Nutraceuticals in Alternative and Underutilized Fruits as Functional Food Ingredients: Ancient Species for New Health Needs

Dario Donno, Maria Gabriella Mellano, Alessandro Kim Cerutti, Gabriele Loris Beccaro
University of Turin, Grugliasco, Turin, Italy

1 Nutraceuticals, Functional Foods, and Food Supplements

Food provides nutrients that nourish the human body and keep it in proper working order. However, early civilizations also established that certain foods confer additional health benefits to humans as they can prevent and treat various diseases (Aluko, 2012). Therefore people have tried to achieve a better quality of life by eating more vegetables, fruits, and other plant foods; taking dietary supplements or nutraceuticals; or using nutritional therapy or phytotherapy to replace official medicines. Moreover, the availability of regional regulatory bodies has spurred intense global research and development with the aim of identifying new bioactive compounds that could be used to formulate functional foods and nutraceuticals (Pisanello, 2014). Hence the demands for nutraceuticals, phytonutrients, and their therapeutic services; manufacturers; marketers; and related licensed professionals have also increased accordingly (Bagchi et al., 2004).

The term “nutraceutical” was coined by combining the terms “nutrition” and “pharmaceutical” by Stephen Defelice, chairman of the Foundation for Innovation in Medicine (New Jersey, 1989). Nutraceuticals were described as “food or part of a food that provides health benefits and are used for prevention or treatment of a disease” (Srivastava et al., 2015). They currently represent the fastest growing segment of the food industry with a market estimate of USD 6–60 billion (Prabu et al., 2012), growing at 5% per annum; however, this is unfortunately likely to attract irresponsible market entrants and products that do not deliver. Nutritionists must therefore ensure that the correct balance is struck between the exploitation of this lucrative business and adequate consumer protection. There is still much ignorance and confusion about nutrition in the minds of the general population. In the Western world ~70% of the population would buy specific foods to reduce the risk of diseases; however, they do not want to be faced with constant decisions about

Alternative and Replacement Foods
http://dx.doi.org/10.1016/B978-0-12-811446-9.00009-5

the health benefits of different foods or diets. Many are unwilling or unable to follow dietary guidelines, which is perhaps not surprising as dietary reference values take little or no account of today's busy lifestyles (Hardy, 2000).

The concepts of nutraceuticals, dietary supplements, functional foods, medical foods, and dietary supplements are confusing and the terms are often used interchangeably. Nutraceuticals are described as products extracted, purified, or produced from a plant, animal, or marine source (e.g., antioxidants from blueberries, elk velvet, or fish oils), or produced from dried, powdered, or pressed plant material. They have a demonstrable physiological benefit or provide protection against chronic diseases (Prabu et al., 2012; Srivastava et al., 2015). Dietary supplements have more defined health roles, as vitamins, minerals, herbs, botanicals, amino acids, and other dietary substances are intended to supplement the diet by increasing the total dietary intake of these ingredients (Roberfroid, 2002). Dietary supplements are not intended to treat or cure disease (Gibson and Makrides, 2000) whereas nutraceuticals are used in the prevention or treatment of diseases. The concept of functional foods is different from that of nutraceuticals and they can be defined as food products that are used in the standard diet to provide beneficial health effects in addition to the traditional nutritional effects (Whitman, 2001). It is possible to link the key concepts (i.e., health, technology, and nutrition) with the main players in the functional food research and development process (i.e., food technologists, nutritionists, and specialists) (Fig. 9.1). The combination of skills possessed by these different players is essential for the development of innovative nutraceutical products. These products must present higher quality standards (and organoleptic properties) compared to corresponding conventional products and aim to maintain well-being (Bigliardi and Galati, 2013).

A diet containing high levels of fruits and vegetables has been associated with a lower risk of chronic diseases because, in addition to their high vitamin and mineral content, these foods also contain compounds with health-protective effects, in particular antioxidant and antiinflammatory compounds (Donno et al., 2013b). In plant biochemistry these molecules are secondary metabolites (i.e., chemical compounds produced within the plants that are not directly involved in the normal growth, development, or reproduction of the organism). Some are found to defend against diseases, predators, ultraviolet radiation, parasites, and oxidants; some facilitate the reproductive process (e.g., serving as attractive smells and coloring agents); and others are important for interspecies competition (Kaur and Das, 2011). These same metabolites provide beneficial health and wellness effects in humans and animals. Evidence from epidemiological, in vitro, in vivo, and clinical studies has consistently demonstrated that a diet rich in plant foods can reduce the risk of some degenerative diseases including diabetes, obesity, inflammatory disorders, cardiovascular complications, and cancer (Cheung and Mehta, 2015).

Inflammation is a protective measure produced by an organism to eliminate an injurious stimuli. The use of antiinflammatory substances can be an effective tool in the therapeutic treatment of diseases; in this context, medicinal plants and their isolated compounds are used globally in folk medicine to treat different inflammatory conditions (De Cassia Da Silveira et al., 2013).

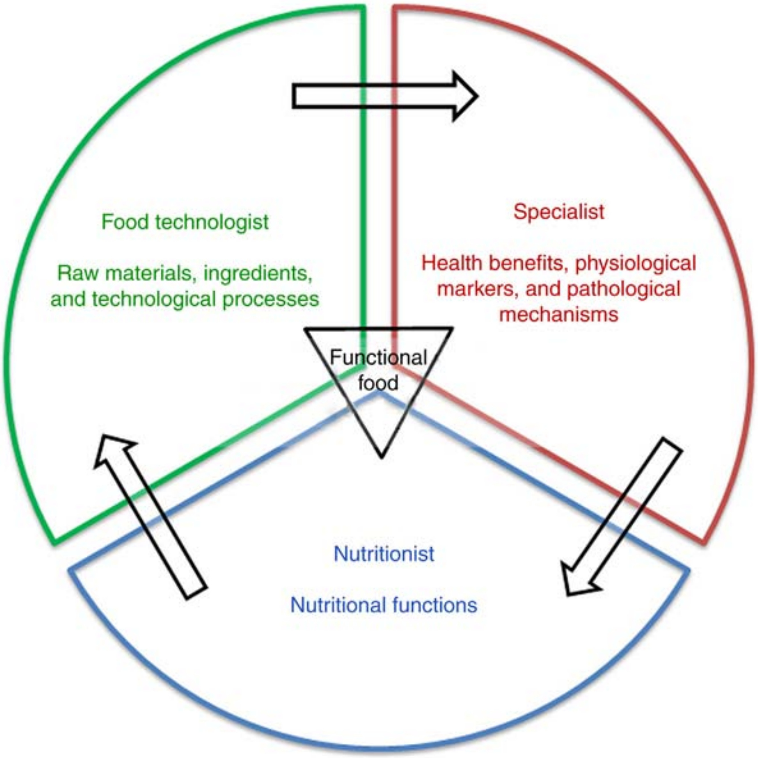

Figure 9.1: The Three Main Participants Involved in the Research and Development of Innovative Nutraceutical Foods.

Source: Bigliardi, B., Galati, F., 2013. Innovation trends in the food industry: the case of functional foods. Trends Food Sci. Technol. 31, 118–129.

In the continuous search for new natural bioactive antiinflammatory molecules, fruits are increasingly being seen as a rich source due to their essential oils and their isolated components, the monoterpenes (Dell'Agli et al., 2013). Moreover, polyphenols are the most abundant bioactive compounds reaching values of 1 g/day in the diet, approximately 10-times higher than vitamin C intake (Arts and Hollman, 2005; Scalbert et al., 2005). The quantity and quality of polyphenols in plant foods may vary significantly according to intrinsic and extrinsic factors such as plant genetics, soil composition, growth conditions, stage of maturity, and postharvest conditions (Jeffery et al., 2003). The dietary intake of phenolics is greatly affected by the eating habits and preferences of individuals (Shahidi and Ambigaipalan, 2015). Simple phenolics (i.e., hydroxycinnamic acid conjugates and flavonoids) are important constituents of fruits, vegetables, and beverages. These compounds show a wide range of antioxidant activities in vitro and are thought to exert protective effects against major diseases such as cancer and cardiovascular disease (Rice-Evans et al., 1997). In the plant kingdom, antioxidant compounds can range from simple molecules (e.g., vitamin C and phenolic acids) to highly polymerized compounds (e.g., tannins). Phenolic compounds can be divided into many different classes (Fig. 9.2); however,

