 3% of the RDA of magnesium to an adult's diet. Magnesium is a very abundant cation in the human body and is a cofactor for many enzymes. Magnesium deficiency is difficult to diagnose, but the subclinical deficiency, which is thought to be widespread, is associated with CVD (Di Nicolantonio et al., 2018). Zinc is more abundant in cheese and a portion of cheddar could supply ~12% of daily requirements. There is a significant body of literature that reports a positive link between zinc intake and CVD; however, excess zinc could lead to higher incidences of CVD (Milton et al., 2018). Thus, it may be prudent to balance zinc intake from cheese with other dietary sources to ensure that recommended levels are not exceeded. Cheese is a good source of phosphorus, which is involved in many housekeeping functions and a portion of cheddar cheese can supply up to 35% of the body's daily needs. There is a concern based on epidemiological studies that excess serum phosphorus levels are linked to CVD (Menon & Ix, 2013) and thus, as for zinc, this needs to be considered when including cheese as part of an overall diet.

While the role of calcium in bone health is well documented, its role in cardiovascular health is less clear-cut (Reid et al., 2017). Calcium plays a critical role in smooth muscle contraction and is a cofactor for enzymes involved in blood coagulation thus, is critical for a healthy cardiovascular system. However, excess serum calcium can lead to deposition in the vascular system, which is a consistent feature of vascular disease and is predictive of adverse cardiovascular events. With growing consumer awareness of the benefits of calcium for bone health, there is a trend toward the use of calcium supplements. However, there is a body of evidence that consuming calcium in such a format may result in vascular disease, possibly associated with temporary high levels of serum calcium postingestion of the supplement. On the contrary, observational studies of dietary calcium do not demonstrate a consistently adverse effect on cardiovascular health. Thus, obtaining calcium from the diet rather than supplements is to be encouraged (Reid et al., 2017). It is also important to consider that calcium in cheese is in a highly bioavailable format with up to 26% of the calcium present in cheese being absorbed (Recker et al., 1988), which is equivalent to the bioavailability of calcium direct from milk. This is significantly more than what is reported for a range of other foods rich in calcium (Weaver & Heaney, 2006). This implies that with an RDA in the region of 1,000 mg that a typical 50g portion of cheddar cheese could supply ~9% of the daily adult needs.

Unlike the other minerals, most of the sodium in cheese arises from the addition of salt, and sodium chloride toward the end of the manufacturing process for most cheeses. Salt addition has a number of important contributions to the quality of the final product including inhibition of microbial activity and activity of various enzymes, assists in syneresis, affects protein hydration, and affects flavor directly by imparting a "salty flavor" and indirectly by its action on microbes and enzymes (Fox et al., 2017c). The final level of sodium in most cheeses ranges from 300 to 1000 mg 100 g^{-1} with the typical level in cheddar being 670 mg 100 g^{-1} (O'Brien & O'Connor, 2017). Sodium is essential to health and is involved in a range of functions including nutrient absorption and maintaining fluid balance. Excess sodium intake is linked to increased blood pressure and CVD; however, there is continued debate as to what constitutes a safe level of sodium and how best to achieve it (Cook et al., 2020). While there continues to be debate about the desirable level of sodium in the diet, the RDA is usually set around 2,400 mg. Thus, a 50g portion of cheddar would contribute 335 mg, which equates to ~14% of the RDA. Thus, as for zinc and phosphorus consumers should take this into consideration when including cheese as a component in their overall diet.

5.6 Vitamins

Cheese is also a good source of vitamins, in particular fat-soluble vitamins including vitamins A and B12 (O'Brien & O'Connor, 2017). From the perspective of CVD; vitamins B_6, B_9, B_{12}, C, E and carotene are the most significant (Debreceni & Debreceni, 2014). Carotene and vitamin E, which are both present in cheese, are important as they have antioxidant activity. There is no specific RDA for carotene but intakes of up to 180 mg are common and the RDA for vitamin E is 15 mg; however, their levels in cheese are significantly below these levels and thus, cheese as a source for these vitamins is unlikely to have a significant impact on CVD. Likewise, the RDA for vitamins B_6 1.3 mg, B_9 400 μg, and D 15 μg are significantly higher than levels found in cheese, so also not likely to impact significantly on the development or prevention of CVD. However, vitamin B_{12} levels in cheese range from ~1.0 to 2.0 μg 100 $g^{-1,}$ and thus a 50g portion of cheese can supply from 20% to 40% of the RDA of 2.4 μg and thus, could impact positively on prevention or development of CVD.

5.7 Bioactive compounds in cheese

In addition to the primary nutrients present in cheese mostly arising from the cheese milk, cheese can also contain a number of bio-active compounds that include bioactive peptides (BAP), products of amino acid metabolism including γ-amino-butyric acid (GABA), and fat metabolism including conjugated linoleic acid (CLA), polar lipids, and products of microbial metabolism including exopolysaccharides (EPS).

BAP are peptides that are encrypted within the casein molecules in the cheese matrix that are released as a consequence of the various proteolytic and peptidolytic enzymes present within the cheese during manufacture and ripening and within the gastrointestinal tract during digestion. The peptides are reported to have a range of health protective effects including antimicrobial, antihypertensive, immunomodulatory, analgesic, and antioxidant (Santiago-López et al., 2018). Many studies have demonstrated the activity of BAP in *in vitro* models, but a demonstration *in vivo* where the peptide has to be stable during gastrointestinal digestion and in most cases be absorbed and remain stable in the bloodstream to gain access to its target more difficult (Egger & Ménard, 2017). Survival of BAP in cheese is further complicated by the fact that cheese is a dynamic product where proteolysis is ongoing throughout ripening. From the perspective of CVD and cheese, BAP induces blood pressure lowering effects has received the most attention. The mechanisms by which BAP are proposed to induce lower blood pressure include; (i) inhibition of the angiotensin-converting enzyme (ACE-inhibition), (ii) increased endothelial nitric oxide synthase activity, (iii) increased endothelial nitric oxide production, and (iv) inhibition of renin. The most intensive studies of these are the ACE-inhibition tri-peptides Ile-Pro-Pro (IPP) and Val-Pro-Pro (VPP), which have undergone a number of human intervention trials and subsequent meta-analysis the outcome of which demonstrate a significant reduction in systolic and diastolic blood pressure (Fekete et al., 2015; Nongonierma & FitzGerald, 2015).

Catabolism of amino acids is an important process for flavor development during cheese ripening (Ganesan & Weimer, 2017). The full extent of amino acid catabolism and its contribution to cheese flavor is still being resolved but it is interesting to speculate that some of the small molecules produced by this process may also have health protective effects. One such molecule γ-aminobutyric acid (GABA) is produced by the decarboxylation of glutamate (Santiago-López et al., 2018). GABA is an inhibitory neurotransmitter in the mammalian central nervous system and has several well-established physiological functions including antihypertensive, lipid serum modulation, immunomodulation, antidiuretic, and tranquilizing effects. Levels in cheese vary and are dependent on cheese type, microbiome and ripening conditions, and duration. There are also significant variations of GABA levels reported within particular cheeses types in different studies (Diana et al., 2014; Lacroix et al., 2013). However, levels in excess of 300 mg kg^{-1} have been reported for many kinds of cheese suggesting that a typical 50g portion would supply sufficient GABA to have physiological effects with regard to the management of hypertension (Diana et al., 2014).

CLA is a dietary polyunsaturated fatty acid naturally found in milk and dairy products. A number of factors, including diet, influence its level and it was found in higher levels in milk from grass-fed cows (O'Callaghan et al., 2016) and cheeses made from grass-fed milks (O'Callaghan et al., 2017) as discussed above, and levels in cheese may also be enhanced by the presence of lactic acid bacteria capable of producing it. Two forms of CLA are naturally present (cis-9, trans-11 and trans-10, cis-12) and their levels in cheese made from milk from grass-based production systems were reported as 1.45 and 0.1 g 100 g^{-1} respectively. CLA has received much attention as a bioactive with a range of health-protecting effects including research that it can act to reduce blood pressure (DeClercq et al., 2012). However, confirmation that CLA can reduce blood pressure in humans as a consequence of consuming it in cheese needs to be established. In addition, as discussed above the biochemical changes and the development of diverse microbiomes in different cheeses during ripening can have a positive impact on the composition of polar lipids in cheese leading to improvements in the antithrombotic properties of cheeses (Lordan, Walsh, et al., 2019).

Some lactic acid bacteria used in cheese manufacture have the capacity to produce EPS, which are a diverse group of polysaccharides that vary in their composition, chain length, and physiochemical characteristics (Lynch et al., 2018). EPS falls into two main categories, heteropolysaccharides, which are composed of a number of different monosaccharide types, or homopolysaccharides, which consist of only a single monosaccharide type. EPS has received much attention as it imparts desirable textural and techno-functional characteristics to fermented milk and cheese. However, a number of health-promoting characterizes have been associated with EPS, including modulation of immune function and promoting the growth of beneficial bacteria in the gastrointestinal tract. It is also known that β-glucans from oats can positively influence cardiovascular health by lowering serum cholesterol levels. The mechanism is not fully understood but it may be that cholesterol is bound by the β-glucans and thus, excreted or that it may be an indirect effect brought about by β-glucan acting as a prebiotic and promoting growth of endogenous microbes with bile salt hydrolase activity. As some lactic acid bacteria, strains can produce β-glucans the potential of cheese manufactured with such strains is receiving attention. Cheddar and Swiss-type cheese were manufactured with a β-glucans producing *Lactobacillus mucosae* strain as an adjunct culture. The adjunct remained viable in both kinds of cheese and had little overall impact on the cheese composition or key quality indices (Ryan et al., 2015). However, whether consumption of these cheeses results in changes in serum cholesterol levels or composition has yet to be established.

6. Human randomized control trial (RCT) studies

As discussed above, many cohorts and randomized controlled trials have been undertaken to explore the relationship between cheese consumption and CVD and recent analysis of these studies are indicating a neutral or positive effect of cheese consumption on a number of metabolic markers including those associated with CVD (Timon et al., 2020). While cross-sectional cohort studies may sometimes be difficult to interpret as dairy is often monitored as opposed to cheese, it is particularly interesting to note that in a number of recent RCTs where cheese is directly tested with respect to other dairy products or a combination of dairy product components, that cheese emerges as conveying positive impacts on biomarkers of CVD in excess of the other test products.

A crossover RCT in 2004 investigated three isoenergetic diets containing either milk, butter, or cheese where 35% of the energy was derived from fat, with 20% coming from milk fat. The cheese used in this study was Samsø, a hard Swiss-type cheese originating in Denmark and all other food items were constant and identical between the three strands of the study. The diets on each of the three strands of the study, which lasted 3 weeks included either whole milk, butter, or cheese provided to 14 healthy young men. It was observed that fasting blood serum LDL-C concentration was significantly higher following the butter diet than the cheese one, with a borderline significant difference in total cholesterol ($P = 0.054$), while whole milk increased serum LDL-C levels similarly to butter (Tholstrup et al., 2004). A similar crossover study, this time using Jarlsberg cheese, a Norwegian variety of Swiss-type cheese with 22 individuals (9 men and 13 women) aged 23−54 years with isoenergetic diets containing equal amounts of fat and protein containing either cheese, butter plus calcium caseinate or butter plus egg-white protein, each for 3 weeks. The key observations were that total cholesterol was significantly lower after the cheese diet than following the butter plus calcium caseinate diet; however, no significant change in the LDL/HDL cholesterol was observed (Biong et al., 2004).

In 2005 a crossover RCT using mature cheddar cheese was undertaken. The study consisted of 19 free-living mildly hypercholesterolaemic volunteers (14 men and five women) who each consumed 40 g of dairy fat per day as either butter or cheese for a 4 week period. The individuals self-selected the remaining components of their diet from a carefully constructed set of foods with similar energy intake and body weights were recorded throughout the study. The results obtained demonstrated that total cholesterol and LDL-C were significantly higher following the butter-containing diet than with cheese, leading the authors to suggest that dietary advice regarding cheese consumption may need to be modified (Nestel et al., 2005).

These three crossover RCTs reported lower blood serum cholesterol levels when cheese was included as a component of the diet as compared to butter where the overall fat intake and total energy of the diets were controlled. In addition, lactose and casein levels in the butter diet were standardized to be equivalent to the cheese diet in the study by Tholstrup et al. while in the study by Biong et al. casein levels were standardized. The cholesterol content of butter is 227 mg 100 g^{-1} (Kolarič & Šimko, 2020) while that of cheddar is 100 mg 100 $g^{-1,}$ and that of Samsø is estimated to be 90 mg 100 g^{-1}, similar to the Swiss cheese Emmental (Table 8.1). In the Tholstrup et al. study subjects consumed 93g butter and 305g cheese, which equates to 211 and 274 mg cholesterol respectively for butter and cheese. Similarly, in the Biong et al. study where 52g butter and 150g cheese were consumed, cholesterol intake was 118 and 135 mg respectively for butter and cheese. In the study by Nestel et al. the diet was standardized to 40g of dairy fat, which equates to 116g cheddar cheese (based on fat content of 34.4 g 100 g^{-1}, Table 8.1) and 49g butter (based on fat content of 81 g 100 g^{-1}), which equate to 111 and 116 mg of cholesterol respectively for butter and cheese. It is interesting to note that in each study dietary cholesterol intake from the test foods was always higher in the cheese diet than in the butter diet and for cheese ranged between 39% and 91% of the previous level of 300 mg per day below which it was suggested cholesterol intake should be maintained (Krauss et al., 2001), while blood serum cholesterol levels were lower on the cheese diets. In addition, it is to be expected that the SFA content of cheese and butter is essentially identical as the fat in both cases originates in the milk. Therefore, the mechanism by which cheese consumption results in reduced cholesterol levels is not clear. One hypothesis is that the high calcium levels in cheese form calcium soaps with the fat, which are less prone to digestion and absorption and thus, are excreted in the feces. This hypothesis has supported a meta-analysis of RCT that demonstrated higher excretion of fat in the feces in the presence of increased calcium intake, in particular, if the calcium was part of a dairy product (Christensen et al., 2009).

To further investigate this hypothesis and to endeavor to confirm the findings of Tholstrup et al. an RCT was established with 49 participants who consumed diets supplemented with either Samsø or salted butter (Hjerpsted et al., 2011). The trial involved a 2-week run-in period on a habitual diet and two intervention periods each of 6 week separated by a washout period of 2 week on the habitual diet. The subjects, aged between 22 and 69 years were grouped based on their normal energy intake levels (low, medium, and high), which were then used to establish the dairy fat content of the test diets, which equated to 13% of their energy requirement. Based on this analysis subjects with medium energy requirements consumed 143 g of cheese or 47 g of butter per day. Fatty acid analysis of the cheese and butter was undertaken and while statistical analysis was not reported, no obvious numerical differences were apparent. Calcium levels were also measured with 834 mg 100 g^{-1} reported for Samsø cheese and 19 mg 100 g^{-1} for butter. Subjects were tested for fasting blood serum cholesterol levels and fecal fat excretion following the intervention. The results obtained indicated that total serum cholesterol, LDL-C, and HDL-C were lower following the cheese intervention, and consumption of cheese did not increase total cholesterol or LDL-C levels compared to the 2-week run-in period; however, fecal fat excretion did not differ between either of the two treatments. Thus, this study supports the observations from the previous study but does not provide any evidence to support the fat excretion hypothesis.

Subsequently, another study was designed that included three diets, one containing 120 g/d of the semi-hard cheese Klovborg made using cow milk, one containing semi-skimmed milk, and a control diet containing butter. Each of the diets was similar in terms of total energy and energy density; protein, carbohydrate, and fat content including amounts of SFA, MUFA, and PUFA. The critical difference in the three diets was with respect to their calcium content. The cheese and milk diets each had 1172 and 1143 mg of calcium of which 810 and 781 mg were dairy calcium. However, the control diet containing butter had only 362 mg of calcium of which none was from a dairy source. The subjects were young to middle-aged healthy men. The diets associated with each of the three arms of the trial were consumed for a 14-day period, with a 14-day washout period between each arm. The results obtained indicated that total cholesterol and LDL-C were lower with both the cheese and semi-skimmed milk diets than in the control containing butter, which is in agreement with the observations from the other RCT described above. However, during this trial, fecal fat excretion was greater during both the cheese and semi-skimmed milk diets than during the control diet containing butter. The authors suggested that this observation may be linked to the larger amounts of total and dairy calcium in the cheese and milk diets (Soerensen et al., 2014) and thus, provides evidence to support the fat excretion hypothesis.

A meta-analysis was undertaken to evaluate RCT that examined the effect of cheese consumption on blood lipids that included these five studies (de Goede et al., 2015). These RCTs were selected on the basis that they compared the consumption of cheese and butter both of which have similar ratios of PUFA and SFA (P/S ratio). Their analysis indicated that compared to butter, cheese intake significantly reduced total cholesterol by 5.2%, LDL-C by 6.5%, and HDL-C by 3.9% but had no effect on triglycerides. However; when other studies are included, cheese intake did increase total cholesterol and LDL-C compared to tofu or fat-modified cheese, but there was insufficient data to compare the intake of cheese on cholesterol to that of other foods.

Subsequent to this meta-analysis two further studies comparing the impact of SFA from butter and cheese on cholesterol levels have been published. In the first of these Brassard et al. (2017) compared five diets involving (i) a diet containing SFA from cheese, (ii) a diet containing SFA from butter, (iii) a diet rich in MUFA, (iv) diet rich in PUFA and (v) a high-carbohydrate, low-fat diet. All of the diets were standardized for energy, protein, fiber, cholesterol, and sodium. Both the cheese and butter diets were matched for SFA content, while MUFA, PUFA, and carbohydrates replaced SFA in the other diets. Olive oil and corn oil was used to enrich the diets for PUFA and MUFA while carbohydrate was obtained from the food components of the diet as well as from added sugar. The level of calcium was higher in the cheese diet than in the other four diets, which had approximately similar levels of calcium. Each of the diets was consumed for 4 weeks followed by a 24-day washout period. The subjects were men and women aged 18−65, with a mean Body Mass Index

(BMI) of 30.7 and HDL-C concentrations below the age- and sex-specific 75th percentile to exclude individuals with high HDL-C concentrations. In total, 92 subjects completed at least one arm of the trial while 64 completed all five. The observed outcomes were that serum HDL-C levels were similar following the cheese and butter diets, but were significantly higher than following consumption of the high carbohydrate diet. Following the cheese diet, LDL-C levels were lower than after the butter diet but were higher than that observed following either of the high carbohydrate, PUFA, or MUFA diets. It was noted that the baseline values for LDL-C significantly impacted the increase observed in LDL-C with the increase in LDL-C being significantly greater with butter than cheese only among individuals with high baseline LDL-C levels. The conclusions from this study suggest that the food matrix modulates the impact of SFA on blood cholesterol and that this may be exacerbated in individuals with high baseline LDL-C levels and that these factors need to be taken into consideration when reviewing existing literature and planning new RCTs.

To further investigate the potential impact of the food matrix Feeney et al. (2018) undertook a parallel-arm trial that involved four arms with three different intervention diets including (i) a full-fat cheddar cheese diet, (ii) a reduced fat cheddar cheese diet supplemented with butter and (iii) butter supplemented with calcium caseinate powder plus a calcium supplement ($CaCO_3$). The fourth arm of the trial required participants to initially complete a 6-week run-in period where they excluded all cheese from their diet and they then embarked on the full-fat cheddar cheese diet. The three intervention diets were matched for total energy, protein, fat, and calcium. The full-fat Cheddar diet included 120g Cheddar cheese, the reduced-fat cheddar diet included 120g cheese and 21g butter, while the butter diet was composed of 49g butter plus 30 g calcium caseinate plus the $CaCO_3$ supplement. Each intervention was for a period of 6 weeks. The subjects were free-living and other than limiting consumption of other dairy products to 50 ml milk per day they had the freedom to select the remaining of their dietary needs according to their normal dietary preferences. The subjects were slightly overweight adults with a BMI $\geq$25 aged $\geq$50 years of which 46% were male. The subjects were randomly assigned to one of the four interventions. A total of 164 subjects completed the study of which 46 consumed the full-fat cheese diet, 45 under the low-fat cheese diet, 42 under the butter diet, and 31 under the full-fat cheese diet following a 6-week cheese-free run-in period. The key observation from the trial was that total cholesterol and LDL-C decreased in a stepwise-matrix manner between the groups with significantly lower total and LDL-C post-intervention when all the fat was contained within the cheese matrix compared to when it was all consumed as butter (Fig. 8.4). It was interesting to note that total and LDL-C increased during the 6-week run-in period in the group that initially underwent a 6-week period where they excluded cheese from their diet but that both total and LDL-C decreased again following consumption of full-fat cheese. Unfortunately, the

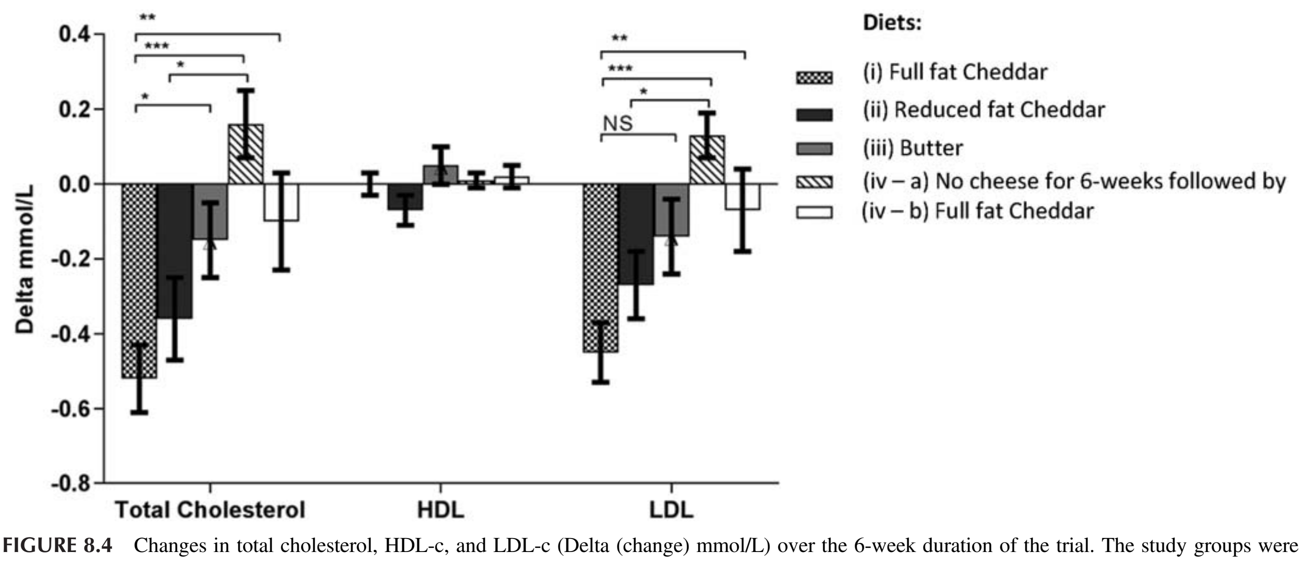

FIGURE 8.4 Changes in total cholesterol, HDL-c, and LDL-c (Delta (change) mmol/L) over the 6-week duration of the trial. The study groups were subject to diets containing (i) full-fat Cheddar cheese, (ii) reduced-fat Cheddar cheese supplemented with butter, (iii) butter supplemented with calcium caseinate powder plus a calcium supplement (CaCO3), and (iv—a) no cheese for 6-week followed by (iv—b) full-fat Cheddar cheese. Data presented as mean differences ± SEM. Significant differences were observed between groups (ii) and (ii) ($P = 0.031$) and groups (i) and (iii) ($P = 0.008$) for total cholesterol. For LDL-c, significant differences were observed between groups (i) and (ii) ($P = 0.016$), (i) and (iii) ($P = 0.015$), and (i) and (iv) ($P = 0.009$). NS $P \geq 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. *(Adapted from Feeney, E. L., Barron, R., Dible, V., Hamilton, Z., Power, Y., Tanner, L., Flynn, C., Bouchier, P., Beresford, T., Noronha, N., & Gibney, E. R. (2018). Dairy matrix effects: Response to consumption of dairy fat differs when eaten within the cheese matrix—A randomized controlled trial.* The American Journal of Clinical Nutrition, 108*, 667—674. https://doi.org/10.1093/ajcn/nqy146).*

numbers completing this arm of the study was significantly lower than required by the study design so the finding lack statistical power so no definitive conclusions can be made from this component of the study. The overall conclusion from this study was again that dairy fat when consumed in the form of cheese, even in large amounts (120 g/day) resulted in lower total cholesterol and LDL-C and that supplementing the diet with other key components of the dairy matrix including protein and calcium in other formats did not reverse this observation, highlighting the significance of the cheese matrix.

7. Role of the food matrix

Nutrition research has tended to follow a reductionist strategy focusing on evaluating the effect of a single nutrient or group of closely related nutrients on a particular health or nutrition effect. Little attention was directed toward the food within which the nutrient was delivered, indeed, in many cases, the nutrient was delivered in a purified form in a capsule, beverage, etc. As knowledge of nutrition and its impact on health advances, it has become ever more apparent that this strategy does not always yield satisfying results or knowledge. A good example of the limits associated with the reductionist strategy is the proposed link between SFA consumption and CVD. Very little attention was directed toward the food within which the SFAs were embedded nor the fact that SFAs themselves are a heterogeneous group of molecules with different structures and functions and that different SFAs may have different impacts on cardiovascular health and development of CVD (Lordan, Tsoupras, & Zabetakis, 2019).

As a consequence, there is now an emerging consensus that food and its nutritional effects need to be considered at the whole food level and this has led to the development of the Food Matrix concept. The food matrix has been defined by the USDA as "the nutrient and non-nutrient components of foods and their molecular relationships, i.e., chemical bonds, to each other" (https://agclass.nal.usda.gov/glossary.shtml; Donovan, & Goulet, 2019) In this definition, the nutrients are the protein, fat, carbohydrate, vitamins, minerals, etc in the food, while the nonnutrients refer to the physical structure of the food and how it is held together with chemical bonds. The form of the nonnutrient component, or more simply its physical structure and the dispersion of nutrients within it will together impact the way the food is digested and the nutrients released, absorbed, and metabolized, ultimately defining the nutritional and health properties of the food. This is particularly relevant in the context of dairy, as milk and products made from it have very complex physical structures and contain a very diverse range of nutrients. For example, fat in milk is contained in globules surrounded by the milk fat globule membrane, while casein proteins are packaged into micelles and whey proteins take up a globular formation. The fat globules, micelles, and globular whey proteins are suspended in the serum phase of the milk composed of water and minerals and it is the interaction of all these components that help to maintain the overall milk structure. When milk is converted into cheese or other products this structure is altered and a new one is established.

Recently, due to the complexity of the dairy matrix, an expert group was formed to review the literature with a view to coming to a combined view/consensus on the potential of the dairy matrix to influence nutritional and metabolic outcomes (Thorning et al., 2017). The key observations from the group from the perspective of CVD were that (i) research evidence to date does not support a positive association between intake of dairy products and risk of cardiovascular diseases, (ii) intervention studies have indicated that the metabolic effects of the whole dairy may be different than those of single dairy constituents when considering the effects on cardiometabolic disease risk and (iii) dairy structure and processing methods may enhance interactions between nutrients in the dairy matrix. This led to the overall conclusion that the "nutritional value of dairy products should be considered as the bio-functionality of the sum of nutrients within dairy matrix structures."

As discussed above, 18 primary cheese types are recognized, which lead to in excess of 1000 varieties arising from changes in the make and ripening procedures (Beresford et al., 2001). These different cheese types vary in their core composition (Table 8.1) but will also contain different microbiomes, levels of minerals, and vitamins, in addition to the amount and range of bioactive compounds. As discussed above, the milk types used and processing parameters applied will impact the nutritional properties of the cheese. These same factors that influence the nutrient content of the cheese will also influence the nonnutrient i.e., the physicochemical structure of the cheese i.e., the matrix.

The impact of milk composition and processing parameters on the cheese matrix was recently reviewed (Feeney et al., 2021). The authors clearly outline how different parameters impact the overall cheese structure; for example how heat treatment of the milk will lead to whey protein denaturation and thus, incorporation of whey protein into the cheese; how the method of coagulation will impact the gel structure, how the size of the curd particles formed after cutting the curd, the rate at which the curd whey mixture is agitated, the temperature to which it is cooked and the pH at which curd particles are separated from the whey, will all impact on the moisture and mineral content of the cheese; how curd handling processes such as "cheddering" and stretching will impact the distribution and structure of fat, protein, and moisture within the body of the cheese and how ripening conditions will impact on protein hydrolysis, lipolysis, the development of the microbiome and generation of bioactive molecules. These interactions and outcomes have been extensively studied in the context of the key quality indices of cheese including its flavor and techno-functional characteristics; however, the impact of the resulting structural differences on the digestion of the cheese mass during oral-gastrointestinal transit is only now being elucidated. For example, it was demonstrated that milk gels formed due to the action of chymosin but not for gels resulting from acidification formed compact protein aggregates under the acidic conditions experienced in the stomach and that the hydrolysis of protein and release of amino acids from chymosin gels was slower than from acid gels (Barbé et al., 2013; Floury et al., 2018).

Texture properties are very well studied in cheese and recently a correlation between textural properties and cheese matrix disintegration was reported (Fang et al. 2016a, 2016b; Guinot et al., 2019; Sharma Khanal et al., 2020). Two studies involved the selection of a range of different cheese types in an effort to have examples representing the diversity of texture properties reflecting their respective manufacturing procedures. In one of these studies the textural and *in vitro* digestibility of five commercial kinds of cheese, stabilized Camembert, smear cheese, young and aged cheddars, and string cheese mozzarella were examined. The results obtained indicated that cheese disintegration at the end of the *in vitro* gastric digestion phase was affected by texture and composition, with elastic cheese such as mozzarella being more resistant to digestion than ripened and soft cheeses with high-fat content such as Camembert and aged cheddar. In addition, the extent of protein hydrolysis correlated with the degree of disintegration (Fang et al., 2016a). A similar study selected nine commercial kinds of cheese consisting of examples of young and aged cheddar, regular and light cream cheese, Parmesan, feta, Camembert, mozzarella, and sliced processed cheese. Following the *in vitro* gastric phase of digestion Camembert and mozzarella displayed the lowest level of matrix disintegration with aged cheddar, and regular and light cream cheeses displayed the highest levels. The textural parameters of springiness, cohesiveness, and hardness were negatively correlated to the rate of cheese disintegration during *in vitro* gastric digestion. Fatty acids were quickly released during the first 30 min of duodenal digestion but slowed thereafter. During duodenal digestion, the free fatty acid release was highest from mozzarella, light cream cheese, and sliced processed cheese, in addition, the fat globule size, and fat and calcium content seem to be important factors determining the rate of lipolysis (Guinot et al., 2019). Two studies have focused on specific cheese types, cheddar, and mozzarella, which vary in fat content. One study involving full and reduced fat cheddars (33% and 21% fat respectively) and mozzarellas (20% and 14% fat respectively) indicated that fat content and level of proteolysis both positively affect cheese disintegration and that the textural parameters of hardness, adhesiveness, resilience, and chewiness negatively correlated to cheese disintegration (Fang et al., 2016b). A subsequent study (Sharma Khanal et al., 2020) investigated full- and low-fat cheddar cheeses. They again observed that cheese matrix disintegration and protein release was directly influenced by cheese composition and textural properties with levels of disintegration and protein release being higher from the full-fat cheddar.

Milk source and milk pretreatments were also demonstrated to impact the behavior of cheese during simulated digestion. Lipids in cheese manufactured from goat milk were observed to be more digestible than lipids from cheese made from cow milk (Asensio-Grau et al., 2019). In cheese, fat is contained within globules or pools entrapped within the protein network. The disintegration of the protein network is required to release the fat globules and provide access to the lipolytic enzymes that will further degrade them via lipolysis. A number of studies have reported that the degree of matrix disintegration correlates with fat release and lipolysis (Guinot et al., 2019; Lamothe et al., 2012). While homogenization is not typically applied to milk prior to cheese manufacture a study using directly acidified model cheeses did incorporate it into the manufacturing protocol (Lamothe et al., 2017). The results obtained indicated that fat was released faster from the cheese matrix made using homogenized milk. The more rapid release was not related to faster matrix disintegration, which the authors proposed implied that the degree of lipid dispersion was more important for fat release than the disintegration of the matrix.

As discussed above, many kinds of cheese undergo a period of ripening during which many physiochemical changes occur in the cheese that impacts the texture and structure of the cheese. Differences in the rate of matrix degradation between aged and mild cheddars were observed in both the gastric and duodenal phases of simulated digestion, with the mild cheddar being more resistant to duodenal digestion (Lamothe et al., 2012). In another study using a Spanish cheese manufactured using a mix of cow, sheep, and goat milk, cheeses matured for 240 days displayed more extensive digestibility of proteins and lipids than a mild cheese aged for 30 days (Asensio-Grau et al., 2019).

While there is significant agreement between most of these studies it is interesting to note the differences observed with respect to the disintegration of Camembert in the studies of Guinot et al. (2019) and Fang et al. (2016a). Guinot et al. (2019) highlight that disintegration was measured differently between the two studies and this may account for the different observations. In addition, Fang et al. (2016a) indicate that they used "stabilised" Camembert, which resulted in a high curd pH resulting in higher calcium concentration in cheese and firmer texture, while Guinot et al. (2019) do not make such a specification. Stabilized Camembert incorporates a number of process modifications including curd washing relative to traditional Camembert. Other process modifications such as the incorporation of homogenization were also demonstrated to affect fat release (Lamothe et al., 2017). Other studies highlight that cheese is a dynamic food undergoing changes throughout ripening that impact on its rate and level of digestion (Asensio-Grau et al., 2019; Lamothe et al., 2012) and such changes may influence the overall digestion and absorption of cheese components and thus may influence the outcome of studies directed to assess the impact of cheese on biomarkers of CVD. Finally, whether the reported differences are the result of different research methodology or subtle but significant differences in the cheese, highlights that establishing a consistent methodology and strict guidelines for cheese description are required so that future studies can be compared and contrasted.

8. Conclusion

In conclusion, in the context of this manuscript the term cheese describes a broad range of dairy foods unified by being manufactured from milk that involves the formation of a protein gel network of the casein micelles within which milk fat globules are entrapped, this gel is then further manipulated to exude moisture and hence concentrate the protein (casein) and fat components of milk. Manipulations to the making technology can lead to the production of >1000 different types that differ in terms of composition and nutritional value. The degree of variation is so great that in many cases attempting to make blanket claims as to the nutritional value or health benefits of cheese based on an analysis of a specific variety may not be practical or indeed advisable.

However, in broad terms, we can conclude that cheeses of most varieties are good sources of protein and vitamins including $B_{12,}$ and are energy dense. Cheese is also a good source of minerals including calcium, phosphorus, zinc, and sodium, all of which are required in a healthy diet but, if taken in excess may have negative consequences for CVD. There is a growing body of evidence indicating that cheese contains a number of bioactive molecules many of which may be positive affecters on CVD. Most cheeses are composed of 20%–35% fat, comprising ~66% SFA and for this reason, there was a view that cheese consumption was likely to lead to elevated blood serum cholesterol and thus, have a role in inducing CVD. However, there is increasing evidence from epidemiological studies and RCTs that this is not the case and that consumption of cheese may have neutral or indeed positive effects on blood cholesterol levels and in particular on LDL-C and on LDL-C/HDL-C levels. However, these studies have only investigated the impacts of a few varieties of Swiss-type and cheddar cheeses and have largely focused on the effects of cheese relative to butter on serum cholesterol levels. This approach is acceptable as it is focused on trying to understand the possible mechanism for the observed cholesterol reduction; however, more studies of longer duration examining the effect of cheese consumption relative to other nondairy components of the diet are required to fully understand the role of cheese in modifying cholesterol levels. In addition, these studies need to be extended to a broader range of cheeses before it will be clear whether any changes observed are cheese variety related or more broadly associated with cheese.

There is a growing consensus in nutrition research that the traditional reductionist approach does not fully explain the variation observed by the impact of different foods on health and nutrition, and that more attention needs to be given to the food in which the nutrients are delivered. This has given rise to the concept of the food matrix and it is well established that a wide variety of structure and structure-function attributes can be assigned to different cheese varieties. Research is now demonstrating that different cheeses behave differently during simulated gastric digestion and that this may impact how their nutrient content is released and absorbed, with consequences for the role of cheese consumption on the development of CVD.

Many groups are exploiting the observations that cheese exerts positive health effects and have sought to develop cheeses designed to provide such benefits to varying levels of success. These can include low-fat or salt-reduced cheeses, cheeses made from different milk sources, or cheeses enriched in bio-actives or containing a modified microbiome. These may provide a commercial opportunity or be used to help elucidate the mechanisms by which cheese can deliver positive health effects; however, the key challenge at present is to build on the observations with traditional cheese types to encode a range of nutritional and health benefits.

In summary, there is increasing evidence that dairy fat consumed within a cheese matrix does not adversely affect blood cholesterol levels or profiles and that cheese can be included as a component in an overall balance, healthy and nutritious diet.

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Chapter 9

Fermented milk, yogurt beverages, and probiotics: functional products with cardiovascular benefits?

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1. Introduction

Cardiovascular diseases are the leading cause of death and morbidity worldwide (Timmis et al., 2019). It is well-established that a maladaptive diet and lifestyle are core risk factors for the development of CVD (Lordan et al., 2019a; Tsoupras et al., 2018; Yu et al., 2018). Long-term metabolic disruptions as a result of prolonged exposure to an unhealthy diet can lead to low-grade systemic inflammation that leads to atherosclerosis. Over time, atherosclerotic plaques develop that can occlude blood vessels leading to ischemia or plaques can erode and rupture leading to major cardiovascular events such as myocardial infarction or stroke (Libby, 2006; Lordan, Nasopoulou, et al., 2018; Lordan, Tsoupras, & Zabetakis, 2021).

Previously, dairy products were considered one of the main foods that may contribute to the development of atherosclerosis and subsequent CVD as a consequence of their high saturated fatty acid (SFA) content. Diets that are characteristically high in SFA increase low-density lipoprotein (LDL) levels, which is a risk factor for CVD (Lordan, Nasopoulou, et al., 2018). However, recent research questions the role of SFA in the development of CVD as many foods that contain high levels of SFA seem to exhibit a neutral or positive association with cardiovascular health (Lordan & Zabetakis, 2017a, 2017b; Lordan, Nasopoulou, et al., 2018). Indeed, two large epidemiological studies have associated both SFA and dairy product consumption with a lower risk of stroke (Dehghan et al., 2017, 2018). In particular, it has been proposed that fermented dairy products may confer greater health benefits than nonfermented dairy products due to the presence of probiotic species and their respective metabolites of the potential for biological consequences (Companys et al., 2020, 2021; Zhang et al., 2020). One such dairy product is yogurt and related beverages.

Yogurt is a popular food around the world that comes in many forms, some with a runny texture that can even occur as a beverage and others that have a thick texture that is almost akin to soft cheese. Some of these products have been discussed in Chapter 7. Historically, yogurt production occurred as a result of the need to preserve milk, which dates back to at least 7000 BCE when it is known that Egyptians consumed fermented milk beverages including *laban khan* and *laban rayed* (or leben). Around 5000 BCE, *dadhi* a milk product similar to modern-day yogurt was mentioned in the Vedas, ancient Hindu scripture where it was documented that it had therapeutic properties. However, it wasn't until the 8th century in Turkey that the origin of the modern-day word yogurt is thought to have originated where it appeared as *yoghurut* (Chandan et al., 2017).

Fermented dairy products have long been associated with health benefits for regular consumers. Indeed, Ellie Metchnikoff first theorized that fermented products may convey health benefits by affecting the gut microbiota in the early 1900s (Metchnikoff, 1908). Yogurt is now part of many dietary guidelines as a source of protein, vitamins, minerals, and calcium. However, in recent years, yogurts have been used to make functional foods and beverages.

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Some of the main traditional fermented milk beverages include kefir, koumiss, ayran/doogh, buttermilk, and various other drinking yogurts. However, there are also value-added beverages (e.g., high protein beverages, carbonated beverages, and sports beverages) and the so-called functional beverages, which can be enriched or supplemented beverages (minerals, fish oils, vitamins, polyphenols, etc.) (Turkmen et al., 2019). Consumer environmental concern, animal welfare awareness (Aydar et al., 2020), and the fact that a significant number of the world population is lactose intolerant (Gupta & Abu-Ghannam, 2012) led to the development of plant-based dairy-alternative fermented drinks.

The concept of functional foods started back in the 1980s in Japan, where the Japanese government observed that the consumption of certain foods resulted in the reduction of the impact of numerous disease risk factors, improving the health status of the aging population resulting in decreased cost associated with their care (FSAI Ireland, 2007). Japan was the first country that defined and regulated functional foods through the "foods for specified health uses" (FOSHU) regulation (Iwatani & Yamamoto, 2019). In EU legislation there is no clear definition for functional foods, although they are regulated by existing food legislation such as the Novel Food Regulation (European Parliament and Council, 2015), the Nutrition and Health Claims Regulation (European Parliament and Council, 2006), the Food Information to Consumers (European Parliament and Council, 2011), and the Foods for Specific Groups regulation (European Parliament and Council, 2013). According to EFSA, functional foods are considered foods that beneficially affect one or more target functions in the body beyond providing the basic nutrients (FSAI Ireland, 2007). The beneficial effect is linked to an improved state of health and well-being and/or reduction of risk of disease. These foods are consumed as part of a healthy diet, and they are not a pill, a capsule, or any form of dietary supplement (European Commission, 2010). According to this definition yogurt and dairy or nondairy fermented drinks are considered functional foods and for this reason, they will be discussed in this chapter. Dairy products command over 40% of the functional food market (Turkmen et al., 2019). Growth in the yogurt markets is expected to continually increase with a forecast compound annual growth rate (CAGR) of 6.4% between 2020 and 2025 (Glanbia, 2021), and yogurt beverages specifically are expected to have a CAGR of 4.8% with the fastest growing and largest markets being Europe and the Asia−Pacific markets. Trends also indicate that lactose-free, low sugar, novel flavors, dairy alternatives, and drinkable products were among the most popular yogurt trends in the market in 2021 (Glanbia, 2021).

This chapter describes the production of the most known and consumed dairy fermented drinks and the main compounds linked to the bioactivity of these drinks with a focus on the cardioprotective effect will be discussed. The main sensory characteristics will also be presented and finally the regulation related to health claims associated with these products. Plant-based dairy alternative fermented drinks are also briefly discussed.

2. Yogurt production

Yogurt is a fermented milk product produced by lactic acid bacteria (LAB), mainly produced using *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus*. Indeed, various other adjunct cultures have been developed that include various LAB such as *L. acidophilus, L. casei, L. helveticus,* and *Bifidobacterium* spp. Commercially, yogurt is produced from the heat treatment of milk that contains extra nonfat milk solids at 85°C for 30 min, which is then cooled to 42°C where starter cultures (2%) are used to inoculate the milk. The inoculated milk ferments until it reaches a pH between 4.4 and 4.6 or acidity of 0.9% is attained after approximately 4 h. The resulting yogurt is then cooled to 4°C to halt the fermentation process preventing excessive production of lactic acid culture growth (Chandan et al., 2017). This yogurt base is then ready for consumption as is or it can be used to produce fruit or flavored yogurts, or indeed other components can be added for the production of functional foods and beverages. An overview of typical yogurt production is summarized in Fig. 9.1.

3. Fermented milk and yogurt beverages

Fermented milk beverages or yogurt drinks are produced using a yogurt mix with reduced milk solids leading to a fermented milk product with low viscosity. There are various classifications of fermented milk products, but they can be broadly summarized according to Tamime (2002) as outlined in Table 9.1.