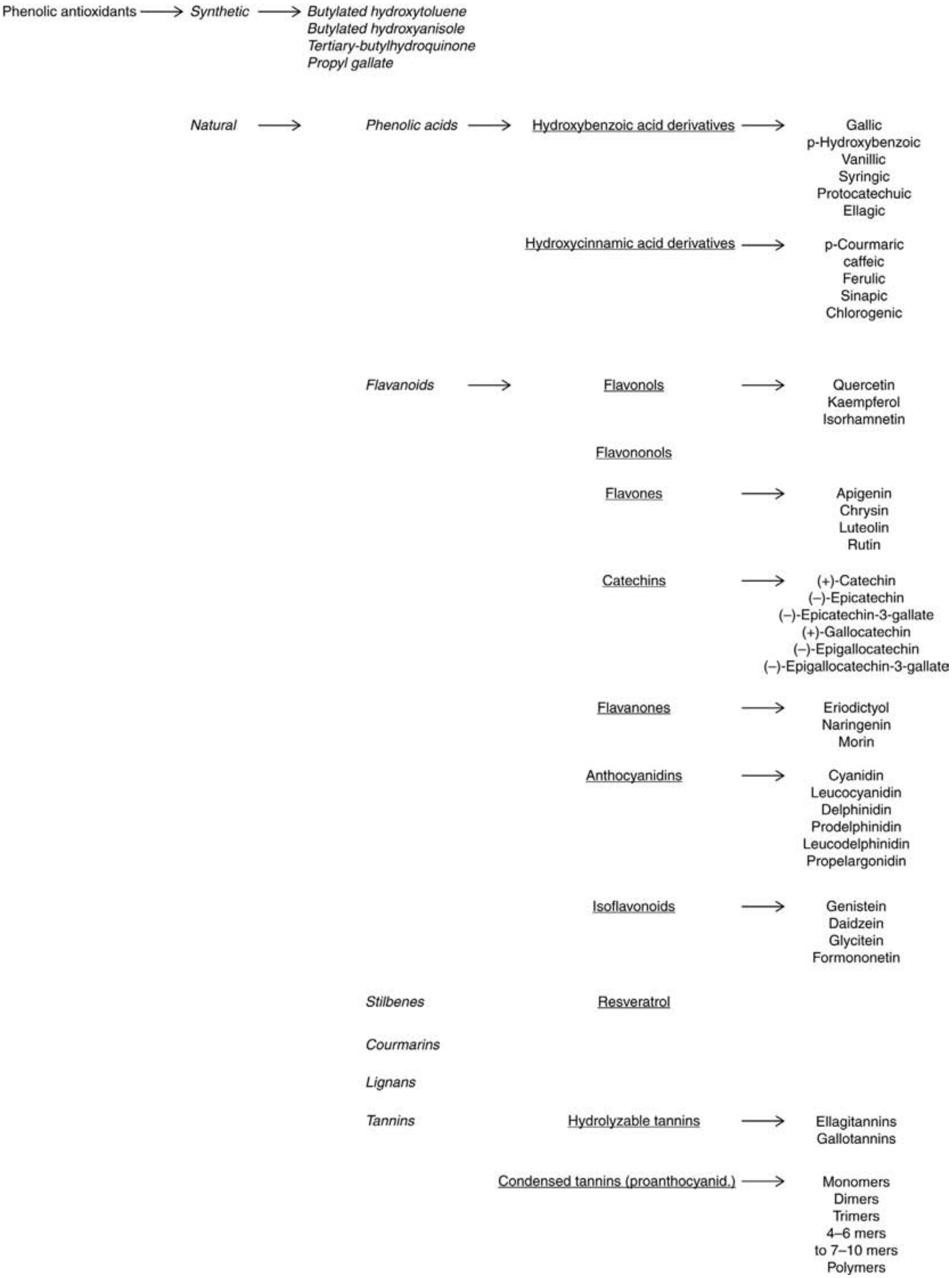

Figure 9.2: Classification of Phenolic Antioxidants.

Source: Shahidi, F., Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects—a review. J. Funct. Foods, 18, Part B, *820–897.*

flavonoids and phenolic acids are the most abundant in plant materials (Tabart et al., 2011; Tabart et al., 2012). These low molecular weight secondary metabolites exhibit excellent antioxidant properties. Moreover, organic acids are also considered as antioxidants with multiple uses in pharmacology (Eyduran et al., 2015); however, their specific mechanisms of action can vary depending on both the structure and the chemical environment (Komes et al., 2011).

2 Fresh Fruit and Derived Products: New Sources for Health-Promoting Agents

Nutraceuticals on the market today consist of both traditional and nontraditional foods. Traditional nutraceuticals are natural whole foods with new information about their potential health qualities. Many (if not most) fruits, vegetables, grains, fish, dairy, and meat products contain several natural components that deliver benefits beyond basic nutrition. Even tea and chocolate have been noted to contain beneficial-health attributes in some studies. Conversely, nontraditional nutraceuticals are foods resulting from agricultural breeding or with added nutrients or ingredients (Prabu et al., 2012).

Wild food plants have become very important for the food industry as they can be used to replace synthetic chemicals and nutraceuticals (Sadia et al., 2014); however, the nutritional, economic, and sociocultural potential of neglected and underutilized natural food resources have yet to be fully exploited and are suffering from a lack of research interest (Beccaro et al., 2015; Donno et al., 2014). For example, data on the antioxidant properties of several plant resources, in particular plants not used in nutrition and medicine, are still lacking. Therefore studies on these plants may be of interest in the search for novel sources of natural antioxidants, functional foods, and nutraceuticals (Donno et al., 2015c). Many alternative and underutilized fruits such as *Asimina triloba* (L.) Dunal, *Crataegus azarolus* L., *Lycium barbarum* L., *Morus nigra* L., and *Amelanchier canadensis* (L.) Medicus are presented in this chapter as novel sources of health-promoting agents.

2.1 Asimina triloba *(L.) Dunal*

The pawpaw (*A. triloba* L. Dunal) is a tree fruit native to the temperate woodlands of the Appalachian region. It belongs to the Annonaceae family, which includes 120 genera and over 2100 species. *A. triloba* is the only temperate species as the rest of the family thrives in tropical or subtropical climates. During the growing season, the pawpaw has a whitish to light-green color that turns yellow–brown at maturity (Donno et al., 2014). The ripe fruit is highly aromatic and the banana-like flesh surrounds two rows of large bean-shaped brown seeds. Pawpaw fruits were traditionally consumed by Native Americans, then by European explorers and settlers, and today they are consumed by local populations in rural areas. Despite some consumer and professional interest, the high perishability of the fruit has been a major factor in slowing the development of this fruit in a larger market (McLaughlin, 2008).

Pawpaws contain significant levels of phytochemicals and unique compounds that may promote health. The fruits are comparable to apples and oranges since they are rich in polyphenolic compounds, vitamin C (30–200 mg kg^{-1}), magnesium (1000–1400 mg kg^{-1}), iron (60–80 mg kg^{-1}), copper (2–8 mg kg^{-1}), and manganese (20–30 mg kg^{-1}). Their antioxidant activity may also be comparable to that of more extensively studied fruit. Moreover, pawpaw fruits are also a good source of potassium (3000–3800 mg kg^{-1}), essential amino acids (mean value of 40 mg kg^{-1} of protein), riboflavin (0.06–0.15 mg kg^{-1}), niacin (10–12 mg kg^{-1}), calcium (500–800 mg kg^{-1}), phosphorus (400–500 mg kg^{-1}), and zinc (10–12 mg kg^{-1}). They are comparable to other important tropical fruits; for example, they contains levels of polyphenols that are similar (caffeic acid and vitamin C) or higher (ellagic acid, ferulic acid, and quercetin) than mango (*Mangifera indica* L.). The levels of antioxidant compounds are also similar to other tropical fruits such as guava, papaya, banana, and ananas (Kobayashi et al., 2008; Kral, 1960).

Some alternative food medicines and products that contain extracts of pawpaw (e.g., Paw Paw Cell Reg, Graviola Max, Royal Graviola, and Graviola liquid extract) have been reported to exhibit antitumor efficacy in animal models and in a limited number of clinical studies (Coothankandaswamy et al., 2010).

2.2 Crataegus azarolus *L.*

Azarole fruits (*C. azarolus* L.) have attracted attention in the food, nutraceutical, and medical fields because of their widely reported health benefits (e.g., they reduce the risk of cardiovascular diseases). The systematics of the *Crataegus* genus have been problematic because of hybridization, introgression, polyploidy, and apomixis. Considerable revisionary work has led to a substantial reduction in the number of species that can plausibly be recognized as *Crataegus*, with global estimates now suggesting there are 150–200 species, which is a much more manageable number to handle in studies of chemical variation within the genus (Bahri-Sahloul et al., 2009).