As demonstrated in Fig. 9.1, the manufacturing steps for yogurt beverages are similar to yogurt, but the agitation step to break the coagulum following the fermentation process differs slightly (Chandan et al., 2017). However, the production of yogurt beverages can be challenging due to the separation of the whey in the products. These issues can be remediated using stabilizing agents such as alginates, carboxymethylcellulose, gelatins, ispaghol, locust bean gum,

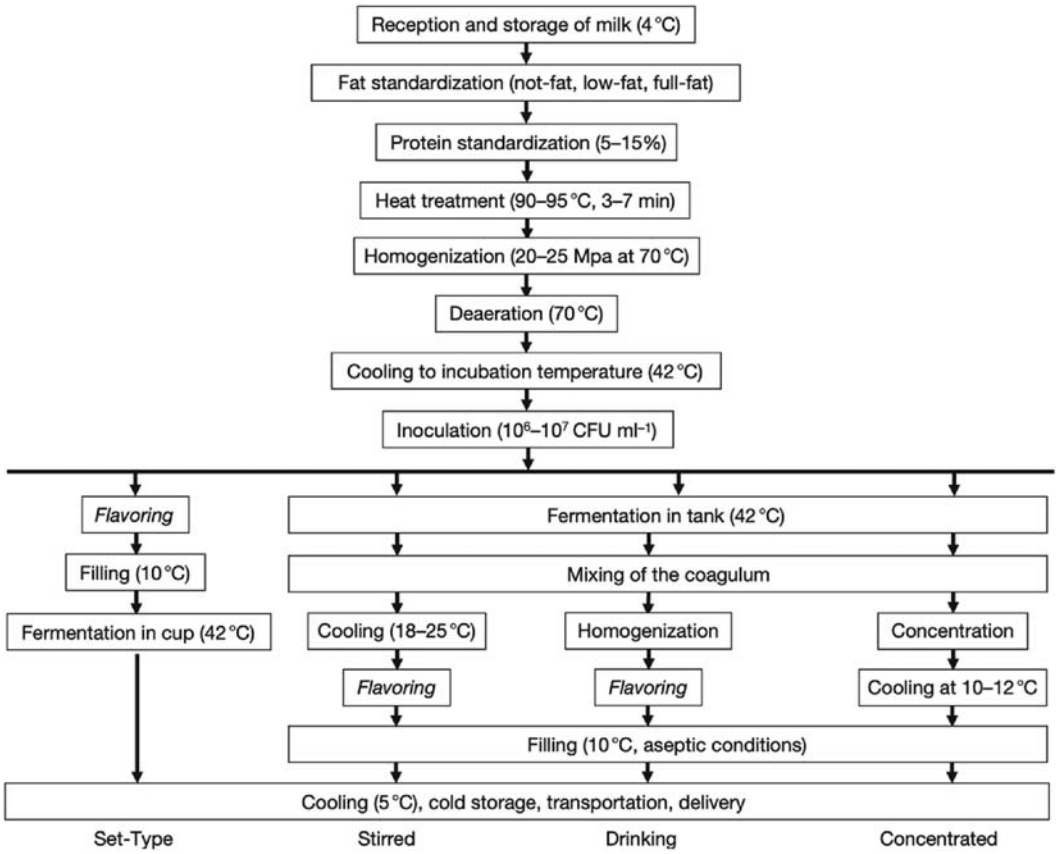

FIGURE 9.1 A basic schematic of the manufacture of basic yogurt products, including beverages. *Reproduced with permission from Corrieu and Béal (2016).*

and pectins (high methoxyl for beverages, low methoxyl for yogurts), sago starch, xanthan gum, and chitosan (Chandan, 2017; Tasneem et al., 2014). These stabilizers can also be manipulated to optimize the sensorial properties of products including their texture, consistency, and mouth feel. Yogurt beverages usually consist of 1.5% fat, 9% milk nonfat solids, 4%–8% sugar, 0.5% stabilizer, and if fruit syrup is used it can be anywhere between 5% and 15% (Chandan, 2017; Chandan et al., 2017).

The majority of yogurt drinks that are mass-produced in Europe and North America tend to be made with bovine milk. However, elsewhere, more traditional fermented milk products tend to be produced from either bovine, ovine, caprine, or camelid species. Indeed, traditional fermented milk and yogurt beverages are widely distributed globally. For example, Turkey is known for its traditional yogurt drink *ayran* commonly consumed in the summer. Ayran is produced by mixing yogurt with water and salt in 30–50 g 100 g^{-1} and 0.5–1 g 100 g^{-1} respectively. Although increased demand for ayran means that modern manufacturing has modified the production of ayran (Altay et al., 2013). Turkey is synonymous with several other fermented milk products including torba yogurt, kurut, kefir, and the fermented milk drink, koumiss (Kabak & Dobson, 2011). Doogh is another popular traditional Iranian fermented milk beverage also manufactured by diluting yogurt with the addition of salt (Esfandiari et al., 2013). Doogh has been associated with several health benefits and has been adapted to produce functional foods (Karami, 2018). Indeed, in many African countries, the production of traditional fermented milk beverages is of significant dietary and socio-economic importance. For example, Ethiopia is known for several traditional milk products including a fermented milk product known as ergo and defatted buttermilk called *arrera*, which is a byproduct of *kibe* production, a traditional butter made from ergo (Gonfa et al., 2001). There are endless traditional and ethnic fermented milk and yogurt beverages that exist in many cultures. While conventional dairy products tend to dominate the markets, the search for healthy wholesome food products with additional health benefits can change consumer markets. One such example of a fermented milk product that has gained significant interest from Western consumers is *kefir*.

TABLE 9.1 A simplistic representation of the types of fermented milk beverages that exist (Robinson et al., 2002; Tamime, 2002).

Type of fermented milk	Examples[a]
Lactic fermentations	**1.** Mesophilic fermentations examples: • Cultured buttermilk, • Villi • Filmjölk • Långofil • Tätmjölk • Nordic ropy milk • Ymer • Maziwa lala **2.** Thermophilic fermentations examples: • Bulgarian buttermilk • Yoghurt • Zabadi • Skyr • Labneh • Dahi **3.** Therapeutic or probiotic beverages examples: • "Bio" fermented milk • Acidophilus milk • Yakult • ABT • Onka • Vitfit • Cultura-AB • Flora ProActiv (Phytosterols)
Yeast—lactic fermentations	Examples: acidophilus yeast milk, kefir, koumiss
Mold—lactic fermentations	Examples: Villi

[a]*Examples may be present in more than one category due to the complex microbial composition.*

3.1 Kefir and probiotics

Over the last few decades, consumers have become more health conscious and seek products that might confer additional health benefits beyond their basic nutritional value, potentially due to their probiotic or prebiotic content. Indeed, yogurt drinks have become extremely popular as a vehicle for probiotic consumption (Mishra et al., 2018). *Kefir* is one yogurt-like beverage that has garnered significant attention due to its potential health benefits (Prado et al., 2015). The origin of kefir and indeed the word kefir is disputed in the literature. Kefir seems to have originated in the Balkans and in the Caucasus, Mongolian, and Tibetan mountains. In the latter regions, it was thought that kefir grains may have been passed from generation to generation as a source of wealth over 4000 years ago. However, the word kefir is thought to originate from the word *Keif*, a Slavic word meaning "well-being" as those who consumed *kefir* associated it with health and a sense of well-being (Farnworth, 2006; Rosa et al., 2017) or the Turkish word *keyif* of the same meaning (de Oliveira Leite et al., 2013; Lopitz-Otsoa et al., 2006).

Kefir can be produced from fermenting milk with either traditional kefir grains (Fig. 9.2), commercial freeze-dried grains, a specific set of starter cultures, and the product that is left after the removal of the kefir grains (Bensmira et al., 2010). Kefir grains can even be preserved wet, dry, or lyophilized (de Oliveira Leite et al., 2013). The kefir grains produce acidified fermented milk that is carbonated or effervescent-like and can contain small amounts of alcohol. Kefir grains are formed by a diverse set of microbes that create a polysaccharide and protein matrix that contain a community of bacterial and fungal species that are symbiotic in association with the kefir grains that are essential to the characteristic kefir fermentation (Marsh et al., 2013; Lordan, Nasopoulou, et al., 2018). The grains have an overall appearance similar to mini cauliflower that tends to be an off-white color varying in size from 0.3 to 3.5 cm in diameter (Garrote et al., 2010; Chen, Shi, et al., 2015; Rosa et al., 2017). This fermentation process is known to result in the production of bioactive metabolites and distinctive changes to the milk. These include bioactive peptides, antibiotics, bacteriocins, lactic acid,

FIGURE 9.2 An image of fresh dry kefir grains. *Reproduced with permission from* Conde-Islas et al. (2019).

exopolysaccharides, alterations to the fatty acid content, and the production of low levels of alcohol (de Oliveira Leite et al., 2013; Rosa et al., 2017).

There are three main ways to produce kefir. The first is the artisanal or traditional method where milk is inoculated with kefir grains, and it is allowed to ferment at 20−25°C for 18−24 h. At the end of the process, the grains can be sieved out and used again with fresh milk, whereas the kefir produced is stored at 4°C until consumption. The other two methods to produce kefir tend to occur in commercial settings. The "Russian Method" is where kefir is produced on a large scale using a process that begins with the percolating of the previous fermentations to keep the kefir in continual production. The other main commercial method follows a similar process but uses pure starter cultures isolated from kefir to inoculate the milk. An optional maturation process of maintaining the kefir at 8−10°C for approximately 24 h can contribute to the organoleptic properties of the kefir, although not performing this step leads to an atypical flavor profile. Kefir is mainly produced from the milk of bovine, ovine, and caprine species (Beshkova et al., 2002; de Oliveira Leite et al., 2013; Prado et al., 2015).

The microbial composition of kefir is highly diverse with symbiotic associations (Marsh et al., 2013; Prado et al., 2015; Slattery et al., 2019). Many of the species include lactobacilli, lactococci, streptococci, acetic acid-producing bacteria, and various yeasts (Plessas et al., 2017; Rosa et al., 2017). However, the microbial composition of kefir is highly variable depending on numerous factors including geographic location and cultural condition (Marsh et al., 2013), which means the nutritional content and the putative biological activities likely differ as a consequence. These interesting biological interactions and associations have been expertly reviewed by Rosa et al. (2017).

Kefir has been associated with numerous beneficial health effects (de Oliveira Leite et al., 2013; Guzel-Seydim et al., 2011; Prado et al., 2015; Rosa et al., 2017; Slattery et al., 2019). Notably, kefir consumption may have consequences on cardiovascular health (Lordan, Nasopoulou, et al., 2018). Kefir has been associated with favorable changes in blood lipid profiles (Maeda et al., 2004; Tung et al., 2018; Urdaneta et al., 2007), exhibited antiinflammatory effects (Rosa et al., 2017), a reduction of lipid deposition (Santanna et al., 2017), and amelioration of endothelial dysfunction, and hypertension (Brasil et al., 2018; de Almeida Silva et al., 2020; Silva-Cutini et al., 2019) in animal models. However, in a clinical study of kefir consumption in mild hypercholesterolemia men over a 4-week period, there was no significant change in the serum total cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol, or triglycerides (St-Onge et al., 2002). In contrast, kefir intake decreased fasting glucose, HbA1C, and lipid levels in patients with type II diabetes mellitus (Ostadrahimi et al., 2015).

Some of the biofunctional properties of kefir could be associated with the bioactive peptides produced during the fermentation of milk with the Kefir grains (Amorim et al., 2019). A study by Amorim et al. (2019) found that the antihypertensive activity could be attributed to 35 peptides that exhibited angiotensin-converting enzyme (ACE) inhibition *in vitro*. Ebner et al. (2015) identified 16 bioactive peptides, which were mainly released from casein during fermentation by the microflora. These peptides showed ACE-inhibitory, antioxidant, antithrombotic, mineral binding, antimicrobial, immunomodulating, and opioid activity. Liu and Pischetsrieder (2017) have evaluated the formation and degradation of bioactive peptides during gastrointestinal digestion by monitoring changes in the peptide profile in a model of oral, gastric, and small intestine digestion of kefir. They found that casein-derived peptides with ACE inhibitory activity increased after combined digestion, suggesting that physiological digestion of kefir might promote the formation of bioactive peptides.

Kefir overall is viewed as a biofunctional product with a long history of health benefits. While market trends show that the perception of kefir as a healthy food has driven increased consumption and increased its market value (projected CAGR of 5.8%, 2016–2026; Mordor Intelligence, 2021), research in the field is still in its infancy. Indeed, according to PubMed, only 682 articles relating to kefir have been published between 2000 and 2020 (PubMed, 2021a). Of these, only approximately 20 articles contained trials with human subjects. Therefore, despite being an exciting field of research, there is a significant gap in the literature supporting many of the health claims attributed to kefir consumption. However, many of these health benefits are attributed to the diversity of probiotics present within the kefir.

Probiotics are live bacteria that confer benefits to the host when adequate amounts have been consumed (Hill et al., 2014). These products have the potential to beneficially alter the human gut microbiome. However, research has also shown that dead bacteria and their components may also confer probiotic effects (Plaza-Diaz et al., 2019). *Lactobacillus* and *Bifidobacterium* are the main strains of lactic acid bacteria (LAB) widely used as probiotics in functional foods and nutraceuticals. Probiotic products come in various forms. These include foods such as kimchi, sauerkraut, kefir, kombucha, miso, pickles, and yogurt along with supplements that can contain multiple strains or single strains of probiotic microbes. Indeed, probiotic milk-based beverages have dominated markets in North America, Europe, and the Asian-Pacific countries (Merenstein et al., 2010). In recent years, next-generation probiotics (NGP), which are akin to pharmacological grade supplements, have garnered attention for their potential beneficial effects against digestive disorders, allergies, metabolic diseases, and even infectious diseases via their potential antiinflammatory effects (O'Toole et al., 2017; Lordan, Rando, & Greene, 2021; Brahma, Naik, and Lordan, 2022). Many dairy products such as yogurt and cheese contain probiotics. Indeed, many dairy beverages have been specifically developed and marketed as probiotic yogurt drinks characterized by a select few or multiple strains of LAB, some of which have been identified in Table 9.1.

Some of the health benefits of fermented milk have been attributed to their probiotic composition, which in turn has led to the development of specialized probiotic milk drinks. These types of products have been associated with benefits to immune health, including reducing antibiotic-associated diarrhea (Dietrich et al., 2014; Wenus et al., 2008) and reducing the incidence of infectious diseases (Merenstein et al., 2010). Research in this field is continuing to grow with over 30,000 articles published between 2000 and 2020 (PubMed, 2021b).

3.2 Plant-based dairy alternatives

In recent years, there has been a trend toward the development of nondairy milk and yogurt-like products, mainly due to animal welfare and environmental concerns, leading to increasing consumer trends for sustainable diets (Aydar et al., 2020) and because a significant number of the world's population is lactose intolerant (Gupta & Abu-Ghannam, 2012). These products can be described as suspensions of dissolved and disintegrated plant materials, resembling bovine milk in appearance (Mäkinen et al., 2016). These nondairy products can be classified as:

- cereal-based, such as rice, oat, spelt, and corn;
- legume-based, such as soy, peanut, lupin, pea, chickpea;
- nut-based such as almond, coconut, walnut, hazelnut, cashew, pistachio;
- seed-based, such as sesame, sunflower, flaxseed, hemp;
- pseudo-cereal-based, such as quinoa, amaranth, and teff (Sethi et al., 2016).

They can be consumed as produced or they can be used to produce fermented milk, yogurts, and other dairy-like products, albeit with additional processing. These dairy alternatives are rich in a number of bioactive compounds, such as phenolic compounds found in peanuts (Settaluri et al., 2012), β-glucans found in oats (Deswal et al., 2014), lignans and tocopherols found in sesame (Namiki, 2007), resistant starch and amylose found in chickpea (Osorio-Díaz et al., 2008; Wang et al., 2018), and isoflavones found in soybeans (Omoni & Aluko, 2005).

There are several challenges when developing plant-based dairy alternatives. For example, depending on the source of protein, the physicochemical properties will affect the processing as well as the attributes of the final product. Another challenge is the low consumer acceptability due to the sensory properties of plant-based proteins, such as their taste but also color and texture. As revealed from a market screening conducted by Grasso et al. (2020) in order to increase their acceptability numerous additives are incorporated such as hydrocolloids, flavorings, sugar, and colors (Grasso et al., 2020). Despite these additives, these alternative milk are generally perceived as "healthier" by consumers, and these products are often marketed to promote a different health benefit, as consumers are trending away from animal-based products over concerns about lactose, fat, and cholesterol (Stall & Adams, 2017; Lordan, Nasopoulou, et al., 2018). The nutritional properties of these alternatives to milk can be highly variable and tend to depend on the plant source, processing, and fortification. Indeed, these products are not nutritionally similar to dairy products, often inferior, and as such, they are not considered a part of the dairy food group in nutritional guidelines (Jeske et al., 2017; Lordan, Nasopoulou, et al., 2018; United States Department of Health and Human Services, 2017).

In order to minimize the use of additives such as emulsifiers and hydrocolloids, and to improve the stability of plant-based dairy alternatives, a number of novel processing methods can be applied (Aydar et al., 2020). These include ultrasound, pulsed electric field, high-intensity ultrasound irradiation, ohmic heating, and ultrahigh and high-pressure homogenization and their combination with the aim to inactivate enzymes and microorganisms, reduce the viscosity and increase stability (Aydar et al., 2020) while maintaining their nutritional profile.

Most of the plants used for the dairy alternatives are low in protein. And while other plants such as legumes contain high levels of proteins, they do not contain all essential amino acids when compared to the protein content of dairy sources. Additionally, the presence of trypsin or other antinutritional factors can inhibit protein digestion (Tangyu et al., 2019). The challenge of the amino-acids composition can be addressed by using a combination of sources, a process known as protein complementation, while fortification is employed to address the issues associated with the lack of some vitamins such as B_{12}, B_2, D and minerals such as zinc, iron and calcium (Sethi et al., 2016; Zhang et al., 2007).

Another challenge faced when producing plant-based dairy alternatives is the fact that most of the valuable nutrients present in the original plant are lost during processing, especially the lipophilic ones. Moreover, the levels of the different macronutrients are quite low compared to dairy products. Additionally, the presence of antinutrients results in reduced bioavailability of some of the minerals such as calcium, iron, or zinc. Fermentation, with the use of probiotic bacteria, can enhance the bioavailability of calcium and other minerals and can reduce the antinutrient content (Aydar et al., 2020), improving the nutritional properties of products containing plant-based proteins. Indeed, dairy alternatives may become important sources of probiotics in the future with the development of soymilk, rice milk, and coconut milk probiotic beverages. However, the efficacy of probiotics obtained from dairy alternatives has yet to be fully studied as highlighted by Rasika et al. (2021).

An additional benefit of fermentation is the improvement of the sensory properties of the plant-based dairy alternatives as reviewed by Tangyu et al. (2019). These products before processing and without the incorporation of additives exhibit low consumer acceptance. The presence of phenols and tannins depending on their molecular weight (Drewnowski & Gomez-Carneros, 2000) can result in bitter or astringent taste, and the presence of volatile compounds derived from the oxidation of plant lipids can be responsible for off-flavors. The color of most of these products is different from the white color of the dairy drinks, due to the different colors of the plant. The texture can also be grainy, the viscosity can be thin with unsatisfactory mouthfeel, which does not resemble the mouthfeel of dairy products (Tangyu et al., 2019).

The most common nondairy fermented drinks traditionally consumed around the world are cereal-based drinks. For example, boza, which is consumed in Bulgaria and Turkey is produced through the fermentation of a variety of cereals such as barley, oat, rye, millet, maize, wheat, or rice. Kvass is a fermented rye bread drink consumed in Russia, Amazake, a precursor of sake is a sweet fermented rice beverage, pozol produced from maize grains is commonly consumed in south-east Mexico (Marsh et al., 2014). There are other nondairy fermented drinks that are not based on cereals, such as Kombucha, which is produced through fermentation of sugared black tea with tea fungus (Jayabalan et al., 2007) and water kefir is a fermented beverage generated through sucrose fermentation of solutions with different dried and fresh fruits (Farag et al., 2020). There have also been strides to use plant-based alternatives as carriers of probiotics, but with the added complication of having to find the right composition of probiotics in order to avoid poor survival of microbes in nondairy substrates and to prevent the occurrence of off-flavors (Pontonio & Rizzello, 2021). Finally, combinations of dairy milk and plant-based proteins have been explored for the preparation of fermented drinks with improved nutritional (increased levels of essential amino acids) and sensory properties (increased preference) (Akin & Ozcan, 2017).

Overall, there has been a dearth of research on the health effects and nutritional quality of alternatives to milk. However, soy milk has been popular for over 25 years and so the research is more widely available, but yet to be established. For instance, soy protein is thought to reduce blood pressure (Liu et al., 2012) and exert hypocholesterolemia effects (Jenkins et al., 2010), but the data appears to be inconsistent to date (Messina, 2016). A recent report utilizing data from the United States Department of Agriculture Branded Food Products Database has highlighted that the nutrient density of various plant-based beverages is highly variable, fortification patterns are inconsistent, and there is a need for standardization of nutrient density in these alternative products (Drewnowski, 2021).

As the plant-based alternatives market grows and consumption increases, it is important that nutritional research of these products continues and expands. The long-term consequences of consuming these products are yet to be determined. While there may be some benefits to consuming plant-based alternative products, it is also likely that due to processing and additives, there could be some negative effects of their consumption. Research going forward needs to consider the effects of their consumption on the potential development of noncommunicable diseases such as cardiovascular diseases and their effect on consumers with preexisting conditions (Borin et al., 2021; Lordan, Nasopoulou, et al., 2018).

3.3 Yogurt beverages as functional foods and carriers of bioactive compounds for cardiovascular health

Yogurt has long been a source of vital nutrients important for general health. In recent years many have explored the benefits of yogurts for cardiovascular health (Mickinley, 2005; Lordan, Nasopoulou, et al., 2018). Yogurts have also been a source of or a vehicle for bioactive components, whether occurring as part of the yogurt production process (e.g., probiotics, polar lipids) or added in before, during, or after production (e.g., supplemented with protein powders, vitamins, probiotics, prebiotics, bioactive components, etc.) (Krasaekoopt et al., 2003; Lordan et al., 2019c, 2020; Megalemou et al., 2017; Ng et al., 2018; Robertson et al., 2016; Yildiz & Ozcan, 2019). Yogurts have been associated with numerous functional benefits such as antioxidant properties, antimicrobial properties, and a source of probiotics, and they are a source of naturally formed and added bioactive lipids and proteins, all of which may benefit cardiovascular health. Functional yogurts have also been produced that have the capacity to lower cholesterol levels due to the addition of plant sterols and stanols (Mickinley, 2005; Marette et al., 2010; Lordan, Nasopoulou, et al., 2018; Sarkar, 2019). Various versions of these yogurt beverages are available commercially, while others are being researched and produced for the functional foods' markets. Yogurt consumption appears to be favorably associated with lower risks of obesity, metabolic syndrome, and cardiovascular diseases (Eales et al., 2016; Lordan & Zabetakis, 2017a, 2017b; Sayon-Orea et al., 2017; Dehghan et al., 2018). Therefore, it has been proposed that yoghurt-based beverages may also exert beneficial health effects in a similar manner.

However, research on yogurt-based beverages are less widely available and it confounded by the fact that so many different and varied products to choose from. Some products contain high omega-3 fatty acids (from flaxseed oil or fish oil) or conjugated linoleic acids, others are high in minerals such as calcium and iron, and many contain specific bioactive components with a specific health target in mind such as the presence of phytosterols to reduce cholesterol, probiotics to promote immune health or melatonin to promote sleep (Sharma, 2005). While most of these beverages tend to resemble milk or yogurt and tend to be nutrient-dense, others may be high in additives and sugars related to their processing and flavor enhancement. These additions may have negative consequences for health and may negate any potential health benefits of consuming these products. Therefore, it is imperative that further innovations in the field try to preserve the preexisting health benefits of their products before altering their nutritional content.

4. Fermented drinks as a source of bioactive peptides

Ongoing research on milk proteins has demonstrated their health benefits throughout the life course, starting from early childhood up to older age. These health benefits are attributed mainly to the essential amino acids present in milk proteins and to the fact that they are a source of bioactive peptides as demonstrated in numerous studies (Nguyen et al., 2020) and reviews (Korhonen, 2009; Nongonierma & FitzGerald, 2015a). Bioactive peptides are protein fragments of low molecular weight comprised of 2−20 amino acids (Martinez-Villaluenga et al., 2017; Nongonierma & FitzGerald, 2016), which are generated during the processing, fermentation or hydrolysis of proteins through the action of proteolytic enzymes (Korhonen & Pihlanto, 2006). Research has shown that they "have a positive influence on physiological and metabolic functions or conditions of the body having a positive effect on human health" (Sánchez & Vázquez, 2017).

Particularly in the case of fermented drinks, LAB synthesize cell-surface proteinases to hydrolyze milk proteins, whey, and caseins, releasing in this way numerous peptides (Fitzgerald & Murray, 2006; Leclerc et al., 2002). When released these peptides, depending on the peptide sequence, molecular weight, and the peptide composition exhibit various biofunctional properties (Aguilar-Toalá et al., 2017). As reported in numerous studies and reviews (Raveschot et al., 2018) they exhibit antioxidant, ACE inhibition, mineral binding, antidiabetic, satiating, immunomodulating, opioid, or antimicrobial (Nongonierma and FitzGerald 2015b, 2016). The peptide functionality depends on the degree of hydrolysis of the protein, which is affected by the conditions of fermentation, such as the starter cultures used, the substrate (source of protein), the time of fermentation, or the fermentation temperature (Chakrabarti et al., 2018).

In relation to the starting culture, Ahn et al. (2009) have found that the use of different *Lactobacillus* sp. can lead to the production of peptides from whey with varying ACE inhibitory activity. Specifically, *Lactobacillus helveticus* is known to produce a number of endopeptidase, aminopeptidase, and cell-envelope proteinases (Chen, Li, et al., 2015), which can release peptides, which inhibit ACE. Indeed, ACE has an important role in regulating blood pressure because it hydrolyzes the decapeptide angiotensin I and produces the potent vasoconstrictor octapeptide angiotensin II. Moreover, ACE hydrolyzes the peptide bradykinin, which is a potent vasodilator (Yust et al., 2003). The ACE inhibitory peptides may therefore have the ability to lower blood pressure *in vivo* by limiting the vasoconstrictory effects of angiotensin II and by potentiating the vasodilatory effects of bradykinin (Murray & FitzGerald, 2007). Chai et al. (2020) has conducted a comprehensive review on the peptides formed during the fermentation of milk using *Lactobacillus helveticus*, highlighting the specific strains of *Lb. helveticus* might produce peptides with higher ACE inhibitory activity. The most well-reported peptides with such activity are valyl-prolyl-proline (Val-Pro-Pro) and isoleucyl-prolyl-proline (Ile-Pro-Pro) peptides. The review also concluded that for each strain there are optimum time and temperature fermentation conditions (Chai et al., 2020). Other bacteria species might result in the production of peptides with different properties. For example, the use of *Lactococcus lactis* for the fermentation of nonfat reconstituted milk resulted in peptides that showed *in vitro* antithrombotic and hypocholesterolemic activity (Rendon-Rosales et al., 2019). Hypocholesterolemic peptides have been shown to suppress cholesterol and lipids in the blood through different mechanisms. For example, they could stimulate the secretion of bile acids, modify lipid metabolism in the liver, or affect hormones and cholesterol receptors (Howard & Udenigwe, 2013; Maestri et al., 2016). The combination of LAB and yeast for the production of fermented drinks will lead to the production of products with a sour taste and slightly alcoholic due to the production of lactic acid and ethanol respectively (Tagliazucchi et al., 2019). This fermentation will result also in the production of bioactive peptides with ACE inhibitory activity (García-Burgos et al., 2020).

The substrate, such as the type of milk used in the production of fermented dairy products has also an effect on the bioactive peptides generated because the composition and relative distribution of the milk proteins differ depending on the type of milk (Guha et al., 2021). For example, camel milk does not contain β-lactoglobulin, sheep milk contains more protein compared to cow milk, while goat milk contains higher β-LG and lower α-LA compared to cow milk. It has been shown that the same bacteria species or even strains can lead to the generation of different peptides when hydrolyzing casein from cow, goat, sheep, camel, or mare milk (El-Salam & El-Shibiny, 2013; Raveschot et al., 2018). The fermentation of plant-based dairy alternatives, depending on the source of protein can result in a variety of peptides. Peptides with antihypertensive activity have been produced during the fermentation of soymilk (Kesika et al., 2021; Tsai et al., 2006) (Kesika et al., 2021; Tsai et al., 2006; Iwaniak et al. 2021) in their work present an overview of the available databases of bioactive peptides. Specifically, a list of bioactive peptides from a variety of fermented foods can be found in the FermFooDb (Chaudhary et al., 2021), which is a manually curated database of bioactive peptides that contains information about the peptides and the process of fermentation.

Some of the challenges related to the bioactive peptides are that most studies proving their functionality have been conducted *in vitro*, which does not take into consideration their biovailability. In other words, whether they remain active after oral ingestion and during GI digestion and absorption they reach the target site intact (Tagliazucchi et al., 2019; Vermeirssen et al., 2004; Xu et al., 2019). Therefore, it is important to investigate whether they survive the gastrointestinal tract during digestion. Researchers have tested the fate of peptides after stimulated gastrointestinal digestion or permeation through Caco-2 cells. While there are only a few studies indicating the absorption of bioactive peptides in humans and animals (Xu et al., 2019), more studies investigating their fate in humans after digestion need to be conducted (Nongonierma & FitzGerald, 2015b). The dearth of relevant studies could be attributed to the difficulty of analyzing the small molecular weight compounds in complex biological fluids, or the fact that many peptides are generated from various food sources during digestion, and it is difficult to find the cause-and-effect relationship between the bioactive peptides consumed predigestion (Nongonierma & FitzGerald, 2015b).

5. Nutrition and health claims

According to the EU legislation and specifically Regulation (EC) No 1924/2006, which was recently amended by Regulation (EU) No 1047/2012, a nutrition claim means any claim that states, suggests, or implies that a food has particularly beneficial properties due to (i) the energy it either provides, or it provides at reduced rates or increased rates, or it does not provide, and (ii) due to the nutrients it contains, contains in reduced or increased proportions or does not contain. For food to bear a nutrition claim it needs to contain energy or certain nutrients at specific levels as described in the ANNEX of Regulation (EC) No 1924/2006. Therefore, fermented milk depending on their source can bear claims related to the protein content, i.e., they can be either "sources of" or "high in" protein, they can either be "sources of" or "high in" certain minerals and vitamins. In the case of dairy products, since milk contains a number of beneficial compounds (Thorning et al., 2017), they can be "high in" or "sources of" proteins, minerals (calcium, phosphorus, and magnesium), water-soluble vitamins (B_{12} and riboflavin), fat-soluble vitamins (A, D, and K_2), and specific lipids (Tagliazucchi et al., 2019). In the case of plant-based drinks, they could also bear the claim "source of fiber". Moreover, at the reformulation stage of these products vitamins and minerals are usually added therefore they can also bear claims related to these.

As discussed in this chapter, a number of bioactive compounds can be found in fermented drinks, such as polar lipids, probiotics, and bioactive peptides, However, whether or not these drinks can bear health claims will depend on the country, since the authorization of the claims differs depending on the regulatory system. Diaz et al. (2020) have summarized the health claims in Japan, the USA, and the EU showing the differences between the regulatory systems in the different regions (Domínguez Díaz et al., 2020). In the E.U., a health claim is any statement that implies a relationship between food and health, and according to Regulation (EC) No 1924/2006, the European Commission authorizes different claims based on the scientific opinion of the European Food Safety Authority. There are three types of claims; (i) the "function claims" (article 13 of the regulation), which either relate to the growth, development and functions of the body, or they refer to psychological and behavioral functions or they refer to slimming or weight-control, (ii) the "risk reduction claims" (or Article 14(1) (a) claims) on reducing a risk factor in the development of a disease, (iii) "Claims referring to children's development" (Article 14(1) (b) of the regulation).

Health claims of specific compounds in the E.U. undergo an authorization process, which is based on the scientific opinion of the EFSA. A compound not authorized in the EU might be authorised in the States or in Japan or China, because of the differences in the regulatory systems and the differences in the authorization processes (Chalamaiah et al., 2019). Japan, as mentioned at the beginning of the chapter, was the first country that proposed the term functional foods (Domínguez Díaz et al., 2020), and their functional foods or foods with health claims are categorized as Foods for Specified Health Use (FOSHU). There are three types of health claims related to functional foods in Japan; foods for specialized health issues, foods with functional claims, and foods with nutrient functional claims (Koirala & Anal, 2021). For approval and authorization, the FOSHU product must clearly demonstrate its effectiveness on the human organism based on strong scientific evidence (clinical studies) as well as the absence of any safety issues. The approved FOSHU can be: a) Regular/ordinary FOSHU, b) Qualified FOSHU, c) Standardized FOSHU and d) Reduction of disease risk FOSHU. A comprehensive description of the authorization process of functional foods and health claims in Japan can be found in Iwatani and Yamamoto (2019) and in Domínguez Díaz et al. (2020). In the USA health claims are those that express a relationship between a food component or a food supplement and a disease or a health-related condition, and they can be classified into structure/function claims and health claims. The structure/function claims do not need Food and Drug Administration premarket approval. In all cases, the wording of the claim needs to be specific and approved during the authorization process.

In the EU there are health claims associated with protein such as "protein contributes to the maintenance of bone", "Protein contributes to the growth or maintenance of muscle mass" or "Dietary proteins help to maintain muscle mass of elderly people" (EFSA Panel on Dietetic Products and Allergies, 2010a). Others associated with calcium such as "Calcium is needed/important for the structure of bones/healthy bones", "Calcium is needed for normal nerve function" (EFSA Panel on Dietetic Products and Allergies, 2010a), or vitamin D for example "Vitamin D contributes to the normal function of the immune system of children" (EFSA Panel on Dietetic Products and Allergies, 2015) or Vitamin D contributes to the normal function of the immune system and healthy inflammatory response" (EFSA Panel on Dietetic Products and Allergies, 2010b).

Currently, there are no health claims approved for probiotics or bioactive peptides in the EU due to several reasons. For example, due to the lack of data proving the substantiation of the claim, due to the claims not being sufficiently defined, or in most cases due to insufficient evidence showing a cause-and-effect relationship (Pen+Tec Consulting, 2019). On the other hand, in Japan probiotic fermented drinks and yogurts have FOSHU approval (Iwatani & Yamamoto, 2019). The same applies to health claims associated with bioactive peptides. In the EU there are no health claims authorized for bioactive peptides due to the lack of data on their safety, their fate in the gastrointestinal tract, and the lack of substantial evidence from human studies (Chalamaiah et al., 2019). Specifically, VPP and IPP enzymes with ACE inhibitory activity as demonstrated in several studies reported earlier in this chapter, have not been approved by the EU Commission. This was based on the scientific opinion of EFSA, which concluded that there was no cause-and-effect relationship between the consumption of IPP and VPP and the maintenance of normal blood pressure. In Japan, however, these peptides derived from bonito and sardine have received FOSHU approval, and have been on the market as natural and safe blood pressure-lowering dietary supplements (Chalamaiah et al., 2019). In the case of polar lipids, there are a number of groups working toward the substantiation of the health claims associated with their antiinflammatory properties and antithrombotic properties in dairy products that so far have demonstrated *in vitro* and *ex vivo* antithrombotic activity (Lordan et al., 2019b, 2019c; Megalemou et al., 2017; Ronan Lordan, Nasopoulou, et al., 2018; Ronan Lordan and Ioannis Zabetakis, 2017), which are due to be assessed in clinical trials. While no polar lipid products have received a health claim to date, another product containing bioactive compounds with antithrombotic activities has been successful. Fruitflow is a tomato-based nutraceutical that has demonstrated safety and efficacy for maintaining normal platelet homeostasis in humans (O'Kennedy et al., 2017).

6. Sensory properties of fermented drinks

Consumption of fermented milk will be influenced by consumer acceptability, which is linked to the sensory properties of the products (Gomes et al., 2013; Muir & Hunter, 1992). Research so far has shown that the sensory properties of fermented drinks depend on their composition, protein and fat content, the starter cultures used, and the substrate (dairy or plant proteins) used for fermentation. The main sensory attributes usually investigated are related to taste, aroma, and mouthfeel. There are five basic tastes: bitter, sweet, sour, salty, and umami. Specifically, in dairy fermented drinks, sourness can be associated with the formation of lactic acid during fermentation (Ganatsios et al., 2021) and bitterness can be associated with the presence of various peptides released during proteolysis of the proteins (Zhao et al., 2016). Lately, researchers have been trying to establish whether a sixth basic taste exists, the taste of fat. Despite the fact that data shows that the interactions between long chain fatty acids and specific receptors in taste bud cells elicit physiological changes that affect both food intake and digestive functions, these findings are not definitive to establish that fat is the sixth basic taste (Besnard et al., 2016). In fermented products, the presence of lipids regardless of whether fat is considered the sixth basic taste or not will be responsible for their characteristic flavor. This is due to the free fatty acids formed during the lipolysis of fat. Fermented milk products, in particular, have higher levels of free fatty acids compared to milk. Moreover, the source of milk (cow, sheep, camel) or the fermentation conditions will affect the profile of free fatty acids and consequently the flavor of the fermented dairy drink (Farag et al., 2020).

Muir and Hunter (1992) had developed a vocabulary for the characterization of fermented dairy products including odor related attributes such as intensity, sour, fruit, buttery, yeasty, creamy, sweet, flavor-related attributes such as sour/acid, fruit, buttery, creamy, rancid, salty, bitter, lemon, sweet, chemical. To characterize aftertaste, they used attributes such as bitter, sour/acid, and other, and finally, for the texture they used firmness, creaminess, viscosity, sliminess, chalky, mouth-coating, and curdy character (Muir & Hunter, 1992). From the investigation of the relationship between these sensory attributes and the physicochemical characteristics of the drinks, they found that there was a relationship between pH and acid flavor, bitter flavor, rancid flavor, and bitter after-taste, while protein to solids-not-fat ratio was shown to affect chalky mouthfeel of fermented drinks.

The source of milk will affect the sensory properties of the fermented drink. When comparing dairy fermented drinks from cow's milk with goats' and sheep's milk there are a number of differences. In terms of flavor, the profile of goat's milk is more intense compared to cow's milk (Dimitrellou et al., 2019). For example, in the case of kefir, buffalo's milk resulted in the production of kefir with improved sensory properties (overall acceptability, appearance, odor, and taste) compared to cow's milk (Gul et al., 2018) due to the differences in fat content, and casein micelle size between the two types of milk. Depending on the type of milk, differences in the textural properties have been reported (Dimitrellou et al., 2019) such as consistency, cohesiveness and viscosity, lower hardness, adhesiveness, extension forces, and higher susceptibility to syneresis, or lower firmness (Miocinovic et al., 2016). Sheep milk when compared to goat and cow's milk exhibit higher adhesiveness and hardness compared to the other types of milk (Domagała, 2009). The source of milk can also affect the color of the final product, for example, addition of goat's milk results in greater whiteness (Vargas et al., 2008) compared to sheep milk (Domagała, 2009).

The type of microorganisms used to produce the fermented drink and the fermentation conditions will have an effect on the sensory properties of the final product. For example, in the case of kefir yeasts are responsible for the formation of several compounds such as peptides, amino acids, ethanol, and CO_2 that contribute to kefir's flavors and aroma. As mentioned earlier lactic acid bacteria will lead to the formation of lactic acid, which will be responsible for the sourness in fermented dairy drinks (Farag et al., 2020). The temperature of fermentation will affect the viscosity of kefir; for example, higher fermentation temperatures will result in higher viscosities and reduced acceptability of the final product (Barukčić et al., 2017).

In the cases of plant-based fermented dairy alternatives, the presence of phenolic compounds can contribute to astringency, or to bitterness depending on their molecular weight (Drewnowski & Gomez-Carneros, 2000). The presence of sugars or fruit juice can compensate for the unpleasant taste of bitterness and astringency. In terms of mouthfeel, the main attribute associated with fermented drinks is viscosity, as mentioned for the dairy fermented drinks as well. This is affected by the total solids and the protein content of the substrate used (Gomes et al., 2013; Küçükçetin et al., 2011). The presence of fiber in plant-based fermented drinks improves the mouthfeel. Depending on the raw plant material the color of the final product can be greenish, greyish, or brownish (Tangyu et al., 2019).

Understanding which sensory properties will drive consumer acceptability of the product will guide product development. Therefore, it is important to use the right sensory test to characterize the sensory attributes and to determine the drivers for the acceptability of the fermented milk. A number of methods and their advantages are presented in the comprehensive review conducted by Cruz et al. (2010). Most of the tests presented in that review are static tests, where sensory attributes are measured at one point. While most studies use static methods to evaluate fermented dairy drinks, a recent study conducted by Esmerino et al. (2017) assessed the dynamic sensory profile of three categories of fermented dairy products using a variety of temporal methodologies such as temporal dominance of sensations (TDS), progressive profiling (PP), and temporal check-all-that-apply (TCATA) (Esmerino et al., 2017). In the case of TDS, assessors are being asked to choose at certain time points the most dominant sensation they experience from a list of predetermined attributes (Pineau et al., 2009). In the case of progressive profiling, assessors score the intensity of specific attributes at predefined moments (Jack et al., 1994), while in the case of temporal check-all-that-apply participants are presented with a list of sensory attributes and are asked to continuously select from that list the attributes that describe the product during the evaluation (Jaeger et al., 2017). Esmerino et al. found that sour taste was confused in many cases with bitterness and for this reason bitterness was the predominant taste of the fermented dairy products, however fermented flavor and sourness are the many characteristics of the tested products (Esmerino et al., 2017). Creaminess, however, was not a predominant perception despite the fact that other researchers such as Janiaski et al. (2016) found it an important attribute influencing liking of fermented dairy beverages. Textural properties such as creaminess were scored in the case of the TCATA method. These findings suggest that the choice of method to evaluate the temporal information on the samples can have a significant impact on the characterization of the products (Esmerino et al., 2017) guiding in this way product development.

7. Conclusions and future directions

Innovations in dairy technology and functional foods have led to the rise in the production and consumption of consumer beverages worldwide. The opportunity to produce functional foods from yogurt beverages is endless, and as highlighted in this chapter, these products can be used to deliver beneficial health benefits via their intrinsic bioactive components and additive bioactive compounds. However, research in this area in relation to health benefits varies in rigor and quality, and some drinkables such as kefir, are still in their infancy. Moreover, there is a lot of interest in the production and consumption of dairy-alternative fermented beverages, due to the growing environmental awareness of the consumers. The specific question of whether these products are beneficial for cardiovascular health is still ongoing. It is likely that yogurt-based beverages low in sugar may indeed confer cardioprotection via the presence of bioactive lipids, peptides, and other microconstituents. It is important that along with the innovation in the sector that research on the health benefits of these products continues to grow. More studies investigating the biovailability of the bioactive compounds present in fermented milk and dairy alternatives and yogurt beverages need to be conducted. The scientific evidence provided through these studies will impact the regulatory framework associated with these products, leading to the provision of clear information through labeling to the consumers. When developing functional foods, such as fermented dairy or dairy-alternative beverages, their sensory properties need to be considered to ensure their consumption by the end user. Therefore, research needs to be conducted in order to ensure that functional foods will be acceptable to the target consumer group while they retain their biofunctional properties.

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Part III

Marine food

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Chapter 10

Seafood and shellfish

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Abbreviations

ACE Angiotensin—converting enzyme
ALA α Linolenic acid
As Arsenic
Ca Cadmium
CVD Cardiovascular diseases
DHA Docosahexaenoic acid
EAA Essential amino acids
EFSA European Food Safety Authority
EPA Eicosapentaenoic acid
FDA Food and Drug Administration
FOSHU Food for Specific Heath Uses
GHP Good hygiene practices
HACCP Hazard analysis critical control point
HDL-C High-density lipoprotein cholesterol
Hg Mercury
HPP High-pressure processing
IQF Individual quick freezing
K Potassium
LCPUFA Long chain polyunsaturated fatty acids
LDL-C Low-density lipoprotein cholesterol
Mg Magnesium
MUFA Monounsaturated fatty acids
Na Sodium
NPN Non protein nitrogen
Pb lead
PCB Polychlorinated biphenyls
PCDD Polychlorinated dibenzodioxins
PUFA Polyunsaturated fatty acids
ROS Reactive oxygen species
SBP Systolic blood pressure
SHR Spontaneously hypertensive rats

1. Introduction

Seafood is one of the most highly traded foods internationally and although global capture fisheries production is unlikely to increase, aquaculture is growing considerably (Smith et al., 2010). Fish and shellfish comprise at least 15% of the consumption of animal protein globally, while it should be noted that the importance of seafood is not only specific to good quality animal protein, but also as a main source of essential omega-3 fatty acids (Hibbeln et al., 2007; Smith et al., 2010).

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In addition, seafood products are believed to be vital food products for populations and countries of the third world since they are rich in macronutrients (Roos et al., 2007). Furthermore, shellfish in general are thought to be a notable commercial good that is highly valued for its financial importance (FAO, 2009, 2016).

Shellfish comprise a group of invertebrate species that dwell in water and can be broadly differentiated mainly in crustaceans and mollusks. Crustaceans are exoskeleton-bearing aquatic invertebrates with hard chitin shells and with visibly segmented bodies. This type is comprised of species such as shrimps, lobsters, and crabs. On the other hand, mollusks can be described as soft-bodied invertebrates, which can be further divided into bivalves, gastropods, and cephalopods. Common examples of such animals are mussels, oysters, clams, sea snails, squid, cuttlefish, and octopus. In addition to crustaceans and mollusks, shellfish also include the animal phylum of *Echinoderms*. Although they are not consumed as often as mollusks and crustaceans, sea urchins and sea cucumbers are quite popular in many parts of the world (Fabricant, 1998). In contrast to their name, shellfish are not considered to be fish. This can be clearer if one considers that crustaceans belong to the phylum of *Arhropoda*, which also includes arachnids and insects (Telford et al., 2008).

1.1 Financial importance

Shellfish compose a large part of the total fisheries production globally. Similar to fish, their nutritional and economical importance is exceptional. To begin with, according to FAO (2016), the global demand for shellfish in regard to quantity represented 38% of total seafood traded in 2013 but it should be noted that at the same time shellfish also represented 63.7% of the commercial value of the total seafood production (FAO, 2016). Furthermore, it appears that the demand for seafood products is increasing across the world and thus the importance of shellfish and shellfish products might follow the same pattern. Based on FAO, in 2014 a total of 146.3 metric tons of seafood were used for human consumption. This corresponds to a global per capita seafood consumption of 20.1 kg. Comparing this to the data of 2013, which showed consumption of 1.8 kg of crustaceans, 0.5 kg of cephalopods, and 2.6 kg of other mollusks per capita (FAO, 2016; Venugopal & Gopakumar, 2017), it can be commented that a significant quantity of seafood products are provided by shellfish. The general data indicate that shellfish, in contrast to fish products, represent a smaller quantity of the commercial landings in metric tons, but are responsible for a larger part of the commercial value. More specifically, according to NOS (2011), in the Gulf of Mexico shellfish top, the economic value list of species fish landings while finfish top the weight or tonnage list. This is also true for the rest of the US.