Crataegus fruits and flowers are used for medicinal purposes; specifically, the fruits are not only consumed fresh and dried but are also used to produce jams, marmalades, and syrups. The weight of the fruit ranges from 2 to 8 g and they are variable in size (up to 25 mm diameter) and color (yellow to bright red to black). There are 1–3 large seeds in the center of each fruit and each contains on average 15 g of total sugar, 1.5 mg of total acidity, 30 mg of vitamin C, 11 mg of Ca, 10 mg of P, 1 mg of Fe, 160 mg of K, 7 mg of Mg, 0.2 mg of Cu, 0.25 mg of Mn, 2 mg of Na, 1 g of protein, and 13 g of carbohydrate per 100 g (Bignami et al., 2003).

Several bioactive phytochemicals have been isolated from the fruits of European and Asian *Crataegus* species. Their high contents of flavonoids, proanthocyanidins, catechins, phenolic acids, essential oils, and terpenoids explains their use as natural therapeutics in the treatment of neurodegenerative diseases, some types of cancer, disorders of the immunological system, and cardiovascular diseases. Extracts from *Crataegus* spp. exert a wide range of pharmaceutical properties, especially on the cardiovascular system, including cardiotonic, antiarrhythmic, hypotensive, hypolipidemic, and antioxidant activities. The use of fruits in the treatment of heart ailments dates back to the late 1800s and numerous laboratory tests and clinical trials have demonstrated their efficacy in the treatment or prevention of cardiovascular diseases (Belkhir et al., 2013).

2.3 Lycium barbarum *L.*

Goji is a solanaceous deciduous shrub native to China, Tibet, and other parts of Asia, and its bright orange–red ellipsoid berries are 1–2 cm long. The two closely related species, *L. barbarum* L. and *Lycium chinense* Miller, have been historically used as food and medicinal plants in China and other Asian countries. Their highly similar anatomy and tissue structure makes differentiation based on morphological and histological analyses very delicate. The most common name for the goji berry is “wolfberry,” and it derives from the character “gou” as it is related to the one that means wolf. The fruits are collected from late summer to autumn, dried in the shade until the skin shrinks, and then exposed to the sun until the outer skin becomes dry and hard and the pulp remains soft (Donno et al., 2015a, 2016a).

Lycium fruits are a rich source of nutrients and phytochemicals including organic acids, sugars and polysaccharides, carotenoids (zeaxanthin), vitamins (i.e., vitamin C, vitamin B complex, and vitamin E), flavonoids, phenolic acids, minerals, trace elements (i.e., zinc, iron, calcium, selenium, germanium, and phosphorus), 18 amino acids (including eight essential amino acids), alkaloids, and fats (Amagase and Farnsworth, 2011).

Studies indicate that the goji fruit has positive effects on aging, neuroprotection, general well-being, metabolism and energy expenditure (i.e., glucose control in diabetics), glaucoma, immunomodulation, and cytoprotection, as well as having antitumor and antioxidant properties. *L. barbarum* and *L. chinense* fruits are widely used in traditional Chinese medicine and they can also be sold as dietary supplements or classified as nutraceuticals for prolonged and safe use (Donno et al., 2016b). Some authors report that the health-promoting agents in goji fruit might confer beneficial health effects by alleviating oxidative stress and counteracting free radicals (Donno et al., 2016b; Potterat, 2010).

Lycium spp. fruits are most often incorporated into complex herbal preparations at 6–18 g/100 g of dried material. If decoction is used then scientific references indicate a dosage of 5–15 g/100 mL of goji, equivalent to 25–120 g of fresh berries. Research recommends 30 mL of decoction four times daily (120 mL/day), which is equivalent to approximately 150 g of fresh berries. Goji has been used as a food and herbal medicine for over 2500 years without any toxic effects; however, there have been two case reports of possible interactions between goji fruit tea and warfarin (Coumadin) (Amagase et al., 2009; Larramendi et al., 2012).

2.4 Morus nigra *L.*

The mulberry (*Morus* spp., Moraceae family) has become domesticated over thousands of years, adapting to tropical, subtropical, and temperate zones in the Northern Hemisphere, and growing in a wide range of climatic, topographic, and soil conditions. There are 24 species of *Morus* (about 100 known cultivars) including the white mulberry (*Morus alba* L.), black mulberry (*M. nigra* L.), and red mulberry (*Morus rubra* L.). *M. nigra* has juicy fruits with a nice color and a unique, slightly acidic flavor (Donno et al., 2015c).

Mulberries are sweet fruits and they play an important role in the food industry due to their high levels of bioactive compounds (mulberry fruits can vary in terms of their chemical composition and antioxidant properties). For this reason, mulberries can be considered as a good source of nutrients and antioxidant compounds (especially anthocyanins and polyphenols) and they can also provide nutritionally useful amounts of minerals (Liang et al., 2012).

Mulberry trees have historically been cultivated for their leaves, which can be used as food for silkworms. Mulberry fruits are currently consumed as both fresh and processed products (e.g., juices, fruit salads, and dried fruits) due to their nutritive value. The production and consumption of mulberry fruits is rapidly increasing, largely due to their aromatic taste, nutritional value, and biological activities. One of the most important bioactive constituents of mulberries are the anthocyanins. These pigments have dual value: first, they constitute an integral part of the sensory attributes since their levels and various forms pertain directly to the coloration of the final product; and second, they are thought to possess diverse biological properties and therefore are considered as secondary metabolites with potential nutritional value. Several researchers have studied the contents of phenolics (i.e., flavonoids and anthocyanins) and carotenoids in mulberry extracts (Sánchez-Salcedo et al., 2015).

Mulberry fruits are traditionally used as worming, hypoglycemic, emetic, or laxative agents. In Chinese herbal medicine the mulberry fruit has been used as a folk remedy to treat several diseases including oral and dental diseases, diabetes, hypertension, arthritis, and anemia. Several studies have confirmed that mulberries may have positive health effects in humans due to their phenolic composition, and this is especially true for people with type 2 diabetes mellitus. Similarly, mulberry leaves have also shown promising biological effects (Calin-Sanchez et al., 2013).

2.5 Amelanchier canadensis *(L.) Medicus*

The genus *Amelanchier* (family Rosaceae) is subdivided into approximately 25 species (e.g., *Amelanchier alnifolia*, *Amelanchier arborea*, and *A. canadensis*) and is widespread in North America and in parts of Europe and Asia. *A. canadensis*, also known as the shadblow serviceberry, ripens in early June and is widely used in the landscape trade. The serviceberry is native to North America (Western and North Central United States, Alaska, and Western Canada), but is less well-known and rarely grown in Europe (except Scandinavian countries) despite its edible fruits, frost resistance, and decorative value (Stushnoff, 1991).

The maroon–purple fruits of *A. canadensis* are similar to those of *A. arborea* (i.e., glabrous, wax-coated pomes of 7–15 mm diameter). In the last two decades there has been growing interest in using the sweet serviceberry in the food industry in fresh fruits, pies, pastries, preserves, jams, jellies, spreads cereals, and snack foods, in particular in Canada and the United States (Michigan). The fruits have also been added to cider, wine, beer, and tea (Cazares-Franco et al., 2014).

The important health benefits of serviceberries, especially their ability to act as a good source of minerals (e.g., manganese, magnesium, iron, calcium, potassium, and copper) and carotenoids (e.g., lutein), has been emphasized in the literature. Moreover, the fruit is also rich in nutraceutical compounds, especially phenolic compounds (e.g., anthocyanins, chlorogenic acid, catechins, and rutin). *Amelanchier* spp. seed oil may serve as a potential dietary source of tocopherols, sterols, and unsaturated fatty acids (Juríková et al., 2013; Ozga et al., 2007).

Indigenous North Americans used different parts of the serviceberry plant for medicinal purposes; for example, in Canada the fruits were used as a juice for treating stomach and intestinal ailments. Eye drops and eardrops were also prepared from ripe serviceberries. The boiled bark can be used as a disinfectant while the root infusion can be used to prevent miscarriage after injury. Native American communities also prepare a tea from the twigs to administer to women after birth, while a tonic from the bark is given to women after delivery to hasten discharge of the placenta. Moreover, several cultivars of *Amelanchier* spp. were found to possess free radical scavenging activity (due to their relatively high anthocyanin content) and antiviral activity against enteric coronavirus. Finally, serviceberry fruits show antidiabetic properties (due to their aldose reductase inhibitor activity) and exhibit the ability to regulate lipid metabolism and energy expenditure (Bakowska-Barczak and Kolodziejczyk, 2008; Bakowska-Barczak et al., 2007).

3 Genetic, Agronomic, and Environmental Factors

The type of sample determines the sampling protocol and how the samples are handled. The main considerations are the degree of homogeneity of the material and the possible variation in the compound content, not only between different materials but also between different samples of the same material. The cultivar, origin, season, and growth conditions will also affect the bioactive content (Nedović et al., 2015). It is essential that the sampling protocol and the number of collected samples reflects the composition of the whole sample. The time between sample collection and analysis should be as short as possible and the protocol should minimize any effects that may cause undesirable losses prior to analysis (Mitra, 2004).

Therefore identification is an important step in the collection of wild plants, even if it is often overlooked by the nutraceutical companies: in the European market only 20% of food product plants are collected in germplasm repositories (Fowler, 2006). Moreover, an individual plant species may have different cultivars with different genetic characteristics, chemical compositions, and health effects on the efficacy of the final product and consumer health (Kerslake and Menary, 1985).

The fruit harvest period can vary across different sampling sites and years (Dvaranauskaitė et al., 2009; Lakusic et al., 2011). The development of tree species is also influenced by natural factors (endogenous or exogenous) as well as by human factors. These factors can influence the chemical composition of plant organs and the respective derived products (Tibaldi et al., 2011).

Food quality is not only dependent on intrinsic characteristics (i.e., genetic factors) but is also closely linked to the pedoclimatic conditions in the collecting area. Indeed, plants growing in more suitable areas have greater potential in terms of product quantity and quality (Vegvari et al., 2008). Knowledge of environmental characteristics, such as the best pedoclimatic conditions for plant development, can help identify the best raw material to be used for fresh plant material and derived functional foods (Rates, 2001).

Each species has its own requirement in terms of altitude, latitude, mean temperature, average annual rainfall, light availability, and physic-chemical soil properties. Climatic conditions have direct effects on the physiological processes and phenology of the plant (e.g., growth, flowering, and fruit ripening) thus they can also affect the availability of essential metabolites for the biosynthesis of active compounds (Vegvari et al., 2008). A plant can almost completely lose the ability to synthesize specific bioactive molecules outside of its own habitat (VV.AA., 2012). Therefore the use of advanced agrotechniques alongside dedicated genetic improvement allows tree species to fit better with the cultivation site and can also improve the final product quality (Capasso et al., 2006).

Assessing the chemical composition of fruit is very important because it allows the functional product to be standardized. Each nutraceutical product should have the same qualitative and quantitative chemical composition in every production batch in order to maintain quality, safety, and efficacy (Ong, 2004). The relationship between these products and the different factors such as their origin, the pedoclimatic conditions (e.g., soil, latitude, altitude, mean temperature, light intensity, and rain frequency), and the agronomic techniques used means that standardization should first extend to the raw materials through the selection of plants with the best bioactive compound content. The subsequent transformation process and the use of standardized methods, together with analytical laboratory controls, contributes to the production of a final product with reproducible and consistent chemical and physical characteristics (i.e., bioactive compounds, appearance, texture, density, and solubility) (Khan, 2006).

4 Identification and Quality Control of Phytochemical Biomarkers

Considering that food composition is affected by many factors, and that compounds are not uniformly distributed within and between samples of a given food, statistically valid sampling and sample preparation are of utmost importance to obtain representative and homogeneous samples (Rodriguez-Amaya, 2001). The main causes of data inaccuracy are that only a single sample lot is analyzed per food and that errors are not observed between intralaboratory and interlaboratory evaluations in which the same homogenized samples are analyzed. The selection, number, handling, and preparation of samples prior to extraction are important factors that can affect data quality. Sample preparation may consist of multiple steps such as drying, homogenization, sieving, extraction, preconcentration, derivatization, and hydrolysis (Luthria, 2006).

Qualitative and quantitative studies of bioactive compounds mostly rely on selecting the proper extraction method (Smith, 2003). Extraction is the first step in the study of bioactive compounds and is crucial to the final result. The five main objectives of extraction are: (1) to extract targeted bioactive compounds from complex samples, (2) to increase the selectivity of analytical methods, (3) to increase the sensitivity of bioassays by increasing the concentration of targeted compounds, (4) to convert the bioactive compounds into a more suitable form for detection and separation, and (5) to provide a strong and reproducible method that is independent of variations in the sample matrix (Nedović et al., 2015). Therefore extraction should be carefully optimized and performed. The polarities of the different bioactive compounds, which vary significantly due to their conjugation status and their association with the sample matrix, determines the solvents to be used in the extraction (Sasidharan et al., 2011). When selecting solvents for the extraction of bioactive compounds, the molecular affinity between the solvent and solute, mass transfer, use of a cosolvent, environmental safety, human toxicity, and financial feasibility should also be considered (Nedović et al., 2015). Optimizing the extraction parameters not only increases the extraction efficiency of the analyte of interest but also reduces the solvent consumed and the waste generated during the extraction process, thus making it more environmental friendly (Chemat et al., 2012). Specific pretreatments (e.g., storage under modified atmosphere, microbial and chemical acidification, pasteurization, and utilization of depolymerizing enzymes) are sometimes needed to preserve the raw materials and extract biomolecules; moreover, additional procedures (e.g., rotary shaking, stirring, and sonication) can also be used to improve the extraction (Laufenberg et al., 2003).

During identification and characterization of bioactive substances, the critical point is usually the selection of the correct measuring method. Spectroscopic methods usually permit crude identification of the bioactive compounds present in food, but in most cases the specific composition can remain obscure. However, spectrophotometry remains very useful for the

Table 9.1: In vitro antioxidant capacity assays.

Chemical Basis	Method
Hydrogen atom transfer	Oxygen radical absorbance capacity Total radical trapping antioxidant parameter Crocin bleaching assay Inhibited oxygen uptake Inhibition of linoleic acid oxidation Inhibition of LDL oxidation
Stable radical formation	Diphenyl-1-picrylhydrazyl
Single electron transfer	Trolox equivalent antioxidant capacity Ferric ion reducing antioxidant power Copper(II) reduction capacity Total phenols assay by Folin–Ciocalteu reagent
Other reactions	Total oxidant scavenging capacity Inhibition of Briggs–Rauscher oscillation reaction Chemiluminescence Electrochemiluminescence

Source: Huang, D., Ou, B., Prior, R.L., 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53, *1841–1856.*

evaluation of antioxidant activity (Table 9.1). Detailed information about the composition of food requires additional methods, such as chromatography and mass spectrometry, to fully characterize the structures of the molecules in the samples (Nedović et al., 2015). Technological innovation in analytical instruments has allowed increasingly sophisticated qualitative and quantitative assessments of the chemical composition of nutraceutical foods (Cordell, 2011). Chromatographic techniques are mainly used to analyze fresh foods and derived products. Chromatography offers a very powerful separation ability, such that the complex chemical components in the extracts can be separated into many relatively simple subfractions. Furthermore, the recent approaches of applying hyphenated chromatography and spectrometry such as high-performance liquid chromatography–diode array detection (HPLC–DAD), gas chromatography–mass spectroscopy (GC–MS), capillary electrophoresis–DAD, HPLC–MS, and HPLC–nuclear magnetic resonance (NMR), can provide additional spectral information. This is very helpful for qualitative analysis and for online structural elucidation (Liang et al., 2004; Steinmann and Ganzera, 2011). More specifically, HPLC is currently the most widely used analytical method when combined with different detectors (e.g., diode arrays, mass spectrometry, and fluorescence) (Donno et al., 2012). It is regarded as the gold standard in the authentication of nutraceuticals, functional foods, pharmaceuticals, and herbal medicines due to its precision, sensitivity, and reproducibility despite its high instrumental cost, relatively long analytical time, and large organic solvent consumption (Donno et al., 2015b).

Identification and quality control of the food material can be performed using marker compounds: a chemical constituent of a raw material, drug substance, or food product that is used for identification or quality control purposes, especially when the active constituents are not known or identified. The active compounds are responsible for the intended pharmacological activity or synergistic therapeutic effects (Ong, 2004). It is necessary to determine most of the phytochemical constituents of analyzed products to ensure the reliability and repeatability of pharmacological and clinical research, as well as to understand the bioactivities and possible side effects of active compounds and to enhance quality control (Liang et al., 2004). Chromatography combined with a suitable detection technique offers a powerful tool for separating the individual compounds and developing a characteristic profile of the sample, otherwise known as a fingerprint. Combining a chromatographic separation system online with a spectroscopic detector has become the most important approach for identifying or confirming the identity of target and unknown chemical compounds (Cuadros-Rodríguez et al., 2016).