1.2 Nutritional value

In general, shellfish are appropriate food for a well-balanced diet and offer a diverse profile of macronutrients. To begin with, shellfish can have a higher protein content when compared to finfish (Venugopal & Gopakumar, 2017). More importantly, shellfish are a good source of essential amino acids (EAA) since species such as the Norway lobster, red and pink shrimp provide threonine, valine, methionine, isoleucine, leucine, phenylalanine (Rosa & Nunes, 2004). In addition to the protein and amino acid profile, shellfish are a great source of lipids. Shellfish have a low content of crude lipid but it should be noted that they contain a considerable amount of essential omega-3 Polyunsaturated Fatty Acids (PUFAs) such as the eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexanoic acid (DHA; C22:6 n-3) (Bergé & Barnathan, 2005; Hossain & Takahashi, 2012). Additionally, the ratio of the n-6 to n-3 PUFAs is lower than 1 which is important since studies have shown that imbalances of n-6 to n-3 PUFAs ratio can lead to the production of inflammatory eicosanoids and cytokines, with proinflammatory action in animals and humans (Kim et al., 2007; Simopoulos, 2002). The lower the value of the n−6/n−3 PUFA ratio in food, as in the case of shellfish, the better the antiinflammatory health outcome (Simopoulos, 2008). Finally, shellfish can be considered a source of vitamins such as B12, D3, and minerals such as sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and zinc (Zn) (Bogard et al., 2015; Dong, 2001, pp. 4−8; Gopakumar, 1997; Souci et al., 2008, p. 1300; USDA, 2012).

1.3 Processing methods

Food processing methods can be dated back to even prehistoric ages. It can be said that food processing was probably developed through trial and error in order to enhance food digestibility, and safety and prolong food shelf-life. Basic processing methods such as slaughtering, cooking, sun drying, salting, preservation, and fermenting have been used from ancient times until the beginning of the 19th century. Therefore, food processing has been slowly evolving along with humanity across the centuries and one could suggest that it has been an important tool for the formation of societies and urbanization.

Nevertheless, it was at the beginning of the 19th century that food processing started to adopt a different approach and one that would be more similar to today's processing methods. Early in the 19th century, Nicolas Appert invented vacuum bottling through the process of boiling in the interest of prolonging food shelf-life. It should be noted at this point that during his effort he was not aware of the presence of microorganisms. It was later in the 19th century that Louis Pasteur would describe the presence of microorganisms and that would propose the pasteurization method, which is still widely used today.

Shellfish are highly perishable and thus require immediate and careful handling. Due to the diverse group of species, the handling and processing might differ. Despite that, there are specific processes that are followed by most. This chapter will not describe in detail the processes, but it will provide information on the effect of various processes on shellfish product quality and nutritional attributes. In general, shellfish products undergo processes such as chilled storage, individual quick freezing (IQF), and thermal treatments such as blanching cooking, or steaming. Chilled storage and freezing extend the shelf-life of shellfish products. The former can prolong shelf-life up to 6−12 days depending on the species (Huss et al., 2007; Tantasuttikul et al., 2011) while the latter extends the shelf-life of the products significantly. At this point, it should be stated that IQF is an expensive processing step when compared to other freezing methods and thus is often used for products of high value. As it has been already stated in Section 1.1, a large part of seafood production revenue can be attributed to shellfish. As a result, products such as shrimp, scampi tail meat, squid fillets, oysters, clams, and scallops use IQF. Although frozen, extended frozen storage will incur a steady decline in the product quality characteristics. More specifically, frozen storage is known to lead to the denaturation of proteins, which can affect the moisture content of products such as shrimps (Saskia & Ruth, 2014). Furthermore, this change in moisture content can impact the sensory properties of the products since it leads not only to changes in viscoelasticity but also a decrease in total nitrogen content (Binsi et al., 2007). One of the most important effects of prolonged frozen storage of shellfish is the oxidation of PUFA. This has both an effect on the sensorial properties and the nutritional profile of the product (Venugopal, 2005).

In addition to the chilling and freezing processes used in shellfish, various heat treatments are also used. Thermal treatments are widely used in the food industry in the interest of food safety, stability, and shelf-life extension. Nevertheless, it is now common knowledge that thermal treatments have undoubtedly an effect on the proximate composition and nutrient profile of the products. Thermal treatments can have both positive and negative effects on products. Typically, cooking has a pleasant effect on the sensorial properties of most products and the same could be said for shellfish but cooking's effect on nutritional value generally has the opposite effect (Maulvault et al., 2012). In shellfish blanching, boiling, cooking, pan frying, steaming, and in general thermal treatments have an effect on proximate analysis (Cheng & Chengchu, 2014; Venugopal, 2005). The effects of boiling and steaming are not significant, but heating in the presence of oxygen gives leads to the oxidation of PUFA. Furthermore, any thermal treatment of shellfish leads to losses in carotenoids, degradation of astaxanthin, losses in iodine and bromine, and partial degradation in thiamin, pyridoxine, and cobalamin (Babu et al., 2008; Huss et al., 2007; Mesko et al., 2016; Venugopal, 2005; Yang et al., 2015). Finally, it could be said that thermal processing can have also a positive effect on the protein digestibility since during denaturation proteins reverse in their primary structure, which increases their bioavailability and digestion (Venugopal, 2005).

Canning is a process that is also used for the processing of shellfish. In contrast to other thermal heat treatments such as pasteurization, canning offers a stable final product with an extended shelf life and without the need for refrigeration. This is due to the fact that canning, unlike most thermal treatments, aims at the inactivation of both spores and vegetative cells. Therefore, canning follows a much harsher heat treatment when compared to other thermal processing methods. This is the reason that canned products are generally considered by the consumer as of reduced quality and nutritional value. One would expect that the effect of canning on shellfish products would follow similar losses of nutrients. Nevertheless, the canning of shellfish seems to have a comparable effect on nutritional value as the processes of boiling and steaming. Therefore, canning is mostly responsible for nutrient losses in water-soluble vitamins such as B_1 and $B_{6,}$ while it does not have a drastic impact on protein and lipid content.

In summary, food processing has been continuously developing and contributing to the production of new food products and advanced food safety. Processing of shellfish is desirable since it extends the shelf-life of the products and reduces foodborne diseases however it negatively impacts the nutrient profile of shellfish products. It has been also determined that a lot of waste products due to the processing of shellfish are rich in bioactive compounds and can be used for the formulation of functional foods and nutraceuticals (Harnedy & Fitzgerald, 2013; Kim et al.,2006; Menon et al., 2015). Therefore, optimization of processing can lead to significant improvements in the nutrient profile of shellfish products.

1.4 Health risks associated with shellfish

Over the past decades, the term food safety has turned into an important scientific discipline that aims to reduce foodborne illnesses through various methods and techniques. More specifically, a number of fundamental and vital regulations, practices, and policies have been implemented throughout the development of the field of food safety. Examples of such policies, practices, and regulations are the food labeling regulations (e.g., EC No 1169/2011), good hygiene practices (GHP) and hazard analysis critical control point (HACCP), policies on biotechnology and food, and guidelines for the management of governmental import and export inspection and certification systems for foods. As previously described, although regulations and policies might differ among countries, the main principles of food safety and its scientific basis remain the same and thus global principles on food safety remain similar through collections of standards and guidelines such as Codex Alimentarius. Nevertheless, it is evident that foodborne illnesses and food hazards cannot be completely avoided. Different products and based on their nature tend to display various safety concerns.

Shellfish and shellfish foodstuffs are a category of foods that have been broadly researched and a number of plausible hazards have been identified. Analogously to other food categories, shellfish and shellfish products have been associated with 3 different types of hazards namely environmental, intrinsic, and process-related hazards.

1.4.1 Environmental hazards

1.4.1.1 Microbiological hazards

Environmental hazards describe a broad group of risks such as microbial pathogens, biotoxins, chemical pollutants, pesticides or pesticide residues, heavy metals, and parasites. Initially, one common hazard that is frequently discussed with shellfish products is pathogenic microorganisms. Multiple food pathogens such as *Salmonella* spp., *Campylobacter* spp., *Vibrio parahaemolyticus*, *Vibrio cholera*, *Clostridium botulinum* Type E have been identified and isolated from both crustaceans and mollusks (Brands et al., 2005; Di Pinto et al., 2008; Newton et al., 2014; Park et al., 2019; Van et al., 2007; Wilson & Moore, 1996). All of the above microorganisms are vegetative cells, with the exception of *C. botulinum* Type E, which produces endospores, that cause foodborne illnesses and some of them have been determined to present high mortality rates (Amagliani et al., 2012).

1.4.1.2 Heavy metals and chemical compounds

Pollutants have been considered frequent hazards in seafood products. Environmental pollution already exists due to natural phenomena, but it has also been determined that human activities also contribute to the total pollution status of seawaters and thus marine and freshwater animals (Baki et al., 2018; Djedjibegovic et al., 2020; Stankovic et al., 2012, pp. 311−373). One of the most common hazards to fish and shellfish products is considered to be heavy metals. Common elements that have been considered frequent hazards in the food chain include mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As) (Stankovic et al., 2012, pp. 311−373). Heavy metals can cause a large number of detrimental effects and can affect all ages of people due to the phenomenon of bioaccumulation (WHO, 2015). Mercury is now very well-known even to the public for its effects and its connection to seafood. Although mercury can be found in its main elemental form, a number of different forms can be found. Mercury forms organic and inorganic molecules such as methylmercury, ethyl mercury, and mercuric chloride.

In addition to heavy metals, pollutants also include substances known as dioxins. Dioxins are mainly polychlorinated biphenyls (PCBs) and similar behaving compounds such as polychlorinated dibenzo - p—dioxins (PCDDs). Both groups of substances have been proven to be carcinogens and also bioaccumulate similar to heavy metals (Nevalainen et al., 2020; WHO, 2015).

Finally, another type of hazardous chemical compound that has been determined in shellfish is biotoxins. Biotoxins in shellfish are typically the result of the accumulation of toxic compounds in shellfish as a result of the food chain. Biotoxins have been part of the legislation of food safety authorities. European Food Safety Authority (EFSA) has already produced legislation on marine biotoxins through Regulation (EC) 853/2004 as well as other regulations that help the implementation of the aforementioned regulation (EFSA, 2010). Furthermore, EFSA and Codex Alimentarius have already classified 5 groups of biotoxins namely saxitoxin, okadaic acid, domoic acid, brevetoxin, azaspiracid based on their chemical structure as well their maximum concentrations and limits (EFSA, 2009).

1.4.2 Process related hazards − allergens

Shellfish represent a food category that is known for allergenic potential. This is an intrinsic hazard of this specific food type. Food allergies are mainly caused by protein molecules that are called antigens and trigger the response of the immune system. That type of response is usually named Type I allergy. The allergic reaction to food such as shrimp and crab is usually caused by specific molecules such as tropomyosin, arginine kinase, hemocyanin, paramyosin, troponins, and other molecules (Fernandes et al., 2015; Sathe & Zaffran, 2016).

Nevertheless, these substances do not constitute all the food allergies that are triggered by shellfish products. A common process-related hazard in regard to shellfish allergies is considered to be the use of metabisulfite. Sulfites are food allergens and they are labeled as such in food products e.g., in the European Union EC 1169/2011 (Chapman et al., 2006). Residual sulfite concentrations remain in the food products after their treatment in metabisulfite, which is used in order to avoid melanosis of products such as lobster and shrimps. The residual sulfites can lead to a food allergic reaction therefore proper labeling and reduction in the concentration of metabisulfites are required in order to avoid that kind of allergic reaction. An additional process-related hazard is also a hazard that has been already analyzed in environmental hazards.

Food pathogenic organisms have been determined to be the cause of a number of foodborne illnesses in shellfish products. Several processes have been set in place in order to inactive (e.g., thermal treatment) or deter the growth and or production of the toxin of certain microorganisms (e.g., pH reduction). Therefore, inadequate processing of shellfish products can be the cause of foodborne diseases (Leroi, 2014).

1.5 Functional foods, bioactive compounds, and shellfish

Across the centuries, food is believed to have the capacity for preventative action against diseases, healing effects, and sometimes the ability to reduce the severity of various diseases or symptoms (Leach, 1989). Although this approach, especially in its historical form could not be scientifically proven, one can see that from the 1990s and on the trends in consumers and thus in the food industry began to shift. During the late 90s, consumers began to approach foods in a completely different manner. Foods ceased to be a way of solely satisfying hunger or providing the necessary components for nutrition. In stark contrast, foods have now turned into a completely new system that could assist people in ameliorating health and improving wellness. According to Milner (2000), the relatively new science of nutrition, other than aiming for a balanced diet, is also ambitious in incorporating the optimization of well-being and reduction of risks in various chronic diseases. This viewpoint aided drastically in the establishment of novel food and pharmaceutical products namely functional foods and nutraceuticals.

Functional foods were first introduced in Japan during the 1980s with the addition of a new food category, Foods for Specified Health Uses (FOSHU), which was also expanded in legislation by the Ministry of Health, Labor and Welfare (Shimizu, 2003). Functional foods quickly gained a reputation across Europe and the United States. This led to the introduction of legislation that can vary between countries or continents. According to the EFSA functional foods are considered as "a food, which beneficially affects one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. A functional food can be a natural food or food to which a component has been added or removed by technological or biotechnological means, and it must demonstrate their effects in amounts that can normally be expected to be consumed in the diet." (Duttaroy, 2019). Moreover, further legislation was implemented with the regulation EC 1924/2006 that set the basis for health claims.

Similarly, to the Food and Drug Administration (FDA) of the USA, functional foods are defined and regulated through the dietary supplements health and education act (DSHEA) of 1994. More specifically, since there is no statutory description of functional foods from the FDA, both neutraceuticals and functional foods are regulated through the DSHEA according to their intended use or they might be regulated through several existing options (Martirosyan & Singh, 2015; Ross, 2000).

Due to differences in the legislation between separate countries and continents, there are no unique criteria for functional foods that can be applied globally, but an informative outline is provided by (Castillo et al., 2018).

- Natural food with improved composition by employing particular agronomical conditions.
- Food including a health-promoting component.
- Food from which a component has been removed to produce less adverse effects on health.
- Food in which the nature of one or more of its components has been chemically improved for obtaining health benefits.
- Food in which the bioavailability of one or more of its components has been increased to improve the assimilation of a health-promoting component.

As mentioned earlier, shellfish comprise a vast array of products and are widely consumed around the world. Similar to other seafood products, shellfish are composed of an extensive assortment of macro- and micronutrients.

As stated in the definition of functional foods according to EFSA, functional food must have adequate nutritional value and should be consumed as part of a normal diet. According to the data provided in Section 1.2, it is evident that shellfish's nutritional profile and its use as a regular food is adequate in order to be characterized as a functional food. Shellfish are also known to contain a number of bioactive compounds that can be used in order to ameliorate the health status of people or combat and reduce the risks of a number of chronic diseases.

2. Shellfish products and their bioactive compounds on diseases

Shellfish contain a substantial number of bioactive compounds that can have a positive effect on people's wellness as well as combat chronic diseases. Due to the variety and diversity of the compounds found in shellfish, this chapter will be categorized according to the macro and micronutrient types of the bioactive components.

2.1 Proteins and protein hydrolysates

Shellfish similarly to seafood is considered to be an excellent source of good quality protein, with good bioavailability and digestibility and rich content in EAA. Proteins, and especially those of good quality, are considered essential macronutrients and are vital both for the physiological function as well the wellness of people. Firstly, it must be noted that shellfish have a satisfactory content of EAA. Subsequently, shellfish can be considered as a good substitute for meat, dairy and fish products. More specifically, shellfish have a protein efficiency ratio better than casein and above 90% (Hamed et al., 2015; Venugopal, 2005). Nevertheless, the importance of shellfish proteins and protein hydrolysates is not only based on their nutritional value. Recently, it has been suggested that during the digestion of proteins in humans, the proteins are broken down to oligopeptides or even smaller peptides such as tri- and di-peptides with attractive physiological properties such as antitumor, antidiabetic neuroprotective and antihypertensive mode of action or to have a preventative action against chronic diseases (Cheung et al., 2015; Harnedy & Fitzgerald, 2012; Kim & Wijesekara, 2010; Tsai et al., 2008; Vijaykrishnaraj & Prabhasankar, 2015).

Firstly, several shellfish peptides from clams, mussels oysters, and shrimps (Benjakul et al., 2009; Hai-Lun et al., 2006; Katano et al., 2003; Nii et al., 2008; Tsai et al., 2008; Wang et al., 2003) possess either antihypertensive or inhibitory activity against angiotensin-I-converting enzyme (ACE). The ACE is responsible for converting angiotensin I to angiotensin II, which is a vasoconstrictor, and subsequently, it is implied that ACE plays an important role in the regulation of blood pressure. A number of studies have been also conducted with spontaneous hypertensive rats (SHR), which have shown a reduction in systolic blood pressure (SBP) but more studies and clinical trials should be performed in humans to produce more evidence that can corroborate this antihypertensive and cardioprotective of these peptides (Je et al., 2005; Lee et al., 2010; Nii et al., 2008). In addition to the antihypertensive properties, some peptides from clams have been suggested to possess a hypocholesterolemic effect and bile acid binding capacity (Lin et al., 2010). Another important property of peptides of white shrimps is their antioxidant activity. According to Latorres et al. (2018), hydrolysates of white shrimp showed antioxidant activity against radical sequestration. In addition to white shrimp, other species of shrimp along with peptides from the skin in squids and from fermented mussels have also suggested that there are a number of bioactive peptides with antioxidant properties (Benjakul et al., 2009; Gimenez et al., 2009; Rajapakse et al., 2005). Finally, another interesting suggestion regarding the antitumor and anticarcinogenic properties of shellfish proteins and protein hydrolysates is related to peptides of oysters (Cheung et al., 2015). Such peptides exhibited, cytotoxic activity and *in vivo* antitumor activity toward cancer cells in the prostate, breast, and lungs (Teixido et al., 2012) (Table 10.1).

2.2 Lipids

Cardiovascular diseases (CVD) comprise a group of chronic diseases that have been considered to be the leading cause of death worldwide. CVD includes a number of different conditions such as coronary artery disease, stroke myocardial infarction, and thromboembolic disease (Mozaffarian et al., 2013). Diet and lifestyle are considered to be important parameters both for the progression and prevention of CVD (Martínez-González et al., 2015). Lipids and fat have been always interconnected with CVD. This is a vague statement since omega-3 PUFA's have been shown to have a protective effect while SFA's are suggested to increase the risk (Stone, 1996). The aforementioned action of omega-3 PUFA's is relevant with shellfish due to their proximate composition in PUFA and their favorable low levels of their omega-6/omega-3 PUFA ratio. In addition to their excellent protein content and although shellfish have a low content of crude fat, a large proportion of their fat is comprised of PUFA and MUFA. In general, shellfish have a higher proportion of PUFA than that MUFA or SFA. More importantly, it has been found that the ratio of omega-6 (n-6) to omega-3 (n-3) PUFA in shellfish is

TABLE 10.1 Functionality of each peptide or hydrolysate (name or peptide sequence) from various marine sources.

Bioactive compound/peptide sequence	Marine source	Function	References
Not given	*Litopenaeus vannamei*	ACE	Benjakul et al. (2009)
VVYPWTQRF	*Crassostrea talienwhanensis Crosse*	ACE	Wang et al. (2008)
VRK and YK	*Meretrix lusoria*	ACE	Tsai et al. (2008)
Not given	*Pinctada fucata martencii*	ACE	Katano et al. (2008)
FCVLRP	*Acetes chinensis*	ACE	Hai- Lun et al. (2006)
elisidepsin	*Elysia refescens*	Anticancer	Teixido et al. (2012)
VWYHT	*Plesionika izumiae Omori*	Antihypertensive	Nii et al. (2008)
Not given	*Crassostrea gigas*	Antihypertensive	Je et al. (2005)
Callinectin	*Callinectes sapidus*	Antimicrobial	Khoo et al. (1999)
Not given	*Litopenaeus vannamei*	Antioxidant	Latorres et al. (2018)
NADFGLNGLEGLA	*Dosidicus gigas*	Antioxidant	Rajapakse et al. (2005)
Not given	*Squid*	Antioxidant	Gimenez et al. (2009)

lower than 1 (Passi et al., 2002), which is favorable against inflammation-related chronic disorders, including CVD (Simopoulos, 2008). Moreover, a substantial amount of long chain PUFA (LCPUFA) are the omega-3 EPA and DHA. According to an analysis from a pooling project of 19 cohort studies, it was determined that PUFA's can reduce the risk of CVD by approximately 9%. The effect of the PUFA's toward CVD was studied based on multiple risk factors and biomarkers such as α linolenic acid (ALA), red blood cell levels of EPA, and DHA (EPA plus DHA; the Omega-3 Index) to describe *in vivo* n-3 LCPUFA status, whole blood, whole plasma/serum, or lipid classes from the latter, i.e., phospholipids, cholesteryl esters, triglycerides and/or nonesterified fatty acids (Del Gobbo et al., 2016; Harris et al., 2017). Along with the aforementioned meta-analysis study, more evidence regarding the preventive role of EPA and DHA toward CVD has been suggested such as a reduction in serum cholesterol (Hamed et al., 2015). Moreover, a reduced intake of saturated fat has been suggested to lead to a reduction of CVD and it also seems that the replacement of SFA's with PUFA's might offer the best of the two worlds (Hooper et al., 2020; Sacks et al., 2017). Shellfish appears to be a worthy category of food to be used for the above purpose. Therefore, it can be reasonably suggested that n-3 LCPUFA such as EPA and DHA have the capacity to reduce the risk of cardiovascular diseases (Calder et al., 2014).

In addition to the effect of LCPUFA's toward CVD's, another important role of LCPUFA's such as DHA and EPA is their capacity to reduce inflammation. EPA and DHA have been implied to possess antiinflammatory properties (Simopoulos, 2008). These properties can be attributed to the increased availability of EPA and DHA to be used for the synthesis of bioactive lipid mediators. Normally, the n-6 arachidonic acid is used as the main substrate. This is crucial since the production of eicosanoids from arachidonic acid has been suggested to have a negative impact (Lewis et al., 1990; Tilley et al., 2001). Therefore, EPA and DHA replacing arachidonic acid as a substrate can reduce inflammation. In addition to preventing the formation of eicosanoids such as prostaglandins, thromboxanes, and leukotrienes from arachidonic acid, EPA and DHA produce analogs of prostaglandins, thromoboxanes, and leukotrienes with weaker biological activity (Wada et al., 2007). Additionally, EPA and DHA are also substrates for the formation of resolvins and maresins that are considered proresolving lipid mediators (Bannenberg & Serhan, 2010; Serhan et al., 2008). These proresolving lipid mediators are produced through the ability of DHA and EPA to influence transcription factor activation and gene expression (Calder et al., 2014). More specifically, EPA and DHA have the ability to influence transcription factors such as the transcription nuclear factor κB, peroxisome proliferator-activated receptor-α and γ, and the sterol regulatory element binding proteins in order to form resolvins, protectins, and maresins.

Nevertheless, it should also be mentioned that recent meta-analyses and systematic reviews have stressed that cardioprotective properties of marine sources cannot only be attributed to their rich n-3 PUFA content, rather than to an interplay of several lipid bioactive in the marine food source, and especially in the polar forms of this n-3 LCPUFA when they are found to be bound into bioactive polar lipids (at the *sn*2 position of their glycerol backbone) with strong anti-inflammatory and antithrombotic properties (Lordan et al., 2020). However, more studies are needed to further evaluate the biofunctionality of shellfish polar lipids.

2.3 Pigments

A number of shellfish products attract consumers by their color. A good example of such products is shrimp and lobsters. Their characteristic color can be attributed to carotenoids that can be found in shellfish. Although carotenoids are responsible for this characteristic color of some shellfish, it should be noted that carotenoids are fat soluble pigments produced mainly by plants and algae and are subsequently transferred through the food chain to shellfish.

The main carotenoid found in shellfish is astaxanthin and has a red-orange color due to its chemical structure of a long chain of conjugated double bonds. Other than the color, this chain of conjugated double bonds is also responsible for the biological functions of astaxanthin. More specifically, it acts as an electron donor and thus astaxanthin is considered to be a potent antioxidant. Its potential health benefits have attracted attention over the past few decades due to its capacity to scavenge free radicals. Moreover, apart from its antioxidant properties, astaxanthin has been suggested to have a protective mode of action against CVD, and it may exhibit antiinflammatory, antiaging, antidiabetic properties. It even may have the capacity to control cataracts (Fassett & Coombes, 2011; Fassett et al., 2008; Higuera-Ciapara et al., 2006; Kaulmann & Bohn, 2014). Therefore, the use of astaxanthin in the nutraceutical industry is now in progress.

To begin with, the presence of antioxidants in foodstuff is useful in view of the potential for the occurrence of various reactive oxygen species (ROS), for example, hydroxyl radicals, singlet oxygen, and peroxy radicals. ROS has already a negative effect on foodstuffs considering that they can alter the functionality of proteins, lipids, and carbohydrates and also precipitate reactions that are responsible for the formation of unpleasant volatiles. This effect of ROS can cause a displeasing impact on the sensorial properties of the products. Therefore, the most noteworthy effect of ROS in food products is their ability to reduce the nutrient value of the product or their capacity to give rise to carcinogenic compounds. Subsequently, the relevance of the presence of compounds such as ascorbic acid, polyphenols, and astaxanthins becomes apparent.

Some of the proposed modes of action regarding its antioxidant capacity are its absorption by the plasma lipids and subsequently transport into body tissues such as skin and lipid-based membranes where it offers a protective role against oxidative stress. Additionally, the astaxanthin content of double bonds acts as a radical scavenger toward ROS species such as singlet oxygen and interrupts oxidation reactions (Ambati et al., 2014; Komatsu et al., 2017).

It has been implied that the antioxidant properties of astaxanthin are also responsible for its antiinflammatory action. In general, oxidative stress and inflammation have been linked to various chronic diseases. Other than EPA's and DHA's effect on the nuclear factor κß, astaxanthin has been proposed to mitigate inflammation in macrophages, neutrophils, and lymphocytes while also reducing the accumulation of ROS in lipopolysaccharide-stimulated macrophages. Moreover, it was suggested by a study that astaxanthin affected the splenic macrophages by modulating macrophage phenotypes (Farruggia et al., 2018).

The implication of astaxanthin in a number of biological functions, its mechanism of action, and health benefits are not yet completely validated. Therefore, a brief exploration of some potential uses will be described. To begin with, a number of animal studies have corroborated the capacity of astaxanthin to reduce blood pressure in spontaneously hypertensive rats SHR (Hussein et al., 2005; Mashhadi et al., 2018; Preuss et al., 2011; Yanai et al., 2008). Moreover, some of the aforementioned studies along with meta-analyses suggest an effect of astaxanthin supplementation toward reducing fasting glucose and plasma glucose. Additionally, it is implied also that astaxanthin reinforced the ability of the pancreas to secrete insulin (Ambati et al., 2014; Naito et al., 2004; Ursoniu et al., 2015). As a result, astaxanthin is researched for its capacity to prevent or aid against diabetes mellitus. Furthermore, the protective effect of astaxanthin against cancer has attracted interest with studies indicating that its antioxidant properties could inhibit cancer initiation and proliferation by stopping the cancer cell reproductive cycle and apoptosis enhancement (Ekpe et al., 2018; Santocono et al., 2007). For the opposing view, a meta-analysis of randomized controlled trials demonstrated that the impact of astaxanthin supplementation did not translate to significant differences between control groups and astaxanthin groups for biomarkers such as total cholesterol, low-density lipoprotein cholesterol (LDL-C), total glucose, BMI, body weight, fasting blood sugar, hemoglobin A1c, systolic and diastolic blood pressure. Significant differences were detected only for C reactive protein and high-density lipoprotein cholesterol (HDL-C) (Xia et al., 2020). In conclusion, astaxanthin appears to be a potent bioactive compound with possible applications in functional food and nutraceuticals however extensive research is required for its biological functions and mechanism of action.

2.4 Chitin and chitosan

Chitin and chitosan are natural biopolymers found in the fungal cell wall and in the exoskeleton of arthropods, insects, and crustaceans. Chitin is a polysaccharide that is comprised of randomly distributed β-1,4-N-acetyl glucosamine while its derivative chitosan is a deacetylated polymer of chitin. Chitin and chitosan are widely used across industries for a number of different applications.

With respect to the food industry, chitin and chitosan from edible marine invertebrates have attracted attention regarding their functional applications (Ganesan et al., 2020). According to various studies, chitin and chitosan have been implied to possess a number of different biological functions such as antioxidant, antimicrobial, antiinflammatory, and cancer-preventing properties (Abdelmalek et al., 2017; Ganesan et al., 2020; Joseph et al., 2021; Srinivasan et al., 2018).

In general, a number of applications of chitin and chitosan have been suggested to offer various health benefits. Nevertheless, the substantiation of these claims requires further research and analysis. For this reason, in this chapter, the claims and probable health benefits of chitin and chitosan will be presented.

Chitosan dietary supplementation has been suggested to reduce cholesterol. This effect of chitosan was confirmed in a double-blind randomized placebo-controlled study. More specifically, chitosan was effective in reducing non-HDL-C and LDL-C after a 12-week period of treatment. According to the same study, the effect was more pronounced for individuals with higher baseline cholesterol levels (Spigoni et al., 2017). Similar findings were presented by a meta-analysis of controlled clinical trials. In addition to the reduction of total cholesterol studies have also hinted toward the capacity of chitosan to reduce blood pressure (Moraru et al., 2018). Moreover, more data suggest the promising bioactive functions of chitosan since another meta-analysis indicates that dietary chitosan supplementation led to a reduction in total cholesterol in murine models (Ahn et al., 2021). Furthermore, the relevance of the usage of chitosan to reduce blood pressure was also validated by Huang et al. (2018). In this meta-analysis, it was determined that chitosan could aid in reducing diastolic blood pressure in the short term with a dose above 2.4 g/day. The reduction of blood pressure and cholesterol levels has hinted at the potential of chitosan to have cardioprotective properties and therefore the possibility to be used to combat CVD (Dang et al., 2020). Nevertheless, chitin and chitosan experimental studies, clinical trials, and meta-analyses also suggest that due to limitations such as a low number of randomized clinical trials and at times a low number of individual or animal participants, the results are not clearly validated.

An additional application of chitosan in foodstuffs is its use as an antioxidant. A number of theories have been suggested regarding chitosan's capacity to donate hydrogen atoms (Xie et al., 2001). More specifically, it has been suggested that the hydroxyl groups can react with hydroxyl radicals through the H-abstraction reaction. Moreover, the hydroxyl groups can also react with free amino groups to form stable radical macromolecules. Finally, another suggested mode of action is the reaction between ammonium groups with the hydroxyl groups of chitosan. The radical scavenging capacity of chitosan is linked to the degree of deacetylation as well as the molecular weight of chitosan (Aranaz et al., 2009). Typically, the lower the molecular weight of chitosan the better the scavenging ability (Li et al., 2014). Although chitosan's ability to act as an antioxidant, due to strong inter and intramolecular hydrogen bonds, a reduced ability to interact with hydroxyl radicals has been observed. For this reason, efforts have been made to modify and insert polyphenols groups in chitosan. This appears, to enhance chitosan's capacity to act as an antioxidant (Kim et al., 2018).

Finally, another association of possible health benefits that have been observed over the past decade is its anticancer activity. The suggested activity is related to (i) prevention of tumorigenesis by oral consumption, (ii) induction of tumor cell apoptosis, (iii) immunopotentiation activity in combination with chemotherapy, and (iv) inhibition of tumor metastasis. In this chapter, we will look into the use of dietary chitosan and its potential anticarcinogenic properties (Kim et al., 2018). Currently, a number of possible mechanisms regarding the anticarcinogenic activity of chitosan have been proposed. Examples of these mechanisms are the antiangiogenic mechanism, the cellular apoptotic mechanism, and the immune-enhancement mechanism (Adhikari & Yadav, 2018). Although chitosan and a number of polysaccharides have been researched for their anticarcinogenic properties, this remains to be a very early approach. A number of studies have demonstrated the potential capacity of chitosan as an anticarcinogenic substance (Azuma et al., 2015; Qi & Xu, 2006; Xu et al., 2009) but there are no randomized controlled trials and meta-analyses to support those claims. Finally, the use of chitosan as an anticarcinogen lies mostly in the field of pharmaceutics than functional foods or nutraceuticals.

3. Processing of shellfish products and its impact on nutritional characteristics

Shellfish is a term that describes a diverse group of marine invertebrates and comprises mollusks, crustaceans, and echinoderms. Due to the variation within species, food products can follow various methods of processing. Nevertheless, a number of similarities can be noticed between the processing of a few products. These similarities are more pronounced when two different shellfish species follow for example a canning process or freezing.

Parameters such as food quality, safety, and nutritional value of foods are constantly changing during the shelf life of a product. Processing of foods is widely used for the production of foodstuff in order to transform them into safe products with specific quality and an extended shelf-life. Food processing has both positive and negative effects on products, but its downsides tend to be more pronounced.

To begin with, in this chapter analysis of the main processes that have a more pronounced effect on the nutritional character of shellfish will be provided. Moreover, alternative processing methods and their benefits are going to be described. Finally, the production and processing steps for a number of shellfish species will be described in detail in order to focus on product-specific processes that can impact their nutritional profile.

3.1 Freezing and frozen storage

Freezing of food products is widely used across the food industry. It has been used since ancient times and has the capacity to preserve most types of foods. In general, frozen products are considered to be safe and there is little association between frozen products with foodborne diseases (Archer, 2004).

Frozen shellfish products have become more common ground in the past decades but they are still being sold live at markets. Typically, shellfish are sold as minimally processes products and do not go through an extended processing line. Therefore, shellfish are frequently either washed, sorted, graded, and packed for the live market or sold as frozen products (Venugopal, 2005).

A significant number of frozen shellfish products are commercially available. Examples of such products are frozen shrimps, octopus and squid, mussels, oysters, and clams. In shellfish specifically, freezing is used due to the fact that it allows for an extended quality and longer storage periods. Postharvest handling and preprocessing differ depending on the species due to their anatomy but also processing steps might vary since some types of shellfish require cooking or blanching before freezing.

In terms of the impact of freezing on the nutritional profile of the products, it is not necessarily the main reason for nutritional losses but the effect of freezing as well as the freezing speed can greatly affect the final products' nutritional value. This can be attributed to the damage to the product's structure due to the formation of ice crystals during the freezing process. More specifically, freezing damage occurs mainly due to mechanical action and physicochemical modifications. It is also well established that the freezing time can significantly impact the product. Slow freezing of the product leads to the formation of larger ice crystals in contrast to quick freezing. Therefore, reduced freezing times are preferred to avoid drip losses during thawing. For this processing step, a number of air blast freezers, contact plate freezers, IQF, or cryogens can be used in order to reduce the freezing time.

Finally, it should be noted that prolonged freezing or cold storage can have a negative effect both on the quality and nutritional value of the products. The extended frozen storage of shrimps and shellfish can lead to lipid oxidation such as oxidation of polyphenols, denaturation of proteins, and or the aggregation of myofibrillar proteins. Additionally, the products of lipid oxidation can further interact with proteins and lead to the formation of protein polymers (Baron et al., 2007; Haghshenas et al., 2015; Saeed et al., 2002). These changes can lower the nutritional value and also lead to a reduction in the product quality and value since they are responsible for the darkening of the flesh or black spot formation.

3.2 Thawing

Although the thawing of products is sometimes discussed as a process, one should consider thawing as part of the freezing process. Products are both thawed commercially but also by the consumers. Although there is a lot of research on novel thawing methods (Cai et al., 2019; Liu et al., 2019; Wang et al., 2020), frozen products are typically thawed by the consumer in the fridge or even on the bench.

Drip losses occur in thawed products due to physicochemical changes that occur during freezing. As a result, thawing can cause the leaching of water-soluble micronutrients such as vitamins and minerals. However, the thawing process can induce further damage to the quality and nutrients of the product. This is dependent on the thawing time and temperature as well as the thawing method (Qian et al., 2019). Although research has not been conducted on the effect of thawing in shellfish products, research on other foods indicates that thawing can contribute to the oxidation of proteins or protein cross-linking and increased enzymatic activity (Benjakul & Bauer, 2000; Wang et al., 2020; Xia et al., 2012).

The thawing process is highly dependent on the freezing process. The faster freezing occurs, the lesser the damage in the food structure, and therefore drip losses and subsequent damage in the product are reduced. Nevertheless, thawing methods, time, and temperature can also impact the quality and nutrients of the product.

3.2.1 Shrimp

The processing of shrimp can follow a number of different processes based on the desired final product. Shrimp are susceptible to quality deterioration due to enzymatic or microbiological processes. These processes can initiate rapidly after harvest due to the high moisture content of the product, near neutral pH values, presence of nonprotein compounds (NPN), and the presence of various digestive enzymes (Nirmal & Benjakul, 2011; Nirmal et al., 2020; Pedraja, 1970). Consequently, shrimp processing typically starts right after harvest and is quite frequently onboard the ship. In order to avoid deterioration, the shrimp catch is quickly stored in ice or frozen if possible. At this point, a differentiation should be made for shrimp catches and farmed shrimps. Although, a fresh shrimp catch can be quickly frozen before being transferred to the processing plant similarly to farmed shrimp, in this chapter we will consider that catches are not frozen in order to present both sides.

To begin with, shrimp might be sold as frozen whole shrimp, deheaded, deheaded, deveined, and peeled. Nevertheless, the main processing steps of the products remain similar for all three types of products. The distinction between those products is the step of deheading and additional washing for the deheaded shrimps and the deheading, maturation, peeling, and additional washing for the peeled shrimps. The aforementioned discrepancies can be easily distinguished in Fig. 10.1.

Fresh shrimp is a highly perishable product and therefore follow quick postharvest processing. Shrimp like all shellfish are quickly transferred to chilled storage or ice on board in order to extend their shelf-life and reduce quality losses. The postharvest processing has no effect on the nutritional value of shrimps or in general shellfish. On the contrary, it aids in the preservation of the quality and nutritional character of shellfish.

Right after the arrival of shrimps in the processing plant, the main processing begins. The shrimp are washed, sorted graded and before the washing process begins. The washing of shrimp as in general shellfish takes place with iced water since high temperature is beneficiary for spoilage microorganisms as well as pathogens. Right after washing, weighing,

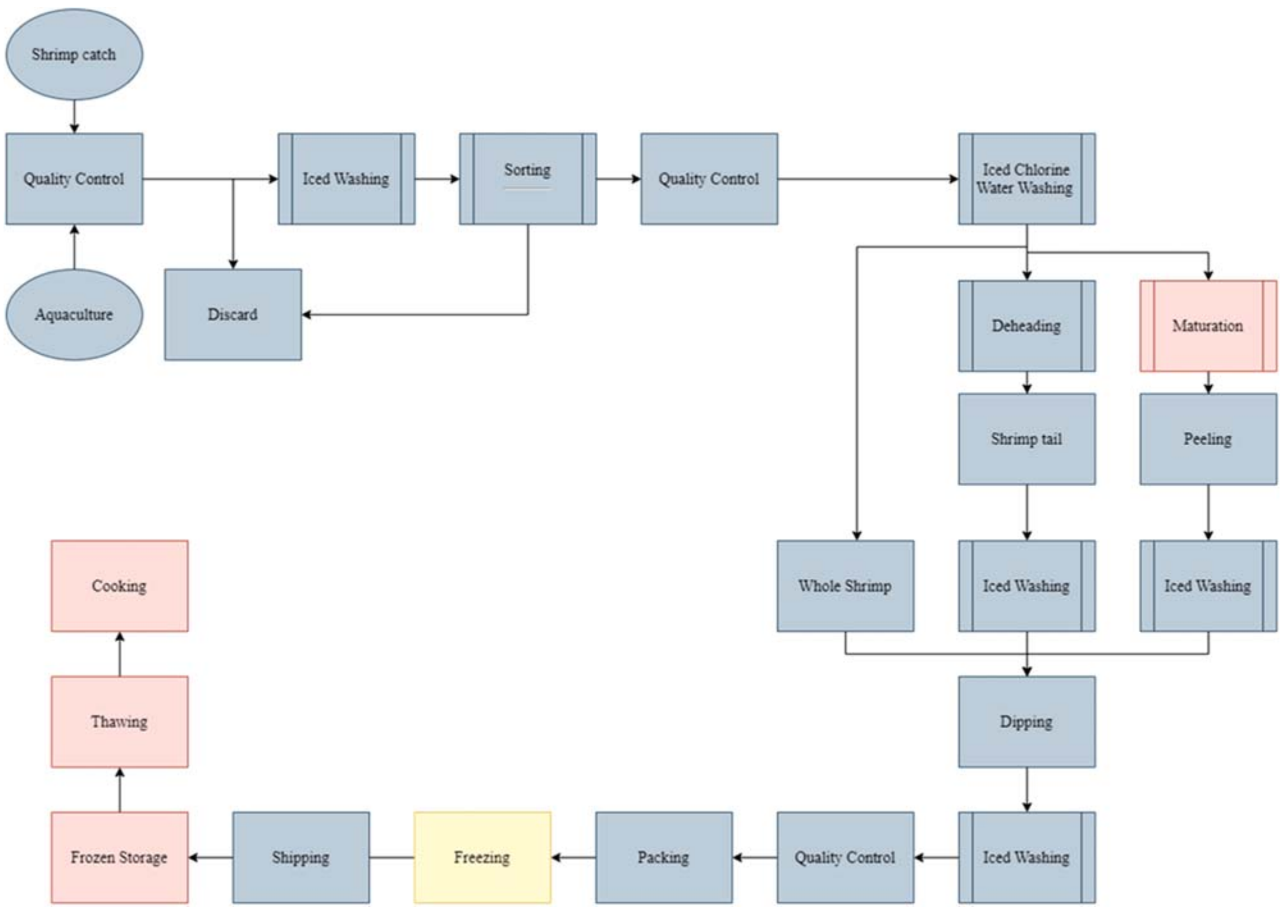

FIGURE 10.1 Frozen shrimp processing.

sorting, and grading, the shrimp have washed once again but with chlorine water. This step is added for hygiene purposes (Anh et al., 2011). From this step on and depending on the final product, the processing of shrimp might vary.

The first processing step that can have an effect on the quality of the shrimp is the maturation process in beheaded and peeled shrimp. In order to separate the shrimp shell and meat, maturation on ice or in brine takes place in a time frame of up to 4 or 5 days. During this step, a number of organoleptic and nutritional parameters are influenced (Dang et al., 2018). A number of different methods have been used for shell loosening in the past such as heat-treatments (blanching and cooking) and the addition of phosphates in the brine. The latter was regulated in the European Union since phosphates can also increase the moisture content of products and thus food fraud. This was regulated under the law EU Commission Regulation 1129/2011. Maturation of shrimp for shell loosening is still used. Nevertheless, novel processing steps are being researched such as high pressure and ohmic heating (Dang et al., 2020). Their application has not been tested however reducing the shell loosening time might prove beneficial also for the nutritional and quality aspects of the shrimps.

Subsequently, dipping with sodium metabisulfite, washing, and packing take place. The product is now ready to be frozen.

3.2.2 Crab and lobster

Frozen crab follows a relatively simple processing sequence but in contrast to shrimp, it requires a cooking step before being frozen. To begin with, right after the arrival of the crab a brief control takes place in order to assess the catch. This mainly takes place to separate different crab species because slight modifications will take place in the boiling step.

The caught crabs are quickly cut in middle in order to remove the carapace. Subsequently, a washing step takes place and the crabs are boiled in a 3% brine at 100°C for about 20–25 min. The species of crab can influence the boiling process since blue crabs are typically cooked at 121°C and for a shorter period of time. Afterward, chilling of the crabs takes place in order to quickly reduce the temperature of the product and the meat is removed and sorted according to their specification. Packing and glazing ensues before freezing and finally, the product is distributed and stored until it reaches the consumer.

Lobsters generally follow a similar processing line to crabs. Frozen lobster can be found as a whole or just the tail of the product. The separation between the two processes is not significant. The freshly caught lobster is sorted and washed. The whole lobster can be then cooked (boiled typically for 10–20 min) while the tail lobster is going to be eviscerated, washed with pressurized water (syringing) to remove dirt, and then dipped in tripolyphosphate additive. Both the whole lobster after chilling and the tail lobster after dipping will be packed and frozen. At this point, it should be noted that the whole lobster can be processed without the boiling step but the thermal processing of the whole lobster aims both at the reduction of the microbial load and the inactivation of enzymes (polyphenol oxidase) that can lead to melanosis.

Similarly, to frozen shrimp, there is no significant effect of the processing steps on the nutrient profile of the products. This can be attributed to the fact that the production of frozen products aims in reducing the quality and nutrient losses of the products. The step-by-step processing of crab and lobster can be seen in Fig. 10.2.

3.2.3 Clams, oysters, and mussels

Similar to the other species of shellfish, clams, oysters, and mussels are available as frozen products. Preparation and processing of clams and oysters into a frozen product follow a comparable process. Both clams and oysters are pressure washed to remove dirt that has adhered to them and sorted according to their size. At this point, broken clams, oysters, and mussels are also discarded.

Right after washing, the clams, oysters, and mussels are steamed to open in the case of clams. The steaming of oysters and mussels is used in order to assist machine shucking. Subsequently, the viscera are removed, and the meats are then washed, sorted, and cut or diced if necessary for the final product. Packaging, glazing, and freezing ensues. In general, this process has no impact on the nutritional characteristics of the products (Fig. 10.3).