If hyphenated chromatography is further combined with chemometric approaches, clear pictures may be developed for chromatographic fingerprints (Bertacchini et al., 2013). The complexity of fresh foods and derived products means that it is necessary to consider many factors for their fingerprint evaluation. The quality of a functional food is closely related to its chemical constituents and their concentrations and may vary slightly according to differences in the climate, cultivation conditions, harvest time, drying, and storage (Hibbert, 2012). To analyze the large amount of generated data, a variety of analytical methods have proven to be useful and versatile tools for the extraction, visualization, and interpretation of the information. In particular, pattern recognition methods as a principal component analysis allows better visualization of the information included in the fingerprints. The original variables are transformed into latent variables to summarize the systemic patterns of variation between the samples (Ieri et al., 2013). Exploratory data analysis is easier if represented as a multivariate data table as a low-dimensional plane.

5 Functional Food Supply Chain and Environmental Sustainability

Sustainability is a broad concept and is considered to be ambiguous because it means different things to different people at different periods of time. As a consequence there are many different definitions of sustainable agriculture; however, most of them are connected to the three pillars of sustainability: society, economy, and environment. For example, Reganold et al. (2001) summarized this concept as “to be sustainable, a farm must produce adequate yields of high quality, be profitable, protect the environment, conserve resources, and be socially responsible in the long term.”

There are a number of indicators that can be used to assess the environmental performance of a production system. Many studies claim that indicators that consider many aspects of the environmental impact at the same time are more useful to address the complexity of agricultural systems (Bastianoni et al., 2007). Therefore some of the most important features of an indicator are its ability to summarize, focus, and condense extensive datasets (obtained from complex environmental parameters) to a manageable amount of meaningful information (Godfrey and Todd, 2001).

Life cycle assessment (LCA) is defined by the International Organization for Standardization (ISO14040:2006) as the compilation and evaluation of the inputs, outputs, and potential environmental impacts of a product system throughout its life cycle. The origin of LCA can be found in the late 1960s within the context of American industry (Hunt et al., 1996) and numerous studies have been carried out to adapt this method to the agricultural sector (Audsley et al., 2003). LCA is now considered a useful tool for comparing alternative food products, processes, or services, and as the background for environmental product declaration (Schau and Fet, 2008). The results of LCAs are commonly presented as impacts in a range of different categories such as global warming, acidification, nitrification, ozone depletion, and toxicity (Pennington et al., 2004). Nevertheless, more importance and consideration is being given to the results in single impact categories. One example is the carbon footprint, which in the LCA approach corresponds to the "global warming potential." This category expresses the number of CO_2 equivalents (CO_2eq., a collective unit for all molecules with a global warming potential) that are associated, directly and indirectly, with all stages of development of the system under consideration. The term CO_2eq. is intended to express all greenhouse gases, with each being weighted according to their global warming potential. The advent of the carbon footprint has made LCA results available to a wider audience (Weidema et al., 2008).

Food production systems have their own specific agronomic requirements. Even different varieties of the same species may have unique requirements in terms of fertilizers, pesticides, water, plantation strategies, and field management, which may result in different environmental burdens (De Gennaro et al., 2012). One exemplary case is the growing of ancient cultivars or forgotten fruits with high nutraceutical values (Donno et al., 2010, 2012). Ancient cultivars are bound to the area they are grown through centuries of slow adaptation to the pedoclimatic conditions, thus resulting in lower agricultural inputs compared to introduced cultivars (Bisht et al., 2007; Jarvis et al., 2008). A lower agricultural input theoretically produces a lower environmental impact; however, in order to be sure about this it is necessary to apply an environmental impact assessment method. A didactical case study was reported by Cerutti et al. (2013), in which the authors investigated the environmental burden of ancient varieties of apple in North West Italy. Hundreds of different cultivars of apple (*Malus domestica* Borkh.) were grown in Italy until the 1950s. After this time the proliferation of commercial cultivars caused the local germplasm to lose importance and be forgotten by growers and consumers. Many ancient cultivars were gradually replaced by commercial ones with low phytochemical and nutraceutical traits. The Italian fruit-growing scene underwent significant change and Golden Delicious is now the most cultivated variety (more than 70% of total orchards); however, the genetic diversity has fortunately been largely preserved thanks to germplasm repositories (Donno et al., 2012).

While Golden Delicious dominates, the ancient apple germplasm of the Piedmont region (Northern Italy) is actually represented by ~350 cultivars, almost all recently described by their nutraceutical, morphological, and agronomic traits (Bounous et al., 2006). The commercial appeal of traditional cultivars is related to their unique quality traits and to the claims of their lower environmental impacts due to being grown on the original agricultural land. Ancient cultivars usually require fewer treatments and field operations per hectare of cultivation in comparison with foreign cultivars because they are more adapted to the pedoclimatic characteristics of the native region.

Cerutti et al. (2013) used LCA methodology to calculate the environmental performance of Grigia di Torriana, Magnana, and Runsé cv., three ancient apple cultivars from Torino and Cuneo provinces. The environmental impacts of these cultivars were compared to that of Golden Delicious. The study was performed in accordance with the ISO 14040:2006 standard series guidelines and with the cradle-to-gate approach as the basis for the life cycle inventory (LCI) (International Organization for Standardization, 1997). Data regarding orchard structure, agricultural inputs, resource consumption, and orchard management practices were obtained directly from the growers who filled in a questionnaire for the 2011 season. In order to consider minor geographical differences, the LCI for each cultivar included the average of three orchards spread throughout the two provinces. Environmental impacts were calculated according to several functional units. Considering the impacts for growing 1 hectare of orchard, the Golden Delicious cultivar showed the lowest environmental performance emitting 6.5 t CO_2eq.; conversely, less emissions were observed in the life cycle of the three ancient cultivars (average 4.9 t CO_2eq.). This result confirms that if low-input agricultural systems are managed properly, and are producing high-value products (including high levels of nutraceutical components), they can be more sustainable than high-input systems (Cerutti et al., 2013).

It could be important to apply the same methodology to some underutilized fruit species (i.e., those of nutraceutical value) in order to evaluate the sustainability of their production and consumption. Worldwide interest in introducing the cultivation of these tree species to promote the differentiation of the cultivated agrobiodiversity and the consumption of new functional foods with excellent nutraceutical properties could also be encouraged by the specific agronomic traits of these species that could be managed with more environmentally friendly agrotechniques (if compared with the most commonly grown fruit species), and by the possible greater sustainability of their production.

6 Alternative and Underutilized Fruit Species and the Healthy-Product Industry

Research carried out in the field of natural bioactive compounds is increasing our understanding of healthy compounds that are naturally available in food, in particular in fruit. This will permit their increased use in foods (instead of artificial drugs), which will in turn increase their quality and added value. New methodologies for the extraction, purification, identification, and quantification of biomolecules using ecofriendly analytical techniques will need to be developed to improve the extraction yields (Chemat et al., 2012; Oroian and Escriche, 2015). With the advent of high-throughput technologies and their applications in food science, the food and beverage industries have been taking steps to transform their traditional R&D and product development processes. The food and beverage industries are now faced with a situation in which they should quickly adapt to new market demands and regulatory updates and be prepared to make expensive and risky decisions as to whether they should proceed with significant investments in the development of functional products (Younesi and Ayseli, 2015).

Alternative and underutilized fruits represent an opportunity for local growers to gain access to specialized markets where consumers lay emphasis on exotic character and the presence of nutrients that are capable of preventing degenerative diseases. The creation of specific horticultural models for nutraceutical fruit production could be an interesting opportunity to obtain a highly standardized raw material for fresh or derived products. In addition, phytochemicals from these fruits could have further applications in the food industry, such as increasing the stability and shelf life of food products. Studies relating to the toxicological analysis of bioactive extracts; the metabolism of bioactive compounds, their bioavailability, and bioaccessibility; and the sensorial and nutritional traits of food products with added bioactive compounds from underutilized fruits should be undertaken (Ayala-Zavala et al., 2011).