3.3 Thermally processed products

Besides frozen shellfish products, a number of thermally processed foods are produced in the industry. Examples of such products can be canned products and cooked and chilled products. The former products are stable products with an extended shelf-life while the latter are considered refrigerated processed foods of extended durability (REPFED). The processing of the aforementioned products differs significantly and thus they are stored under different conditions and for shorter periods of time (Bugallo et al., 2012). Nevertheless, it is more typical for ready-to-eat shellfish products to be cooked and frozen therefore cooked and chilled products will not be discussed in this chapter. Moreover, cooked and

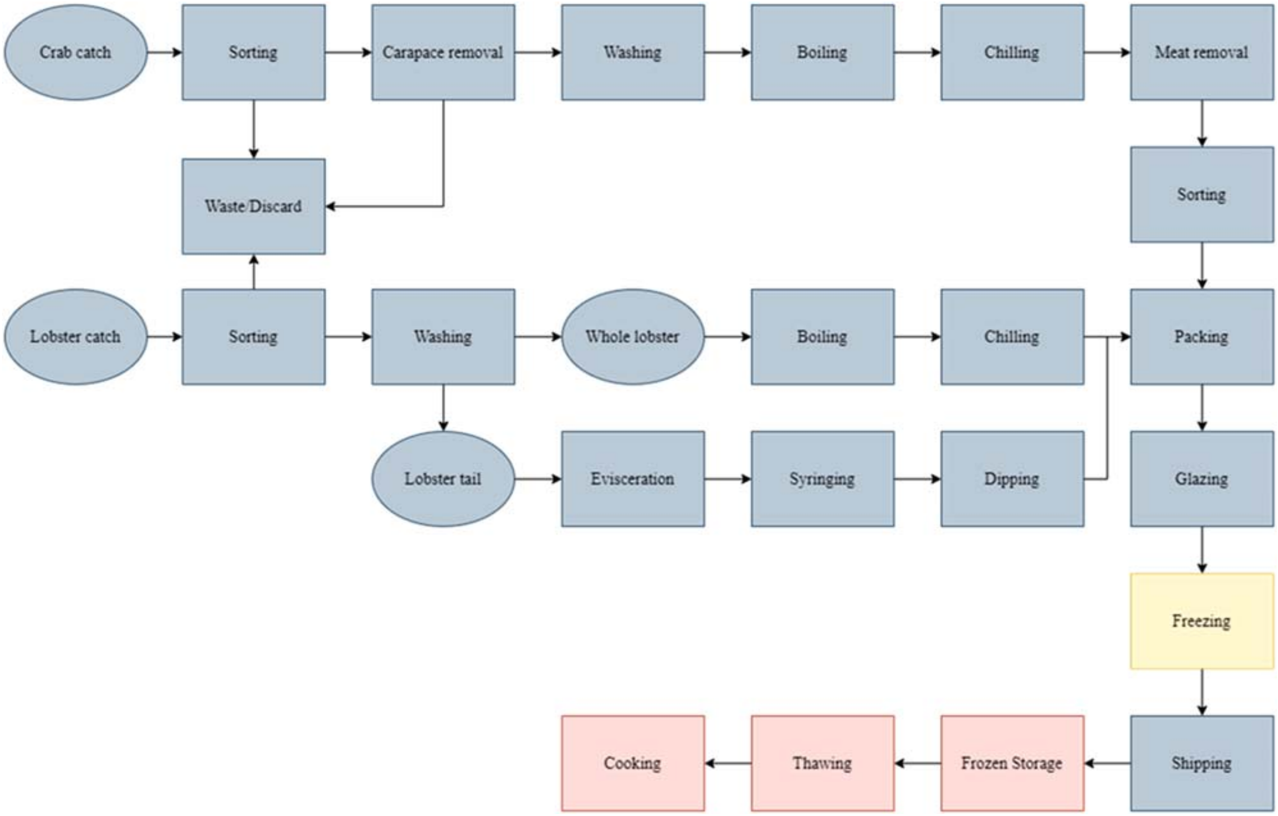

FIGURE 10.2 Processing of frozen crab and lobster.

frozen products typically follow the same processing sequence as frozen products with the inclusion of a boiling or steaming step followed by a chilling step that aims the quick reduction of the product's temperature. An example of such food products was given in the processing of lobster in Section 3.1.2. Consequently, this chapter will be focused on canning.

Canning is widely used in the food industry in order to produce shelf-stable products. The main advantage of canning is that the products do not have to be refrigerated and are readily available and convenient (Awyah et al., 2007). A variety of shellfish products are canned such as clams, oysters, shrimp, and crabs. Although canned foods are convenient, they are occasionally considered as lower-value products. This presupposition is based on the harsh thermal processing of the cans, which is necessary for the inactivation of *Clostridium botulinum*, which is considered the most heat-resistant pathogenic spore.

3.3.1 Boiling and steaming

Before the processing steps and the effect of the sterilization process are described, it is important to explain the effect of two common processes that are widely used both for frozen products and thermally processed products. Boiling and steaming are widely used in the food industry both for cooking or blanching products such as vegetables, cereals, shellfish, and others. In shellfish products, boiling and steaming can be used in order to lightly pasteurize the products, inactivate enzymes, or sometimes even to help open products such as oysters and mussels or even aid with the separation of the meat in products such as shrimp and lobster.

As a thermal process, boiling and steaming should be expected to impact the nutritional profile of shellfish. However, it has been determined that the thermal processing of shellfish in the presence of water does not have a significant effect on the nutrients (Gooch et al., 1987; Kreuzer, 1984; Venugopal, 2005; Su & Liu, 2013). The only significant effect that was determined for boiling was inducing losses in astaxanthin content (Yang et al., 2015). Moreover, it should be taken into account that mild cooking typically benefits the products as the denaturation of proteins leads to an enhancement in their digestibility (Venugopal, 2005). As a result, the process of boiling or steaming is not presented with the red color in the processing flowcharts since their impact on the proximate composition and nutrient profile is insignificant.

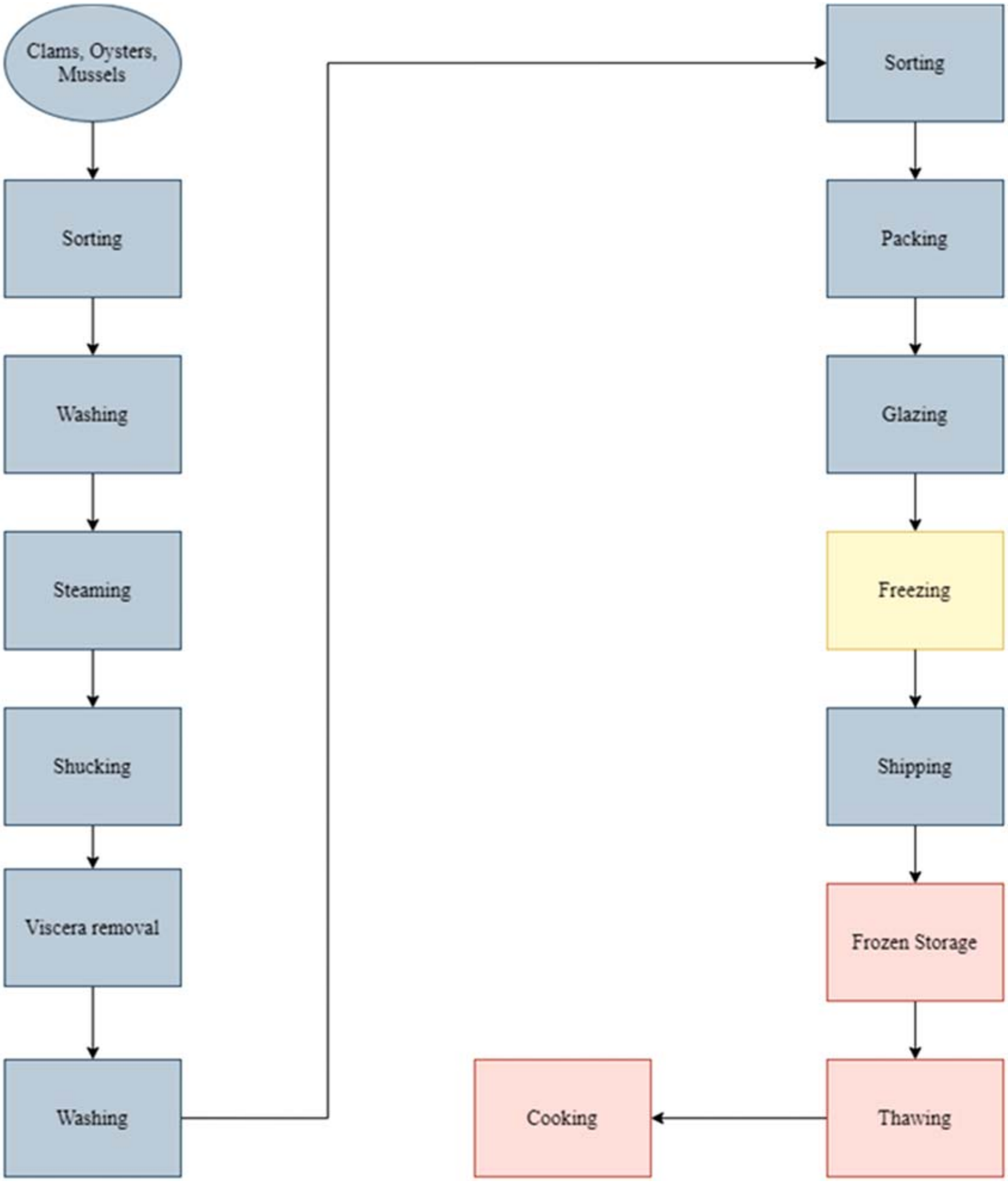

FIGURE 10.3 Frozen clams, oysters, and mussels processing.

3.3.2 Canning

In contrast to boiling and steaming, the sterilization process of canning is more drastic and therefore expected to have a more pronounced effect on the nutritional profile of canned shellfish products.

One advantage that canning offers in this specific case is that canning of shellfish frequently happens in water medium or brines. This offers a level of protection for nutrients since oxidation is not as pronounced as in frying or dry cooking. The most pronounced effect of canning on shellfish products is relevant to the vitamin content. The losses in vitamins can vary depending on the specific vitamin, as well as the conditions of the heat treatment such as time, heat, and medium (Venugopal, 2005). More specifically, water-soluble vitamins are prone to leaching and transferring in the cooking medium. Moreover, thiamin is unstable during heating and vitamin B_6 is inactivated in prolonged heating (Venugopal & Gopakumar, 2017). In addition to vitamins, minerals such as Mg, K, and iodine (I) are also labile to leaching during canning. Furthermore, no significant changes in Vitamin B_{12} were observed during canning (Almonacid et al., 2015).

Protein content seems to be unaffected during canning. To be more precise, the protein content increases but this can be attributed to the reduction of the water content (Almonacid et al., 2015). Furthermore, according to Casales (1988), the amino acid content of the product remains the same.

Similarly to protein content, canning did influence considerably the fatty acid content of mussels and sea urchins. Most importantly, the products show high retention of EPA and DHA (Almonacid et al., 2015; de la Cruz-Garcia et al., 2000).

3.3.3 Cooking

Cooking of products either in the industry or by the consumer is probably the procedure that has the most notable effect on the nutritional profile of shellfish. As previously mentioned, most precooked shellfish products have been heat-treated mostly with boiling or steaming, and thus no significant reduction in the nutrients is expected. On the other hand, cooking raw, frozen, or pre-cooked shellfish products is always part of the food preparation. Common, cooking methods of such products are frying and baking (dry cooking). In general, proteins are not diminished by cooking and their digestibility is enhanced. Nevertheless, drastic heating may lead to a reduction in protein quality and a decrease in amino acids (Ghribi et al., 2017; Kocatepe et al., 2019; Venugopal, 2005).

A more pronounced effect of cooking can be observed for the lipid profile of shellfish. Cooking in the presence of oxygen results in the oxidation of PUFAs. This has a twofold effect on the nutritional profile of the shellfish. Firstly, the oxidation of PUFAs is vital, since they are one of the major macronutrients. Secondly, oxidation of PUFA's is responsible for the formation of oxidative products such as peroxides, which can further react with proteins and thus further reduce nutrients and probably to the production of undesirable substances (Ghribi et al., 2017; Mnari-Bhouri et al., 2010; Venugopal & Gopakumar, 2017). What is more, EPA and DHA are responsible for the protective effect of shellfish toward CVD and other diseases and thus a reduction in those PUFAs reduces the bioactive capacity of shellfish. Moreover, cooking processes such as frying can lead to a decrease in cholesterol and sitosterol content (Ozogul et al., 2015).

Another important compound that is strongly influenced by heat treatments is astaxanthin. As previously stated astaxanthin is a potential bioactive compound and as a result, its retention is vital. Astaxanthin showed a reduction both for processes such as boiling, frying, and microwaving (Yang et al., 2015; Zhao et al., 2006). The greatest reduction was presented in fried shrimp (93.54%), followed by boiling (56.31%) and microwaving (50.06%) after 10 min.

Among other things, additional micronutrients that are influenced by cooking procedures are also B_1 (thiamin), vitamin B_6 and B_{12}, which can be destroyed or lose their biological function (Venugopal, 2005). Moreover, minerals such as iodine and bromine also showed a reduction of about 43% (Mesko et al., 2016).

In conclusion, cooking shellfish is the process that influences the most the nutrient profile of such foods. In general, wet cooking such as boiling and steaming seems to induce the least amount of losses in nutrients. In contrast, dry heating where oxygen is present undermines the product's proximate composition drastically. According to the literature, the cooking method that seems to depreciate the product's nutrients the most is frying. In addition to the diminishment of nutrients, it also gives rise to new compounds that either further deteriorate the product or they can have a negative effect on health.

3.4 High pressure processing

High-pressure processing (HPP) has been used in a number of products due to the fact that it can be more advantageous in certain cases than thermal treatments. HPP has proven quite useful for the juice industry since it has been found to offer additional advantages to microbial safety. More specifically, it has been found to offer higher stability and retention for a number of compounds in different products such as antioxidants, phenols, vitamin C, and carotenoids (Jacobo-Velazquez & Hernadez-Brenes, 2012; Nunez-Mancilla et al., 2013; Keenan et al., 2012). In addition to specific micronutrients, HPP seems to be more favorable in the retention of the total nutritional value of products.

In relation to shellfish, HPP has already been used for products such as oysters, clams, crab, and lobster. HPP has gained a lot of traction due to its multifaceted effect on products such as clams and oysters. To be more specific, clams and oysters treated with HPP have an extended shelf life and increased microbiological safety, and reduction of enzymatic activity and are no longer in need of shucking. Therefore, one could say that HPP, influences both safety and spoilage parameters (Gyawali et al., 2019; Linton et al., 2003; Wang et al., 2016; Ye et al., 2013) while it can also reduce labor costs for shucking (Kingsley, 2014).

However, the versatility of HPP can be extended with regard to the quality and nutritional profile of food products (Mujica-Paz et al., 2011). HPP is a nonthermal processing method and thus affects the properties of foodstuff to a lesser extent. So far HPP has been shown not to affect the quality aspects of raw clams and oysters. Clams and oysters that have been processed with HPP show no difference in texture and taste when compared to raw products (Mermelstein, 1997).

Furthermore, HPP and its effect on the nutritional profile have been researched for a number of products. So far it has been shown that it is an advantageous method when compared to thermal treatments. Nevertheless, similar studies have not been conducted for products such as shellfish and thus there is no data available. In conclusion, HPP seems to be a favorable processing method for shellfish since it influences safety, quality, and labor costs but it also looks promising for the retention of nutrients.

4. Seafood products or food products using shellfish constituents

Although shellfish's proximate composition and content of macro- and micronutrients show promising for the preparation of functional foods, there are not a lot of products that are advertised as functional foods. In contrast, there is extended research on the utilization of byproducts in the nutraceutical industry or their use in food products (Binsi, 2018; Harnedy & Fitzgerald, 2012; Olsen et al., 2014; Ruocco et al., 2016; Venugopal, 2018). As a result, shellfish products have been treated more as a potential source for the production of functional products or nutraceuticals but not as complete products with the capacity to promote health and combat diseases. A hypothesis that could offer some explanation on this matter is that shellfish are considered to be high-value products that are already widely sold worldwide and thus making use of the by-products of their production is an easier or more profitable option.

A functional product that applied for a health claim to EFSA was Symbiosal. It was a salt product that contains 3% chitosan and claimed that it has the capacity to combat hypertension. EFSA denied the health claim due to limitations that were observed in the clinical studies but also for the lack of explanation of the mechanism of action (EFSA, 2015; 2018). However, this product once again made use of the by-products of shellfish.

The literature on bioactive compounds from shellfish and their use as nutraceuticals is extensive. So far, few considerations of shellfish products have been done as a complete functional food. Based on the existing regulations, food can be considered a functional product either through the addition of bioactive compounds or on its own. Therefore, shellfish products can apply for health claims without any additional processing as long as the data support the claim. Moreover, research on processing methods can reduce losses of the already present bioactive compounds, which can further increase the capacity of shellfish products to either promote health or counter various diseases.

Shellfish are comprised of a number of different species. Their proximate composition, macro, and micronutrients are considered to be of excellent quality in terms of nutritional value. Additionally, a number of bioactive compounds have been observed and are extensively researched. Consequently, shellfish is a promising food category for the preparation of complete functional foods with the potential to combat multiple diseases. As a result, more research on the production methods of shellfish, usage of their by-products, and effects on human health is required.

5. Conclusion

Shellfish are a valuable product from both an economic and nutrition standpoint. They have been used for thousands of years and now, more than ever, their significance with regards to their content of beneficial bioactive compounds, has attracted additional research. Thus far, shellfish foodstuff are considered to be complete products offering vital macro and micronutrients essential for our diet. As a result, their inclusion in a healthy diet is indisputable. Nevertheless, numerous studies and meta-analyses suggest that the bioactive compounds of shellfish can or have the potential to combat various chronic diseases that are influencing a substantial proportion of the world population. Such diseases are CVD, inflammation, diabetes mellitus, and cancer. Additional research on shellfish and their constituents along with advances in processing methods may lead to the formulation of new functional foods with the capacity to substantially improve the wellness of the human population.

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Chapter 11

Fish-derived functional foods and cardiovascular health: An overview of current developments and advancements

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1. Introduction

Cardiovascular diseases (CVD) are the leading cause of death globally accounting for almost 18 million deaths per annum (Timmis et al., 2018). Lifestyle and diet are modifiable risk factors and are key to a healthy cardiovascular system and the prevention of CVD (Tsoupras, Lordan, et al., 2019). Diet in particular is critical to the prevention of disease whereby the consumption of seafood is known to exert protective effects on our cardiovascular health (Yu et al., 2018). Fish consumption has long been associated with favorable health outcomes (Li et al., 2020; Mohan et al., 2021). Fish are abundant and there are over 34,000 species of vertebrate animals found in fresh and salt water around the world (Parenti & Weitzman, 2021). The edible fraction of fish differs in appearance, composition, and nutritional properties. Fish provide 3.3 billion people with almost 20% of their average per capita intake of animal protein (The State of World Fisheries and Aquaculture 2020, 2020). According to the FAO (2018) declaration for sustainable fisheries and aquaculture, the term "Fish" includes all aquatic food production groups, including mollusks, crustaceans, and other aquatic animals, but excludes aquatic mammals, reptiles, seaweeds, and other aquatic plants (The State of World Fisheries and Aquaculture 2020, 2020). This chapter will largely focus on freshwater, saltwater fish, and farmed fish. Chapter 10 will discuss functional foods in relation to other seafood, including shellfish.

Once caught, fish require preservation, storage, and processing. During fish processing, a significant amount of byproducts are produced including the heads, skin, trimmings, fins, viscera, frames, and sometimes muscle, which are currently wasted, underutilized, or used to produce low-value-added products such as fish meal and fish silage (Shahidi et al., 2019). Depending on the species of fish, up to 50% of the whole fish can be wasted or characterized as a byproduct (Shahidi et al., 2019), while other studies and reports claim that up to 75% of the catch is considered a waste or byproduct (Pastoriza et al., 2004; Shahidi et al., 2019). Along with the waste generated during the processing of fish, a significant amount of bycatch is also discarded. Bycatch are organisms other than the target species, including fish outside the legal size range, overquotas, or fish discarded for other reasons (Burgess et al., 2018; Rocha Camargo et al., 2021). Considering the large amounts of underutilized fish byproducts and bycatch produced by the seafood sector, finding alternative uses and value-added products for human consumption is critical to preventing food waste, economic losses, malnutrition, and hunger.

Functional food is a complex term defined by Granato et al. as industrially processed or natural foods that, together with a well-balanced diet, have potential positive benefits on human health beyond their nutritional properties (Granato et al., 2017). Production and consumption of functional foods intend to develop a strategy to help reduce the risk for noncommunicable diseases (NCD), such as the above-mentioned cardiovascular disorders. The popularity of this type of food

$ Equally contributed to the chapter.

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product among consumers has significantly increased in the past decade due to general population awareness of the relationship between diet and health and thus, consumers are seeking food choices that fit their lifestyle and provide positive health outcomes. This fact has been exacerbated as a result of the global health crisis derived from the Coronavirus (COVID-19) pandemic, with people concerned about their health, and general well-being, and consequently, an increase in the demand for preventive health and immune-boosting food bioactive, supplements, and nutraceuticals (Galanakis et al., 2020; Grant & Lordan, 2021; Lordan et al., 2021). This interest in foods with functionality has led to the creation and acceleration of an emerging functional foods market that is growing by approximately 10% per year (Hilton, 2017). Moreover, dietary guidelines support and promote the intake of vegetables, nuts, dairy, and fish intake in our diets. The latter contains bioactive lipids and peptides with health-promoting effects. Fish lipids are rich in monounsaturated fatty acids (MUFA; e.g., oleic acid), omega-3 polyunsaturated fatty acids (e.g., n-3 PUFA; linolenic (L), eicosapentaenoic (EPA), and docosahexaenoic acid (DHA) acids), and bioactive polar lipids (e.g., phospholipids, sphingolipids, and glycolipids) (Tierney et al., 2019). Dietary polyunsaturated neutral and polar lipids found in a variety of foods, including fish and seafood, are of particular interest as they exhibit potent inhibition or modulation of the signaling pathways of key proinflammatory and prothrombotic mediators (Tsoupras et al., 2020). Additionally, meta-analysis has shown that marine EPA and DHA have a triglyceride-lowering effect due to the activation of peroxisome proliferator-activated receptors (PPARs) that promotes the expression of a protein involved in fatty acid oxidation, inhibition of fatty acid incorporation into triglycerides, and reduction of hepatic very low-density lipoprotein (VLDL) synthesis (Eslick et al., 2009; Shearer et al., 2012). Due to the demonstrated beneficial properties of PUFAs, their use as supplements has increased; however, the omega-3 intake in the Western region is lower than the recommended guidelines (Bates et al., 2014). A collaborative strategy to enhance the consumption of these bioactive compounds, in addition to the food sources, is the PUFA fortification of food or PUFA-based functional foods. The use of olive pomace as a feed ingredient for sea bream and sea bass has been shown to enrich the fish diet with bioactives against CVD polar lipids (Nasopoulou et al., 2011) and this is further discussed in Chapter 5.

Another way to develop functional food products containing biofunctional ingredients from fish is the incorporation of bioactive peptides derived during the hydrolysis of proteins of fish byproducts and fish bycatch. As revealed from a number of *in vitro* studies and animal studies, these peptides have cardioprotective effects such as antihypertensive activity (Jensen & Mæhre, 2016). Only a small number of human clinical trials have been conducted so far investigating the cardioprotective effect of fish proteins, and most of them have shown an effect on blood lipids (Jensen & Mæhre, 2016).

In this chapter, the role of the seafood sector in the production of functional foods for cardiovascular health is discussed. In particular, developments in the field of functional marine lipids, green extraction methodologies, bioactive peptides, and consumer-related issues are discussed.

2. Functional marine lipids and cardiovascular diseases

Dietary patterns that include regular fish consumption, such as the Mediterranean diet, have consistently been associated with favorable cardiovascular health outcomes in both epidemiological studies and clinical trials (Petsini et al., 2018; Prato & Biandolino, 2015). These observations have largely been attributed to the intake of their polyunsaturated fatty acids (PUFA) content, specifically their omega-3 fatty acids content ($\omega 3$ long-chain fatty acids) (Petsini et al., 2018). Eicosapentaenoic acid (EPA) and DHA are the most well-known and studied omega-3 fatty acids, which are abundant in seafood (Fig. 11.1). However, there are other omega-3 fatty acids such as alpha-linolenic acid (ALA), which are more commonly associated with plant-based sources such as flax seeds, soy products, chia seeds, and rapeseed (canola) oil (Fig. 11.1).

While marine oil consumption is synonymous with cardiovascular health, marine oils have also previously been investigated for their effects on metabolic syndrome (Pedersen et al., 2010), obesity (Thorsdottir et al., 2007), rheumatoid arthritis (Petersson et al., 2018), cancer (Werner et al., 2017), and various other noncommunicable diseases that are characterized by a systemic inflammatory state (Tierney et al., 2019), with varying outcomes reported. Marine oils have been investigated for decades since the observations of the Greenlandic Innuits who consumed diets that were characterized by high intakes of fats from seal flesh and fish (Bang et al., 1971). These populations had an unusually low incidence of heart disease, which was contrary to theories associating fat intake with CVD (Lordan et al., 2019a, 2020). Indeed, it is still contested today whether these associations were accurate (Fodor et al., 2014). Several studies were established to determine the effect of n-3 PUFA intake on cardiovascular health and the multibillion fish oil supplement industry was born (Fodor et al., 2014). In 2019, the omega-3 fish oil supplements were worth 5.8 billion USD globally, with a compound annual growth rate of 8.4% forecast for the period between 2020 and 2027, in which the United States accounts for 77% of the total revenue (Grand Review Research, 2020). The potential health benefits of PUFA from fish oils are well documented and fish consumption is encouraged in most national dietary guidelines (Raatz et al., 2013). The potential cardioprotective benefits of fish-derived PUFA are summarized in Fig. 11.2 as per Lordan et al. (2020).

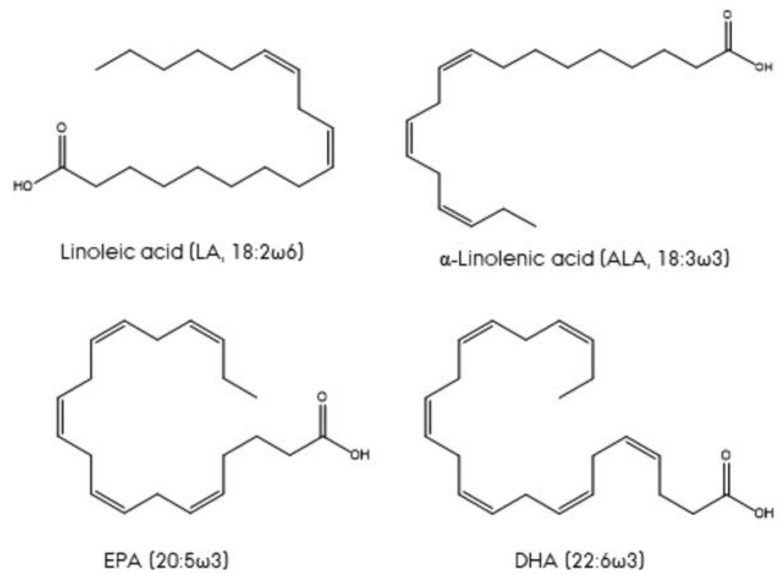

FIGURE 11.1 The most common examples of polyunsaturated fatty acids (PUFA), including those highly abundant in fish such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA).

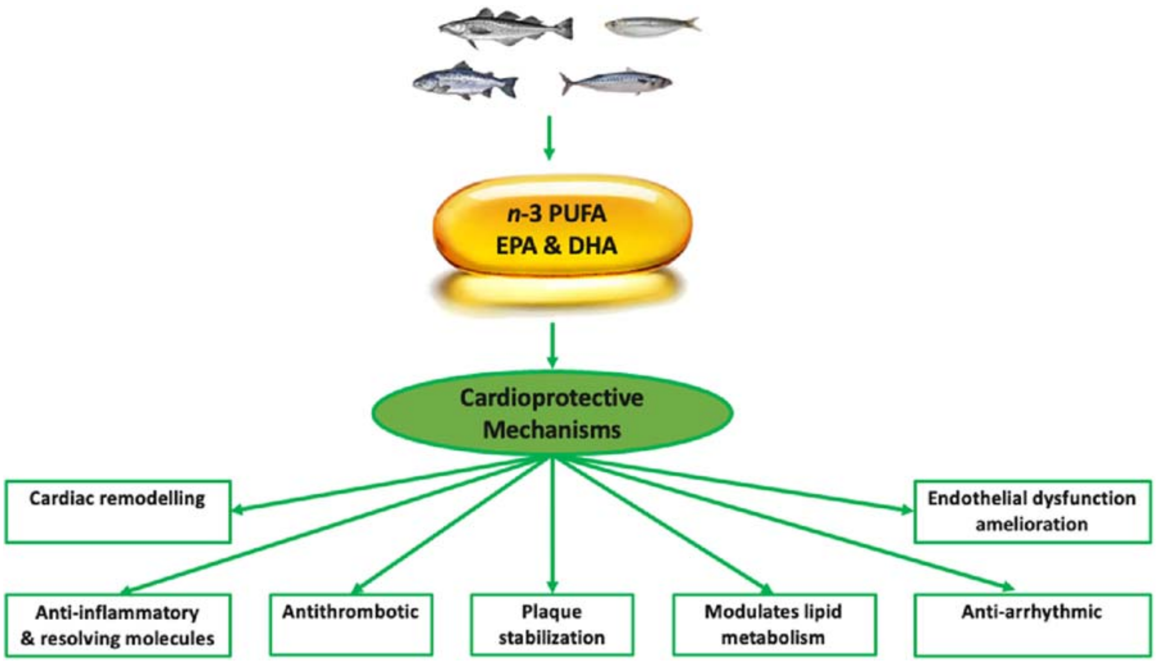

FIGURE 11.2 The potential cardioprotective effects of fish-derived n-3 PUFA. *Adapted with permission from Lordan et al. (2020).*

While n-3 PUFA seems to provide multiple cardioprotective benefits, there are other lipid microconstituents in fish that may exert health benefits (Lordan et al., 2018). Indeed, several clinical trials and meta-analyses show that n-3 PUFA consumption seems to exert protective cardiovascular effects as recently reviewed (Lordan et al., 2020), however, some have failed to show a benefit of n-3 PUFA intended to reduce cardiovascular risk. For example, in a recent meta-analysis of trials examining the antiarrhythmic effects of n-3 PUFA, it was shown that supplementation >1 g/day was associated with an increased risk of atrial fibrillation (Gencer et al., 2021). Overall, it would seem that due to differences in trial designs, trial endpoints, type of n-3 PUFA formulations used, dosing regimens, trial power, etc. that it isn't clear what effect n-3 PUFA has on cardiovascular morbidity or mortality and the recent spate of discordant results does not clarify these issues (Farukhi et al., 2021).

One explanation for these failed trials is the possibility that other lipid microconstituents may play a role or that the n-PUFA used in these trials were neutral lipids, present as triacylglycerols and free fatty acids, rather than in the more bioavailable polar lipids that bare n-3 fatty acids (Lordan et al., 2020). Indeed, polar lipids (phospholipids, sphingolipids, and glycolipids) are present in fish, shellfish, and algae (Shiels et al., 2021). Polar lipids have been associated with a potential cardioprotective effect by exerting antiinflammatory (Lordan et al., 2020), antithrombotic effects (Dwivedi & Pomin, 2020), and antiatherosclerotic effects (Liu et al., 2021) that are not significantly affected by cooking (Nomikos et al., 2006; Redfern et al., 2021). One potential mechanism that may explain some of the observed cardioprotective effects of fish or fish oil consumption may be mediated by the platelet-activating factor (PAF). PAF is a proinflammatory phospholipid mediator implicated in a variety of conditions characterized by inflammation, including anaphylaxis, cardiovascular diseases, cancer, neurological diseases, and infections. However, PAF is also an important mediator of everyday biological processes including day-to-day cell signaling and protection from pathogenic insults, and it plays a role in female and male reproductive biology via its receptor (PAF-R) (Lordan et al., 2019c). PAF synthesis and degradation is tightly regulated by a set of enzymes. In situations of dysregulated inflammatory signaling or systemic inflammation PAF levels increase, which leads to the upregulation of various inflammatory cytokines, thus contributing to the inflammatory state (Tsoupras et al., 2018). In atherosclerosis, PAF is thought to play a contributory role to all stages of atherosclerosis from initiation, and development of lesions, all the way to plaque rupture. However, it is thought that PAF antagonists such as polar lipids may act as inhibitors of the PAF pathway reducing the inflammatory milieu resulting from the engagement of polar lipids with the PAF-R (Lordan et al., 2019b, 2019c). These polar lipids are abundant in marine foods, particularly fish. Indeed, there has been an effort over the last few years to identify these antiinflammatory and antithrombotic polar lipids in numerous fish and processing byproducts including salmon, herring, and boarfish (Tsoupras, Lordan, et al., 2019) Indeed, these polar lipid extracts have demonstrated potent anti-PAF effects (Tsoupras, Lordan, et al., 2019) and they are the subject of clinical trials for the development of nutraceuticals (www.ClinicalTrials.gov: NCT03603769 and NCT03866265).

As people tend to be more health conscious and purchase more "wellness" products nowadays (Food Insight, 2020; Lordan, 2021) it is likely that the market value may increase beyond forecasted values. In Europe, omega-3 products market is forecasted to reach USD 14.61 billion by 2025 growing at a CAGR of 7.0% during the forecast period (2016−26), with an expected increase due to the change in EU regulation and specifically the adoption of Regulation (EU) 2016/127 making DHA a mandatory ingredient in infant formula from 2020 (Mordor Intelligence, 2020). While fish oil products may be beneficial to health, the industry has received criticism about how fish oils are extracted at an industrial scale using toxic materials that have significant environmental consequences. In section 2.1, the recovery of cardioprotective fish lipids using green extraction technologies is discussed.

2.1 Recovery of fish lipids by green extraction methodologies

Conventional fish oil extraction involves the use of high temperatures and toxic solvents during the process. This practice compromises fish oil quality due to possible degradation of the labile compounds, such as PUFAs, as well as the environment, given that the solvents used are usually hazardous chemicals. At an industrial scale, the classical fish oil extraction method used is known as 'wet reduction' by which fish oil, water, and meal fractions are separated by cooking, pressing/centrifugation, decantation, and drying (Bimbo & Crowther, 1992). In the last decade, research has focused on the development of a strategy to integrate green extraction methods that ensure the maintenance of the fish oil quality and safety, the increase of the extraction process efficiency (in terms of solvent usage and energy consumption), and the minimization of the environmental impact. Among the green extraction techniques with the potential industrial application are enzymatic (EA), mechanical ultrasound (UAE), microwave (MAE) assisted extractions, and supercritical fluid extraction (SFE).

Enzymatic hydrolysis involves the use of food-grade exogenous proteolytic enzymes, normally alkaline or neutral proteases, and relatively low temperatures to promote the release of the oil from the fish proteins. Oliveira et al. compared the fatty acid composition (quality) of oil extracted from yellowfin tuna byproducts by enzymatic hydrolysis, physical (pressing), and traditional chemical solvent methods (Oliveira et al., 2017). It was demonstrated that the enzymatic assisted oil contained higher levels of EPA and DHA fatty acids and presented the lowest acidity and peroxides indexes in comparison with the traditional extraction methods. In order to obtain a balance between the extraction yield and quality of oil, it is necessary to adjust the parameters including the type of enzyme, temperature, enzyme concentration, pH, and reaction time required. Optimum pH is dependent on the enzyme used. An increase in the enzyme concentration and temperature, as limiting factors of the enzyme activity, improves the extraction yield (Routray et al., 2017); however, extreme pH and temperatures should be avoided because of the denaturation of the enzymes that can occur (Alfio et al., 2021).

One drawback of enzymatic hydrolysis is the low oil recovery yield in comparison with traditional extractions; however, the combination of the enzymatic treatment with another novel processing technique has led to better results. Zhang et al. investigated the recovery and quality of oil from yellowfin tuna heads by enzymatic hydrolysis after a high-pressure pretreatment (Zhang et al., 2021). Ultrahigh pressure moderately disrupts the biological tissue and denatures the fish proteins, exposing its peptide bonds and therefore, being more susceptible to proteolytic enzymes. Higher extraction yields in samples obtained by high-pressure pretreatment following enzymatic hydrolysis than just the enzymatic treatment (Zhang et al., 2021). Although it is a promising green strategy, it must be emphasized that the extraction product is an unrefined oil that contains nontriglycerides and other components that require a refining process before being considered an edible fish oil.

Ultrasound (UAE) and microwave-assisted extraction (MAE) consist of the use of mechanical waves and microwaves, respectively, to warm the solvent in contact with the fish biomass resulting in cell membrane disruption therefore, intracellular lipids are released and available for extraction. The advantages of these methods are a short extraction process, use of low temperature, the possibility of extraction automatization, and high reproducibility; however, special care must be taken as the heat generated could induce oxidation processes reducing oil quality. Although these assisted extraction methods are widely employed in the extraction of food components, their use in fish oil extraction is scarce. Afolabi et al. evaluated the role of extraction time (10−50 min), microwave temperature (50−90 °C), and energy (600−1000 W) in the oil recovery yield and quality of eel lipids extracted using MAE and ethanol. The combination of 800W, 30 min, and 60 °C resulted in the highest extraction yields as higher values in these parameters caused the overheating and degradation of the samples (Afolabi et al., 2018). Moreover, Costa and Bragagnolo demonstrated that the moisture content of the fish samples plays an important role as water absorbs microwave energy and results in an increase in matrix temperature and pressure leading to cell disruption and an increase in the extraction efficiency (Costa and Bragagnolo, 2017).

Supercritical fluid extraction (SFE) is the most widely environmentally friendly technique used. The most common solvent employed is CO_2 because it has accessible critical conditions ($T_c = 304.15$ K; $P_c = 7.38$ MPa). It is considered safe, does not leave any residue in the extracts, does not require high temperatures, and it displaces the oxygen during extraction, preserving thermolabile compounds such as PUFAs (Alfio et al., 2021). It provides higher extraction yields and high oil quality extracts with fewer impurities than conventional solvent extractions (Grosso et al., 2015). Rubio-Rodríguez et al. developed a coupled extraction-fractionation SFE-CO_2 that improved the extracted oil quality by removing free fatty acids from the oil (Rubio-Rodríguez et al., 2012). One of the drawbacks of SFE-CO_2 is its unsuitability to extract polar compounds (e.g., polar lipids); nevertheless, the possibility of adding a cosolvent, such as alcohol, can overcome this limitation and improve the extraction efficiency. Subra-Paternault et al. successfully extracted phospholipids-enriched extracts from scallop byproducts using a two-step fractionation SFE-CO_2 process. In the first step, the nonpolar lipids were removed with CO_2 as a solvent, and subsequently, CO_2 + 2-propanol (50:50 wt%) as cosolvent efficiently separated phospholipids enriched mixtures (90 g/100 g of extracts) (Subra-Paternault et al., 2015). The main parameters to consider during SFE-CO_2 oil extractions are the moisture content of the fish biomass, particle size, temperature, pressure, CO_2 flow rate, and use of cosolvent. Rubio-Rodríguez et al. analyzed the influence of moisture content and particle size in the SFE oil extraction efficiency from hake byproducts, demonstrating that the water content should be under 20% and that the freeze drier, which weakens the cell wall structure, increases the extraction efficiency over air-drying (Rubio-Rodríguez et al., 2008).

Green extraction processes, despite being a promising environmental-friendly alternative to successfully extract fish oil in the industry, the high costs and initial investment limit their use in the fish oil industry. However, it is important that further investment and research continue to support the adoption of green extraction techniques, not only for the health and environmental impact but also for the superior quality lipids extracted and efficiency in the sector.

3. Fish proteins and their properties

Fish and fish products are rich in protein and they can provide up to a third of the average daily recommended protein intake (Muthukumarappan & Swamy, 2017). Levels of a protein depend on the species and 15%−25% of them are found in the muscles of the fish. There are three main groups of proteins in fish: (1) myofibrillar (2) sarcoplasmic (3) and stroma proteins, comprising 70%−80%, 20%−30%, and 3% of total muscle proteins, respectively (Petricorena, 2014). Myofibrillar proteins are high-salt soluble proteins consisting mainly of myosin followed by actin and tropomyosin. Sarcoplasmic proteins are considered enzymes and the stroma proteins consist mainly of collagen and elastin. Fish protein is easily digestible with more than 95% digestibility (Usydus et al., 2009) and a biological value reaching 75, meaning that 75% of the nitrogen consumed is absorbed and retained in the human body. It is of higher quality compared to the meat protein derived from other animals, but inferior to milk and eggs. Fish protein has a stable composition of essential amino acids, with slight deficiencies of methionine and threonine, and an excess of lysine (Lozano & Hardisson, 2003).

3.1 Marine bioactive peptides

Recently there is increased interest and research on the peptides derived from fish proteins. Like in the case of peptides derived from other protein sources, these can be generated from the proteins during processing, fermentation, or through enzymatic hydrolysis, chemical hydrolysis, or solvent extraction (Sridhar et al., 2021; Wang et al., 2017). Regardless of the protein source, these peptides, although they exhibit bioactivities *in vitro*, it is difficult to prove their biological function *in vivo* and for this reason, health claims associated with them have not been approved in the E.U. Compared to peptides derived from other animal sources, marine-derived peptides can be resistant to proteolysis by gastrointestinal proteases, which presents an advantage over other peptide sources due to steric interactions (Pavlicevic et al., 2020). Their bio-functional or techno-functional properties will depend on the source of protein, hydrolysis conditions such as the enzyme used, pH, hydrolysis time, temperature, and enzyme to substrate ratio since these conditions will result in the generation of peptides with varying molecular weight and amino acid sequences.

Marine bioactive peptides are usually generated through enzymatic hydrolysis of the protein-rich fish processing byproducts or bycatch as shown in Fig. 11.3. The general process involves the mincing and homogenization of the protein-rich waste with water, addition of enzymes (proteases) at controlled temperature and pH depending on the specific enzyme (Vijaykrishnaraj & Prabhasankar, 2015), followed by enzyme inactivation. This can be achieved either by

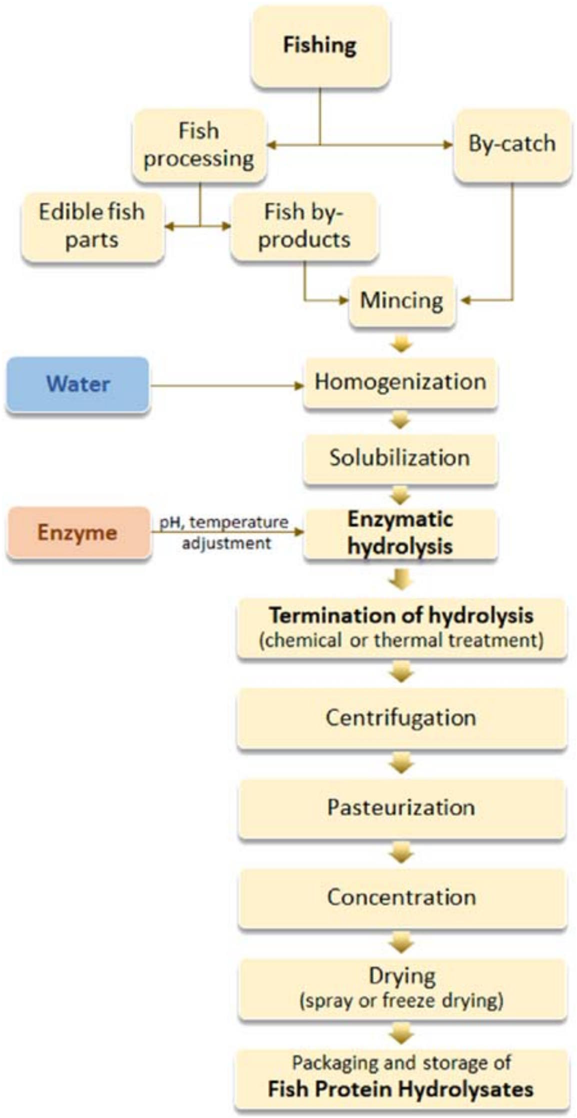

FIGURE 11.3 Schematic representation of fish protein hydrolysates generated through enzymatic hydrolysis. *(Adapted from He et al., 2013).*

elevated temperatures or by changes in the pH outside the optimum range for the enzyme. The next steps are the separation of the particles and the oil phase from the aqueous phase, usually through centrifugation, followed by pasteurization, depending on the desired quality attributes of the final product (Petrova et al., 2018). In many cases, preconcentration takes place before drying of the aqueous phase resulting in the generation of the dry fish hydrolysate powder (Gevaert et al., 2016; He et al., 2013). In some studies, aqueous fractions are further purified or fractionated in order to isolate specific peptides with different molecular weights and subsequently different biofunctional and techno-functional properties (He et al., 2013).

Depending on the conditions under which enzymatic hydrolysis takes place, the fish protein hydrolysates, when compared to the intact protein, will exhibit improved techno-functional properties such as emulsification, solubility, oil binding capacity, water binding capacity, foaming capacity, emulsification capacity (He et al., 2013). Therefore, they can be used as ingredients in a variety of products to improve sensory, and physicochemical properties. Hydrolysis results also in improved biofunctional properties as observed by many researchers and as presented in numerous reviews published in the field (Heffernan et al., 2021; Lordan et al., 2011; Yathisha et al., 2019; Šimat et al., 2020). For example, bioactive peptides with antioxidative, antihypertensive, antithrombotic, anticancer, antiobesity, antimicrobial, and immunomodulatory properties have been reported and tested *in vitro* and *in vivo* (Heffernan et al., 2021; Rustad & Hayes, 2012; Sridhar et al., 2021). Bioactive peptides with antioxidative, antihypertensive, and other cardioprotective effects can also be generated during the normal digestion of fish proteins (Jensen & Mæhre, 2016).

Cardiometabolic syndrome (CMS) is defined as a cluster of several risk factors such as insulin resistance, impaired glucose tolerance, dyslipidemia, hypertension, and central adiposity (Abachi et al., 2019). A number of natural products, including bioactive peptides, from different protein sources, have been investigated for the prevention and treatment of the CMS-associated risk factors inclusive of hypertension (Abachi et al., 2019). Yathisha et al. highlighted peptides rich in arginine, valine, and leucine, with low molecular weight (<1 kDa) and shorter chain length (<20 amino acids) exhibited high antihypertensive activity, *in vitro* and *in vivo* (Yathisha et al., 2019). Antihypertensive activity of these peptides is shown through the inhibition of the angiotensin-I-converting enzyme. This enzyme regulates blood pressure as it promotes the conversion of angiotensin I to the potent vasoconstrictor angiotensin II. Moreover, this enzyme inactivates the vasodilator bradykinin, which is a blood pressure-lowering agent (Karami & Akbari-adergani, 2019). These processes result in increased blood pressure (Darewicz et al., 2014), while ACE inhibitors, which are the most-studied food peptides from various protein sources, could potentially lower blood pressure (Darewicz et al., 2014). The first study investigating fish-derived peptides with ACE inhibitory activity was published in 1986 and was on fish protein hydrolysates produced from sardine and hairtail (Suetsuna & Osajima, 1986). Since then many studies have been published on the bioactive peptides from different types of fish meat and fish processing waste (Ghalamara et al., 2020). Abachi et al. (2019) conducted a comprehensive review of the studies investigating the peptides generated from different fish, hydrolysis conditions, peptide characterization, and peptide structure (Abachi et al., 2019).

It has been reported that dietary proteins with low ratios of methionine−glycine, and lysine−arginine, such as soy and fish protein, favor a hypocholesterolemic effect. Several studies have investigated the effect of fish protein hydrolysates on serum lipids of hypercholesterolemic diet rats (Ben Khaled et al., 2012; Ben Slama-Ben Salem et al., 2018; Drotningsvik et al., 2016; Wergedahl et al., 2004). Khaled et al. have suggested that the hypolipidemic effect of fish protein hydrolysates derived from sardinelle might be due to their abilities to lower serum total cholesterol, triglycerides, low-density lipoprotein cholesterol levels as well as to their antioxidant activities preventing the lipid peroxidation process (Ben Khaled et al., 2012). Furthermore, while there are numerous studies investigating the potential management of type II diabetes mellitus using bioactive peptides from milk, there is a dearth of evidence on the antidiabetic properties of peptides derived from fish (Xia et al., 2017). Antidiabetic activity can be demonstrated through the response of insulin-regulated glucose metabolism and dipeptidyl peptidase IV (DPP-IV), α-amylase, and α-glucosidase activities (Xia et al., 2017). Ktari et al. (2014) have shown that oral administration of zebra blenny fish protein hydrolysates, decreased the α-amylase activity in that of high-fat-high-fructose feed rats compared to the high-fat-high-fructose diet group (Ktari et al., 2014).

4. Production of functional foods from fish bioactives

Many of the functional food products being developed incorporate bioactive lipids into a food substance or simply create nutraceuticals from the oils directly. The enrichment of foods with n-3 PUFA oils and powders has been the subject of interest for decades. However, the difficulty of these approaches is that it is difficult to balance function with the sensory properties of the final products due to the possibility of fishy tastes and odors (Kolanowski & Berger, 2009, pp. 39−49). Another difficulty in this field is the fact that n-3 PUFA is susceptible to oxidation, hence the necessary addition of other natural antioxidants to prevent oxidation in the formulation of functional foods (Jamshidi et al., 2020; Shehzad et al., 2021).

Therefore, novel approaches are required, including the encapsulation of fish oils using nano-liposomes or spray-drying or manipulation and formulation of emulsions for the purpose of incorporating them into food products for human consumption (Cáceres et al., 2008; Encina et al., 2016; Judge et al., 2007; Ojagh & Hasani, 2018). On many supermarket shelves, it is easy to find foods with added n-3 PUFA such as EPA or DHA in milk, bread, cereals, and a host of dairy functional foods and beverages. In the United States, there are different kinds of milk such as Horizon Organic or Fairlife with added DHA and vitamin D in some of their products (Fa!rlife, 2021; Horizon, 2021), although nowadays to prevent the risk of unfavorable organoleptic properties algal oils are used instead. Indeed, whether for direct consumption or their inclusion in functional foods, the fish oil market was worth \$1905 million (USD) in 2019 with a forecasted CAGR of 5.8% between 2019 and 2027 (GlobeNewswire, 2021). As people tend to be more health conscious and purchase more "wellness" products nowadays (Lordan, 2021), it is likely that the market value may increase beyond forecasted values.

The direct addition, emulsification, or microencapsulation of fish oils are the techniques most commonly used to fortify foodstuffs. Special attention needs to be paid to fish oil oxidation. Lipid oxidation involves the presence of oxygen and other initiators including temperature, free radicals, and light or metal ions, among others. It reduces the nutritional and sensory quality, as well as the safety of PUFA-enriched food due to the formation of new compounds that contribute to off-flavors, reduces their shelf-life, and even compromise their safety depending on the type of oxidation compounds formed (i.e., oxygenated α,β-unsaturated aldehydes) (Guillen & Goicoechea, 2008; Martínez-Yusta et al., 2014; Vidal et al., 2015). In order to overcome this limitation, in addition to controlling the environmental conditions of the product by means of monitoring the temperature, exposure to light and oxygen, and controlling the processing parameters, research has been focused on how to protect PUFAs from degradation in fortified food. As a possible strategy, the use of antioxidants has earned the scientific community's attention. For instance, Qiu et al. investigated the antioxidant effect of ferulic acid and lyophilized ferulic esters (methyl-, ethyl- and dodecyl ferulate) in fish oil-enriched milk (Qiu et al., 2017). Likewise, the utilization of brown seaweed extracts (*Fucus vesiculosus*) as a source of natural antioxidants in granola bars fortified with fish oil-in-water emulsions was demonstrated to reduce lipid oxidation in a 10-week period when concentrations 0.5%−1% algae extract in the emulsion was added (Karadağ et al., 2017). An extended revision of the most recent advances in the fortification of food products with fish oils including a description of the main challenges of fish oil incorporation in food and the most recent techniques, advantages, and limitations of the effects of adding fish oil in foods has been recently published (Jamshidi et al., 2020).

Regarding bioactive peptides, a number of commercial fish protein hydrolysates already exist in the market, mainly in Japan. While the market share of fish peptides compared to milk-derived peptides is relatively small, there are a number of commercial products already available in the market (Rustad & Hayes, 2012). An example is the antihypertensive dietary supplement Valtyron, which is a sardine muscle hydrolysate with ACE inhibiting properties (Gevaert et al., 2016; EFSA, 2010). It is generated through the hydrolysis of sardine protein using a *Bacillus licheniformis* alkaline protease (Matsui & Kawasaki, 2000). Another commercial product is SEACURE, a fish protein concentrate used as a dietary supplement with immunomodulatory properties (Duarte et al., 2006). Katsuobushi, also known as bonito flakes, is a dietary supplement and also functional food since it can be used as an ingredient in a number of dishes (Gevaert et al., 2016). It is produced through the fermentation of bonito fish and it supports healthy blood pressure (Takenaka et al., 2021). Other dietary supplements derived from fish with ACE inhibition properties are Protensin (Knowde, 2021) and Tensideal (Compalia, 2021). Gevaert et al. (2016) summarize the main products commercially available in Europe, USA, and Japan and classify them based on their claimed physiological activities.

5. Sensory attributes of fish hydrolysates

Hydrolysis of proteins results in the formation of peptides with a bitter taste as reported in many studies. This undesirable taste is one of the challenges faced when developing functional foods containing these peptides as ingredients (Rustad & Hayes, 2012). Bitterness depends on the starting material or the degree of hydrolysis. For example, Dauksa et al. have reported that the presence of bile in whole fish and fish viscera, the fat and ash content could be responsible for the bitter taste of fish protein hydrolysates (Dauksas et al., 2008, pp. 101−114). While on the other hand, the content of total amino acids and hydrophobic amino acids did not correlate with bitterness (Dauksas et al., 2008, pp. 101−114). Other researchers have found that bitterness is associated with hydrophobicity, degree of hydrolysis, molecular weight, proline residues, type of enzymes, and amino acid sequences (Idowu & Benjakul, 2019). Idowu and Benjakul have reviewed the main methods used to debitter fish protein hydrolysates, such as extraction with alcohol, treatment with activated carbon, Maillard reaction, use of cyclodextrin, chromatographic separation, and enzymatic hydrolysis with exopeptidase and plastein reaction (Idowu & Benjakul, 2019), leading to hydrolysates with a decreased bitter taste. However, they have also noted that these methods might lead to changes in peptide structure and loss of some peptides leading to subsequent changes in the biofunctional properties of the fish protein hydrolysates. Therefore, attention should be paid when these methods are applied. On the other hand, a number of studies have explored the use of fish protein hydrolysates as flavor enhancers (Muzaddadi et al., 2016; Witono et al., 2019) due to the presence of peptides with MSG-like flavor (Noguchi et al., 1975).

5.1 Factors influencing the consumption of fish and fish-related functional foods

The average consumption of seafood in Europe is 24.35 kg per capita (European Commission, 2019), while the global consumption is 20.3 kg per capita (Cantillo et al., 2021; FAO, 2018). Like in the case of most foods, consumption is affected by consumer perception and acceptability. Consumer perception of a certain food product and its acceptance or rejection is affected by multiple factors (Costell et al., 2009). In the case of fish, Verbeke et al. have highlighted a number of motives and barriers associated with fish consumption that are linked to the consumer characteristics, such as attitudes, consumer involvement, lifestyle, food habits and experience, sociodemographic characteristics, health and diet beliefs and convenience among others (Verbeke et al., 2007). In relation to sociodemographic characteristics, it is evident that consumption differs among regions and countries. An illustrative example is given by Menozzii et al. comparing Hungary and Portugal (Menozzi et al., 2020) where consumption in Hungary is 5.6 kg and in Portugal is 56.8 kg ("The EU Fish Market," 2019). This is affected by availability, and tradition within countries, but also by beliefs formed throughout the life course (Claret et al., 2014). Despite the fact that fish meat is considered healthy meat, fish consumption is negatively affected by the perception that fish can be a source of contaminants such as polychlorinated biphenyls, dioxins, organochlorines pesticides, some heavy metals, and other environmental toxic substances (Conte et al., 2014).

In most cases, food choices and consumption is strongly associated with product characteristics. Many studies have been conducted that explore consumer perception relating to the differences in acceptance depending on the source of fish whether they are farmed or wild (Claret et al., 2014). Sensory perception is one of the main driving forces leading to the consumption of most foods including functional foods, whereby fish is not the exception. Carlucci et al. (2015) reported that the main drivers of fish consumption are sensory perception (taste, smell, and texture) of fish, perceived health benefits, and fish-eating habits (Carlucci et al., 2015). On the other hand, the main barriers to fish consumption are again sensory perception but in this case sensory rejection or dislike, health risk concerns, high price perception, lack of convenience, lack of availability of the preferred products, and lack of knowledge in selecting and preparing the product (Cantillo et al., 2021). The country and region where one lives will also determine the species consumed (Apostolidis & Stergiou, 2012). A recent review of the existing literature on the factors affecting the consumption of fish has identified that females, older people, those highly educated, with higher income, living with a partner consume seafood more frequently (Cantillo et al., 2021).

Carlucci et al. (2015) have reviewed product characteristics that affect the consumption of fish such as the country of origin and they compared imported versus domestic fish (Carlucci et al., 2015). Or the preservation method and they compared chilled, frozen or canned, salted, and smoked. They also investigated the effect of product development by comparing traditional versus innovative products. Moreover, significant research is being conducted lately on the perception of farmed versus wild fish (Claret et al., 2014; Hoque & Alam, 2020; López-Mas et al., 2021), and research shows that although consumers prefer wild fish they consume farmed fish due to the differences in price (Carlucci et al., 2015). Another factor that has attracted significant research interest is the effect of eco labeling or sustainability on the consumption of fish (Menozzi et al., 2020). Menozzi al. (2020) have reported that consumers' willingness to pay for sustainable products, with nutrition and health claims, depends on the country and the type of fish. Understanding the factors affecting the consumption of fish and fish products can help design educational strategies, marketing strategies, and policies in order to promote their consumption. Furthermore, identifying which sensory characteristics of the products drive consumption will help the development of foods and particularly functional foods acceptable to consumers. In this way, the intake of the bioactive compounds will be ensured through the consumption of sensory-acceptable functional foods.

6. Conclusions

CVD is a global cause of excess morbidity and mortality. Supporting healthy dietary and lifestyle choices by promoting the consumption of fish is of significant importance to reaching the protein and n-3 PUFA requirements of a healthy diet. However, there is significant waste in the fishing and aquaculture industries. Therefore, finding useful approaches to reduce waste and create value-added products from byproducts and bycatch from these industries is essential. Fish-derived bioactive lipids and peptides can be utilized to create functional foods targeting CVD prevention through dietary means due to the antihypertensive, antiinflammatory, and antithrombotic lipid and peptide microconstituents within. However, the development of fish oil products, in particular, is not always considered environmentally friendly, and therefore modern green extraction technologies will be key to growth in the fish-derived functional foods sector. Certainly, greater research and investment into the fish-derived functional foods sector is required to drive innovation for a healthy future. Moreover, it is necessary to consider consumer perception toward the different functional foods developed in order to ensure their consumption.

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Part IV

Beverages

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Chapter 12

Functional properties of the fermented alcoholic beverages: Apple cider and beer

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1. Introduction

Fermented alcoholic beverages have been used throughout history by a variety of cultures for the preservation of foods and juices, for their organoleptic and intoxicating properties, and for several other proposed at the time health benefits (Phillps, 2014). During the consumption of alcoholic beverages such as cider and beer, the ethanol content is absorbed by diffusion within the gastrointestinal tract before entering the blood and further into the tissue. Alcohol is one of the most widely consumed recreational drugs worldwide due to its availability, relaxing effect, intoxicating capacity, and for some potential health benefits. However, alcohol can be considered a psychoactive drug and therefore must be treated with respect. Alcohol has been the subject of deep historical debate relating to both its good and bad properties in various cultures throughout history (Ferreira & Willoughby, 2008). Regular alcohol intake has both risks and benefits. Even though several beneficial functional health properties of alcoholic beverages have been proposed, overconsumption of alcohol has been tightly associated with detrimental health effects, along, which are being exacerbated by the potential for addiction to intoxication. Indeed, the overconsumption of alcohol has been linked to and responsible for many traffic accidents, health disorders, violent crimes, and as a result, increased morbidity and mortality (Rajendram et al., 2005).

The excessive drinking of alcohol can have toxic effects on a variety of organs and tissues such as the liver, heart, brain, lungs, and kidneys (Agarwal & Seitz, 2001; Rajendram & Preedy, 2009). Alcohol not only can affect the body physically but also mentally as it is considered a psychoactive drug, with excessive consumption or “binge drinking” causing mood swings, slow thought processes, and violent outbreaks/self-destructive behavior. Doll and Peto (1981) have initially suggested that approximately 35% of cancer deaths in the United States were potentially avoidable by the modification of diet, including 2%−4% alcohol consumption, while this percentage might be as low as 10% or as high as 70% depending on these modifications. Since then, large epidemiological studies, such as the Nurses’ Health Study (NHS) and the EPIC study have also indicated that consumption of alcohol that is higher than the recommended upper limits (i.e., more than one drink per day) is associated with increased risk for several chronic disorders including specific types of cancer like oral, esophageal, liver, and breast cancer (Mostofsky et al., 2016; Ubago-Guisado et al., 2021).

On the other hand, the same epidemiological studies NHS and EPIC have emphasized that protective health benefits against cardiovascular diseases (CVD), some cancers, and several other conditions that can be derived from adherence to healthy diets, such as the Mediterranean diet, a key constituent of which is light to moderate consumption of alcoholic fermented beverages (Mostofsky et al., 2016; Ubago-Guisado et al., 2021). More specifically, moderate intake, defined as up to 1−3 units of alcohol per day (Bamforth, 2002), is associated with a lower risk of hypertension, myocardial infarction, stroke, sudden cardiac death, gallstones, cognitive decline, and all-cause mortality. Moreover, analyses using repeated assessments of alcohol over time and deaths from all causes in the NHS study, further indicated that women with low to

Functional Foods and their Implications for Health Promotion. https://doi.org/10.1016/B978-0-12-823811-0.00013-4

moderate intake and regular frequency (>3 days/week) had the lowest risk of mortality compared with abstainers and women who consumed substantially more than one drink per day (Mostofsky et al., 2016). Similar findings for lower cardiovascular risk have been reported in several studies (Costanzo et al., 2011; De Gaetano et al., 2002).

The outcomes of the benefits of moderate consumption of alcoholic beverages from epidemiological studies are in accordance with the first reported randomized trial on the benefits of the Mediterranean diet versus a classic "Westernised" diet, the Lyon Diet Heart Study (de Lorgeril et al., 1994, 1999). Within this study, several health benefits were observed in the group following the Mediterranean diet, which could not be explained by any difference in low-density lipoprotein (LDL) cholesterol between the groups. Instead, the proposed mechanisms behind the observed effects were that the beneficial properties of compounds present in foods and alcoholic beverages of the Mediterranean diet can reduce platelet aggregation and lipid peroxidation (de Lorgeril et al., 1999). This study provided evidence and proposed mechanisms supporting previous epidemiological studies that coined the controversial term "French Paradox" (Renaud & de Lorgeril, 1992), for the observed low incidence of mortality due to ischaemic heart disease in France, despite similar levels of population smoking, saturated fat intake, serum cholesterol levels, and blood pressure, with other countries that have higher rates of CVD (Burr, 1995). These benefits have been associated with the antioxidant and anti-inflammatory compounds found in alcoholic beverages like wine, which is widely consumed in France (Burr, 1995).

Several other studies have also indicated that other fermented beverages, including beer and apple cider, have also been found to contain similar biofunctional antioxidant and anti-inflammatory compounds. Indeed, it has been proposed that moderate consumption of such functional fermented alcoholic beverages may provide a plethora of health benefits such as a decrease in oxidative stress, thrombosis, and inflammation, lowering of LDL cholesterol, and increase in high-density lipoprotein (HDL) cholesterol levels and functionality, with a subsequent improvement of cholesterol ratios, decreased stress and improvement of moods, reduced risk of coronary heart disease, ischaemic stroke and total mortality (Sierksma et al., 2004; Rimm et al., 1999; Klatsky et al., 2001; Moran et al., 2021; Rotondo et al., 2001; van Tol & Hendriks, 2001; Rendondo et al., 2018; de Gaetano et al., 2016; Cousin et al., 2017; Tsoupras et al. 2020, 2021a; Xi et al., 2017).

It is now well-established that a J-shaped curve depicts the association of alcohol consumption, with all-cause of mortality and especially with CVD (Fig. 12.1), indicating that low to moderate consumption of fermented alcoholic beverages may provide protection against chronic disorders (i.e., 20%−30% reduction of CVD risk) in comparison to people that abstain their intake, while on the other hand overconsumption above a reversion point has been associated with higher mortality rates (de Gaetano et al., 2016; Redondo et al., 2018).

However, it has yet to be established whether abstainers may benefit from a change in their dietary habits by taking on moderate alcohol consumption. The consumption of alcohol can be displayed in "units" or "drinks", which depend on the amount of ethanol in the beverage. This measurement can be used to indicate "light-moderate" or "heavy" drinking in terms of the measurement of ethanol consumed in a day (Dufour, 1999; Kalant & Poikolainen, 1999). Light-moderate drinking is the consumption of one or two alcoholic drinks per day whereas heavy drinking is the consumption of

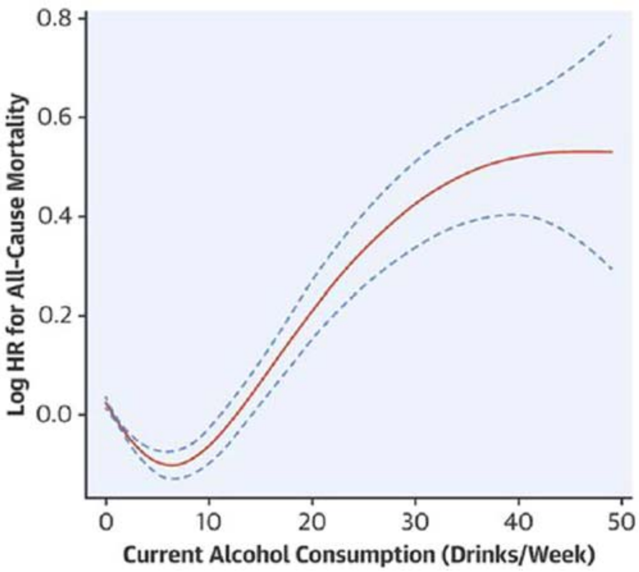

FIGURE 12.1 A graph of the J-shaped curve representative of the relationship between alcohol intake (Drinks/Week)) and all-cause mortality. *Reproduced from* *Xi et al. (2017)*

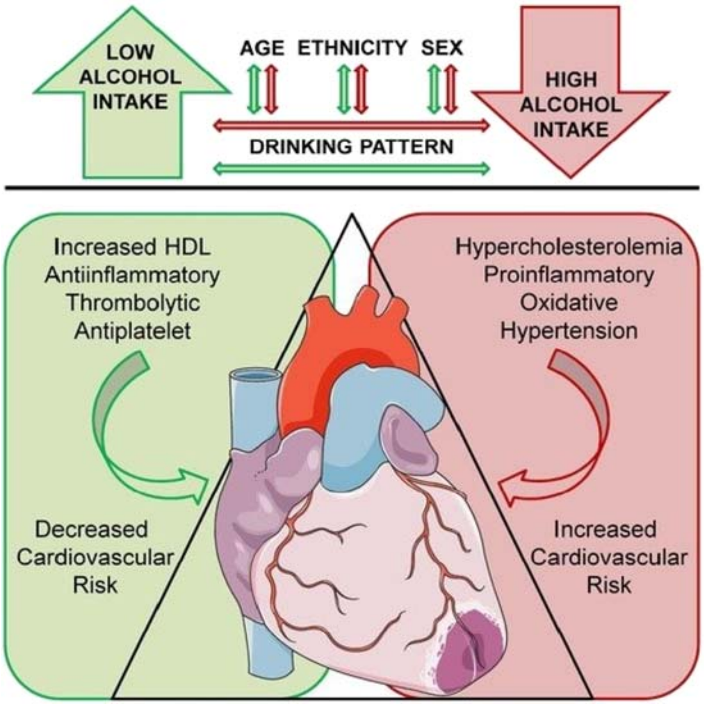

FIGURE 12.2 Biological mechanisms and risk factors underlying cardio-protection by low−moderate alcohol consumption and harmful effects by high intake of alcohol. *Reproduced from Chiva-Blanch and Badimon (2019).*

three or more alcoholic drinks a day. Specifically, the amount of alcohol that is considered light is 30 g/day of ethanol and anything above this is considered heavy drinking.

The literature shows that the controlled intake of alcohol has a positive effect on various pathways in the body that may protect and prevent coronary heart disease (Gaziano et al., 2000; Leighton et al., 1997; McKee & Britton, 1998; Rimm et al., 1999). Fig. 12.2 displays the benefits of moderate alcohol consumption on the biological pathways involved in atherosclerosis and CVD (Agarwal, 2002; Chiva-Blanch & Badimon, 2019; Srivastava et al., 1994; Goldberg and Soleas 2001; Rotondo et al., 2001).

The health benefits of fermented beverages like apple cider and beer, when consumed in moderation, are now well defined. The functional properties of these beverages are tightly associated with their high content in anti-inflammatory and antioxidant functional phenolics, with lower but considerable levels of highly biofunctional polar lipid bioactives with antithrombotic and anti-inflammatory properties (Lordan et al. 2019a, 2019b; Tsoupras et al. 2020, 2021a). Indeed, the production process and fermentation can also affect their probiotic functional properties on gastrointestinal microbiota and perceived health outcomes (Cousin et al., 2017; Lordan et al., 2019a; Moran et al., 2021).

The fermentation process that creates alcoholic beverages have been altered and adapted throughout history and to this day in the industry to create consistent, full flavor, and safe products. These products are naturally rich in bioactive components, but nowadays with the growth of the functional foods and nutraceuticals industries (Chopra et al., 2022), some companies have taken this a step further to create products with these same attributes but with the added benefit of biofunctional components. The general process involves the employment of yeasts that mainly consume and convert the sugars of the medium to alcohol, while several biofunctional compounds and probiotics are also present in the fermented final product if unfiltered. Fermented beverages and especially nondairy probiotic beverages are believed to be the next functional foods for probiotic delivery, with the advantages of lacking dairy allergens such as lactose and allergenic proteins and having a vegan-friendly status. This chapter will review alcoholic beverages, apple cider, and beers, with a focus on their antioxidants and anti-inflammatory functional properties.

2. Apple cider

Apple cider can be defined as a fermented alcoholic beverage made from apple juice. Styles of cider are extremely diverse and not easy to categorize, depending on the type of apple juices used and the degrees of sweetness, from extra dry to sweet, and alcohol content usually ranging from 1.2% to 8% (v/v). Apple cider is one of the oldest known beverages as apple trees have been cultivated since ancient times and a number of ancient documents cite alcoholic beverages made from apples. By the beginning of the ninth century, cider drinking was well established in Europe, and in the 11th century cider consumption became widespread in England, and orchards were established specifically to produce cider apples. Since then, cider has become one of the most popular alcoholic beverages in Europe. The worldwide popularity of cider has also grown, especially in North America and Australia (Cousin et al., 2017). The popularity of the drink is due to its sweet, refreshing taste, along with the availability of apples all year round (Wojdylo, 2008).

Apple cider production has the same basic first stages as the production of apple juices, but with the additional steps required to ferment the apple juice into cider (Fig. 12.2) (Cousin et al., 2017). The fermentation process varies due to differences in the apple variety, the fermentation procedures, added ingredients, and production processes (e.g., pasteurization, fermentation parameters, etc.). The apple variety choice is what gives the cider its specific flavors and aromas. However, these choices can also affect their composition of nutrients and bioactive components (Moran et al., 2021; Tsoupras, Moran, Pleskach, et al., 2021). Once the apple varieties have been chosen the process can begin. Cider apples are usually harvested between the months of September and December and stored for up to 10 days to allow to "sweat", a procedure, which makesthe apples easier to grind and increases the sugar content. They are then squeezed to a pulp and further pressed to release the juices that will be used in fermentation, leaving behind a compacted substance known as pomace. This pomace also contains multiple bioactive components, which will be discussed further in Chapter 5.

The juices are then transferred to the vat house (4−16°C) for an 8-week two-stage fermentation process (Fig. 12.3). At this stage, the apple juice is usually sterilized of all-natural yeasts that are found on the apples themselves, in order to ensure the natural yeasts do not begin to ferment as the flavors and aromas can become unpredictable between batches, although some artisanal producers do retain the natural yeasts to make unique products. After this, specific yeast can be added and any other ingredients or substrates can also be added, such as sugar and other components to produce a specific cider flavor, including honey, berries, or other varieties of herbs, fruits, or spices (Crowden, 2008.). This product is then left to ferment for a specific amount of time and is closely monitored. The initial stage involves the conversion of the apple's natural sugars by yeast to ethyl alcohol followed by the second stage involving the conversion of the natural malic acid into carbon dioxide by the lactic acid bacteria (Cousin et al., 2017). The juice is siphoned off from the thick sediment left known as lees and is put through a filter process to clarify it further and remove any suspended particles. The cider can slowly mature over a period of several months and when ready it can be put through several separate filtering processes, chilling, followed by further filtering and carbonation, before finally being packed and readied for distribution.

Although apple cider can be simply defined as fermented apple juice, the process and specifications can be much more complex than its definition. Several parameters affect cider production, such as the variety of apples, growth conditions, apple cultivar, ripening stage, fermentation type, yeast strains, equipment, additives used, and methods employed (Laaksonen et al., 2017). Indeed, yeast strains and yeast-available nutrients are crucial factors for the functional properties, nutritional value, and taste characteristics of apple cider (Al Daccache et al., 2020; Le Quere et al., 2006). There are specific yeast species that are associated with cider fermentation. In countries such as Ireland, Spain, and France, cider is mainly produced from naturally developing yeast species, derived from the fruit or sometimes from the surface of the processing equipment, stimulating alcoholic fermentation (Lorenzini et al., 2019; Morrissey et al., 2004; Valles et al., 2007). The most prevalent yeast species in alcoholic fermentation has been shown to be the indigenous *S. cerevisiae*, which is also known as a budding yeast for its ability to bud off the parent cell. It is used in cider, beer, and wine making and is used as a model eukaryotic cell in the fields of molecular and cellular biology. The use of isolated strains of *S. cerevisiae* is an essential strategy for maintaining the quality and reproducibility of the cider fermented beverage.

Apart from *S. cerevisiae*, several other yeast species can supplement *S. cerevisiae* and thus enhance flavor complexity and aroma intensity, which can be used in both wine and cider fermentation. Although most of these strains cannot perform complete alcoholic fermentation individually, a mixture of these strains together with *S. cerevisiae* delivers a desirable method for alcoholic fermentation using the correct inoculation methods (Lorenzini et al., 2019; Morrissey et al., 2004; Valles et al., 2007).

2.1 Apple cider composition, functional compounds, and associated health benefits

Apples are widely accepted as a healthy fruit with an abundance of healthy beneficial functional compounds such as fiber, antioxidants, tannins, and other phenolics, vitamins, and minerals, while recently it was also found that apples, apple juice, and cider products also contain such molecules, as well as several lipid bioactive and essential fatty acids with anti-inflammatory functional properties (Moran et al., 2021; Tsoupras et al. 2021a, 2021b). Due to the fermentation process, apple cider has a slightly different composition of biofunctional compounds and associated biological properties than apple juice (Tsoupras, Moran, Pleskach, et al., 2021). Indeed, the variety of apple cider products also varies considerably due to

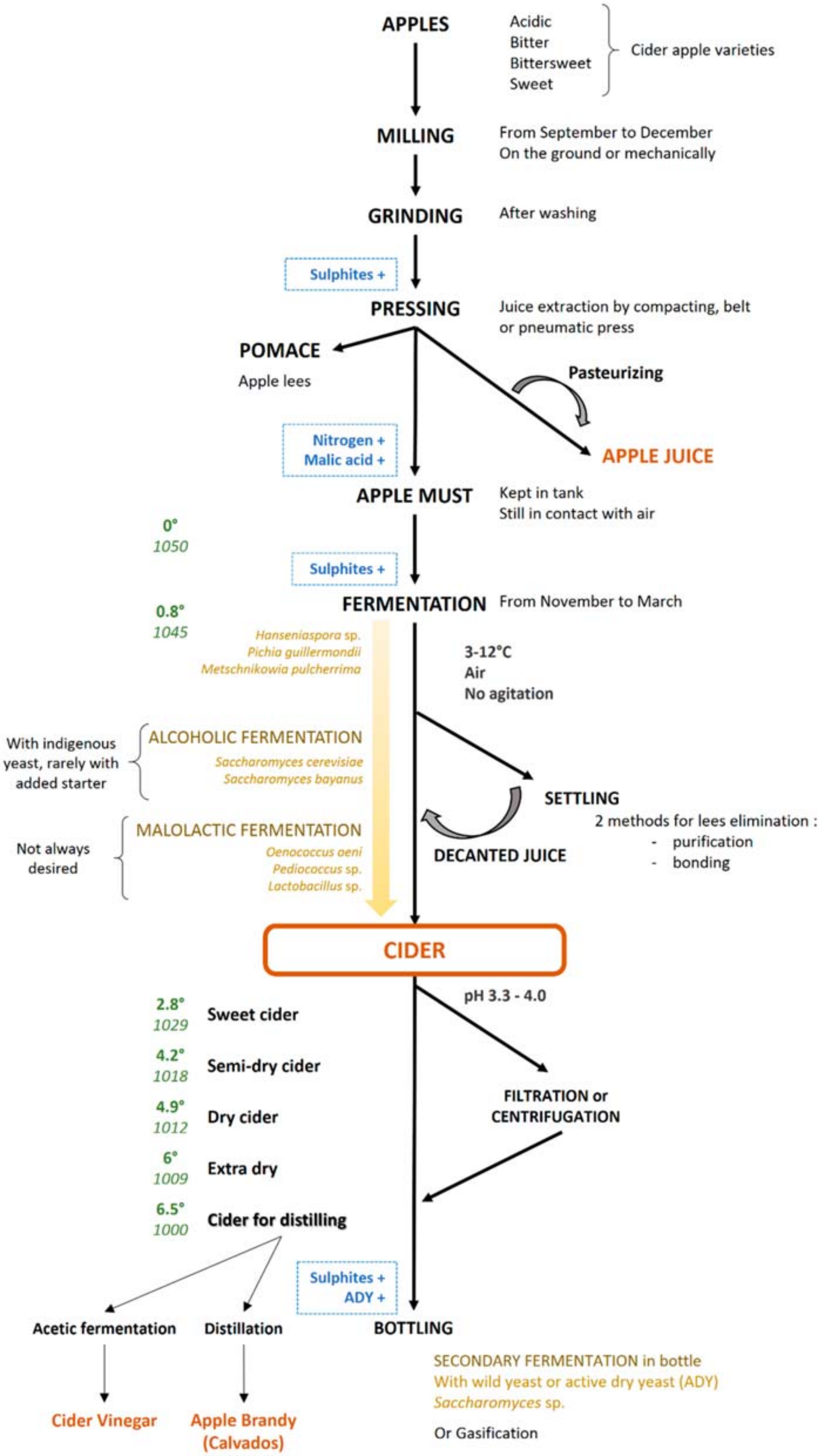

FIGURE 12.3 Cider-making process in France. Legend: +, optional addition; alcohol degree; density; *ADY*: active dry yeast. *Reproduced with permission from* Cousin et al. (2017).

the array of apple varieties and yeasts that can be used, thus leading to a variety of potential organoleptic and biologically consequential composition alterations to the final product (Moran et al., 2021; Tsoupras, Moran, Pleskach, et al., 2021).

In relation to the sensory properties of apple cider, tannins are a well-known natural phenolic compound utilized for its astringency and bitter taste (Ma et al., 2014), and thus they are primarily used in fermented cider and other alcoholic fruit beverages, such as grape fermentation to wine (Ashok & Upadhyaya, 2012). Apple cider production utilizes these types of compounds to bring a unique taste and aroma. Apart from that, the process of fermentation was historically used as a way of preserving food and beverages, however in more recent years, health benefits resulting from this method have been shown to be beneficial for the immune system (Ozen et al., 2015), the digestive system (Didari et al., 2015), the nervous system (Wang et al., 2016), and the cardiovascular system (Astrup, 2014; Sanlier et al., 2019; Wong et al., 2006). Other health benefits of such fermented beverages are believed to be related to their microbial content and their effect on the gastrointestinal system (Marsh et al., 2014). Overall, the fermented apple cider product contains bioactive compounds and probiotics, with several health benefits, and thus it has been proposed as a potential functional beverage.

2.1.1 Cider hydrophilic compounds

The carbohydrates found in apples and apple juice are primarily short-chain sugar molecules such as fructose, sucrose, and glucose (Karadeniz & Ekşi, 2002) whereas the long-chained dietary fibers are mainly found in the plant cell wells (Englyst & Englyst, 2005) that are removed during the squeezing process. The sugars found in natural raw apple juice are considered healthy for their medium to high glycemic index in small portions, however, the juice and cider industry tends to add sugars in order to produce specific sweet and refreshing flavors. The cider industry also adds sugars to improve the fermentation process, which in itself affects the carbohydrates composition of the beverage. The yeasts consume the sugars found in the juices (natural or added) to produce ethanol in the cider.

Vitamins and minerals are an important aspect of eating fruit and vegetable, however, processing can severally affect their bioavailability. For example, vitamin C is significantly decreased due to the pasteurization of the juice (Suárez-Jacobo et al., 2011). Minerals found in apples vary significantly due to their type, environment, and ripeness. These minerals include nitrogen (0.4%−0.8%), phosphorus (0.3%−0.5%), and potassium (~1.2%) (Campeanu et al., 2009), which have been shown to possess health beneficial properties. However, there is little research on the bioavailability of these minerals after juicing, fermenting, and pasteurization.

In relation to the anti-inflammatory, antiplatelet, and antioxidant effects of the apples and the apple cider compounds, the hydrophilic components have been reported to exhibit little to no bioactivity in these biological pathways (Dutta-Roy et al., 2001).

2.1.2 Cider phenolics and lipids with functional properties and health benefits

In contrast to the water-soluble extracts of apples that showed very little to no antiplatelet effects, it was recently found that apples, apple juice, and apple cider contain several polar lipid bioactives, which have shown strong anti-inflammatory and antiplatelet functional properties (Moran et al., 2021; Tsoupras et al. 2021a, 2021b). In addition, the copresence of less hydrophilic phenolics with an intermediate amphipathic polarity in the polar lipid extracts of these apple extracts may provide additional antioxidant benefits too, since phenolics are a well-defined class of functional compounds against oxidative stress and associated disorders (Lee 2003; Lordan et al., 2019c; Tsoupras et al. 2009, 2018).

Although there are low amounts of bioactive polar lipids in apple juice and cider, still these lipid bioactives have shown strong protection against platelet aggregation induced by potent inflammatory and thrombotic mediators such as the platelet-activating factor (PAF), as well as against well-established platelet agonists like adenosine diphosphate (ADP) (Moran et al., 2021; Tsoupras et al. 2021a, 2021b). Several amphiphilic polar lipid bioactives have been found in apple products (juice and cider) and by-products, with phosphatidylcholine (PC), phosphatidylethanolamine (PE), and glycolipids exhibiting the strongest anti-inflammatory and antiplatelet potency (Tsoupras et al. 2021a, 2021b). It was also found recently that these polar lipid bioactives in both apple juice, cider and cider by-products predominantly contain bio-functional monounsaturated fatty acids (MUFA), such as the omega-9 (n-9) oleic acid (C18:1n9), and the essential omega 3 (n-3) and omega 6 (n-6) polyunsaturated fatty acids (PUFA), alpha-linolenic acid (ALA; C18:2n3), and linolenic acid (LA; 18:2n6), with favorably low levels of the n-6/n-3 PUFA ratio (Moran et al., 2021; Tsoupras et al. 2021a, 2021b).

These findings further support the anti-inflammatory potency of the apple cider polar lipid bioactives, since the lower the values for the n-6/n-3 PUFA ratio in a food or beverage the better the anti-inflammatory health benefits of this product (Simopoulos et al., 2008). Moreover, the free fatty acids forms of both the n-9 MUFA oleic acid and the n-3 PUFA ALA found in apple cider have on their own shown several anti-inflammatory and antithrombotic functional health properties too, which also can add to the overall antithrombotic and anti-inflammatory potential of apple cider.

It should also be noted that apple cider fermentation seems to affect the structures, fatty acid composition, and subsequently the structure-activity relationships of the cider polar lipid bioactives (Moran et al., 2021; Tsoupras, Moran, Pleskach, et al., 2021). In addition, higher amounts of these polar lipid bioactives were observed in the apple wastes (apple pomace) that are by-products produced during the production process of apple cider (Tsoupras, Moran, Byrne, et al., 2021), further suggesting that valorization of apple cider by-products may provide functional lipid compounds to be used as fortifying agents in other foods to potentially enhance their health benefits, along with reducing the environmental footprint of these wastes.

However, further human trials will establish whether these apple cider polar lipid bioactives and their biofunctional fatty acid content can also protect humans *in vivo* too, as alluded to in the introduction, other components of alcoholic beverages may negatively offset these potential benefits if consumed in excess. Nevertheless, the presence of such bioactive lipids in apple cider and its by-products opens an exciting avenue of research for the development of potential functional products with potential anti-inflammatory and antithrombotic properties.

An important subgroup of amphipathic molecules found in high amounts in apples and their products, juice, and cider are several less-hydrophilic phenolic compounds (Riekstina-Dolge et al., 2014) with strong anti-inflammatory and anti-platelet properties (Tsoupras, Moran, Pleskach, et al., 2021), along with strong antioxidant, metal chelating, and free radical scavenging activities (Lamy 2016). This large subgroup includes compounds such as flavonoids, tannins, and phenolic acids like benzoic acid, as well as hydroxycinnamic acids that contribute to cider aroma (Lea, 1995) and procyanidins that participate in enzymatic browning and polymerization, which is accountable for the bitterness and astringency of apple juices and ciders (Lea & Arnold, 1978).

Apart from their functional properties on cider sensory characteristics, apple cider phenolics have been found to display several bioactive functions too, with the most well-established being their antioxidant potency. The beneficial antioxidant effects and associated health benefits of phenolics have been reviewed thoroughly (Lee 2003; Lordan et al., 2019c; Tsoupras et al. 2009, 2018). The literature displays a clear correlation between diets rich in fruits and polyphenols and the reduction of inflammatory manifestations linked to chronic diseases such as atherosclerosis (Woodside 2013). These compounds contain beneficial health properties to prevent and treat chronic diseases such as CVD (Habauzit 2012) and maybe even cancer despite the tumultuous relationship between the two (Huang 2009; Tsoupras et al., 2009). The absorption, bioavailability, and utilization of these compounds have been studied throughout the literature (Cory, 2018) and the nutritional content of the apple show that they contain compounds that affect inflammatory mediators, however, there is little research on how much of these compounds are left behind during the juicing process (Francini, 2013).

The phenolic content of four apples or of 20 glasses of apple juice (long life) is comparable to that contained in an average 150 mL glass of wine (Paganga et al., 1999), It seems that apple cider still contains an abundance of bioactive antioxidants with a variety of phenolic compounds too (Riekstina-Dolge et al., 2014). Like apples, there is a correlation between the phenolic content and antioxidant ability in cider, with procyanidin B_2 displaying the strongest antioxidant ability of the cider polyphenols (Lobo et al., 2009), while it was previously shown through blood and urine testing that cider polyphenols are taken up and metabolized in the human body (DuPont et al., 2002).

2.1.3 Other cider compounds and probiotics with functional properties and health benefits

Apple beverages including cider can be sources of probiotics, due to which several functional properties and health benefits have been documented for apple cider. For example, apple cider has also demonstrated antiviral capabilities in a study against poliovirus 1, through its probiotics' content (Konowalchuk & Speirs, 1978). Spent cider yeast, a by-product of cider production, was used successfully as dietary supplementation in an animal model, where it beneficially alters the gut microbiome to enhance gut functions and reduce *Salmonella* and *Escherichia* carriage in the animals' gut (Upadrasta et al., 2013). The exopolysaccharides (EPS) produced by lactic acid bacteria during the fermentation of cider have previously been shown to possess anticarcinogenic and cholesterol-lowering abilities (Salazar et al., 2008). The lactic acid bacteria strain *Pediococcus parvulus* isolated from cider produced the EPS, 2-substituted (1,3)-D-glucan which was shown *in vitro* to possess some probiotic properties such as the ability to adhere to human enterocyte cells (Russo et al., 2012), and to regulate macrophages (Fernández de Palencia et al., 2009) with anti-inflammatory response capabilities during activation (Notaraigo et al., 2014). The potential probiotic properties demonstrated by the lactic acid bacteria strains used in cider fermentation suggest the possible probiotic potential that cider may possess if they survive processing. Thus, studies on apple fermentation beverages, including apple cider, comprise a promising field of research with great potential for available new, healthy and pleasant functional products on the market (Cousin et al., 2017).

3. Beer

3.1 Beer production

Beer is one of the most popular drinks worldwide. Like cider, the brewing of beer is an ancient process dating as far back as 8000 years. However, it is only technological advancements over the past 150 years or so that brings us to a modernized practice of several methods of brewing we are now familiar with (Bamforth, 2002), resulting in many unique types of beer such as ale, stout, and lager, which are the most popular and common types of beer brewed worldwide. The ingredients in beer are often altered to produce unique properties and characteristics in specific types (Nogueira et al., 2017). The basic ingredients and substrates include malted grain like barely (*Hordeum vulgare*), hops (*Humulus lupulus*), yeast (i.e., *Saccharomyces cerevisiae*), and water (Seefeldt et al., 2011). Though the core ingredients remain the same, beer all over the world can vary due to the variety of malted grain selected, grain roasting time, the temperature used, yeast strain, and pH utilized in the brewing process. These parameters can all have an impact on the quality and composition of the end product yielding several hundreds of identified compounds that contribute to the sensory properties and nutritional value of beers (Buiatti, 2008; Gerhäuser, 2005). Some of these compounds originate in raw materials like malt and hops while some are the result of yeast metabolism of fermentable sugars. Additional substrates and ingredients may be added depending on the brewer; however, these are not needed and are generally added to improve flavor or the brewing process.