The development of new functional products with intended health claims requires concrete scientific criteria. To meet the current stringent regulations for functional products, the food industry must approach this topic using integrative approaches. Integration of complex biological knowledge relevant to human health and diseases into the functional product development process could not only increase the capacity of innovative product development, but could also support informed decision making (Younesi and Ayseli, 2015). Food science is a blend of many scientific and engineering disciplines, such as chemistry, biology, agricultural science, and chemical engineering, which is applied to provide a better understanding of food and its transformation and to deliver safe and desirable food products to the consumer (Smithers, 2016).

In addition, economic analysis of the extraction processes and marketing of natural bioactive extracts should be contemplated. If food products are to be produced for use in the nutraceutical and pharmaceutical industries, there will be certain criteria that production system must meet: for example, it must clearly be economical, environmentally safe, and not have an impact on the environment (Barnes and Prasain, 2005; Cerutti et al., 2014).

A source of bioactive substances should be identified and cultivars of the same species must be considered in order to choose the highest and the most stable concentration of natural compounds (Fowler, 2006; Joubert et al., 2008). Once this source has been identified, a constant and stable supply of plant material should be secured. This means that an agronomic system for biomass production should be developed in order to allow the full expression of the genetic capability of the cultivar (Kerslake and Menary, 1985; Khan, 2006). Moreover, climate and soil conditions should fit with the species requirements. The impact of fertilization and irrigation on the biomass production and its chemical constituents should be understood and the economic growth and cultivation of the raw material should generally be explored (Lakusic et al., 2011). In the future, a plant production of secondary metabolites may be controlled with different growth regulators according to good agricultural practices used in this agronomic production (Donno et al., 2013a). The successful production of biomass should include the development of appropriate harvest techniques and storage processes and mechanization of the harvest process should be addressed in order to maintain an economic system of production (Spinelli et al., 2012). The integral exploitation of the entire food production supply chain could have economic benefits to producers and a beneficial impact on the environment, leading to a greater diversity of products for human consumption. These products represent a new class of functional foods that have not been completely exploited and that could also contribute health benefits to consumers. Due to the safety and limitations surrounding the use of synthetic antioxidants, natural antioxidants and nutraceuticals obtained from edible sources (i.e., underutilized fruit species and derived products) are alternative sources of interest (Shahidi and Zhong, 2015).

In conclusion, further studies on the isolation of bioactive compounds using complementary methods and their effects on biological status in animal models and human subjects are needed to evaluate their potential benefits. Moreover, it is necessary to further confirm lack of toxicity and bioavailability of these natural sources (Moure et al., 2001): the use of isolated nutraceuticals as dietary supplements or functional food ingredients may also be helpful in order to promote the human health, reduce the disease risk, and improve the efficacy of these materials.

References

Aluko, R.E., 2012. Functional Foods and Nutraceuticals. Springer, New York, NY, USA.

Amagase, H., Farnsworth, N.R., 2011. A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of *Lycium barbarum* fruit (goji). Food Res. Int. 44, 1702–1717.

Amagase, H., Sun, B., Borek, C., 2009. *Lycium barbarum* (goji) juice improves in vivo antioxidant biomarkers in serum of healthy adults. Nutr. Res. 29, 19–25.

Arts, I.C.W., Hollman, P.C.H., 2005. Polyphenols and disease risk in epidemiologic studies. Am. J. Clin. Nutr. 81, 317S–325S.

Audsley, E., Alber, S., Clift, R., Cowell, S., Crettaz, P., Gaillard, G., Hausheer, J., Jolliet, O., Kleijn, R., Mortensen, B., 2003. Harmonisation of environmental life cycle assessment for agriculture: final report, concerted action AIR3-CT94-2028. CE DG VI-Centre de documentation.

Ayala-Zavala, J.F., Vega-Vega, V., Rosas-Domínguez, C., Palafox-Carlos, H., Villa-Rodriguez, J.A., Siddiqui, M.W., Dávila-Aviña, J.E., González-Aguilar, G.A., 2011. Agro-industrial potential of exotic fruit byproducts as a source of food additives. Food Res. Int. 44, 1866–1874.

Bagchi, D., Preuss, H.G., Kehrer, J.P., 2004. Nutraceutical and functional food industries: aspects on safety and regulatory requirements. Toxicol. Lett. 150, 1–2.

Bahri-Sahloul, R., Ammar, S., Grec, S., Harzallah-Skhiri, F., 2009. Chemical characterisation of *Crataegus azarolus* L. fruit from 14 genotypes found in Tunisia. J. Hortic. Sci. Biotechnol. 84, 23–28.

Bakowska-Barczak, A.M., Kolodziejczyk, P., 2008. Evaluation of saskatoon berry (*Amelanchier alnifolia* Nutt.) cultivars for their polyphenol content, antioxidant properties, and storage stability. J. Agric. Food Chem. 56, 9933–9940.

Bakowska-Barczak, A.M., Marianchuk, M., Kolodziejczyk, P., 2007. Survey of bioactive components in Western Canadian berries. Can. J. Physiol. Pharmacol. 85, 1139–1152.

Barnes, S., Prasain, J., 2005. Current progress in the use of traditional medicines and nutraceuticals. Curr. Opin. Plant Biol. 8, 324–328.

Bastianoni, S., Pulselli, F.M., Castellini, C., Granai, C., Dal Bosco, A., Brunetti, M., 2007. Emergy evaluation and the management of systems towards sustainability: a response to Sholto Maud. Agric. Ecosyst. Environ. 120, 472–474.

Beccaro, G.L., Bonvegna, L., Donno, D., Mellano, M.G., Cerutti, A.K., Nieddu, G., Chessa, I., Bounous, G., 2015. *Opuntia* spp. biodiversity conservation and utilization on the Cape Verde Islands. Genet. Resour. Crop Evol. 62, 21–33.

Belkhir, M., Rebai, O., Dhaouadi, K., Congiu, F., Tuberoso, C.I.G., Amri, M., Fattouch, S., 2013. Comparative analysis of Tunisian wild *Crataegus azarolus* (yellow azarole) and *Crataegus monogyna* (red azarole) leaf, fruit, and traditionally derived syrup: phenolic profiles and antioxidant and antimicrobial activities of the aqueous-acetone extracts. J. Agric. Food Chem. 61, 9594–9601.

Bertacchini, L., Cocchi, M., Vigni, M.L., Marchetti, A., Salvatore, E., Sighinolfi, S., Silvestri, M., Durante, C., 2013. The impact of chemometrics on food traceability. Chemom. Food Chem. 28, 371–410.

Bigliardi, B., Galati, F., 2013. Innovation trends in the food industry: the case of functional foods. Trends Food Sci. Technol. 31, 118–129.

Bignami, C., Paolocci, M., Scossa, A., Bertazza, G., 2003. Preliminary evaluation of nutritional and medicinal components of *Crataegus azarolus* fruits. Acta Hortic. 597, 95–100.

Bisht, I., Mehta, P., Bhandari, D., 2007. Traditional crop diversity and its conservation on-farm for sustainable agricultural production in Kumaon Himalaya of Uttaranchal state: a case study. Genet. Resour. Crop Evol. 54, 345–357.

Bounous, G., Beccaro, G., Mellano, M., Torello Marinoni, D., Cavanna, M., Botta, R., 2006. Antico germoplasma piemontese di melo: caratterizzazione genetica e proprietà antiossidanti dei frutti. Italus Hortus 13, 101–104.

Calin-Sanchez, A., Martinez-Nicolas, J., Munera-Picazo, S., Carbonell-Barrachina, A., Legua, P., Hernandez, F., 2013. Bioactive compounds and sensory quality of black and white mulberries grown in Spain. Plant Foods Hum. Nutr. 68, 370–377.

Capasso, F., Grandolini, G., Izzo, A.A., 2006. Fitoterapia: Impiego Razionale delle Droghe Vegetali. Springer, Milan.

Cazares-Franco, M.C., Ramirez-Chimal, C., Herrera-Hernandez, M.G., Nunez-Colin, C.A., Hernandez-Martinez, M.A., Guzman-Maldonado, S.H., 2014. Physicochemical, nutritional and health-related component characterization of the underutilized Mexican serviceberry fruit *Malacomeles denticulata* (Kunth) G. N. Jones. Fruits 69, 47–60.

Cerutti, A.K., Beccaro, G.L., Bruun, S., Bosco, S., Donno, D., Notarnicola, B., Bounous, G., 2014. LCA application in the fruit sector: state of the art and recommendations for environmental declarations of fruit products. J. Clean. Prod. 73, 125–135.

Cerutti, A.K., Bruun, S., Donno, D., Beccaro, G.L., Bounous, G., 2013. Environmental sustainability of traditional foods: the case of ancient apple cultivars in Northern Italy assessed by multifunctional LCA. J. Clean. Prod. 52, 245–252.

Chemat, F., Vian, M.A., Cravotto, G., 2012. Green extraction of natural products: concept and principles. Int. J. Mol. Sci. 13, 8615–8627.

Cheung, P.C.K., Mehta, B.M., 2015. Handbook of Food Chemistry. Springer, Berlin, Heidelberg, Germany.

Coothankandaswamy, V., Liu, Y., Mao, S.C., Morgan, J.B., Mandi, F., Jekabsons, M.B., Nagle, D.G., Zhou, Y.D., 2010. The alternative medicine pawpaw and its acetogenin constituents suppress tumor angiogenesis via the HIF-1/VEGF pathway. J. Nat. Prod. 73, 956–961.

Cordell, G.A., 2011. Phytochemistry and traditional medicine: a revolution in process. Phytochem. Lett. 4, 391–398.

Cuadros-Rodríguez, L., Ruiz-Samblás, C., Valverde-Som, L., Pérez-Castaño, E., González-Casado, A., 2016. Chromatographic fingerprinting: an innovative approach for food "identitation" and food authentication: a tutorial. Anal. Chim. Acta 909, 9–23.

De Cassia da Silveira, E., Sa, R., Andrade, L.N., De Sousa, D.P., 2013. A review on anti-inflammatory activity of monoterpenes. Molecules 18, 1227–1254.

De Gennaro, B., Notarnicola, B., Roselli, L., Tassielli, G., 2012. Innovative olive-growing models: an environmental and economic assessment. J. Clean. Prod. 28, 70–80.

Dell'Agli, M., Di Lorenzo, C., Badea, M., Sangiovanni, E., Dima, L., Bosisio, E., Restani, P., 2013. Plant food supplements with anti-inflammatory properties: a systematic review (I). Crit. Rev. Food Sci. Nutr. 53, 403–413.

Donno, D., Beccaro, G.L., Mellano, M.G., Canterino, S., Cerutti, A.K., Bounous, G., 2013a. Improving the nutritional value of kiwifruit with the application of agroindustry waste extracts. J. Appl. Bot. Food Qual. 86, 11–15.

Donno, D., Beccaro, G.L., Mellano, M.G., Cerutti, A.K., Bounous, G., 2014. Chemical fingerprint as nutraceutical quality differentiation tool in *Asimina triloba* L. fruit pulp at different ripening stages: an old species for new health needs. J. Food Nutr. Res. 53 (1), 81–95.

Donno, D., Beccaro, G.L., Mellano, M.G., Cerutti, A.K., Bounous, G., 2015a. Goji berry fruit (*Lycium* spp.): antioxidant compound fingerprint and bioactivity evaluation. J. Funct. Foods 18 (Part B), 1070–1085.

Donno, D., Beccaro, G.L., Mellano, M.G., Torello-Marinoni, D., Cerutti, A.K., Canterino, S., Bounous, G., 2012. Application of sensory, nutraceutical and genetic techniques to create a quality profile of ancient apple cultivars. J. Food Qual. 35, 169–181.

Donno, D., Boggia, R., Zunin, P., Cerutti, A.K., Guido, M., Mellano, M.G., Prgomet, Z., Beccaro, G.L., 2015b. Phytochemical fingerprint and chemometrics for natural food preparation pattern recognition: an innovative technique in food supplement quality control. J. Food Sci. Technol., 1–13.

Donno, D., Cavanna, M., Beccaro, G.L., Mellano, M.G., Torello-Marinoni, D., Cerutti, A.K., Bounous, G., 2013b. Currants and strawberries as bioactive compound sources: determination of antioxidant profiles with HPLC-DAD/MS. J. Appl. Bot. Food Qual. 86, 1–10.

Donno, D., Cerutti, A.K., Prgomet, I., Mellano, M.G., Beccaro, G.L., 2015c. Foodomics for mulberry fruit (*Morus* spp.): analytical fingerprint as antioxidants' and health properties' determination tool. Food Res. Int. 69, 179–188.

Donno, D., Galizia, D., Cerutti, A.K. 2010. Fruit nutraceutical value in ancient apple cultivars grown in Piedmont (Northern Italy). XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on Environmental, Edaphic, and Genetic Factors Affecting Plants, Seeds and Turfgrass. 940, 131–138.

Donno, D., Mellano, M.G., Cerutti, A.K., Beccaro, G.L. 2016a. Simbiosi tra agro-farmaceutica e piccoli frutti opportunità per le microfiliere locali. Riv. frutticoltura e di ortofloricoltura. 6, 34–37.

Donno, D., Mellano, M.G., Raimondo, E., Cerutti, A.K., Prgomet, Z., Beccaro, G.L., 2016b. Influence of applied drying methods on phytochemical composition in fresh and dried goji fruits by HPLC fingerprint. Eur. Food Res. Technol. 242, 1961–1974.

Dvaranauskaitė, A., Venskutonis, P.R., Raynaud, C., Talou, T., Viškelis, P., Sasnauskas, A., 2009. Variations in the essential oil composition in buds of six blackcurrant (*Ribes nigrum* L.) cultivars at various development phases. Food Chem. 114, 671–679.

Eyduran, S.P., Ercisli, S., Akin, M., Beyhan, O., Gecer, M.K., Eyduran, E., Erturk, Y.E., 2015. Organic acids, sugars, vitamin C, antioxidant capacity and phenolic compounds in fruits of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry genotypes. J. Appl. Bot. Food Qual., 88.

Fowler, M.W., 2006. Plants, medicines and man. J. Sci. Food Agric. 86, 1797–1804.

Gibson, R.A., Makrides, M., 2000. n−3 Polyunsaturated fatty acid requirements of term infants. Am. J. Clin. Nutr. 71, 251s–255s.

Godfrey, L., Todd, C., 2001. Defining thresholds for freshwater sustainability indicators within the context of South African Water Resource Management. 2nd WARFA/Waternet Symposium: Integrated Water Resource Management: Theory, Practice, Cases. Cape Town, South Africa. Second WARFA/Waternet Symposium: Integrated Water Resource Management: Theory, Practice, Cases, Cape Town.

Hardy, G., 2000. Nutraceuticals and functional foods: introduction and meaning. Nutrition 16, 688–689.

Hibbert, D.B., 2012. Experimental design in chromatography: a tutorial review. J. Chromatogr. B 910, 2–13.

Hunt, R.G., Franklin, W.E., Hunt, R., 1996. LCA: how it came about. Int. J. Life Cycle Ass. 1, 4–7.

Ieri, F., Martini, S., Innocenti, M., Mulinacci, N., 2013. Phenolic distribution in liquid preparations of *Vaccinium myrtillus* L. and *Vaccinium vitis idaea* L. Phytochem. Anal. 24, 467–475.

International Organization for Standardization, 1997. Environmental Management: Life Cycle Assessment: Principles and Framework, ISO.

Jarvis, D.I., Brown, A.H., Cuong, P.H., Collado-Panduro, L., Latournerie-Moreno, L., Gyawali, S., Tanto, T., Sawadogo, M., Mar, I., Sadiki, M., 2008. A global perspective of the richness and evenness of traditional crop-variety diversity maintained by farming communities. Proc. Natl. Acad. Sci. USA 105, 5326–5331.

Jeffery, E.H., Brown, A.F., Kurilich, A.C., Keck, A.S., Matusheski, N., Klein, B.P., Juvik, J.A., 2003. Variation in content of bioactive components in broccoli. J. Food Compost. Anal. 16, 323–330.

Joubert, E., Richards, E.S., van der Merwe, J.D., De Beer, D., Manley, M., Gelderblom, W.C.A., 2008. Effect of species variation and processing on phenolic composition and in vitro antioxidant activity of aqueous extracts of *Cyclopia* spp. (honeybush tea). J. Agric. Food Chem. 56, 954–963.

Juríková, T., Balla, S., Sochor, J., Pohanka, M., Mlcek, J., Baron, M., 2013. Flavonoid profile of saskatoon berries (*Amelanchier alnifolia* Nutt.) and their health promoting effects. Molecules 18, 12571–12586.

Kaur, S., Das, M., 2011. Functional foods: an overview. Food Sci. Biotechnol. 20, 861–875.

Kerslake, M.F., Menary, R.C., 1985. Varietal differences of extracts from blackcurrant buds (*Ribes nigrum* L.). J. Sci. Food Agric. 36, 343–351.

Khan, I.A., 2006. Issues related to botanicals. Life Sci. 78, 2033–2038.