Beers can be subcategorized depending on the yeast used in fermentation, including whether a top-fermenting yeast or a bottom-fermenting yeast is used. A top-fermenting yeast such as *Saccharomyces cerevisiae* produces ales. *Saccharomyces cerevisiae* ferments at warm temperatures. Due to the short fermentation period and the warm environment, the resultant ales generally contain a unique and complex range of flavors that are generally described as fruity, which can be attributed to the yeast-derived esters. These ales include a range of beer beverages such as stout, porters, dark ales, and pale ales, which vary significantly in taste, flavor, and aroma. In contrast, *Saccharomyces pastorianus* is a bottom-fermenting yeast that produces lager-style beers. These beers are produced at cooler temperatures and generally contain lower amounts of hops. This leads to a less bitter taste, dry and light final product, which is lower in calories and alcohol percentage due to less fermentable substrates within the process.

The fermentation and production of beers are considered to be much more complicated than cider or wine due to the number of substrates used and the number of alterations that must be set in place to produce a specific beer such as ales or lagers (Fig. 12.4). There are many steps where these modifications can take place, however, the process remains relatively consistent following the pattern of malting, milling, mashing, mash filtration, boiling, fermentation, maturation, and packaging. Malting is the process of converting the barley seed into malt. This process begins with steeping, the stage where water is added to the seeds and allowed to soak. These water-soaked grains are then left in a humidity-controlled room for 4−6 days, allowing them to take in water and begin to germinate. This germination triggers the release of alpha and beta amylases, glucanase, proteinases, and phosphatase, which are degradative enzymes. This allows the enzymes to alter the malt by separating and breaking down the carbohydrates in the endosperm into smaller chain sugars. These sugars can then be consumed by the yeast in the fermentation process to convert the compounds into alcohol.

The mashing stage occurs by crushing the malted barley, again releasing the simple sugars in the seeds to be used in the fermentation process. After 60 min there is very little starch remaining in the grains, which are now known as brewers spent grain (BSG). This is then heated to 75°C, which allows the wort to separate easily. The separation occurs in an artificial filter where the water containing the nutrients and the substrate (mashed barley and hops) are passed through. The filtered liquid is then cooled, and yeast is added to begin fermentation.

Even though beer is one of the most widely consumed alcoholic beverages it has been the subject of wide criticism for its association with a tendency for people to overconsume or succumb to alcoholism with potentially detrimental health effects. Nevertheless, moderate consumption of beer has been shown to produce similar health beneficial properties to that of wine by reducing the risk of certain chronic diseases such as diabetes, coronary diseases, and overall mortality (de Gaetano et al., 2016; Ferreira & Willoughby, 2008; Mukamal & Rimm, 2008; Redondo et al., 2018). The alcohol within the beer is one of many health-beneficial components of beer, although this alcohol is most likely the primary ingredient that contributes to atherosclerosis (Li & Mukamal. 2004; Tolstrup & Grønbaek, 2007). Other beneficial health properties can be attributed to the hop and cereal-related compounds that are free of sugar, cholesterol, or fat, the inclusion of phenolic compounds with strong antioxidant and anti-inflammatory properties, soluble fiber, magnesium, and other micronutrients (Bamforth, 2002).

Nonalcoholic beers have also displayed similar health benefits without the negative properties that are associated with alcohol consumption. It has been described that nonalcoholic beer has several nutrients derived from its ingredients including vitamins, minerals, proteins, carbohydrates, and antioxidants that make beer a potential functional supplement

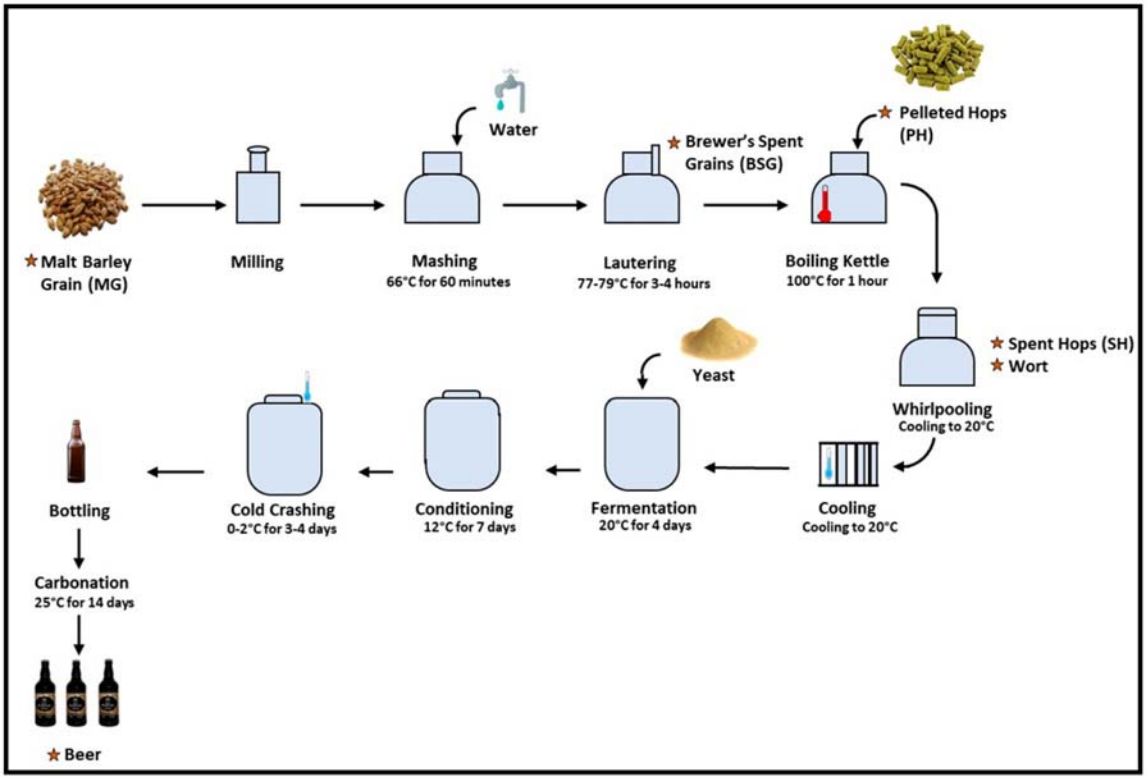

FIGURE 12.4 A schematic of the brewing process for the production of Irish red ale outlining the sampling points for the brewing materials, by-products, wort, and beer as highlighted with a star. *Reproduced with permission from Lordan et al. (2019a).*

(Vazquez-Cervantes et al., 2021). For example, similarly to classic beers, nonalcoholic beers contain also several functional hops' components and the bioactive melanoidins, which can provide beneficial effects for health in these beers without the presence of alcohol (Sharma et al., 2021; Vazquez-Cervantes et al., 2021). Overall, traditional - and nonalcoholic beer consumption appear to have similarities and differences with respect to their effects on the cardiovascular system (Olas & Bryś, 2020). More randomized clinical trials are needed to determine this.

3.2 Beer composition, functional compounds, and associated health benefits

3.2.1 Beer composition and nutrients

Though the core ingredients remain the same, beer all over the world can vary due to the different raw materials selected and the brewing method applied, which have an impact on the quality and composition of the product, yielding several hundreds of identified compounds that contribute to flavor, aroma, texture, foam stability, and nutritional value of beers (Buiatti, 2008; Gerhäuser, 2005). Some of these compounds originate in the raw materials, while some are the result of yeast metabolism of the fermentable sugars, as well as added ingredients to enhance the sensory and functional properties of beer. Beer is primarily composed of water (90%) with alcohol, carbon dioxide, and all other other compounds and nutrients being dissolved/suspended in it, such as carbohydrates, inorganic salts, organic acids, hop derivatives, and some B vitamins (Buiatti, 2008). Beer generally ranges in alcohol content from 1% to 6% depending on the type of beer with craft beers ranging in the higher alcoholic volumes. Carbohydrates make up roughly 3%−4% of these macro nutrients in the form of dextrins, monosaccharides, oligosaccharides, and pentosans. These can alter with recipes and during fermentation, they are utilized to produce ethanol and a range of other products, such as carbonyl compounds, esters, aldehydes, and organic acids.

The beers' bitterness and fruity after taste are from the added hops' derivatives, more specifically the α-acids (humulones) and β-acids (lupulones). Iso-α-acids vary from brewer to brewer with regard to their specifications and added ingredients. Generally, they will range between 15 mg/L in an average American lager to roughly 100 mg/L in a bitter English ale (De Keukeleire, 2000). These organic acids are usually a product of yeast and its fermentation, whereas the inorganic components of beer are metal cations, anions, and trace metals, which among the B vitamins are crucial to the growth of the yeast originating in the embryo and aleurone layer of the malted grain (Muy-Rangel et al., 2018), as well as contributing to the final product's clarity and flavor. These components can be derived from the ingredients or substrates added during the brewing process (Anderson et al., 2019). Apart from aldehydes and organic acids produced during fermentation, other organic components found in beer such as phenolic compounds, esters, ketones, and sulfur compounds, also affect the sensory characteristics of beer, while the increased levels of some of these compounds as a result of reactions during the brewing process can cause various undesirable end-products (Buiatti, 2008).

3.2.2 Beer bioactive phenolics

With respect to the health benefits of beer, most of the research studies to date have focused on the phenolic compounds found in beer, such as xanthohumol and its metabolites, and their antioxidant potency with subsequent cardiovascular protection (Arranz et al., 2012). In beer, several polyphenols are derived from the malt (two-thirds) and the hops (one-third) (Buiatti, 2008). The primary source of a beer's antioxidant capacity (55%−88%) is the result of the phenolic composition including ferulic acid (50%), syringic acid, (+)-catechin, caffeic acid, protocatechuic acid, and (−)-epicatechin (Zhao et al., 2010). This data relates only to the commercial larger beverages however it does give a representation of the significance of these phenolic compounds. These compounds vary from beer types such as ales, lagers, or crafts and recipes (brewer's specifications).

The composition of lagers versus ales can vary significantly and it was found that the nonhopped lagers produced a greater abundance of phenolic compounds in their final product in comparison to their hopped counterparts (Marova et al., 2011). Beers produced with ingredients with an abundance of polyphenols increase the overall phenolic content of the product. Such ingredients are generally fruit or fruit-based (Juice). Nardini and Garaguso (2020) observed the bioactive compounds found in fruit and nonfruit beers. Their results showed that cherry beers had the highest abundance of phenolic compounds followed by other fruit beers as expected (Nardini & Garaguso, 2020). It was also found that catechins were the most abundant phenolic compound. The array of phenolic compounds could be linked to the additional sugar content brought in by the fruit. This added sugar content is used to aid in secondary fermentation, which consequently may lead to increased phenolics.

Darker ales such as stout tend to vary in phenolic content, however, the darker color generally has been linked with the concentration of the phenolic compounds and as a result of the malting process (Socha et al., 2017). Hydrolysis seems to be an important issue relating to the bioavailability of several of these phenolics, especially cinnamic acid. This process could potentially occur in the small intestine, which means the number of phenolic compounds in a beer may not necessarily indicate the level of antioxidant capacity and therefore, a greater variety of phenolic compounds may be desirable to benefit from their intake. Consideration also must also be paid to the absorbability and the metabolism of phenolics, for which there is evidence that some phenolics have bioavailability issues, particularly in beer, which has both bound and free phenolics (Crozier et al., 2009; Lordan et al., 2019c; Nardini & Ghiselli, 2004).

Commercial beers are widely consumed, however, the craft beer industry has also vastly grown. Cheiran et al. (2019) have estimated the identification of 57 phenolics and 11 nitrogenous compounds in craft beers. Of these compounds, only 12 of them have been identified in commercial beer, which may indicate the possibility of craft beers having a more diverse and potentially more beneficial content than the widely popular commercial beers.

Nonalcoholic and low-calorie beers have also begun to become popular, however, the phenolic composition of these was lower than their original (Vinson et al., 2003). Some research has been conducted in the area of nonalcoholic or low-calorie beers, which may be an interesting area of research with the emerging popularity due to the focus of many consumers on wellness products or healthier alternatives (Tireki, 2021). Indeed, others just want to enjoy the social aspects of alcohol consumption without the fear of health repercussions or drunk driving laws (Catarino & Mendes, 2011). Further analysis of these beverages is required to fully understand the phenolic composition and randomized trials to determine their possible health benefits. Despite the lack of extensive research, there are indications that these nonalcoholic beverages contain phenolic compounds that may affect pathways related to Alzheimer's disease (Osorio-Paz et al., 2020). These compounds have properties that were shown to reduce cognitive decline, and moderate glucose and lipid metabolism.

Overall, the strong antioxidant and anti-inflammatory potency of bioactive phenolics being present in fermented alcoholic beverages, such as beer, which are rich in such phenolics, further suggest their potential health benefits against several chronic disorders (Tsoupras et al. 2009, 2018). Nevertheless, more studies are needed to evaluate also the effects of the low absorbability and bioavailability of phenolics on their proposed health benefits.

3.2.3 Beer bioactive lipids

The primary source of the protein, lipids, carbohydrates, and polyphenol compounds in beer is the malted barley. Barley contains approximately 3%−4% of lipids at the beginning of the malting process, which is reduced during the malting process and germination due to the hydrolysis of some of these triglycerides to free fatty acids (Anness, 1984). Thus, the lipid content obtained from the malted barley is lost mainly in the spent grain, and consequently, it has been estimated that lipid content in beer can be less than 0.1% (Buiatti, 2008). The variety of barley grown, and the malting and mash conditions affect the lipid content and the fatty acid composition of the malted barley, wort, and beer, with the dominant fatty acids being the SFA palmitic (16:0) and stearic (18:0) acids, the MUFA oleic acid (18:1) and the essential PUFA linoleic (18:2n6) and alpha-linolenic (18:3n3) acids (Bravi et al., 2012; Evans et al., 2013; Gordon et al., 2018).

It has been reported that increased temperatures over 65°C during mashing can increase the concentration of the essential PUFA linoleic (18:2n6) and alpha-linolenic (18:3n3) acids, as the higher temperature allows these fatty acids to be absorbed from the aleurone layer of the barley that is often not absorbed at lower temperatures (Bravi et al., 2012). Once malted, ~80% of the fatty acids still present are either neutral lipids or free fatty acids, while phospholipids and glycolipids make up the remainder (Anness, 1984). These phospholipids have been identified as PC, PI, and L-PC, where PC and PE were identified as the most abundant classes (Anness, 1984; Hough et al., 1982). In addition, lipids extracted from the malt present in wort undergo modification during fermentation by the action of yeast. For example, low concentrations of medium chain length fatty acids were observed in the wort, while during fermentation the short chain fatty acids were catabolized by yeast, which in turn produced some medium chain length fatty acids during fermentation (Taylor & Kirsop, 1977).

Beer lipids have often been associated with adverse effects on beer quality due to the occurrence of off-flavor products, such as the trans-2-noneal produced from the degradation of the long chain linoleic and linolenic acids (Bravi et al., 2012). Indeed, fatty acids in beer also affect the stability of the foam head, an important feature of many beers (Gordon et al., 2018; Wilde et al., 2003). Conversely, recent research has outlined the beneficial roles of beer lipids, such as their important roles in yeast metabolism, where the long chain PUFA contribute positively to the activation of yeast cell growth under anaerobic conditions, contributing to a quicker fermentation (Bravi et al., 2012).

Most importantly, it has also been recently reported that several types of beer (ale, lager, and stout) and related ingredients and by-products, contain bioactive polar lipids with strong antithrombotic and anti-inflammatory properties with potential health benefits (Lordan et al. 2019a, 2019b; Tsoupras et al., 2020). Even though beer contains low amounts of lipids, it was recently found that some beer polar lipids are highly bioactive even in low amounts. Indeed, these polar lipids, which are an abundant fraction of the total beer lipidome, strongly inhibit the activation of human platelets induced by inflammatory and thrombotic mediators like PAF and thrombin (Lordan et al., 2019a, 2019b; Tsoupras et al., 2020). Since both PAF and thrombin are implicated in thrombo-inflammatory manifestations during the onset and development of several inflammation-related chronic disorders (Tsoupras et al., 2009, 2018), the presence of polar lipid bioactive in beer in low amounts, along with the antioxidant phenolics of beer, may provide a rational for the health benefits of moderate consumption of beer.

Furthermore, the analysis of the polar lipid profile of the beer, its raw materials, and by-products, compared with the bioactivity of these against inflammation and thrombosis, showed that during the brewing process lipids undergo changes that affect their biological activity, particularly when the wort is fermented to beer (Lordan et al., 2019a).

Further separation and lipidomic analysis of ale PL bioactives resulted in the structural elucidation of several different subspecies of polar lipid bioactives found in ale, along with structure-activity relationships of their strong anti-inflammatory and antithrombotic properties (Tsoupas et al., 2020). It was found that the most bioactive beer PL were molecules belonging to phosphatidylcholines (PC), phosphatidylethanolamines (PE), and several glycolipids, with strong potency against the inflammatory and thrombotic pathways of PAF (Tsoupras et al., 2020). These beer PL bioactives were also found to contain biofunctional fatty acids, such as the n-9 oleic acid (C18:1n9) and the essential n-3 PUFA ALA, as well as much lower but considerable amounts of the long chain n-3 PUFA, DHA and EPA, at their *sn*-2 position, with favorable low levels of the n-6/n-3 PUFA ratio, which further support the proposed beneficial health effects of PL bioactives from fermented alcoholic beverages against inflammation-related disorders (Tsoupras et al. 2018, 2020), since the lower this ratio the better the health outcome against chronic disorders (Simopoulos, 2008). In addition, the bioactive sphingolipid and glycolipid molecules found in ale, such as specific ceramides and glucosylcerebrosides with sphingosine, phytosphingosine, and dihydrosphingosine bases but also specific monogalactodiglycerides and sphingomyelin species bearing the aforementioned functional fatty acids at their *sn*-2 position, have been reported to possess strong anti-inflammatory and antitumor properties. These compositions add further evidence that these products may contain strong bioactivities against the PAF pathway of inflammatory and thrombotic manifestations associated with the onset and development of chronic disorders (Tsoupras et al., 2018, 2020). Nevertheless, further research is needed to support the *in vivo* health benefits of beer PL bioactives, as well as on the valorization of PL bioactives from by-products produced during the brewing process, such as spent grain, spent hops, and wort.

3.2.4 Beer probiotics

Many fermented beverages, including beer, contain living microorganisms contributing to the fermentation process, of which some are genetically similar to strains used as probiotics with many health benefits, and so these microorganisms have become another focus of attention. Several studies have indicated the health benefits of such probiotics, especially for the improvement of gut microbiota and the prevention of gastrointestinal disorders, including inflammation-related ones (Hod et al., 2017; Marco et al., 2017; Perceval et al., 2019; Szajewska et al., 2020). Among all beer types, in recent years, the consumption of craft beers has gained popularity. Some craft beers are unpasteurized and unfiltered and so they may potentially act as a new vehicle for delivering naturally occurring probiotics from beer or additional probiotics added to the production with associated health benefits (Capese et al., 2018). Indeed, recent research is exploring the development of novel functional beer products with higher efficacy of their probiotic content, including sour beers with high probiotic live counts that exhibit several health benefits is a promising field too (Alcine Chan et al., 2019).

However, beer typically contains hop iso-α-acids, which prevent the growth and survival of probiotic lactic acid bacteria, and thus the use of suitable fermentation strategies is crucial for developing novel functional beer products. For example, there are now studies demonstrating the feasibility of utilizing probiotic lactobacilli as starter cultures in beer brewing (Alcine Chan et al., 2019. Moreover, a probiotic-containing functional wheat beer (PWB) was successfully produced by an axenic culture system with classic *Saccharomyces cerevisiae* in combination with probiotics'-containing functional sour beer (PSB), while the new combination of probiotics in this novel beer promoted antidepressant effects in Swiss webster mice treated with PWB or PSB, respectively (Silva et al., 2021). Finally, the inclusion of probiotic yeast strains with well-established health benefits in mixed starters' cultures (i.e., classic *S. cerevisiae* yeast strains from wort fermentation) increased the antioxidant activity and polyphenols content in the novel beer products, in comparison to beers from single starter fermentations, indicating the influence of including some well-established probiotic yeast strains on these parameters (Capese et al., 2018). Thus, some mixed starter cultures can result in very promising tools to increase the healthy quality of beer, such as the improvement of the antioxidant activity and polyphenols' content of beer (Capese et al., 2018).

Overall, changing the fermentation process and the starter cultures for the production of beer can provide new probiotic content and associated functional properties to the final beer product (Capese et al., 2018), while the valorization of wastes from the beer production, such as the underused malt sprout beer by-products, as substrates for the growth and dehydration of probiotic lactobacilli strains and for lactic acid production, is also another promising field of functional beer products (Cejas et al., 2017; Djukić-Vuković et al., 2016).

3.3 Deriving functional beverages and products from beer and cider with a focus on cardiovascular health

As alluded to in this chapter, the cider and beer industries are undoubtedly a treasure trove of bioactive compounds due to the wide variety of botanical substrates utilized that contain bioactive components that may be extracted or produced due to the various processes utilized in the brewing industry. By-products of both industries, which are further discussed in Chapter 4, show that multiple biofunctional products can be developed from the wastes of these industries that may have applications for human health, animal feeds, and animal health. However, both beverages themselves can be further processed or altered to become functional beverages or even nutraceuticals. Indeed, the tremendous growth of the beer and cider industries at both an industrial level and artisanal level has driven product innovation in recent years (Baiano, 2021; GlobeNewswire, 2021). Furthermore, some novel functional beverages and nutraceutical products have been developed with a particular focus on cardiovascular health benefits (Salanţ;ă et al., 2020).

As previously discussed in this chapter, everyday cider may exert potential anti-inflammatory and antithrombotic properties that may be beneficial for cardiovascular health (Moran et al., 2021; Tsoupras, Moran, Pleskach, et al., 2021). Although it is a field of research in its infancy, the cider industry has the opportunity to develop novel functional beverages and nutraceuticals from the cider itself by altering production processes or adding adjuncts to the processes (Duralija et al., 2021; Moran et al., 2021; Reis et al., 2014).

One approach has been to combine apple juice and other fermented beverages, such as whey-based beverages fermented by kefir, which produces novel beverages with high total phenolic content and antioxidant activity with potential cardiovascular benefits (Cousin et al., 2017). However, most strategies exploited to date look to the use of apple cider vinegar, a product of the apple cider industry.

Apple cider vinegar has gained tremendous attention for its potential antiglycaemic and weight loss benefits in popular culture, although not always supported by high-quality evidence (Launholt et al., 2020; Samad et al., 2016; Hlebowicz et al. 2007). The main difference between cider and apple cider vinegar is the type of fermentation applied. Outside of an industrial setting apple cider vinegar can occur naturally by overextending the fermentation time required to obtain cider, particularly when using the natural microflora of apples to ferment the apple juice. However, generally and at an industrial level apple cider vinegar is produced by a two-step fermentation process, whereby the first step is usually an oxygenated fermentation to produce ethanol, and a second fermentation driven by *Acetobacter* converts the ethanol to acetic acid, which is then allowed to mature in barrels for up to several months (Joshi & Sharma, 2009). The resulting apple cider vinegar can be consumed, or it can be used to produce novel products such as functional foods, beverages, supplements, and nutraceuticals (Bartkiene et al., 2021).

Apple cider vinegar is touted to have multiple cardiovascular benefits by exerting antiglycaemic, antioxidant, immunomodulatory, and anti-inflammatory properties along with aiding with weight loss, Although promising, there have been very few studies beyond *in vitro* or *in vivo* evidence (Abdulrauf et al., 2018; Ahmadifar et al., 2019; Budak et al., 2011; Halima et al., 2019; Tripathi et al., 2020). For example, the administration of apple cider vinegar during calorie-restricted dieting may improve weight loss thus supporting cardiovascular health (Khezri et al., 2018). However, many similar studies investigating the weight loss potential of apple cider vinegar tend to be of low-quality evidence due to poor study methodologies, the risk of bias, a low number of participants, etc. (Launholt et al., 2020). Another study has hinted at the possibility of ergogenic properties in humans that may have benefits for athletes (Chiu et al., 2020). Others have reviewed the bioactive components in vinegar, such as organic acids, polyphenols, melanoidins, and tetramethylpyrazine, for their potential functions of antioxidative activity, regulation of lipid metabolism, liver protection, blood pressure, and glucose control, antifatigue and antitumor properties (Xia et al., 2020). However, further studies are needed to explore the novel functional compounds in vinegar and their molecular mechanisms for health benefits in the future.

Moreover, there is also concern that consumption of apple cider vinegar or supplements containing it can cause tooth decay and damage to the esophagus (Hill et al., 2005; Launholt et al., 2020). Considering there is a widespread belief that apple cider vinegar has potential health benefits due to folk beliefs, cultural beliefs, or traditions, it is important to conduct further research to substantiate or reject these claims. To date, the literature does not support a significant benefit of apple cider vinegar supplementation, but further investment and research in the field may be led to innovative functional beverages or nutraceuticals.

Another interesting side-product of apple cider breweries and vinegar production is the “mother of vinegar”, which is a biofilm composed of an extracellular form of cellulose and acetic acid bacteria on the surface of the vinegar. While its name is derived from its ability to produce vinegar when added to wine, cider, or other alcoholic liquids (Ayikin et al., 2015), the mother of vinegar is also known as *Mycoderma aceti*, a New Latin expression, from the Greek “Μύκητας” (“fungus”) plus “δέρμα” (“skin”), and the Latin aceti (“of the acid”) (Fuchs, 2006). The “mother” of apple vinegar produced by natural acetic acid bacteria was found to contain high iron (Fe) contents and several phenolics with strong antioxidant capacity, such as gallic acid and chlorogenic acid, among others (Ayikin et al., 2015). However, due to the scarce literature further studies are needed to fully evaluate the applications of “mother” vinegar and its bioactive compounds in functional foods and other products of potential benefits, such as supplements of vinegar powder.

In contrast to the cider industry, tremendous strides have been taken by the beer industry to develop value-added and novel products to improve profitability, reduce waste, and become market leaders in novel sectors such as promoting cardiovascular health. In doing so, some companies and researchers have investigated the development of novel functional beverages and nutraceuticals from beer to target cardiovascular health. Many have attempted to exploit the natural composition of beer via the production process to enhance the final products’ phenolic content and antioxidant capacity. Some products have utilized noncereal adjuncts or additives in the beer production process such as fruits, herbs, or spices (Paiva et al., 2021). These adjuncts can be added at various steps of the beer production process such as in the fermentation stage, boiling of the wort, or maturation stage. Zapata et al. (2019) demonstrated the inclusion of macerated quince fruits increased the total phenolic content of the beer and the overall antioxidant capacity. A similar study investigated the addition of legumes (spelt, chickling, and lentils) to various malts to determine which beer had the greatest phenolic and antioxidant content of the beer. In this study lentil and chickling-containing beers seemed to exhibit more favorable properties potentially due to higher isoflavones (Luneia et al., 2018). Paiva et al. (2021) have reviewed various noncereal adjuncts that may beneficially affect the antioxidant properties of beers. Indeed, they show that fruits are the main adjuncts added and that adding the materials to the boiling of the wort may extract the phenolic constituents more efficiently. However, the authors do highlight that although the use of adjunct raw materials as a strategy can successfully increase the phenolic content and antioxidant capacity of the beers, they may not be bioavailable upon consumption (Paiva et al., 2021).

Indeed, it is uncertain whether the potential cardiovascular benefits of their consumption outweigh the potential risks of alcohol beverage consumption due to a lack of human research.

Other strategies to create functional food products for cardiovascular health include the alteration of the fermentation process. One strategy employed has been the production of a brown beer vinegar, which is a nonalcoholic beer-based product rich in polyphenols that is somewhat akin to apple cider vinegar as it utilizes a second fermentation to produce acetic acid. This product exhibited antioxidant properties that may be beneficial for cardiovascular health (Muduru et al., 2018). Another strategy investigated a beer fermented by kefir (Rodrigues et al., 2016), a complex mix of yeasts and lactic acid bacteria highlighted in Chapter 9. In this study, kefir grown in molasses was used to ferment the malt to produce a novel beer. A group of male Wister rats held in normal conditions was administered 1.5 mL of the novel beer by gavage for 30 days along with other groups who have gavaged a control beer modified with aqueous kefiran, kefir fermented molasses, and a 4% (v/v) solution of ethanol in water. After 7 days some animals were subjected to a carrageenan-induced inflammatory reaction or an ethanol-induced gastric ulcer experiment. While all animals had similar circulating polyphenol levels and serum inflammatory markers, there was a notable beneficial difference in the inflammatory and ulcerogenic responses in the group treated with the kefir beer and the control beer modified with aqueous kefiran (Rodrigues et al., 2016). This study indicates that a novel beer fermented with kefir may be protective against proinflammatory insults, which may also be beneficial for cardiovascular health, which is in line with what has been reported relating to the cardiovascular benefits of kefir fermented dairy products as discussed in Chapter 9.

Innovative product developers in the brewing industry also look to exploit the existing composition of beers. For example, xanthohumol (Fig. 12.5) is a prenylated flavonoid almost exclusively associated with beer and its potential benefits thereof (Elrod, 2018, pp. 19–32). Xanthohumol is derived from the female flowers of the hops plant (*Humulus lupulus* L) and is thought to have potential anti-inflammatory, antineoplastic and chemopreventive properties (Harish et al., 2021; National Center for Biotechnology Information, 2021). Multiple novel products have been brought to market or are under investigation that contains high concentrations of xanthohumol within the products.

One study investigated the production of beers with high xanthohumol content using different varieties of hops (Amarillo, cascade, centennial, and Galaxy hop varieties) (Paszkot et al., 2021). This study showed that hopping and fermentation increased the polyphenol content of the wort, but that maturation slightly decreased these levels, which was also true of the antioxidant capacity although each beer still had greater antioxidant properties than the wort itself. Notably, the beer made with the Galaxy hops also had the highest xanthohumol content and the highest antioxidant capacity. Therefore, altering the brewing process and hops selection may increase the xanthohumol and antioxidant capacity and thus the potential cardiovascular benefits of such beer products. However, xanthohumol doesn't just exert antioxidant properties, it has also been shown to inhibit the cyclooxygenase enzymes that are responsible for the synthesis of proinflammatory eicosanoids and it may inhibit interleukin-1β (Elrod, 2018, pp. 19–32). Furthermore, xanthohumol may exert anti-inflammatory effects via influencing pathways that affect TNF-α and NF-kB (Gupta et al., 2014; Rossi et al., 2014). Xanthohumol is also thought to exert cardiovascular benefits via modulating lipid levels and adipocyte function (Elrod, 2018, pp. 19–32; Liu et al., 2015; Miyata et al., 2015) and exerting antithrombotic effects (Luzak et al., 2017; Xin et al., 2017). Hence why there are companies and researchers looking beyond functional beverages alone and also pursuing the use of xanthohumol and beers for the formulation of nutraceuticals intended for the prevention and/or inflammatory manifestations. Indeed, some groups have specifically focused on xanthohumol e.g., Xanohop Gold, MeridiumXN, and others (Betatec, 2021; NutraceuticalsWorlds). However, others have looked at the entire beer composition and one group has microencapsulated a pale ale craft beer by spray-drying the beer to concentrate and stabilize the phenolic compounds. Their novel nutraceutical was stable, exhibited antioxidant properties, and had acceptable sensory properties (Maia et al., 2020), which may have cardiovascular benefits that the authors suggest may be used for a health claim.

Overall, there is tremendous growth and innovation in the beer and cider sectors that are leading to the production of novel functional foods, beverages, and nutraceuticals that may have cardiovascular benefits. However, more investment and research are required to grow this extremely niche area of research and product development.

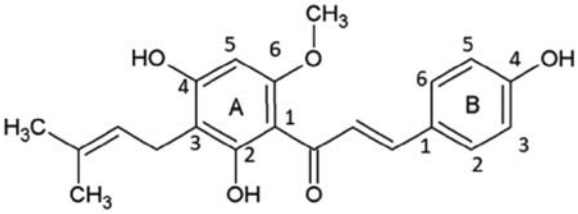

FIGURE 12.5 Structure of xanthohumol. *Reproduced with permission from Harish et al. (2021).*

4. Conclusions

Fermented foods and beverages were among the first processed food products consumed by humans. Their consumption is synonymous in history with everyday life, social events, and their potential health benefits, which is likely why their production methods had not changed much until the advent of the industrial revolution. The production of beverages such as cider and beer nowadays was initially valued because of their improved shelf life, safety, and organoleptic properties. Alcoholic beverages such as apple cider and beer should be treated with respect and consumed in moderation in order to fully reap their health benefits. Over consumption of any ethanol, the product can increase the possibility of diseases and mortality. It is increasingly understood that such fermented beverages can also have enhanced nutritional and functional properties due to the transformation of substrates and formation of bioactive or bioavailable end-products, along with healthy macro- and micronutrients that have been shown to possess long-term beneficial effects. The presence of valuable probiotics may also further enhance the gastrointestinal and anti-inflammatory health benefits of such fermented beverages. Although only a limited number of well-designed clinical studies on fermented beverages have been performed, there is evidence that these foods provide health benefits well beyond the starting food materials. During fermentation, the probiotics responsible for fermentation synthesize vitamins and minerals, modify the current nutrients and produce biologically active phenolics, lipids, and peptides well known for their health benefits, and remove some nonnutrients.

Thus, fermented alcoholic beverages, such as apple cider and beer and their by-products and side-products contain many valuable bioactive compounds, including phenolics, polar lipid bioactives, and probiotics, with potential health benefits, such as antioxidant, antimicrobial, antifungal, anti-inflammatory, antithrombotic, antidiabetic and anti-atherosclerotic functional activities, which can improve health, lower stress levels, lower cholesterol, improve hormone balances, lower the risk of a cardiac event, and a range of other benefits. The design of novel fermented alcoholic beverages, functional beverages or nutraceuticals with improved functional properties and health benefits, by altering the fermentation process and the starter cultures is also another field of modern research on functional foods.

Conflicts of interest

The authors declare no conflict of intertest.

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Chapter 13

Wine bioactive compounds

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1. Introduction

The term wine refers to the natural beverage produced by fermenting the juice of crushed ripe grapes by yeasts. It is an aqueous solution that contains approximately 12%−14% v/v ethanol. It also contains several other chemical compounds including hydrocarbons, glycerol, organic acids, lipids, amino acids, proteins, minerals, esters, ketones, lactones, aldehydes, and phenolic compounds. Red wine differs from other alcoholic beverages due to the presence of several groups of phenolic compounds. However, although moderate wine ingestion is positively correlated with desirable health effects, excessive consumption increases the risk of serious illnesses including liver cirrhosis (Santos-Buelga & González-Manzano, 2011). Several studies have reported a J-shaped relation between alcohol consumption and health, whereby light and moderate drinkers had less mortality risk while abstainers or heavy drinkers were at the highest risk (Di Castelnuovo et al., 2006).

Phenolic compounds are of high technological importance since they are directly related to the sensory and health-promoting properties of wine. It is known that wine phenolic compounds are characterized by a high affinity to proteins. Their binding with mucosal and salivary proteins in the mouth and the resulting precipitation (in the case of proline-rich salivary proteins) are responsible for the organoleptic character of astringency. In addition, their interaction with other biological proteins may account for some of their positive or negative health effects. This chemical property also affects their absorption, metabolism, and bioactivity (Fernandes et al., 2017). Flavonoids, an important group of phenolic compounds, are also strong antioxidants. Their antioxidant activity is responsible for the scavenging of the free radicals *in vitro* and possibly for the prevention of free radical-associated injuries *in vivo*. However, there is a gap between *in vitro* and *in vivo* studies due to the lack of enough epidemiological and *in vivo* human studies concerning flavonoids, digestibility, bioavailability, and interactions (Fernandes et al., 2017).

Red wine, upon ingestion, passes through the oral cavity to the gastrointestinal tract where several metabolic reactions occur with the action of microbiota. Phenolic aglycones may be absorbed by the small intestine; however, most phenolics are in the form of polymers or are combined with other compounds (e.g., glucosides, esters) that can only be absorbed after the action of enzymes or of gut microbiota (Fragopoulou & Antonopoulou, 2020). There is evidence that within the health beneficial compounds of wine the main ones studied are the phenolic compounds and their metabolites, which are produced after consumption (Woo & Kim, 2013). Other polar lipid compounds have also shown promising health outcomes, especially in synergy with wine phenolics, like resveratrol (Fragopoulou & Antonopoulou, 2020).

2. Phenolic compounds from grape to wine

Phenolic compounds are important in wine production because of their sensory characteristics and their influence on wine aging potential. They are considered significant bioactive metabolites involved in the antioxidant, antiinflammatory, antithrombotic, antimicrobial, and antihypertensive activity of wine in human's health after moderate consumption (Apostolidou et al., 2015; Deng et al., 2006; Fernandes et al., 2017; Wang et al., 2006). Primarily synthesized in grapes, they can be extracted in must and wine through winemaking. Chemical interactions during the vinification process, maturation, and storage may alter the initial phenolic profile of grapes, however, a strong relationship between grapes and

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wine composition is apparent and reveals the impact of raw material on the final product. The two large groups of phenolic compounds are flavonoids (flavan-3-ols, anthocyanins, flavonols) and nonflavonoids (hydroxycinnamic acids, hydroxybenzoic acids, stilbenes) (Lingua et al., 2016; Teixeira et al., 2013) (Fig. 13.1).

The most abundant group in grapes and wines are flavan-3-ols, which include monomers such as (+)-catechin, (−)-epicatechin, (+)-gallocatechin, (−)-epigallocatechin, (−)-epicatechin-3-*O*-gallate, and high molecular weight polymers composed by monomers known as proanthocyanidins or tannins. They are located in skins, seeds, and flesh and they are responsible for the astringent and bitter properties of wines as well as for their antioxidant activity (Teixeira et al., 2013; Yilmaz et al., 2015). Another important group, anthocyanins (e.g., delphinidin-3-*O*-monoglucoside, cyanidin-3-*O*-monoglucoside, petunidin-3-*O*-monoglucoside, peonidin-3-*O*-monoglucoside, malvidin-3-*O*-monoglucoside), are located in skins and sometimes in the flesh of red grapes contributing to the color of wines. Although they are considered good antioxidant agents in small fruits (Espín et al., 2000) and their activity has been attributed to their oxonium ion in the C ring (Rice-Evans et al., 1997), their content in grapes has not been correlated or it has been negatively correlated with their antioxidant capacity (Jordão & Correia, 2012; Kyraleou, Koundouras, et al, 2016).

Flavonols, such as quercetin, myricetin, and kaempherol are located in the berry's skin and play an important role in the color (after copigmentation with anthocyanins) and bitterness of wines. They may also act as antioxidants with protective

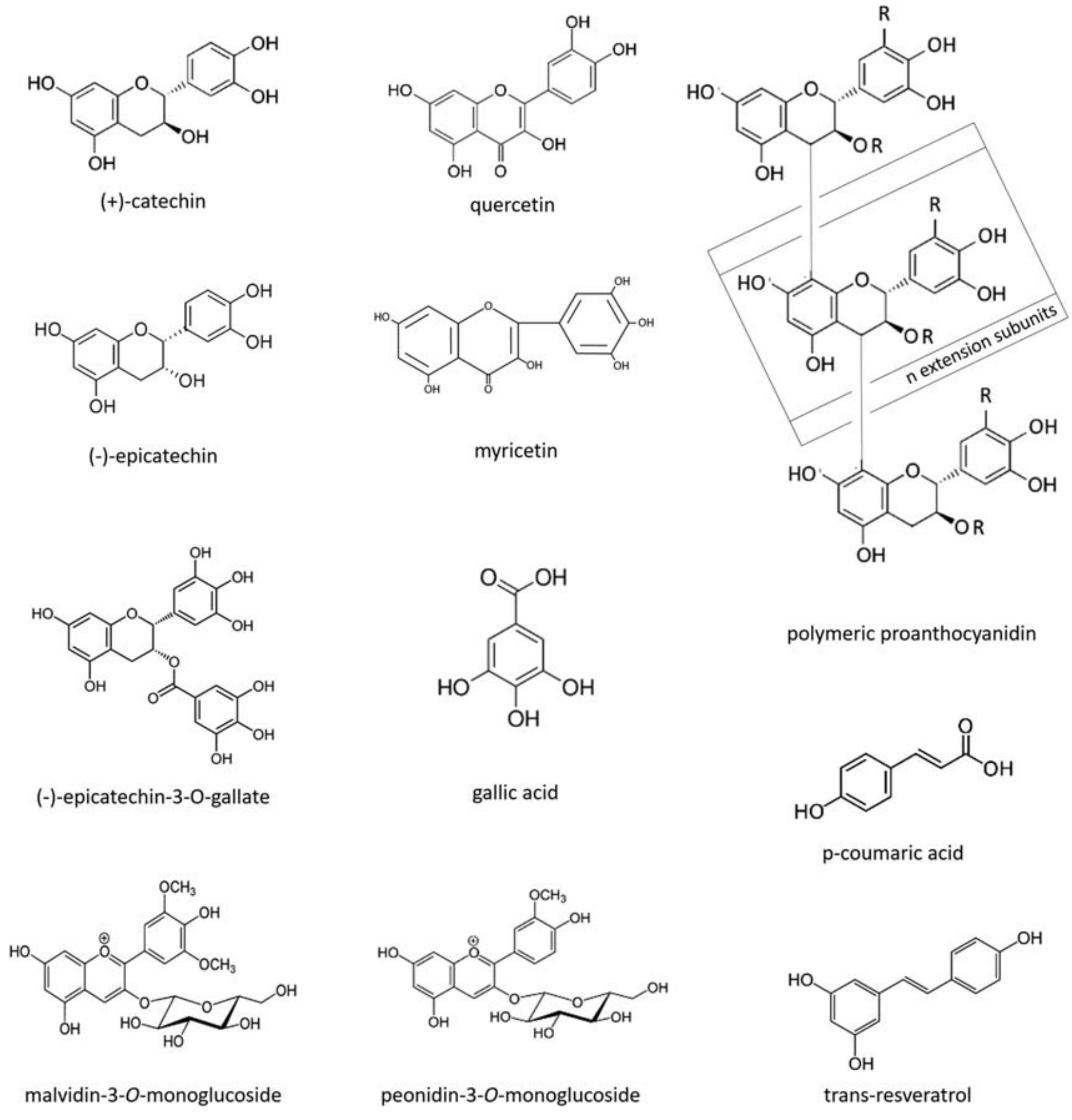

FIGURE 13.1 Phenolic compounds in grapes and wines.

effects on cardiovascular diseases (Perez-Vizcaino & Duarte, 2010). Phenolic acids are divided into hydroxycinnamic acids (p-coumaric acid, caffeic acid, ferulic acid) located in all parts of the berry, and hydroxybenzoic acids (gallic acid, vanillic acid) located mainly in skins and seeds. They contribute to the copigmentation and the esterification reactions that occur during winemaking. There is evidence that phenolic acids have antioxidant properties and they act synergistically with other bioactive compounds elevating their antioxidant activity (Pan et al., 2021). Moreover, stilbenes are increasing during berry maturation and although they are present in low concentrations in wines, there are evidence, particularly for resveratrol, about their protective effects on human's health (Teixeira et al., 2013; Yilmaz et al., 2015). Finally, several studies have shown the strong antithrombotic and antiatherogenic potential of grape skin extracts and wine that are rich in such phenolics (Fragopoulou & Antonopoulou, 2020; Fragopoulou et al., 2004, p. 66).

Wine phenolic content depends on the grapes' phenolic composition, the techniques applied during the vinification process, and the chemical interactions occurred during the maturation and storage of wines (Atanacković et al., 2012; Basalekou et al., 2019; Kallithraka et al., 2015; Kondrashov et al., 2009; Kyraleou, Teissedre, et al, 2016; Lingua et al., 2016).

2.1 Grape phenolic composition

Seeds and skins of grapes are the main sources of phenolic compounds, while the flesh has lower portions, which consist mainly of hydroxycinnamic acids (Teixeira et al., 2013; Yilmaz et al., 2015). Seeds have a greater phenolic content compared to skins or flesh and thus higher antioxidant capacity (Kyraleou et al., 2019; Yilmaz et al., 2015). This could also be associated with the different composition of seeds' proanthocyanidins, including galloylated subunits and low molecular weight flavan-3-ols, which were determined to have antioxidant properties (Plumb et al., 1998; Yilmaz & Toledo, 2004). Yilmaz et al. (2015) observed differences in the peroxyl radical scavenging activity among six individual molecules and determined the higher levels in resveratrol, followed by (+)-catechin, (−)-epicatechin, (+)-gallocatechin, and the lowest levels in gallic acid and ellagic acid.

The phenolic concentration in grapes depends greatly on the grape variety and the environment (geographical location/terroir) but it can be influenced by the climatic conditions (vintage effect) and vineyard management (Downey et al., 2006; Kyraleou et al., 2020; Kyraleou, Kallithraka, et al, 2015; Lingua et al., 2016; Pérez-Magariño & González-San José, 2006).

Differences in phenolic composition can be observed among varieties (Kyraleou et al., 2020; Lingua et al., 2016; Theodorou et al., 2019), for example, grapes of Cabernet Sauvignon and Syrah had higher concentrations of flavonols compared to Merlot, however, Merlot was richer in flavan-3-ols and transresveratrol (Lingua et al., 2016). In general, red grape varieties have a higher content of phenolic compounds compared to white ones while the red vinification process, which includes longer maceration times, favors their almost complete extraction. Vineyard treatments could play an important role in grape phenolic compositions due to the effect on the vine's microclimate and the biosynthesis of phenols in grapes (Downey et al., 2004, 2006). The training system of vines is a way of controlling the temperature and sunlight exposure of the vine's canopy, which in combination are important parameters for biosynthesis and content of phenolic compounds and their extractability from grapes to wine (Downey et al., 2006; Kyraleou, Kallithraka, et al, 2015).

2.2 Vinification process and alcoholic fermentation

The main procedures during vinification include grape processing and alcoholic fermentation of the must, but the techniques applied may differ among the grape varieties, wineries, and the type of wine produced. The latter can also be influenced by the vintage, the grape quality, and the maturity of grapes.

After the harvest, grapes are transferred to the winery in order to complete the vinification process following a number of steps: destemming, crushing, pressing, maceration, and alcoholic fermentation; depending on the type and style of the final product (Fig. 13.2). During destemming and crushing of grapes, phenolic compounds are extracted in low levels from skins, seeds, and stems into the must. At this stage, excessive mechanical damage to grape's parts may increase the extraction of phenolic compounds from stems or seeds and it could lead to undesirable flavors in the wine (Zhang et al., 2020).

2.2.1 Vinification of white grapes

In white vinification extraction techniques, such as cryoextraction, can be applied before pressing the grapes. After fast freezing, whole berries remain for a few hours at low temperatures around zero, depending on the applied technique (Ruiz-Rodríguez et al., 2020). This is considered a "gentle" procedure as it targets skin components while the seeds remain

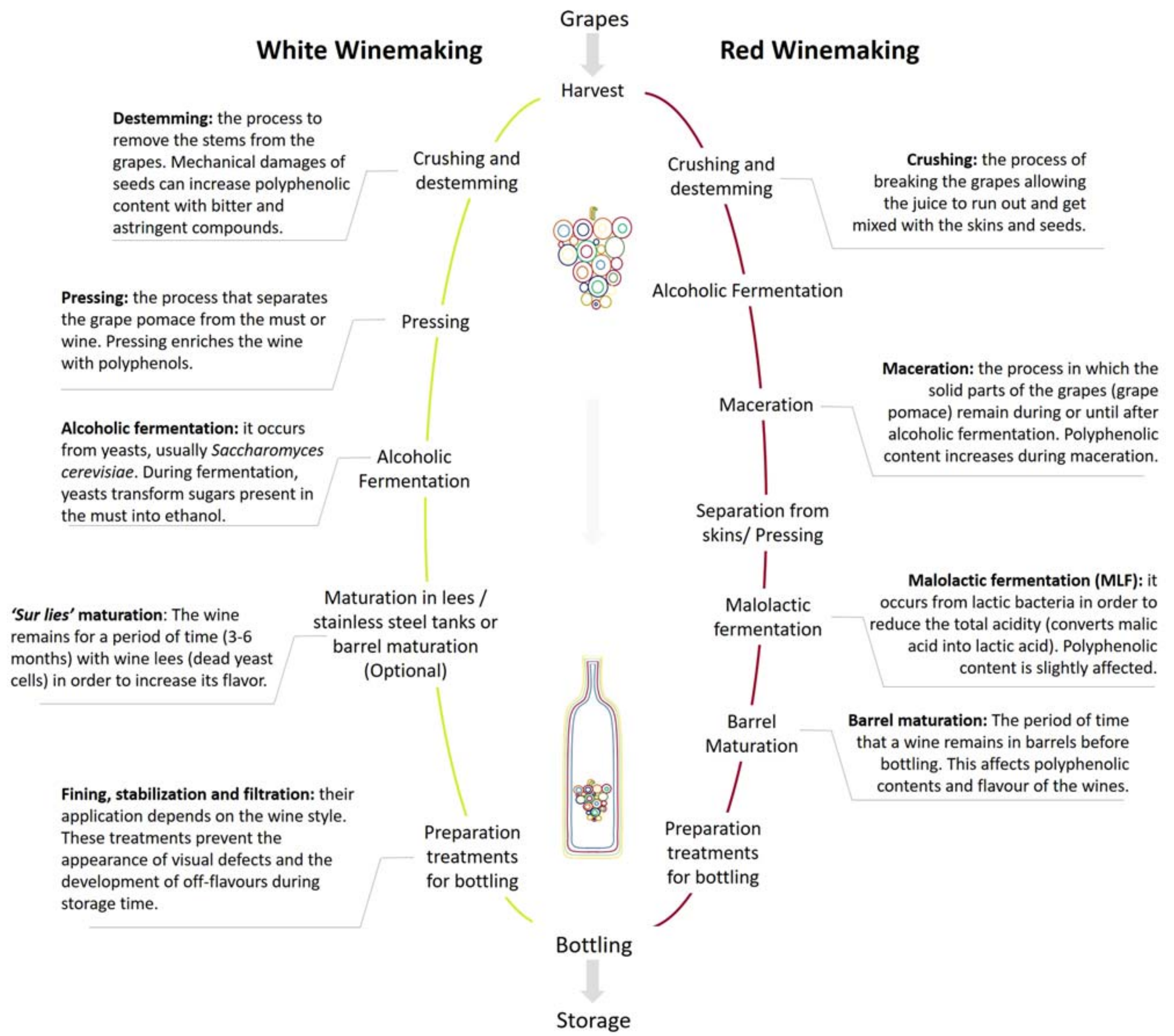

FIGURE 13.2 Main procedures in the white and red winemaking process.

undamaged and less capable to contribute with their astringent and bitter phenols in the must. Then the grapes are pressed and the phenolics, as well as other compounds, are extracted from the different parts of the berries to the must. Intense pressure and for longer time results in musts with an increased phenolic concentration in wines. The high content of phenolic compounds is not considered desirable for fresh white wines since they get oxidized resulting in "brown" color and quality deterioration of the final product. However, their presence is desirable for wines that undergo aging or in the case of different wine styles produced from white grapes. Recently, white wines with extended maceration also known as orange wines were registered in the special wines' category in the International Code of Oenological Practices (2021 Issue) of the International Organization of Vine and Wine (OIV). These wines are produced after long maceration with grape pomace (for at least 1 month) and have a characteristic orange-amber color and a tannic taste. Their phenolic composition, as well as their antioxidant activity, is higher compared to wines produced with the standard white vinification process (Milat et al., 2019).

After pressing, yeasts are added to the must to facilitate alcoholic fermentation. During alcoholic fermentation phenolic content can vary depending on the fermentation conditions, the yeast strain (Rebollo-Romero et al., 2020; Romboli et al., 2015), and the applied treatment or the stage of alcoholic fermentation (Scutarașu et al., 2021). Yeasts, besides being responsible for the completion of alcoholic fermentation, are capable of producing bioactive compounds as secondary metabolites during this stage (hydroxytyrosol and tyrosol). These compounds are characterized by various properties beneficial for health (antioxidant, anticarcinogenic, cardioprotective, and antimicrobial) and their production depends on the yeast stain (Rebollo-Romero et al., 2020; Romboli et al., 2015; Álvarez-Fernández et al., 2018). The concentration of p-coumaric, syringic, gentisic, caftaric, and protocatechuic acids increases with enzymatic treatment in white vinification while the content of other phenolic compounds (ferulic, gallic acids, *trans*- and *cis*-resveratrol) is influenced mainly by the alcoholic fermentation stage and the grape variety (Scutaraşu et al., 2021). In addition to that, enzymes or microorganisms originating from the grape's microflora may play an important role in a number of chemical reactions during alcoholic fermentation that affect the phenolic composition in wines, highlighting the importance of raw material contribution to the composition of the final product (Garde-Cerdán & Ancín-Azpilicueta, 2006; Rebollo-Romero et al., 2020).

2.2.2 Vinification of red grapes

In red vinification, the maceration process that occurs after crashing and during the alcoholic fermentation has a great impact on wine's phenolic composition. During maceration, skins and seeds remain with the must to facilitate the extraction of phenolic compounds from skins and seeds, in order to enrich the wine with color and flavor. The variety's phenolic content, the time of maceration (Del Llaudy et al., 2008), the temperature (Budić-Leto et al., 2003; Vrhovsek et al., 2002) and the intensity and the frequency of "pigeage" (punch down of skins and seeds) during the process (Fourie et al., 2020) have an important impact on the final phenolic composition of must and wines.

The maceration process can last a few days (4−5) and in some cases until the end of alcoholic fermentation. During red alcoholic fermentation, grape's microflora has a higher contribution to the final wine phenolic content compared to standard white vinification due to the maceration process, which promotes additional chemical reactions. Yeasts or other microorganisms originating from the grapes produce metabolites, such as acetaldehyde, which can react with phenolic compounds while yeast cells can absorb anthocyanins resulting in the modification of the phenolic composition and the wine color. Additionally, the yeast's metabolic behavior through its β-glucosidase activity can affect the content of resveratrol (Vrhovsek et al., 1997) and quercetin (Romboli et al., 2015) in red wines.

By lengthening the maturation time, the increased ethanol can cause excessive extraction of compounds from the solid parts of grapes especially those that originated from the seeds. Total phenolics and flavonoids, as well as the concentration of *trans*- and *cis*-resveratrol, are increasing with extended maceration (Fourie et al., 2020; Poklar Ulrih et al., 2020). The total amount of anthocyanins are extracted during the first days of maceration and can slightly increase (Poklar Ulrih et al., 2020) or decrease (Budić-Leto et al., 2003) depending on the duration, the variety's phenolic content or the vinificator used (Poklar Ulrih et al., 2020; Vrhovsek et al., 2002). Higher temperatures at the end of maceration may also increase the total anthocyanins in red wines (Vrhovsek et al., 2002). Total nonflavonoid compounds are extracted on the first days of maceration and remain at the same levels even after a long maceration (Poklar Ulrih et al., 2020). Finally, the separation stage from the seeds and skins after maceration, which is followed by the pressure of the mash, can additionally increase the concentration of phenolic compounds in wines and their antioxidant capacity (Poklar Ulrih et al., 2020).

2.3 Maturation in tanks or barrels

Depending on the style, the wine may undergo a maturation process at the end of fermentation and before the bottling stage. The maturation process takes place in stainless steel tanks, old oak casks, or wooden barrels in order to enhance the flavor or improve the quality of the wine, but it also helps wine's stabilization. At that stage, the wine is racked into the new containers in order to remove the residues of the alcoholic fermentation process defined as grape pomace and wine lees. Grape pomace consists of the grape's parts that remain after the pressing procedure and wine lees are the residues in the bottom of the tank or the barrel, which consists of smaller grape's parts and dead yeast cells. Those fractions contain high levels of phenolic compounds (Antoniolli et al., 2015) because of a partial extraction during maceration or the precipitation of high molecules during clarification techniques and the maturation process. However, a small portion, which is described as "fine lees" still remains during maturation to confer at wine's flavor, however only in the case that high-quality grapes were employed.

2.3.1 Maturation of white wines

White wines have a low content of phenolic compounds and according to the style of the final product they could remain for a few months in contact with wine lees (dead yeast cells), known as *sur lies* maturation, in a stainless still tank or wooden barrel, in order to increase their flavor. Phenolic compounds, especially high molecular weight proanthocyanidins pass through the cell wall and plasma membrane of dead cells to interact with their intracellular components (Mekoue

Nguela et al., 2019). In combination with *bâtonnage* treatments (stirring the lees in the wine), autolysis of yeast cells releases higher portions of phenolic compounds, which increase the complexity of wine flavor (Fornairon-Bonnefond et al., 2002).

The use of wooden barrels instead of stainless steel tanks during maturation confers additional flavor to white wines due to the phenolic compounds extracted from the wood. The phenolic content of wine before maturation also has an impact on the final product. As it was aforementioned, the maceration process increases the concentration of total phenolics in white wines, however, barrel maturation after prolonged maceration can decrease the phenolic content due to chemical reactions (hydrolysis, oxidation), absorption on yeast cells, or formation of macromolecules followed by precipitation. It was observed that for wines after prolonged maceration the content of monomeric flavan-3ols decreased as the maturation time in barrels was increased (Lukić et al., 2015).

2.3.2 Maturation of red wines

On the other hand, red wines, which have a high content of phenolic compounds responsible for the astringent and bitter taste, will have to undergo a maturation process in barrels in order to "soften" their taste. The time of maturation depends on the initial phenolic content and the type of the wine produced and can last from a few months to a few years. After being completed the alcoholic fermentation by yeast, lactic acid bacteria are activated with elevated temperatures in order to decompose malic acid into lactic acid, resulting in lower total acidity of the wine, in a process called malolactic fermentation (MLF). MLF through the β-glucosidase activity of lactic bacteria may reduce the concentrations of anthocyanins and the concentration of tartaric esters of the hydroxycinnamic acids (*trans*-caftaric and *trans*-coutaric acids) along with the rise of their corresponding free forms (*trans*-caffeic and *trans*-p-coumaric acids) linked to lactic acid bacteria metabolism (Hernández et al., 2006). Additionally, MLF has a small impact on resveratrol content (Vrhovsek et al., 1997) but not on monomeric (Hernández et al., 2006), oligomeric and polymeric proanthocyanidins (Vrhovsek et al., 2002).

The maturation process can also alter the phenolic composition (Hernández et al., 2006; Poklar Ulrih et al., 2020; Vrhovsek et al., 2002) in red wines due to interactions between anthocyanins and flavan-3-ols, polymerization of flavan-3-ols or precipitation of high molecules. A high decrease of anthocyanins occurs as a result of the formation of polymeric pigments and adducts with tannins (color stabilization) as well as of monomeric flavan-3-ols, which are polymerized to compose higher molecular weight proanthocyanidins (Budić-Leto et al., 2003; Poklar Ulrih et al., 2020). Therefore, polymeric proanthocyanidins, which are less bitter and astringent compared to low molecular weight flavan-3-ols, are increasing during maturation. Other compounds such as *trans*- and *cis*-resveratrol, and gallic acid are also increasing during maturation (Poklar Ulrih et al., 2020). Despite the great changes during maturation, it was reported that the antioxidant capacity of the wine remained at the same levels as it was at the beginning of the maturation process (Poklar Ulrih et al., 2020). Although the antioxidant activity of a matrix is highly dependent on the type and concentration of phenolic content, it is also the results of synergistic and antagonistic interactions among different phenolic compounds as well as among phenols with other compounds (Lingua et al., 2016). The formation of larger molecules, some of them are precipitating during maturation, with additional extraction of wood components could change the phenolic compounds but might have a smaller impact on the antioxidant activity of the wine.

2.4 Bottling, storage, and aging

The winemaking process (before bottling) also includes treatments such as fining, stabilization, and filtration (depending on the wine style) in order to prevent the appearance of visual defects (oxidation, hazes), the development of off-flavours and to ensure that the wine quality will be maintained during storage time. One of the main targets is to minimize the concentration of phenolic compounds, which are involved directly or indirectly in haze formation, browning development, and astringent or bitter taste. The above treatments are essential for young wines but not for those intended to be matured, as maturation is a natural way of stabilization and clarification and additional treatments should be avoided. In an attempt to prevent the loss of flavor compounds (mainly phenolic compounds), which are extracted during the maturation process and can improve the wine's sensory characteristics during storage time, fewer treatments are applied to those wines.

The winemaking process, packaging type, and storage conditions have a great impact on wine's shelf life and its aging potential. The phenolic content before bottling is a very important parameter affecting the storage period of a wine. Polymerization of flavan-3-ols, copigmentation, and oxidation, may alter, improve or deteriorate, the flavor of a wine. Reactions such as polymerization and copigmentation improve wine's flavor through the reduction of astringent and bitter compounds and stabilize color (shift from bright red to orange-red hues). However, excessive oxidation may lower the quality of the final product. Red wines, without barrel maturation, showed a slight decrease in total phenolic content

(10%–20%) after a few months or a year in the bottle (Budić-Leto et al., 2003; de Souza et al., 2018). However, a longer aging time resulted in a higher reduction of total phenols that might be due to the formation of larger molecules highly expected to precipitate during storage. Mono-varietal wines (without barrel maturation) from Greek red grape varieties, showed a decrease in total phenolic content, from 63.6% to 80.7% after eight years of bottle aging depending on the variety (Kallithraka et al., 2015). Additionally, monomeric anthocyanins diminish during storage time and they may reach up to a reduction of 85% due to their conversion to polymeric pigments or reaction with tannins. However, the usage of amber color bottles may delay these reactions and protect wines' color for a longer time (de Souza et al., 2018).

Storage conditions are additional parameters that influence the wine's phenolic content and the overall quality of the wine. Elevated temperatures may reduce the total phenolic compounds of the wine. However, the influence of temperature on the individual phenolic groups is different (high temperatures may increase total tannins while simultaneously reducing total anthocyanins). High storage temperatures in both red and white wines accelerate the oxidation reactions resulting in a faster aging process, which reduces their shelf-life (Hopfer et al., 2013; Scrimgeour et al., 2015). Based on kinetic models Butzke et al. (2012) suggested that inappropriate storage conditions during shipping could accelerate the aging of a wine by up to 18 months depending on the type and the geographical origin of the wine (Butzke et al., 2012).

In general, during the first year of a wine in the bottle rapid changes occur, which affect mainly its color. In red wines, the red-orange hues increase due to the sharp decrease of monomeric anthocyanins and the formation of new polymeric complexes while in white wines browning hues appear due to the oxidation of flavan-3-ols (monomers and oligomers) and hydroxycinnamates. During storage time, total phenolics, total anthocyanins, stilbenes, and small molecular structure phenols are reduced due to the formation of larger molecules (Budić-Leto et al., 2003; de Souza et al., 2018; Scrimgeour et al., 2015).

3. Winemaking by-products in functional foods

The moderated consumption of wine has been associated with several health benefits (Apostolidou et al., 2015; Fragopoulou & Antonopoulou, 2020; Leri et al., 2020) and it is considered a product with "functional properties" due to its chemical composition. Besides grapes and wines, a high number of by-products derived during vinification are rich in valuable elements such as phenolic compounds, minerals, fibers, and vitamins (García-Lomillo & González-SanJosé, 2017). These can be used to enrich, fortify or create new products with bioactive ingredients in the food sector and have a positive impact on human's health (Teixeira et al., 2014). The concentration and composition of bioactive compounds in winemaking by-products are influenced by the variety, the origin of grapes, the winemaking conditions, and the extraction method (Anastasiadi et al., 2010; García-Lomillo & González-SanJosé, 2017; Khanal et al., 2009). In an attempt to reduce winery wastes through their efficient use as natural resources of health beneficial ingredients (e.g., antioxidants); unripe grapes, shoots, stems, grape pomace, seeds, skins, and other by-products have been evaluated in order to be converted and contribute to value-added products (Bucalossi et al., 2020; Dupas de Matos et al., 2018; Manca et al., 2020).

3.1 Vineyard by-products

Unripe grapes that remain after the application of viticultural techniques are used as acidifying agents in food preparations and sauces (Dupas de Matos et al., 2018; Karapinar & Sengun, 2007). Their phenolic extracts can be used as ingredients for plant-based functional foods preparation to improve their nutritional and antioxidant characteristics (Bucalossi et al., 2020). The juice of unripe grapes has also been proposed as a potential alternative antimicrobial agent at the household level when it is used as a dressing ingredient in vegetables (Karapinar & Sengun, 2007) or as a marinate in meat (Sengun et al., 2020). The antioxidant capacity of functional foods enriched with unripe grapes, their juice, or their extract is influenced by the type of the product and the origin of unripe grapes (variety, maturation stage, environmental factors) (Öncül & Karabiyikli, 2015). Vine leaves, young lateral shoots, and pruning residues have also been investigated and proposed as potential sources of bioactive compounds such as *trans*-resveratrol and other phenolic compounds with a high antioxidant activity that can be used in the food industry (Balík et al., 2008; Fernandes et al., 2013; Jesus et al., 2020). In addition, the use of phenolic extracts from vine pruning residues was suggested for use in functional foods or nutraceuticals due to their anticolorectal cancer potential activity (Jesus et al., 2020).

3.2 Winery by-products

During winemaking, the large portion of stems, grape pomace (skins and seeds), and wine lees represent an economic and environmental problem. A large portion of winery by-products is used for the production of wine/marc spirits or ethanol after distillation, but it can be also used for the production of calcium tartrate, seed oil, and as a source of natural pigments or other phenolic compounds (Braga et al., 2002; Teixeira et al., 2014). The interest in wastes from the wine industry is increasing because of their content of bioactive compounds and their potential use as a source of high-added value antioxidants (Anastasiadi et al., 2010; Balík et al., 2008). Grape pomace, seeds, and skins in different forms (extracts, flour) were proposed to be added as functional ingredients in many products. Some of them are yogurt (Chouchouli et al., 2013; Karnopp et al., 2017; Marchiani et al., 2016; Tseng & Zhao, 2013), salad dressing (Tseng & Zhao, 2013), cookies (Karnopp et al., 2015), biscuits (Pasqualone et al., 2014), sausages (Moradi et al., 2011), fruit candies (Cappa et al., 2015), bakery products and pasta (Iuga & Mironeasa, 2020). Their addition may promote human's health as a source of antioxidant dietary fibers, extend the shelf-life of food products and affect their quality and sensory characteristics (Dabija et al., 2017; García-Lomillo & González-SanJosé, 2017; Karnopp et al., 2017; Marchiani et al., 2016; Tseng & Zhao, 2013). Furthermore, the use of seed and stem extracts has been proposed as potential natural antilisterial mixtures (Anastasiadi et al., 2009).

4. Methods of analysis

Due to the importance of the group of wine phenolic compounds various analytical methods exist for their identification and quantification. Depending on the accuracy of quantification they can be used to provide insight into the average level and quality of phenolic groups or specific concentrations and identities for each phenolic compound. These methods can be applied to grapes before or after maturity, to grape must, and also to wine.

4.1 Spectrophotometric methods

Spectrophotometric methods provide a rough estimation of the total amount of specific phenolic groups in grapes and wines and give some information on their astringency or color intensity.

4.1.1 Estimation of the total phenolic content of wines

The total phenolic concentration of a sample can be estimated through different methods depending on various properties.

The total phenolic index (TPI) or OD 280 value, is one of the most simple and rapid analyses that is performed in order to evaluate the total amount of phenols present in grapes, must, or wine. The basis of this measurement is the phenolic ring's ability to absorb UV light and it can be easily estimated after dilution of the sample with distilled water and a spectrophotometric measurement on a 10 mm optical path at 280 nm. The value can be from 6 to 120 a.u., the higher the value, the higher the number of phenols present in the sample. This method is simple and fast; however, it does not offer precise results and may overestimate the phenolic content due to other compounds that also absorb at 280 nm (Ribéreau-Gayon et al., 2006).

Several colorimetric methods have also been set up for phenolic determination. These methods are based on the reaction of phenols with a chemical reagent and the formation of a colored complex that can be determined spectrophotometrically. However, these methods lack specificity for phenolics since they work better with pure extracts than with complex mixtures such as wine. In addition, the reactions are not specific and other wine compounds could interfere leading to the overestimation of the wine phenolic content (Herderich & Smith, 2005).

One of these methods is the Folin-Ciocalteu. This method is more precise than the TPI and is based on the ability of the phenolic compounds to bound to the Folin-Ciocalteu reagent, a mixture of phosphotungistic and phosphomolybdic acids. This value can be expressed as gallic acid equivalents and it has been highly correlated to the antioxidant capacity measurements in grapes and wines. Other colorimetric assays are the vanillin and the DMACA (4-dimethylamino cinnamaldehyde) methods. However, several parameters such as reagent concentration, reaction time, and temperature as well as the type of acids and solvents may influence the measurements of the former while the latter overestimates the phenolic content in nonpurified samples (Herderich & Smith, 2005). The most widely used is the acid hydrolysis method (LA method), which is based on the ability of proanthocyanidins to partially convert to anthocyanins when heated under acidic conditions. The LA method is easy and reproducible, however, it does not take into account the difference in tannin structures and can also lead to overestimation of the results, especially after barrel or bottle aging (Ribéreau-Gayon et al., 2006).

For this reason and because of their rapid, simple, and robust nature two other precipitation methods, the bovine serum albumin (BSA) and the methylcellulose (MCP) assays are often employed. The BSA tannin assay is based on the ability of proteins to precipitate phenols (Kyraleou, Pappas, et al, 2015) while the MCP method relies on the ability of phenolic compounds to precipitate when after their reaction with a methylcellulose polymer in the presence of ammonium sulfate. The total phenolic content for both methods is estimated as mg (+)-catechin equivalents using a (+)-catechin standard curve (Luis Aleixandre-Tudo & du Toit, 2019). However, both the above methods also lack sensitivity since they do not precipitate the same amount and type of phenolic compounds.

Anthocyanins can be estimated separately from other phenolic compounds due to their importance in the red color of grapes and wines. As anthocyanins can be present in red wine in different forms such as free or combined with phenols, there are different methods to estimate each form. Their total concentration (total anthocyanins, TA) can be assayed based on their ability to change color depending on the pH of the sample, and lose their color by sulfur dioxide (Ribéreau-Gayon & Stonestreest, 1965). Most reliable, especially with wine samples, is the sulfur dioxide bleaching method as the pH-based method is sensitive to the presence of SO_2. Results of both methods can be expressed as absorbance units (A.U.) or malvidin-3-glucoside equivalents (Luis Aleixandre-Tudo & du Toit, 2019; Ribéreau-Gayon et al., 2006). In addition, a variety of parameters such as total anthocyanins, total phenolics, color density, degree of anthocyanin ionization, SO_2 resistant pigments, and wine chemical age (i and ii) can be determined employing the Modified Somers Assay. It is based on the effect of hydrochloric acid, acetaldehyde, and sulfur dioxide addition on anthocyanin structure(Basalekou et al., 2017).

4.1.2 Estimation of the antioxidant activity of wines

Estimation of the antioxidant activity today remains a highly interesting topic as antioxidants are involved in reducing the risk of several diseases, including cardiovascular diseases. Several methods exist for this purpose, differing on the mechanism and reaction conditions, thus determining different classes of antioxidants (Di Lorenzo et al., 2017). Some of these methods focus on the scavenging ability of reactive nitrogen and oxygen species, others focus on the disappearance of a free radical and others use techniques based on the ferric reducing ability of plasma or the abilities of electrogenerated bromine (Muselík et al., 2007). The antioxidant activity in grapes, must and wines can be estimated using the DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox Equivalent Antioxidant Capacity (TEAC), FRAP (ferric-reducing antioxidant power), oxygen radical absorbance capacity (ORAC), 2,2′-azinobis-(3-ethylbenzothiazoline)-6 sulfonic acid (ABTS) and CUPRAC (cupric ion-reducing antioxidant capacity) assays. The most used ones are the ABTS, DPPH, and TEAC assays.

The ABTS assay is based on color change that occurs when the radical cation $ABTS^{\bullet+}$ is reduced to ABTS followed by absorbance measurements at 734 nm (Rivero-Pérez et al., 2008; Roberta et al., 1999). Another free radicals-scavenging activity method is the DPPH assay. An appropriately diluted sample is mixed with DPPH in methanol and the absorbance at 515 nm is measured until the reaction reaches an equilibrium (Villaño et al., 2004). The TEAC method is based on the ability of the antioxidants to scavenge the cation radical, which is generated by the interaction of ABTS with the ferrylmyoglobin radical. The measurement is compared to standard amounts of Trolox (Muselík et al., 2007) and the results can be expressed in mM or μL of trolox per L of wine, using the relevant calibration curve or as % of inhibition between two measurements after a specific time of reaction with the free radicals. For the FRAP assay, a portion of the diluted sample is left to react with the FRAP reagent and the antioxidant activity of the samples is calculated with reference to a standard curve given by a Fe^{2+} solution of known concentrations. Table 13.1 summarizes the antioxidant activity of wines produced by different varieties in relation to their total phenolic content (determined by Folin-Ciocalteu).

4.2 Advanced methods

For a more accurate estimation of the phenolic compounds, sophisticated methods based on liquid or gas chromatography coupled with or without mass spectrometry and recently also spectrophotometry may be used. The most suitable method due to its accuracy, cost-effectiveness, and relevant simplicity has proven to be high-pressure liquid chromatography (HPLC), which is now used for the determination of phenolic compounds (Luis Aleixandre-Tudo & du Toit, 2019). Most HPLC methods require a sample pretreatment step for purification reasons, however, some analyses can be performed by direct injection of the samples, while detection can be easily made with a photodiode array detector provided that the compounds be analyzed absorb in the UV-vis region, e.g., anthocyanins, flavonols. Alternatively, a fluorescent detector may be used (Gómez-Alonso et al., 2007).

Despite its robustness, and its ability to separate a large number of phenolic compounds, HPLC lacks the ability to separate polymeric phenols, pigments, and generally larger molecular structures (Luis Aleixandre-Tudo & du Toit, 2019). This problem may be overcome with the use of mass spectrometry, although this type of instrumentation is highly sophisticated and cannot be used without considerable cost. Recently, fourier transform infrared spectroscopy (FT-IR) has emerged as a promising method for fast and efficient routine analysis of phenolic compounds. It has proven to be

TABLE 13.1 Phenolic content and antioxidant capacity of red and white wines.

Grape variety	Wines/origin	Total phenolics[a]	Antioxidant capacity of wines[b]			References
			TEAC	FRAP	DPPH	
Tinto Fino	Fresh red wines/ Spain	1457–1604 mg/L	–	–	–	Pérez-Magariño et al. (2006)
Cabernet Sauvignon	Fresh red wines/ Spain	1951–2232 mg/L	–	–	–	Pérez-Magariño et al. (2006)
Cabernet Sauvignon	Commercial samples (2–3 year old)	1453–2912 mg/L	7.7–16.6 mmol Trolox/L	7.0–15.2 mmol Trolox/L	–	(Kondrashov et al., 2009)
Merlot	Commercial samples (2–3 year old)	1447–2100 mg/L	7.5–11.2 mmol Trolox/L	6.9–9.7 mmol Trolox/L	–	(Kondrashov et al., 2009)
Merlot	Fresh red wines/ Serbia	999–1208 mg/L	–	–	1.04–1.34 μL/mL	Atanackovica et al. (2012)
Cabernet Sauvignon	Fresh red wines/ Serbia	911–1410 mg/L	–	–	0.68–1.43 μL/mL	Atanackovica et al. (2012)
Pinot noir	Fresh red wines/ Serbia	694–1196 mg/L	–	–	0.58–1.86 μL/mL	Atanackovica et al. (2012)
Prokupac	Fresh red wines/ Serbia	544–1159 mg/L	–	–	0.79–2.71 μL/mL	Atanackovica et al. (2012)
Xinomavro	Fresh red wines/ Greece	1820–2380 mg/L	–	–	1.56–1.62 mmol Trolox/L	Kyraleou, Pappas, et al. (2015)
Blaufränkisch	Fresh red wines/ Slovenia	1622 mg/L	–	–	13.53 mmol $DPPH_2$/L	Poklar Ulrih et al. (2020)
Malvazija istarska	White wine (maceration + barrel maturation)	476–800 mg/L	–	–	4.0–5.8 mmol Trolox/L	Lukić et al. (2015)
Malvazija istarska	White wine (standard vinification + bottle maturation)	285–436 mg/L	–	–	0.9–2.2 mmol Trolox/L	(Lukić et al., 2015)
Malvazija istarska	White wine (standard vinification)	220–390 mg/L	–	–	0.6–1.2 mmol Trolox/L	(Lukić et al., 2015)
Dingač, Babić, Cabernet Sauvignon, Faros, Faros barique, Merlot	1–4 year-old red wines (average)/ Croatia	2193–3183 mg/L	–	22.159–32.280 mmol Fe^{2+}/L	54.6%–82.6%	Katalinić et al. (2004)
Maraština, Pošip, Traminac, Graševina	1–2 year-old white wines (average)/ Croatia	292–402 mg/L	–	2.213–3.856 mmol Fe^{2+}/L	10.3%–16.16%	Katalinić et al. (2004)
Monastrell	Fresh red wines/Spain	496.3–558.3 mg/L	–	–	6.02–6.78 mM Trolox/mL	Mulero et al. (2010)

TABLE 13.1 Phenolic content and antioxidant capacity of red and white wines.—cont'd

Grape variety	Wines/origin	Total phenolics[a]	Antioxidant capacity of wines[b]			References
			TEAC	FRAP	DPPH	
Greek red grape varieties (e.g., Limniona, Mavrotragano, Thrapsa etc)	8 years old red wines/Greece	271−798 mg/L	−	−	0.21−1.31 mmol Trolox/L[c]	Kallithraka et al. (2015)

[a] *Folin-Ciocalteu method: expressed in mg/L gallic acid equivalent.*
[b] *TEAC, trolox equivalent antioxidant capacity; FRAP, ferric reducing/antioxidant power; DPPH, a,a-diphenyl-b-picrylhydrazyl.*
[c] *non published data.*

successful in the determination of grape phenolics extractability, total phenolic and flavonoid concentration, color parameters as well as antioxidant capacity (Garcia-Hernandez et al., 2020; Nogales-Bueno et al., 2017; Silva et al., 2014). Moreover, recent research highlighted its potential for the prediction of the degree of polymerization of tannins, and their characterization in terms of tannin chain composition, a method originally performed with the use of HPLC and a number of various and laborious pretreatment steps (Basalekou et al., 2019). Representative FT-IR spectra of wine samples are given in Fig. 13.3. Thus, the use of FT-IR is promising for many analyses regarding the group of phenolics, given its simplicity and minimum requirements for sample pretreatment, sample volume, and solvent use.

5. Health benefits of wine bioactive compounds

In developed countries, life expectancy is progressively increasing. However, this life extension is followed by a higher prevalence of numerous age-connected and lifestyle-associated diseases such as diabetes mellitus, cancer, cardiovascular and amyloid diseases, in particular, Parkinson's and Alzheimer's diseases. It is not surprising that the research has progressively focused on "prevention" rather than on "cure". In line with this, the recommendations to the population have

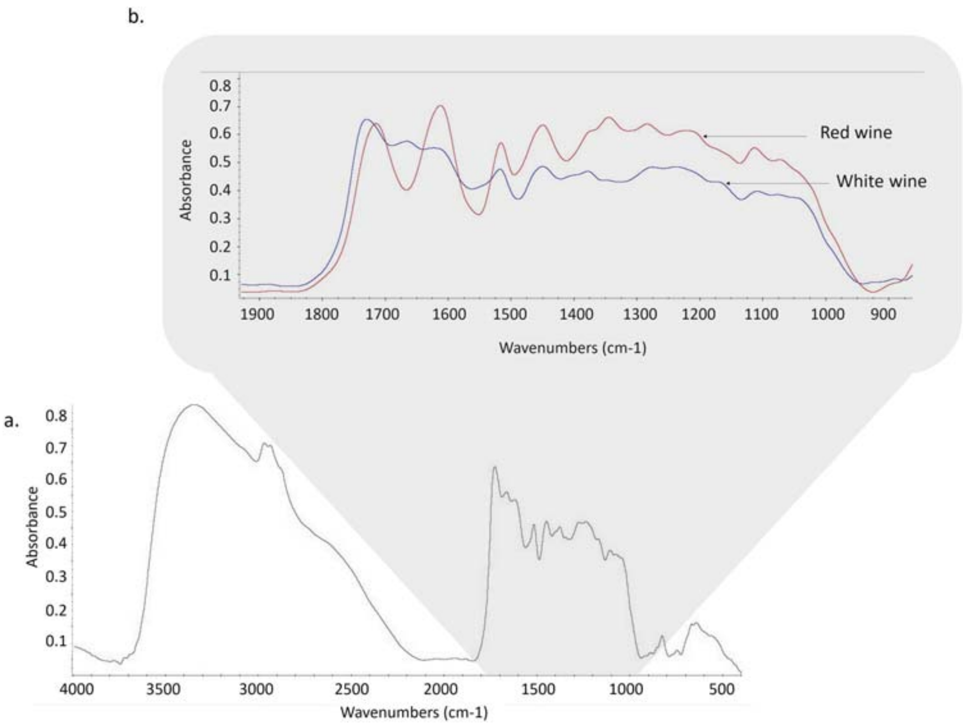

FIGURE 13.3 (A) Typical FT-IR spectra obtained from wine sample. (B) Red and white wine spectra on the fingerprint region.

shifted from "drugs" to a healthier way of living, including exercise both mental and physical, lower calorie intake, and a more appropriate and healthy diet (Leri et al., 2020).

The health benefits arising from the consumption of wine have attracted the interest of the scientific community; indeed numerous clinical studies have revealed that several health benefits of moderate wine consumption can be attributed to the chemical composition of wine and in particular to its phenolic content (Apostolidou et al., 2015; Fragopoulou & Antonopoulou, 2020; Leri et al., 2020). These compounds affect protein homeostasis through different signaling pathways, regulate metabolism, repair DNA, and exert antioxidant activity (Williams et al., 2004). Some wine-derived polar lipid extracts, also rich in phenolics, have also shown great potential against inflammation and thrombosis too, through a synergy of their glycolipids with the phenolics present in these extracts (Fragopoulou & Antonopoulou, 2020; Fragopoulou et al., 2004, p. 66).

Phenolic compounds are a diverse group of compounds sharing common structural characteristics, which are responsible for their similar biological activity in tissues and cells. These effects are mainly due to the alteration of important functions of cells including proteostasis, signaling, homeostasis (energetic, metabolic, and redox) as well as reduction of oxidative stress and control of gene expression (Leri et al., 2020). However, due to the differences in a high number of individual parameters (age, gender, metabolism, presence of microbiota) and variations in wine chemical composition, the conclusions drawn from studies where wine consumption is related to a specific health issue should be cautious. In addition, most of the *in vitro* studies evaluated the protective effects of pure phenolic compounds in extracts where they were present in much higher concentrations than the ones commonly found in wine (Fernandes et al., 2017).

Recent research has provided knowledge on the beneficial effects and the importance of (bio)phenols in the human diet, for maintaining a healthy status. In addition to that, their consumption is recommended as a significant parameter for prevention and even in some cases therapy of numerous diseases. Their action is mostly due to multi-target effects (including the repair of affected homeostatic systems) of these compounds than to a single effect on a specific pathogenesis step. The most studied wine phenolic compounds for their healthy properties are resveratrol (3,5,4′-trihydroxy-*trans*-stilbene), quercetin, myricetin, rutin, kaempferol, malvidin, ferulic acid, and caffeic acid, which are originated from the grapes or are formed during alcoholic fermentation, as well as ellagic acid, which is found in barrel-aged wines (Bravo, 1998).

The most studied biochemical property of wine phenolic compounds is their antioxidant activity and it is due to their ability to bind free radicals inhibiting their generation and reducing thus the free-radical-related injury and the diseases attributed to the oxidized compounds (Rice-Evans et al., 1996). This protective antioxidant effect involves scavenging of various reactive oxygen, nitrogen, and chlorine species, alterations of oxidative pathways, binding with proteins and

TABLE 13.2 Health benefits associated with moderate wine consumption and their phenolic content.

Prevention of diseases	References
Cancer	Kopustinskiene et al. (2020)
Cardiovascular	Fragopoulou and Antonopoulou (2020)
Metabolic syndrome	Leri et al. (2020)
Type-2 diabetes	Xia et al. (2017)
Parkinson's	Fernandes et al. (2017)
Alzheimer's	Ho et al. (2009)
Obesity	Ali et al. (2014)
Biological action	**References**
Antioxidant	Rice-Evans et al. (1996)
Anti-ageing	Visioli et al. (2020)
Antithrombotic	Fragopoulou and Antonopoulou (2020)
Anti-inflammatory	Calixto et al. (2004)
Antimicrobial	Droebner et al. (2007)
Antihypertensive	Perez-Vizcaino and Duarte (2010)
Anti-allergic	Singh et al. (2011)
Phytoestrogenic	Kallithraka (2005, pp. 265−290)

enzymes direct action on signaling paths and several receptors, and interference with epigenetic modulations of chromatin (Ayissi et al., 2014; Cecchini et al., 2013; Halliwell, 2001).

However, wine phenols beyond their antioxidant function may act in various possible ways (Table 13.2) including their interaction (and thus inhibition) with biological proteins such as enzymes, receptors, and even transcription molecules. Their diverse chemical structure may involve them in plenty of physiologic processes, which may have a positive impact on several metabolic and neurodegenerative diseases as well as playing a hormonal role as phytoestrogens (Kallithraka, 2005, pp. 265−290). The biological actions that have been ascribed to these compounds include, among others, promotion of beneficial gut microbiota as well as effects against the formation of amyloid protein aggregates, antimicrobial, anti-hypertensive, inti-inflammatory, antiallergic as well as hypoglycemic, and vasodilator (Calixto et al., 2004; Fernandes et al., 2017; Rigacci & Stefani, 2014).

The beneficial effects of diet phenolic compounds are weakened by their reduced bioavailability and their low intestinal adsorption—digestibility (Leri et al., 2020). Additionally, gut microbiota may modify their chemical structure and bio-transform them (Curti et al., 2019). The beneficial molecular forms of these compounds are probably not only the ingested ones but also their metabolites, which are formed *in vivo* by the intestinal microbiota (Fernandes et al., 2017). However, although their bioavailability is lower in comparison with drugs, when consumed on a regular basis and in abundant quantities they may exert significant mid- or long-term beneficial physiological effects (Visioli et al., 2020). They can also be used as drug ingredients developed to face chronic inflammatory conditions, thrombosis, atherosclerosis, cancer (in combination with anti-cancer drugs) and to reduce the risk of aging-related diseases such as neurodegeneration (Rigacci & Stefani, 2014).

The ingestion of wine in moderation is more beneficial than the ingestion of any other alcoholic drink. However, although wine differs from the rest of alcoholic beverages due to its antioxidant activity and phenolic content, its alcohol content should not be overlooked. All wine health protective effects are related to low to moderate consumption. Heavy alcohol consumption on a daily basis is a high risk for death and disability and the population should not be motivated to start a drinking habit. Excessive alcohol ingestion is positively related to cirrhosis risk and several types of cancers, mainly those that concern the digestive and respiratory tract (Fernandes et al., 2017). There a consistent evidence suggesting a J-shaped relation between alcohol and health (Di Castelnuovo et al., 2006). In this respect, only moderate wine consumption may have a protective health effect compared to complete alcohol abstention and heavy drinking.

Some of the health beneficial effects attributed to the consumption of wine will be discussed below and especially those related to cardiovascular diseases, cancer, diabetes, and neurological disease prevention.

5.1 Cardioprotective effects

Renaud and de Lorgeril (1992) based on epidemiological studies, observed that the mortality due to cardiovascular diseases in the population of France was low despite their diet being rich in saturated fats. This "paradox" was called the "French Paradox" and the authors suggested that the higher wine consumption in France compared with that in most of the rest of Western countries conferred protection from cardiovascular diseases. In fact, according to the results of epidemiological studies, low to moderate consumption of wine is related to 20%−30% less mortality risk and in particular with lower risk of cardiovascular mortality (Fernandes et al., 2017). However, the ingestion of wine ethanol may be accompanied by both harmful and beneficial consequences, depending on the quantity ingested and consumer differences. Moreover, several of the positive effects of wine consumption, regarding CVD prevention, have been partly attributed to its ethanol content. However, there is evidence that the protective effects are not exclusively due to ethanol. Alcohol-free wine exhibited antioxidant and superoxide scavenging properties, which may prevent LDL oxidation and protect endothelial cells against lipid peroxidation (Pal et al., 2004).

This health-promoting effect of wine phenolic compounds was initially attributed to their function as antioxidants, but today it has been suggested that besides their action against oxidative stress they also exert antithrombotic and antiinflammatory effects (Fragopoulou & Antonopoulou, 2020; Fragopoulou et al., 2004, p. 66). Inflammation and platelet coagulation are mainly responsible for atherosclerosis, the major pathogenic process of cardiovascular diseases (CVD).

Atherosclerosis is a process that involves several steps: endothelial dysfunction, low-density lipoprotein (LDL) infiltration, and leukocyte circulation into endothelium, oxidation of LDL, macrophages derived from monocytes, foam cell generation, migration and proliferation of muscle cells, platelet assimilation and as a consequence formation of thrombus. Primary (platelet coagulation) and secondary (aggregation and fibrinolysis) hemostasis participate in this mechanism (Fragopoulou & Antonopoulou, 2020). A phospholipid inflammatory mediator (platelet-activating factor, PAF) and oxidized phospholipids are considered to be responsible for the initiation and prolongation of the atherosclerotic lesion. PAF plays a crucial role in the dysfunction of the endothelium and in platelet aggregation (Choleva et al., 2019;

Demopoulos et al., 2003). Wine bioactive compounds possess cardioprotective effects since they could either have a direct effect on the individual steps of atherosclerosis or modulate the metabolism of glucose and lipids (Xanthopoulou et al., 2017). In more detail, the most well-studied protective effects of wine phenols concern: the function of the endothelium, the reduction of oxidation, the decrease of LDL and reduction of its oxidation, the lowering of blood pressure, the reduction of inflammation and inhibition of platelet activation and aggregation (Apostolidou et al., 2015; Fragopoulou & Antonopoulou, 2020; Fragopoulou et al., 2004, p. 66).