Kobayashi, H., Wang, C.Z., Pomper, K.W., 2008. Phenolic content and antioxidant capacity of pawpaw fruit (*Asimina triloba* L.) at different ripening stages. Hortscience 43, 268–270.

Komes, D., Belščak-Cvitanović, A., Horžić, D., Rusak, G., Likić, S., Berendika, M., 2011. Phenolic composition and antioxidant properties of some traditionally used medicinal plants affected by the extraction time and hydrolysis. Phytochem. Anal. 22, 172–180.

Kral, R., 1960. A revision of *Asimina* and *Deeringothamnus* (Annonaceae). Brittonia 12, 233–278.

Lakusic, B., Ristic, M., Slavkovska, V., Milenkovic, M., Lakusic, D., 2011. Environmental and seasonal impacts on the chemical composition of *Satureja horvatii* SILIC (Lamiaceae) essential oils. Chem. Biodivers. 8, 483–493.

Larramendi, C., Garcia-Abujeta, J., Vicario, S., García-Endrino, A., López-Matas, M., García-Sedeño, M., Carnés, J., 2012. Goji berries (*Lycium barbarum*): risk of allergic reactions in individuals with food allergy. J. Investig. Allergol. Clin. Immunol. 22, 345.

Laufenberg, G., Kunz, B., Nystroem, M., 2003. Transformation of vegetable waste into value added products: (a) the upgrading concept; (b) practical implementations. Bioresour. Technol. 87, 167–198.

Liang, L.H., Wu, X.Y., Zhu, M.M., Zhao, W.G., Li, F., Zou, Y., Yang, L.Q., 2012. Chemical composition, nutritional value, and antioxidant activities of eight mulberry cultivars from China. Pharmacogn. Mag. 8, 215–224.

Liang, Y.-Z., Xie, P., Chan, K., 2004. Quality control of herbal medicines. J. Chromatogr. B 812, 53–70.

Luthria, D.L., 2006. Significance of sample preparation in developing analytical methodologies for accurate estimation of bioactive compounds in functional foods. J. Sci. Food Agric. 86, 2266–2272.

Mclaughlin, J.L., 2008. Pawpaw and cancer: annonaceous acetogenins from discovery to commercial products. J. Nat. Prod. 71, 1311–1321.

Mitra, S., 2004. Sample Preparation Techniques in Analytical Chemistry. John Wiley & Sons, Hoboken, NJ, USA.

Moure, A., Cruz, J.M., Franco, D., Domínguez, J.M., Sineiro, J., Domínguez, H., José Núñez, M.A., Parajó, J.C., 2001. Natural antioxidants from residual sources. Food Chem. 72, 145–171.

Nedović, V., Raspor, P., Lević, J., Šaponjac, V.T., Barbosa-Cánovas, G.V., 2015. Emerging and Traditional Technologies for Safe, Healthy and Quality Food. Springer, Cham, ZG, Switzerland.

Ong, E.S., 2004. Extraction methods and chemical standardization of botanicals and herbal preparations. J. Chromatogr. B 812, 23–33.

Oroian, M., Escriche, I., 2015. Antioxidants: characterization, natural sources, extraction and analysis. Food Res. Int. 74, 10–36.

Ozga, J.A., Saeed, A., Wismer, W., Reinecke, D.M., 2007. Characterization of cyanidin-and quercetin-derived flavonoids and other phenolics in mature saskatoon fruits (*Amelanchier alnifolia* Nutt.). J. Agric. Food Chem. 55, 10414–10424.

Pennington, D., Potting, J., Finnveden, G., Lindeijer, E., Jolliet, O., Rydberg, T., Rebitzer, G., 2004. Life cycle assessment part 2: current impact assessment practice. Environ. Int. 30, 721–739.

Pisanello, D., 2014. Chemistry of Foods: EU Legal and Regulatory Approaches. Springer, Cham, ZG, Switzerland.

Potterat, O., 2010. Goji (*Lycium barbarum* and *L. chinense*): phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. Planta Med. 76, 7–19.

Prabu, S.L., Suriyaprakash, T., Dinesh, K., Suresh, K., Ragavendran, T., 2012. Nutraceuticals: a review. Elixir Pharmacy 46, 8372–8377.

Rates, S.M.K., 2001. Plants as source of drugs. Toxicon 39, 603–613.

Reganold, J.P., Glover, J.D., Andrews, P.K., Hinman, H.R., 2001. Sustainability of three apple production systems. Nature 410, 926–930.

Rice-Evans, C., Miller, N., Paganga, G., 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. 2, 152–159.

Roberfroid, M.B., 2002. Global view on functional foods: European perspectives. Br. J. Nutr. 88, S133–S138.

Rodriguez-Amaya, D.B., 2001. A Guide to Carotenoid Analysis in Foods. ILSI Press, Washington DC.

Sadia, H., Ahmad, M., Sultana, S., Abdullah, A.Z., Keat Teong, L., Zafar, M., Bano, A., 2014. Nutrient and mineral assessment of edible wild fig and mulberry fruits. Fruits 69, 159–166.

Sánchez-Salcedo, E.M., Mena, P., García-Viguera, C., Martínez, J.J., Hernández, F., 2015. Phytochemical evaluation of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry fruits, a starting point for the assessment of their beneficial properties. J. Funct. Foods 12, 399–408.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K., Latha, L.Y., 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr. J. Tradit. Complement. Altern. Med., 8.

Scalbert, A., Johnson, I.T., Saltmarsh, M., 2005. Polyphenols: antioxidants and beyond. Am. J. Clin. Nutr. 81, 215S–217S.

Schau, E.M., Fet, A.M., 2008. LCA studies of food products as background for environmental product declarations. Int. J. Life Cycle Ass. 13, 255–264.

Shahidi, F., Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects: a review. J. Funct. Foods 18 (Part B), 820–897.

Shahidi, F., Zhong, Y., 2015. Measurement of antioxidant activity. J. Funct. Foods 18 (Part B), 757–781.

Smith, R.M., 2003. Before the injection: modern methods of sample preparation for separation techniques. J. Chromatogr. A 1000, 3–27.

Smithers, G.W., 2016. Food Science: Yesterday, Today, and Tomorrow. Reference Module in Food Science. Elsevier, Amsterdam, The Netherlands.

Spinelli, R., Schweier, J., De Francesco, F., 2012. Harvesting techniques for non-industrial biomass plantations. Biosyst. Eng. 113, 319–324.

Srivastava, S., Sharma, P.K., Kumara, S., 2015. Nutraceuticals: a review. J. Chronother. Drug. Deliv. 6, 1–10.

Steinmann, D., Ganzera, M., 2011. Recent advances on HPLC/MS in medicinal plant analysis. J. Pharm. Biomed. Anal. 55, 744–757.

Stushnoff, C., 1991. *Amelanchier* species. Acto Hortic. 290, 549–568.

Tabart, J., Franck, T., Kevers, C., Pincemail, J., Serteyn, D., Defraigne, J.-O., Dommes, J., 2012. Antioxidant and anti-inflammatory activities of *Ribes nigrum* extracts. Food Chem. 131, 1116–1122.

Tabart, J., Kevers, C., Evers, D., Dommes, J., 2011. Ascorbic acid, phenolic acid, flavonoid, and carotenoid profiles of selected extracts from *Ribes nigrum*. J. Agric. Food Chem. 59, 4763–4770.

Tibaldi, G., Fontana, E., Nicola, S., 2011. Growing conditions and postharvest management can affect the essential oil of *Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart. Ind. Crops Prod. 34, 1516–1522.

Vegvari, G., Brunori, A., Sandor, G., Jocsak, I., Rabnecz, G., 2008. The influence of growing place on the rutin content on *Fagopyrum esculentum* and *Fagopyrum tataricum* varieties seeds. Cereal Res. Commun. 36, 599–602.

Sansavini, S., Costa, G., Gucci, R., Inglese, P., Ramina, A., Xiloyannis, C. (Eds.), 2012. General Arboriculture. Pàtron Editore, Bologna.

Weidema, B.P., Thrane, M., Christensen, P., Schmidt, J., Løkke, S., 2008. Carbon footprint. J. Ind. Ecol. 12, 3–6.

Whitman, M.M., 2001. Understanding the perceived need for complementary and alternative nutraceuticals: lifestyle issues. Clin. J. Oncol. Nurs., 5.

Younesi, E., Ayseli, M.T., 2015. An integrated systems-based model for substantiation of health claims in functional food development. Trends Food Sci. Technol. 41, 95–100.