Inflammation is considered a factor that plays a crucial role in the atherogenic process during the onset and development of CVD and of other chronic disorders (Gonçalves et al., 2010; Tsoupras et al., 2018). Several studies (*in vitro*) suggest that wine's phenolic compounds could alter the pathways that eventually lead to inflammation, toward homeostatic levels. It has been shown that these compounds may affect enzymatic activity and are able to inhibit both the expression and the release of cytokines (which are considered proinflammatory compounds) by modifying the transcription factors. Extracted wine phenolics have been reported to directly affect the activity of several enzymes involved in inflammatory processes. Wine phenolics and especially resveratrol, exert an inhibition effect on the proinflammatory (5-lipoxygenase, cyclooxygenase-1) and on PAF biosynthesis enzymes (lyso-PAF-acetyltransferase, PAF-CPT), decreasing circulation of eicosanoids and suppressing PAF synthesis respectively (Tsoupras et al., 2007; Xanthopoulou et al., 2010, 2014). Proinflammatory enzymes metabolize free arachidonic to prostanoids and leucotrienes, which act as proinflammation compounds and initiate in this way the process of inflammation. Magrone and Jirillo (2010) observed that wine phenolic compounds could decrease the secretion of cytokines by reducing the action of a transcription factor (nuclear factor kappa of activated B cells). This factor is involved in the gene transcription of several inflammatory compounds including cytokines. Moreover, extracts from red wine have been shown to have an inhibitory effect on the expression of adhesion molecules and on monocyte chemoattractant protein-1 and macrophage colony-stimulating factor (Calabriso et al., 2016).

Homeostasis consists of an aggregation of platelets (primary), coagulation (secondary), and fibrinolysis (tertiary). This reaction is initiated when a risk (biological, physical, or chemical) exceeds the cellular capacity of homeostasis. The disorganization of the homeostatic system leads to harmful modifications of cellular functions including the fluidity of membranes, and the tertiary structure of proteins including folding and bioenergetics (Naviaux, 2019).

Platelets are key blood elements responsible for the initiation of thrombosis. It has been shown that wine phenolic compounds may significantly modify the ADP-induced aggregation of platelets (Fragopoulou & Antonopoulou, 2020). This result was observed when both red and white grape varieties were studied indicating that grape phenolic compounds are mainly responsible for the biological activity independently from the grape color (Fragopoulou & Antonopoulou, 2020; Fragopoulou et al., 2004, p. 66). Moreover, grape phenols extracted from seeds were shown to reduce platelet aggregation exerting thus a protective effect against atherosclerosis (Ruf et al., 1995).

Nitride oxide (NO) is secreted by the extracellular activation of kinase and kinase *P*38. NO could inhibit the aggregation of platelets and in this way offer protection against atherogenesis through the reduction of the release of monocytes and LDL from the endothelial cells into the arteries walls (Rezaee-Zavareh et al., 2016). Additionally, NO generation is able to reduce the infection rates (such as those attributed to *Chlamydia pneumoniae*), which are positively related to atherosclerosis. *In vitro* studies have shown that wine phenolic compounds could influence the biosynthesis and the secretion of NO by the vascular endothelium (Soleas et al., 1997). Various wine samples were monitored for modulation of the formation of NO in monocytes (Magrone & Jirillo, 2010) and for their effect on platelet activity and thrombosis (Xanthopoulou et al., 2017). In case this vasorelaxant effect of phenols can also be observed *in vivo*, they would be able to help in the maintenance of the patency of the coronary artery and enhance antithrombotic defenses attributed to anti-aggregatory activity of NO.

The exact mechanism by which phenolic compounds inhibit platelet activity is not fully elucidated up to now but it seems possible that these compounds modify the fluidity of the membranes, the affinity of the lignan-receptor, and the signaling pathways within the cells. These effects are possibly mediated by antioxidant mechanisms and modulation of pathways that are related to NO production and release (Chen et al., 2017).

Phenolic compounds have also been found to have an inhibitory effect on pancreatic lipase, a digestive enzyme, and thus influence lipid metabolism and development of CVD. Lipase plays a crucial role in triglyceride hydrolyzation and by inhibiting the activity of this enzyme, grape seed, and oligomneric phenols could limit their efficient digestion and absorption. Cholesterol esterase involved in the duodenal lipid digestion can also be inhibited by phenolic compounds found in grapes and wines as well as other enzymes involved in lipid biosynthesis (Adisakwattana et al., 2010).

Moreover, wine phenolic compounds are well known for their antioxidant and radical scavenger activity. Oxidized LDL may be a key factor in the initiation and progress of diseases, related to oxidative stress, such as atherosclerosis (Castaldo et al., 2019). The inverse relation between CDV incidence and consumption of red wine may be explained by the antioxidant activity exerted by wine phenolics. These beneficial effects include the decrease in oxidized LDL plasma levels and seem to be independent of wine alcohol content (Prasad, 2012).

5.2 Cancer chemoprotective effects

There is scientific evidence that wine phenolic compounds exhibit anticancer action, however, the responsible mechanisms for this activity are not fully understood. Cancer appearance involves abnormal proliferation and the impaired cycle of cells, which are responsible for the growth of uncontrolled cells which are able to metastasize to various organs of the human body (Kopustinskiene et al., 2020). The causes of cancer may be both internal (genetic mutations, inhibition of apoptotic function, oxidative stress, hypoxia) and external (pollution, smoking, exposure to irradiation, ultraviolet rays, and stress) (Blackadar, 2016).

Phenolic compounds may exert various anticancer effects. In more detail, they modulate the activities of the enzymes that scavenge the reactive oxygen species (ROS), induce autophagy and apoptosis, participate in cell-life cycle and lower the proliferation of cancer cells and their invasiveness (Kopustinskiene et al., 2020). Indeed, several studies have shed light on the beneficial role of wine phenolics as cancer protectors, while the antiinflammatory and antiplatelet effects of wine phenolics against the PAF pathways and metabolism seem also to be associated with their potential antimetastatic properties against tumor progression too (Tsoupras et al., 2012). Both *in vivo* and *in vitro* trials reported that wine may exert an inhibitory action on cell proliferation from different tissues such as gastrointestinal, lung, prostate, and oral (Bassig et al., 2012; Song et al., 2015; Yu et al., 2016).

ROS are the byproducts of oxidative phosphorylation in the cells and are generated in mitochondria (Murphy, 2009). They are responsible for the generation of free radicals, which are closely related to oxidative stress and are involved in the appearance of inflammation resulting in various degenerative diseases including cancer. The anticancer activity of the phenolic compounds is based on both antioxidant and prooxidant mechanisms. They can act either directly scavenge ROS and chelate metal ions or indirectly activate antioxidant enzymes, suppress prooxidant enzymes, and enhance the production of enzymes with detoxification action (Youn et al., 2006) In addition, phenolic compounds could target apoptotic signaling factors (activate antiapoptotic proteins while suppressing proapoptotic proteins and caspases) stimulating thus the mechanisms of cell death (Kopustinskiene et al., 2020).

The metabolism of tumor cells is altered by abnormal mitochondrial functions. In more detail, in tumor cells, mitochondria are hyperpolarized, and this property offers them resistance to cell death signaling (Fantin et al., 2006). Most flavonoid phenolic compounds behave as acids (by releasing a proton in basic environments) with pKa values in the range of 6−9. Due to this property, they are able to release in the basic environment of mitochondria (pH 7.8) a hydrogen cation. This chemical function is considered very important in cancer prevention since it protects cells from oxidative stress (Dorta et al., 2005).

5.3 Protection against diabetes mellitus

Diabetes is a group of metabolic diseases where the level of sugar in the blood is higher than the optimum. It is known that α-glucosidase, an enzyme that is responsible for the liberation of glucose from starch and disaccharides, and α-amylase, which hydrolyses polymeric carbohydrates, both play an important role in controlling insulin secretion by lowering glucose release rate, reducing its absorption and suppressing postprandial hyperglycemia (Fernandes et al., 2017). These enzymes are the main enzymes that affect digestion of edible carbohydrates and they are secreted both in the oral cavity and in the small intestine (by the pancreas). Several *in vitro* studies have shown that wine phenolic compounds inhibit the enzymatic activities of both the above-mentioned enzymes (Hiyoshi et al., 2019; Xia et al., 2017).

Moreover, phenolic compounds may have a significant effect on glucose metabolism since they influence several other parameters such as the stimulation of pancreatic β-cells to secret insulin, the reduction of hepatic glucose secretion, the activation of insulin receptors, and the modulation of glucose uptake. Phenolic compounds were able to reduce glucose absorption in the intestine by inhibiting the sodium-dependent glucose transporters, stimulating thus the secretion of insulin, and reducing the glucose output of the liver (Cermak et al., 2004).

Phenolic compounds could also increase the activity of 5′-adenosine monophosphate-activated protein kinase (AMPK), an enzyme that regulates glucose transport and fatty acid oxidation, controlling thus metabolism and reducing the risk of obesity, a condition that correlates positively with diabetes mellitus (Leri et al., 2020). Several studies have demonstrated that phenolic compounds by activating AMPK, suppress liver gluconeogenesis, induce hepatic fatty acid β-oxidation and stimulate glucose transporters in muscle and adipose tissues, resulting thus in the reduction of blood glucose and lipids in the liver and in improving glucose sensitivity (Ali et al., 2014; Rupasinghe et al., 2016).

5.4 Protection against neurological diseases

In addition to cardiovascular and cerebrovascular benefits, moderate alcohol drinking improves social life and other cultural habits that maintain cognitive functions in the elderly. Moderate drinking in elderly people was associated with a lower risk of dementia compared with nondrinking (Deng et al., 2006).

However, besides alcohol, wine contains phenolic compounds, which may also have a protective effect on Alzheimer's disease as was shown in a study where moderate consumption of Cabernet Sauvignon in mice reduced the formation of Aβ peptides and improved spatial memory (Wang et al., 2006). Muscadine wines were also effective in attenuating Aβ aggregation (Ho et al., 2009). Gallic acid and catechin-rich grape seed extracts were able to inhibit cognitive deterioration by reducing the levels of soluble Aβ oligomers in a study contacted by Ono et al. (2008). In addition, other studies revealed that the risk of Alzheimer's disease and of the progress of cognitive decline was much lower in a population of middle age subjects who drank less than three servings of wine per day (but not any other alcoholic drink such as beer) compared with nondrinkers (Luchsinger et al., 2004; Nooyens et al., 2014). Moreover, mild and moderate wine consumption in elderly people (aged over 65 years), showed a negative association with Alzheimer's disease, and this effect was not attributed to other known predictors of dementia such as social, psychological, medical, or familial (Orgogozo et al., 1997).

There is growing evidence of the positive role of dietary phenols to improve cognitive performance and reduce the neuroinflammatory and oxidative stress responses, which occur as a consequence of age and neurodegenerative pathologies. Several mechanisms were proposed for this neuroprotective action of phenolic compounds. It has been suggested that these compounds inhibit amyloid aggregation and fibrillization either by chelating the enzymes involved or by formatting nontoxic complexes reducing thus the Aβ plaque pathology. Inhibition of β- and/or activation of a-secretase might be also involved in their mechanism of action (Vauzour, 2012).

Inflammation is considered an important parameter that contributes to neuronal damage where the formation of both ROS and reactive nitrogen species (RNS) are implicated. Phenolic compounds may protect hippocampal cells from oxidative damage by three mechanisms: increasing intracellular glutathione content (an antioxidant compound), decreasing ROS contents, and inhibiting the Ca^{2+} influx (Ishige et al., 2001). In addition, phenolic compounds may result in improving memory and reducing depression in humans through their circulation in the peripheral and cerebral blood flow (Krikorian et al., 2010). Phenolic compounds and their metabolites may localize in the brain and exhibit both neuroprotective and neuromodulatory effects.

5.5 Other effects related to human health

Phenolic compounds have also been reported to exert antiallergic properties. This effect is due to their interaction with the allergenic proteins, which results in the formation of insoluble complexes (Singh et al., 2011). In this way, they may modulate the allergic reaction by having a direct effect on proteins that are responsible for the allergic sensitization and rendering them hypoallergenic. The protein-bind ability of phenolic compounds is also responsible for their antimicrobial action against bacteria and viruses, which offers protection from various infectious diseases. They could form complexes, both soluble and insoluble with a variety of proteins including enzymes preventing inhibiting thus the multiplication and metabolism of the microorganisms (Droebner et al., 2007).

In addition, wine phenolic compounds and in particular flavonoids have been demonstrated to exert an estrogenic effect due to their structural similarity with estrogens (steroid hormones) (Kallithraka, 2005, pp. 265−290). These compounds (which are called phytoestrogens) are able to elicit estrogen-like actions in tissues by mimicking the animal steroid hormones. In this way, they may initiate estrogen-like effects or act as antagonists to inhibit estrogenic activity (Cassidy, 1999). Among others, they may modulate hormonal secretion by inhibiting the activity of steroid enzymes, stimulating the formation of sex hormones in the liver, and interfering with the secretion of gonadotropins (Kurzer & Xu, 1997). It has been shown that wine flavonoids can be bound to the receptors of estrogens and act as antiestrogens by completing with the endogenous estrogens and suppressing their biosynthesis in cells (Eng et al., 2001). Estrogens have a crucial role in breast cancer development and thus the inhibition of their synthesis may modulate the progression of the disease. Eng et al. (2001) reported that red wine had an inhibitory effect on aromatase, an enzyme responsible for the conversion of androgens to estrogens.

6. Future remarks

Today there is still much work to be completed before we fully understand the nature of wine phenolic compounds. Since in general, only a small proportion of grape phenolic compounds can be found in the resulting wines, it is imperative to improve our knowledge regarding their distinct structures, their extractability, their interactions with other wine chemical compounds, and their structural or compositional modifications. Is phenolic concentration or composition more important for the quality and the health properties of the wine? Which structures are correlated with particularly desirable wine sensory characteristics and health properties and how we can enhance their occurrence in grapes and extraction in wine?

There are also still several questions to be answered concerning the health-protective effects of wine phenolic compounds. Which compounds are the most effective in each disease prevention? What is the relation between their structure and activity? Do they act alone or there is a synergistic effect with other wine microconstituents? What are the mechanisms of their action? What is the required dose and the frequency of consumption? What are their bioactive forms *in vivo* including their metabolites? How their bioaccessibility and stability are modulated after ingestion?

To date, a number of analytical methods can be employed the determination of the wine and grape's total phenolic content. However, it is difficult to compare results that have been measured by the different methods due to their inability to measure directly the phenolic content. In addition, differences in the extraction, isolation, purification, and homogenization techniques may further enhance the variability of the data. The chromatographic methods may separate oligomeric phenolic compounds; however, they do not resolve individual polymeric compounds present in complex mixtures such as wine. The lack of reference compounds also limits their efficient identification and quantification. To better assess the sensory and health properties of phenolic compounds and to be able to modulate both their content and composition in the vineyard and winery, it is necessary to add quantitative methods to the existing protocols for efficient measurements of specific compounds in grapes and wines. These methods should be able to provide quantitative values in a quick and simple way that can be easily implemented for routine analysis of wines.

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Part V

Regulation

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Chapter 14

Functional foods: growth, evolution, legislation, and future perspectives

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1. Introduction

Since the early 1990s, many publications in lay and the specialized press began to discuss functional foods. Indeed, in 1993 the term 'functional food' appeared in *Nature* news magazine in an article highlighting Japan's new initiatives to explore the links between food and medicine (Swinbanks & O'Brien, 1993). Broadly speaking multiple definitions of functional foods exists (Vettorazzi et al., 2020), but there is no universally accepted definition. To paraphrase the Food and Nutrition Board of the National Academy of Sciences, the American Dietetic Association, the Mayo Clinic, and the International Food Information Council (IFIC), functional foods can be defined as modified food ingredients or food that may benefit health beyond its basic nutritional content when consumed at effective levels (American Dietetic Association, 2004; Bagchi, 2014; Havel et al., 1994; Zeratsky, 2022). However, the term functional foods originates in marketing (Henry, 2010) and as such is not defined in any legislation in North America or Europe. For this reason, the definition of functional foods lies in a gray area where some products may be classified as dietary supplements or even nutraceuticals depending on the processing the product has undergone and/or any health benefits they possess. However, functional food is not usually considered a dietary supplement or nutraceutical unless the product's appearance has been altered by processes such as lyophilization, encapsulation, or a similar process that creates an entirely new product with the appearance of a pill, gel tablet, powder, syrup, or alternative form. However, this understanding of functional food is debated by some. Research into functional foods and ingredients has also had environmental impacts whereby food industry by-products that have specific health benefits can be exploited to create value-added products for manufacturers with benefits for consumer health. They can also have environmental impacts by reducing waste and in turn reducing the costs of waste management of these byproducts (Atef & Mahdi Ojagh, 2017; Lordan et al., 2018; Tsoupras et al., 2019).

In this chapter, we further explore the implications of the growth of the functional foods sector with regard to increased research and legislation. We also discuss the broader future of functional foods with regard to cardiovascular health.

2. Functional foods research

Research in the field of functional foods has grown considerably over the last two decades. Indeed, almost 10,000 papers were published in PubMed in 2021 referring to functional foods (Fig. 14.1A). However, the field has come under continual criticism for a lack of human clinical trial evidence, which in some respects is reflected in Fig. 14.1B, whereby trials constituted less than 1% of all published research in 2021. Despite this, it is clear there has been a steady increase in clinical research regarding functional foods over the last decade. ClinicalTrials.gov (www.clinicaltrials.gov) has over 2,500 trials registered that make reference to functional foods on their clinical trials page. However, clinical trials are expensive and may not necessarily be the most practical way to determine the efficacy of a functional food (Forouzanfar et al., 2016).

The rise of new ingredients and products proclaiming to be functional foods led to an urgent call for legislation and control in the 1990s in order to allow the safe distribution and sale of these new products. As such, it was incumbent on the

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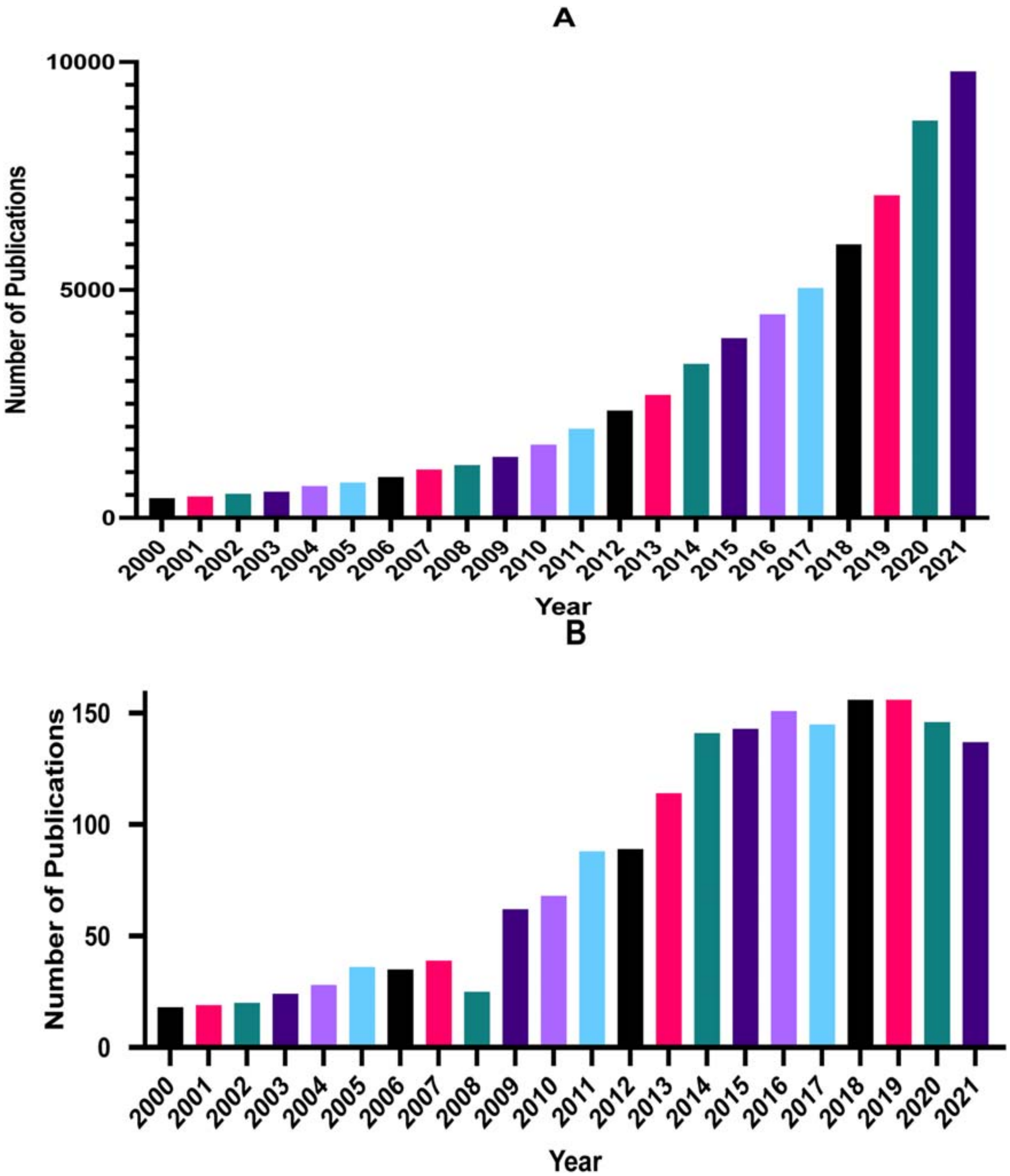

FIGURE 14.1 (A) A graph of the number of publications available in PubMed over the last 2 decades using the search term "functional foods"; (B) A graph of the number of publications available in PubMed over the last 2 decades using the search term "function foods" filtered by "clinical trial" and "randomized Control trial".

United States Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), along with other international bodies to provide guidance and legislation.

Most national regulatory authorities acknowledge the existence and potential benefits of functional foods, but do not recognize or define functional foods in their respective legislations. Instead, other pathways of legislation allow for the application of nutritional content or health claims to support these products on the market, which tend to have a lot of gray areas open to interpretation, an occurrence that is also true of nutraceutical products (Chopra et al., 2022; Santini et al., 2018). It has become increasingly important for businesses that want to succeed in developing novel functional foods to learn and adapt to legal practices, legislations, and procedures that are essential to a product's introduction and success in the market (Wong et al., 2015). This includes procuring intellectual property protection and seeking guidance on compositional specifications, safety laws, labeling laws, etc., at a national and international level. This can make it difficult for some businesses to market their products appropriately and in accordance with legislation.

3. The growth and evolution of functional foods

In humans, food is not only for nutrition, but food also plays an important role in culture, religion, pleasure, and family life. In modern society, we appear to demand even more food. We now expect food to not only provide us with our energy and nutritional needs, but also we expect some foods to provide health benefits that may even stave away some non-communicable diseases (NCD), such as CVD, by reducing risk factors for these diseases.

The link between food and disease prevention and treatment has long been established in Traditional Chinese Medicine of the Zhou Dynasty (1046−256 BCE) and ancient Indian Vedic texts (1500−1200 BCE) (Milner, 2000; Sarkar et al., 2015). These links were established even before Hippocrates (460−370 BCE), the proclaimed Father of Western Medicine, who is often misquoted to have said “Let food be thy medicine and medicine be thy food”. Despite no evidence of this exact statement in the *Hippocratic Corpus* (Cardenas, 2013), it is likely this link was known to Hippocrates and in any case, the sentiment has not changed and the philosophical links between food and medicine have a common origin (Henry, 2010). Generations of people have handed down cultural beliefs that specific foods may be able to treat or prevent certain ailments (Milner, 2000) or that some foods may be negatively associated with health (Lordan et al., 2019), many of which persist to this day. The modern society holds higher standards and with the advent of the industrial revolution and great strides in technological advancements and food science, there is a growing requirement for the innovation of foods that provide more than simply adequate nutrition. In addition, there have been advancements in the exploitation and utilization of food industry by-products and their bioactive compounds as functional ingredients for the production of novel value-added products. This philosophy has driven an increase in research and product development for over 30 years, giving rise to the functional foods market as we know it.

Functional foods are thought to have first been developed in the 1980s in Japan to combat an increase in NCDs associated with their aging population. At the time, regulations were introduced to monitor and approve foods with specified health benefits (Arai, 1996), which was closely followed by a research initiative to further investigate the links between food and health (Henry, 2010; Ohama et al., 2006). Since 1991, Japan follows the Foods for Specified Health Use (FOSHU) regulatory process, which significantly contributes to the Japanese economy (Chopra et al., 2022). In 2015, Japan introduced the Foods with Function Claims (FFC), which are additional regulations less stringent than FOSHU focusing on 14 health benefits (Nutra Ingrediets-asia, 2018). There are now over 1000 products approved by FOSHU or the FFC, which reached total sales of at least $8 billion USD in 2018 alone (Iwatani & Yamamoto, 2019). Globally, the functional foods and beverages market has grown exponentially, and it is expected that the market will grow further from $281 billion USD to $530 billion USD in 2028 with a projected compound annual growth rate of 9.5% (Fortune Business Insights, 2021). There has also been a notable increase in demand for healthy products with superior nutritional profiles, which was particularly heightened as a consequence of the pandemic (Fortune Business Insights, 2021; Lordan, 2021a) due to the link between nutritional status and patient outcomes with respiratory infections (Zabetakis et al., 2020).

The growth in the market has also driven significant innovation, which is an achievement considering the food industry is generally thought to be an area of low research intensity in comparison to other sectors (Bigliardi & Galati, 2013). Innovations have largely occurred due to scientific and technical advancements in food processing and consumer demand for convenience and health products (Beckeman & Skjöldebrand, 2007; Bigliardi & Galati, 2013; Roberfroid, 2000). As outlined in this book, multiple advancements have been made in the area of functional foods with cardiovascular benefits. These include formulations and foods containing bioactive peptides, lipids, and other microconstituents, and products including functional fermented beverages, protein powders, and products derived from by-products of the olive, fish, dairy, and vegetable industries as discussed in various chapters in this book.

In the first six chapters of this book, plant-derived bioactive compounds and functional foods are discussed. Chapter 1 authored by Papastavropoulou and Proestos outlines the important cardiovascular benefits of vegetables, highlighting the various human and epidemiological studies. They also specify some of the many main staples of healthy dietary patterns along with the bioactive compounds responsible for the known health benefits. Growth in the development of functional foods derived from fruit and vegetables has steadily increased due to their favorable nutritional content of vitamins, minerals, trace elements, lipids, and proteins. In particular, the vegetable protein market has grown in prominence due to consumer demands for plant-based products, which reached a market value of USD 9.55 billion in 2020 and is expected to grow with a compound annual growth rate of 7.3% between 2022 and 2027 (Expert Market Research, 2022).

In Chapter 2, Stoikidou and Koidis reviewed many of the bioactive compounds associated with the cardiovascular benefits of tea and coffee consumption, which have been researched extensively in recent years (Niu et al., 2021; Simon et al., 2021; Tsoupras, Lordan, Harrington, et al., 2020). However, innovation in the field has now led to the development of tea (Sajilata et al., 2008) and coffee (Saeed et al., 2019) nutraceuticals, and functional foods, using their bioactive compounds such as polyphenols to improve cardiovascular health. Similar bioactive constituents are found in cocoa, which is discussed in Chapter 3, where the cardiovascular benefits of cocoa and the resulting functional cocoa products were highlighted by Mayorga-Gross and Montoya-Arroyo. In recent years there has been a major interest in the research of beneficial cocoa products for both neurological and cardiovascular health. Indeed, studies are beginning to examine how the processing of cocoa affects bioactive compounds within and how by-products of the industry may be used for innovative products (Cádiz-Gurrea et al., 2020; Campos-Vega et al., 2018; Lemarcq et al., 2020; Oracz et al., 2020; Sioriki et al., 2022).

In Chapter 4, Alves and colleagues discuss the well-established benefits of olive oil and the compounds responsible for their cardioprotective effects, where they also emphasize health claims surrounding olive oil. Olive oil is widely consumed in the Mediterranean and is thought to exert cardioprotective benefits (Martínez-González et al., 2014). In Chapter 5, Karantonis and colleagues discuss the potential development of functional foods derived from the pomaces of the olive oil industry along with the cider, beer, and wine industries, highlighting the potential health benefits that can be obtained from repurposing what would otherwise be considered byproducts of these industries, tackling both environmental problems and health problems. In Chapter 6, Manful and colleagues review the state of research regarding berries, their broad range of bioactive components, and the development of functional foods.

Chapters 7−9 deal with dairy-derived functional foods. In Chapter 7, Aktypis et al. describe the processes involved in yogurt production and the potential health benefits of its consumption, an area of research that is growing due to the potential cardiovascular benefits of yogurt and fermented dairy product consumption (Dehghan et al., 2018; Lordan & Zabetakis, 2017). On the same theme of the benefits of fermented food consumption, Beresford provides an in-depth review of cheese production and its nutritional benefits and implications for human cardiovascular health in Chapter 8. Chapter 9 by Lordan and Dermiki, highlights the many strides made over the last few decades to create functional dairy beverages with cardiovascular and probiotic benefits. Plant-based dairy alternatives and traditional dairy products are also explored. These chapters collectively highlight the important role of the dairy industry in functional food developments. Indeed, the dairy beverage market is expected to increase in growth with a projected compound annual growth rate (CAGR) of 4.8% between 2020 and 2025 with the fastest growing and largest markets being Europe and the Asia−Pacific markets as a result of novel product and health innovations (Glanbia, 2021).

In Chapter 10, Kios et al. review the processing, nutritional value, safety, and development of functional foods from seafood including shellfish. Chapter 11 follows a similar theme, where Vidal et al. discusses the development of fish-derived functional foods with a particular focus on green extraction technologies relating to bioactive lipids and peptides for the promotion of environmentally friendly product development, and the development of fish-derived novel functional foods and nutraceuticals. Both chapters highlight the extensive growth and innovation in the processing and development of functional foods in the seafood sector. Indeed, considering the health benefits of consuming fish (Lordan et al., 2020; Petsini et al., 2018; Tsoupras et al., 2022), it is important that innovation in this field considers consumer preferences and how to increase the consumption of healthy fish products.

The final two chapters of the book, discuss the contentious area of functional food development from alcoholic beverages. Tsoupras et al. outline the potential opportunities to develop functional products from beer and cider and their derivatives in Chapter 12, with a particular focus on the bioactive components that may exert cardioprotective effects within those beverages (Moran et al., 2021; Tsoupras, Lordan, Keefe, et al., 2020). Finally, Basalekou et al. in Chapter 13 focused on the composition of wine, its processing, maturation, and potential cardioprotective properties, which have long been associated with cardiovascular benefits (Fragopoulou & Antonopoulou, 2020).

4. Legislation and monitoring of functional foods

The rise in innovation, production, and consumption of functional foods obviously has benefits to society via improved health and increased growth of value-added products for the food industry. However, regulation of products for human consumption is vital to ensure that the products developed do indeed carry the benefits they purport to exert, and most of all that they are safe to consume. Globally, responsibility for the safety of a food product largely resides with the manufacturer or company that places the product on the market (Vettorazzi et al., 2020). Indeed, as previously alluded to, Japan is one of the only countries to define functional foods under a regulatory category of their own. In the following text, we briefly discuss the strategies adopted by the FDA in the United States and EFSA in the EU for the regulation of functional foods.

4.1 The Food and Drug Administration (FDA)

In the United States, the Food and Drug Administration (FDA) (https://www.fda.gov/) regulates the claims that manufacturers can make about food or supplement products, including the nutrient content of products termed functional foods. As part of this remit, the FDA regulates the labeling and safety of food, beverages, supplements, or derivatives of food brought to market under the Federal Food, Drug, and Cosmetic Act (FD&C Act). This Act was introduced in 1938 and enacted in Chapter 9 of Title 21 of the United States Code (21 USC). This Act has been continuously amended to reflect developments in the industries by various Acts supplemented by Title 21 of the Code of Federal Regulations (21 CFR) (The United States Food and Drug Administration, 2018). Nutraceuticals are not recognized by the FDA but some may fall under the remit of the Dietary Supplement Health and Education Act of 1994 (National Institutes of Health, 1994), and the Food and Drug Administration Modernization Act of 1997 (FDAMA) (The United States Food and Drug Administration, 1997) which supports the FD&C in regulating such products (Lordan et al., 2021). Indeed, the FDA established the Office of Dietary Supplement Programs (ODSP) in 1996 in order to deal with the increased growth and prevalence of supplements to increase surveillance and increase consumer protection (Lordan et al., 2021; Santini & Novellino, 2018). Novel products must submit a New Dietary Ingredient Notification (NDI) to the ODSP for review. However, there is concern that these legislations fail to provide adequate protection to the consumer as they place the responsibility of product safety on the manufacturers, which means products that do not receive proper testing can make it to market and into the hands of the consumer.

Manufacturers do not need to seek approval or register their products with the FDA to begin manufacturing and distributing their products in the United States. Indeed, labels containing information on nutrient content or health claims can be approved based on an authoritative statement from the Academy of Sciences or relevant federal authorities once the FDA has been notified and on the basis, the information is widely known to be true and is not deceptive (Lordan et al., 2021; Santini & Novellino, 2018). While the FDA has the capacity to act on any products deemed unsafe that reach the market, the problem lies in the fact that the product can reach the market in the first place risking consumer safety. The opposite side to that argument is that it would be impossible to monitor all products making it to market considering the growth and innovation of functional foods, dietary supplements, and nutraceuticals over the last few decades.

Just like in Europe, health claims are permitted on products when supported with scientific evidence. These “authorized health claims” have been reviewed by the FDA and are permitted to be present on the labels of food products or supplements to indicate that the product may be beneficial for health or reduce the risk for a disease or health-related condition (The United States Food and Drug Administration, 2022a), which falls under the Nutrition Labeling and Education Act of 1990 (Congress.Gov, 1990). Via this process, health claims are petitioned for by manufacturers or business’s to the FDA, examples of which are available via the FDA’s website (The United States Food and Drug Administration, 2022a). Significant scientific agreement (SSA) among experts supported by all of the publicly available evidence is required in order to achieve approval of an authorized health claim (The United States Food and Drug Administration, 2022a). As part of this process, a ranking system was introduced by the FDA to categorize health claims based on the weight of the evidence provided from “A” (authorized health claims with substantial evidence) to “D” (poor evidence) as explained by Domínguez Díaz et al. (2020). Some of the qualified health claims relating to cardiovascular health are outlined in Table 14.1.

Over the years, the FDA has initiated public and expert consultation on the topic of surveillance, monitoring, and legislating for functional foods (Food Navigator, 2006; Newswise, 2006). However, as of yet, there does not appear to be specific legislation on the horizon for functional foods, and as discussed by Ghosh et al. (2019) there is unlikely to be in the future as further rigor and scientific evidence may be required. This would be in the form of controlled human studies, which are costly. In this scenario, it would be the responsibility of the manufacturer to conduct such studies which may stifle innovation in the functional foods space if finances are tied up in funding clinical trials (Chopra et al., 2022). Further and more detailed information on legislation and monitoring practices of the FDA can be found in the following references Wong et al. (2015) and Bagchi (2014).

4.2 The European Food Safety Authority (EFSA)

In the European Union, the European Food Safety Authority (EFSA) on behalf of the European Commission is responsible for regulating food law and health claims (EFSA, https://www.efsa.europa.eu/en), which was established in law in the EU General Food Law (Regulation (EC) No 178/2002) (European Commission, 2002b). The majority of EFSAs work occurs as a result of a request by EU member states, the European Parliament or the European Commission. Although there are some instances whereby EFSA will carry out scientific assessments on their own accord (known as “self-tasking”) when

TABLE 14.1 FDA qualified health claims relating to cardiovascular diseases, diabetes, and hypertension (The United States Food and Drug Administration, 2022b).

Condition	Qualified health claim
Cardiovascular diseases	Oleic acid and coronary heart disease
	Folic acid, vitamin B_6, and vitamin B_{12} and vascular disease
	Nuts (macadamia, walnuts, and general nut consumption) and coronary heart disease
	Omega-3 fatty acids and reduced coronary heart disease risk
	Unsaturated fatty acids (soybean oil, corn oil, canola oil, and olive oil) for reduced risk of heart disease
Type II diabetes mellitus (T2DM)	High-amylose maize starch and reduced risk of T2DM
	Psyllium husk and reduced risk of T2DM
	Whole grains and reduced risk of T2DM
	Chromium Picolinate and reduced risk of insulin resistance and T2DM
Hypertension	Magnesium and reduced risk of high blood pressure
	Omega-3 fatty acids and reduced blood pressure
	Calcium and hypertension, pregnancy-induced hypertension, and preeclampsia

there are emerging issues in food regulation that requires reviews. This is to ensure a high level of food safety and quality across the EU (EFSA, 2006). However, neither functional foods, nutraceuticals, nor bioactive compounds are specifically regulated at a European level (Vettorazzi et al., 2020) but they may fall into the categories of other preexisting EFSA regulations or laws in member states as simplified in Table 14.2.

There are therefore some concerns that legislation can be overbearing or stifle product innovation in the food and supplement industries despite being essential for the protection of the consumer (Chopra et al., 2022). However, with sufficient evidence, novel foods are listed and authorized according to Regulation (EC) No 2015/2283 (European Commission, 2015) and the Commission Implementing Regulation (EU) 2017/2470 (European Commission, 2017). This list can increase and grow with the authorization of novel foods by the EFSA (Vettorazzi et al., 2020). EFSA guidance on the application process for authorization of a novel food according to Regulation (EU) 2015/2283 was published as a scientific opinion in 2016 (EFSA Panel on Dietetic Products et al., 2016). The guidance outlines the necessary evidence required for inclusion in the authorized novel foods list, which includes the intended purpose and description of the product, history of consumption, the manufacturing process, and composition data among other details. Despite the fact that the safety of a product lies with the business that places the product on the market, EFSA does evaluate products for human consumption that are governed by multiple EFSA guidelines. EFSA evaluates the product's toxicity safety in four main areas including chronic carcinogenicity, genotoxicity, toxicokinetics, and developmental or reproductive toxicity (Vettorazzi et al., 2020). Through these mechanisms, it is possible to bring functional foods safely to market in the EU. While there is less emphasis on ensuring product efficacy, the process of applying for inclusion on the list of novel foods and ingredients does add a layer of protection for the consumer. This is particularly important considering the multiple incidences of consumer extortion through false product claims during the COVID-19 pandemic (Lordan, 2021b; Lordan et al., 2021).

5. Future perspectives

Collectively, this chapter and this book presents a relatively small insight into the many developments that have occurred in the functional food sector over the last three decades. As indicated throughout, innovation is a key part of this success. Indeed, multiple benefits including the reutilization of industry by-products to create value-added products have led to surges in the production of various functional foods and supplements. Certainly, the pandemic has also increased research in the field (Zabetakis et al., 2021). Over the next decade, it is expected that the surge in research and market growth will continue to rise. However, it is important that existing legislation is continually amended and updated to reflect new trends

TABLE 14.2 A general overview of some European food regulations governed by the European Commission and the EFSA.

Regulation	Reference	Year	Implications
Regulation (EC) No. 178/2002 of the European Parliament and of the Council of January 28, 2002 laying down the general principles and 2002 requirements of food law, establishing the European Food Safety authority, and laying down procedures in matters of food safety.	European Commission (2002b)	2002	Establishment of EFSA and European food safety legislation.
Directive 2002/46/EC of the European Parliament and of the Council of June 10, 2002 on the approximation of the laws of the Member States relating 2002 to food supplements.	European Commission (2002)	2002	Laws were established for food supplements. Lists vitamins and minerals allowed in the manufacture of food supplements. Bioactive compounds must appear on the packaging of products, the directive does not include bioactive compounds intended for medicinal applications. Guidance established for vitamins and minerals.
Regulation (EC) No. 1925/2006 of the European Parliament and of the Council of December 20, 2006 on the addition of vitamins and minerals and 2006 of certain other substances to foods.	European Commission (2006)	2006	Established legislation for the compounds allowed in food fortification and supplements. Lists the allowable vitamins, minerals, or other food constituents. Also lists constituents banned or restricted, and lists substances that require investigation.
Regulation (EU) No. 609/2013 of the European Parliament and of the Council of June 12, 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 2013, p. 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No. 41/2009 and (EC) No 953/2009.	European Commission (2013)	2013	Legislation for the control of incorporating amino acids, carnitine, choline, inositol, minerals, nucleotides, taurine, and vitamin into one or more categories of foods for specific consumers including children or individuals with special dietary requirements.
Regulation (EU) 2015/2283 of the European Parliament and the council of November 25, 2015 on novel foods, amending Regulation (EU) No. 1169/2011 of the European Parliament and of the Council and repealing 2015 Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No. 1852/2001.	The European Commision (2015)	2015	Legislation for novel foods that were not consumed widely by humans prior to the May 15, 1997, with some exceptions such as specific food ingredients, additives, flavorings, enzymes, etc.
Commission Implementing Regulation (EU) 2017/2470 of December 20, 2017 establishing the Union list of novel foods in accordance with 2017 Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods.	European Commission (2017), pp. 72–201)	2017	Legislation that authorizes and provides the conditions and labeling requirements for the inclusion of novel food constituents such as *N*-acetyl-D-neuraminic acid, specific algal oils, specific extracts from plant and marine sources, lycopene, and multiple other potentially bioactive food constituents that can be placed on the market within the EU referred to in Regulation (EU) 2015/2283 (European Commission, 2017, pp. 72–201).

Note that this table is simplified and that there are several amendments, corrigenda, and updates to the existing legislation that are not detailed in Table 14.1.
Adapted with permission from Vettorazzi et al. (2020). Additional information and updates are available on the EFSA website (https://www.efsa.europa.eu/en).EFSA does provide specific guidance on health claims relating to cardiovascular health (EFSA Panel on Dietetic Products and Allergies, 2011). In this guidance, EFSA provides a roadmap for the types of health claims that manufacturers can apply to EFSA for their products. These include claims relating to antioxidants and preventing oxidative damage, reduction of postprandial lipids, or maintenance of blood pressure or platelet function. Indeed, there have been some successful applicants such as FruitFlow, which is a tomato-based antithrombotic product that can help maintain platelet homeostasis (O'Kennedy et al., 2017; Tsoupras, Lordan, & Zabetakis, 2020). Examples such as FruitFlow demonstrate that companies can successfully obtain a health claim for their products when sufficient evidence is provided for evaluation.

and product development of functional foods. With new developments in the functional foods sector and scientific advancements, new fields of research may also emerge. New technologies will allow for genetically modified organisms and foods in the sector, which will need new legislation as they develop. Indeed, research in its own right will advance thanks to improvements in nutrigenomics and metabolomics (Daliri & Lee, 2015).

While this chapter has specifically focused on the legislations and regulations of the United States and Europe, it is important to recognize that many other countries and regions have their own forms of regulating functional foods and supplements (Chopra et al., 2022). Legislation surrounding functional foods is complex and in general functional foods are not recognized in legislation in most countries. However, bioactive constituents found within functional foods do tend to be recognized in EU and FDA guidance and legislation and can therefore be used to market products and substantiate health claims for some functional food products. While these legislations tend to have strict labeling laws, surveillance of products for safe human consumption is mostly lacking considering the responsibility for the safety and efficacy of a product tends to lie with the business or manufacturer of the product that is brought to market. It could be argued that EFSA regulations are two strict in contrast to the FDA, as current EFSA guidance places a lot of emphasis on producing clinical data to substantiate a health claim (Daliri & Lee, 2015). Therefore, there is the risk of extortion of customers or even safety concerns, as shown during the COVID-19 pandemic in the United States (Chopra et al., 2022). That isn't to say that authorities aren't overseeing the sector, but there are certain areas that could be improved such as product surveillance and education for product developers and manufacturers in the areas of innovation, health claims, labeling, intellectual property, and more. Should update or additional legislation is introduced in the future, it is also important to balance the restrictions placed on the food industry to prevent the stifling of innovation or increased costs for small businesses that may reduce competition within the markers. Therefore, additional guidance and support may be required from surveillance and legislative authorities (Chopra et al., 2022).

This book sought to gain an insight into the current research and development trends regarding functional foods with a slant toward the development of products aiming to tackle the ever-growing burden of cardiovascular and metabolic diseases on global health. The editors would like to thank all who contributed to the development of this book.

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Index

Note: 'Page numbers followed by "*f*" indicate figures and "*t*" indicate tables.'

M

N

O

P

Functional Foods and Their Implications for Health Promotion

Addresses functional foods, how they work, and how food components promote health

Functional Foods and Their Implications for Health Promotion presents functional foods, from raw ingredients to the final product, and provides a detailed explanation of how these foods work and their impact on health.

This book presents the functions of food against diseases and discusses how healthier foods can be produced. Broken into four parts, this book presents a deep dive into plant-derived functional foods, dairy foods, marine food, and beverages. *Functional Foods and Their Implications for Health Promotion* includes case studies, applications, literature reviews, and coverage of recent developments.

Intended for nutritionists, dieticians, food technologists, as well as students and researchers working in nutrition, dietetics, and food science, this book is sure to be a welcomed resource.

Key Features:

- Uses flow diagrams to highlight the effects of processing on produced functional foods
- Combines information for the production/formulation of the food with data on bioactivities and bioavailability
- Presents whole foods and non-food components and focuses on the functionality and the availability of foods

About the Editors:

Ioannis Zabetakis is a Senior Lecturer and the current Head of the Department of Biological Sciences at University of Limerick, Ireland. His research focuses on the cardioprotective properties of food lipids with a particular emphasis on dairy and marine foods and the development of novel supplements.

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Ronan Lordan is a researcher at the Institute for Translational Medicine, Perelman School of Medicine, University of Pennsylvania. His research focuses on the development of novel prophylactics and therapeutics including nutraceuticals, food supplements, and bioactive microconstituents of natural origin for communicable and non-communicable diseases. His latest research is focused on circadian rhythms and their role in metabolism and aging.

Dipak Ramji is a Professor of Cardiovascular Science at the School of Biosciences in Cardiff University. He is also a Fellow of the Learned Society of Wales. His research focus is on understanding how natural products regulate cellular processes in heart disease with the goal of attaining deeper mechanistic insights and identifying preventative/therapeutic agents.

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