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Green Extraction: From Concepts to Research, Education, and Economical Opportunities

Farid Chemat, Natacha Rombaut, Anne-Sylvie Fabiano-Tixier, Jean T. Pierson, and Antoine Bily

1.1

Introduction

One of the principal aims of sustainable development of green processing is in the teaching of green chemistry in colleges, high schools, and academic laboratories of both developed and developing nations. The researchers from academia and professionals from industry have chosen not to ignore the potential consequences of green chemistry and processes and have realized that they have responsibilities in education, research, and acceptance for industrial implementation of green chemistry (analysis, extraction, synthesis, separation, etc.) [1]. They recognize that their research will affect the future of the planet with the creation of new products and processes that improve the quality of life and reduce environmental hazards [2–4]. The implementation of green chemistry technologies minimizes the use of materials that are hazardous to human health and environment [5], decreases energy and water usage, and maximizes efficiency (Figure 1.1).

Extraction of natural products has been used probably since the discovery of fire. Egyptians and Phoenicians, Jews and Arabs, Indians and Chinese, Greeks and Romans, and even the Mayans and Aztecs, all possessed innovative extraction processes (maceration, alembic distillation, etc.) used even for perfume, medicine, or food. However, during the 1990s, it was not easy to find literature concerning the dispersed efforts for greening the extraction practices. It was necessary to wait for the tremendous development of green chemistry made by the Environmental Protection Agency (EPA) and led by Paul Anastas [3], who published a series of fundamental books from 1994 trying to create a general conscience on the need for green chemistry.

Recent trends in extraction techniques have largely focused on finding solutions that minimize the use of solvent and energy, such as supercritical fluid extraction, ultrasound extraction, subcritical water extraction, controlled pressure drop process, pulse electric field, and microwave extraction. The tremendous efforts made in greening extraction processes can be evaluated through the consideration of books devoted to these aspects as can be seen in Figure 1.2. Theoretical and

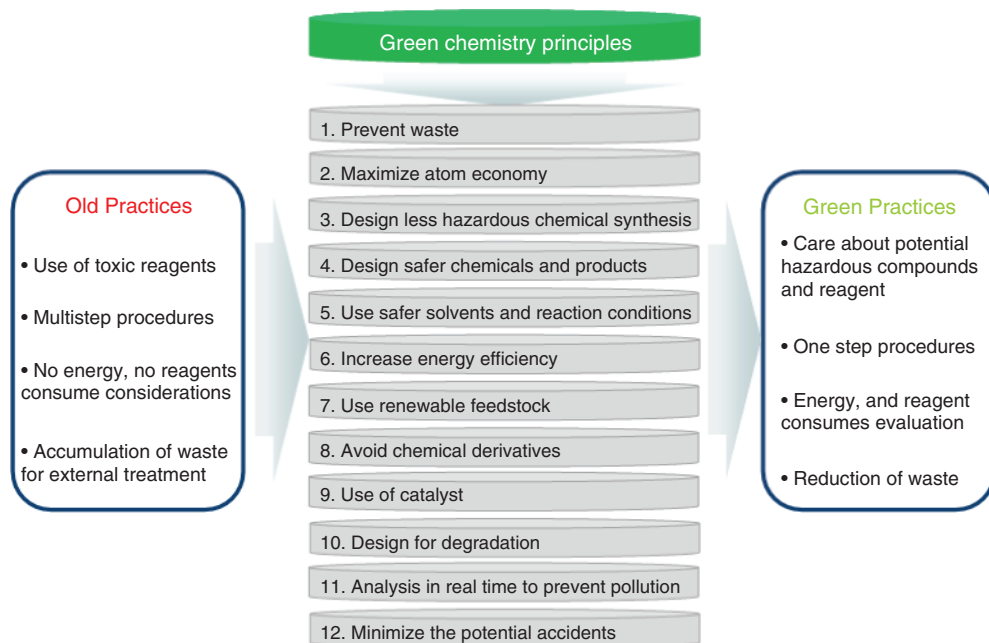
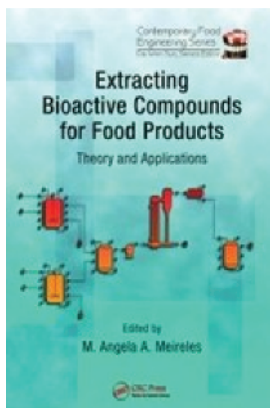


Figure 1.1 Impact of green chemistry in changing industrial and academic practices.

practical efforts are absolutely necessary to convince the members of the chemical societies about the need for such a revolution in our mentality and practice.

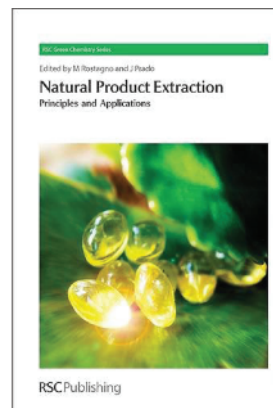
To meet the requirements of the market and to abide by the regulations in force, any extraction process must meet a number of quality criteria, contrary to some popular misconceptions; the “natural” state of an extract is no guarantee of its harmlessness to humans and the environment. In such a changing context, we must now switch over from a simple interest in data analysis to interest in models to a strong consideration of the environmental side effects of our practices as a consequence of the high demands of the extraction processes. This evolution or revolution in the extraction of natural products is summarized in Figure 1.3. Green extraction of natural products could be a new concept to meet the challenges of the twenty-first century, to protect both the environment and consumers, and at the same time enhance competition among industries to evolve more ecologic, economic, and innovative methods. Within the green extraction approach, the concept of the green extract is that of an extract obtained in such way as to have the lowest possible impact on the environment (lower energy and solvent consumption, etc.) and whose eventual recycling would have been planned for (coproducts, biodegradability, etc.). The green extract should be the result of a whole chain of values in both senses of the term, that is, economic and responsible, starting from the production and harvesting of the plant, the transformation process of extraction, and separation together with formulation marketing.



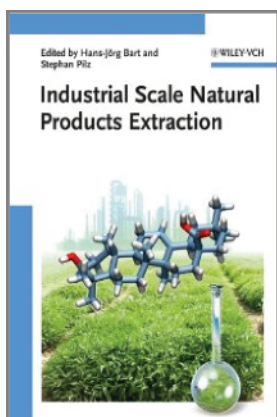
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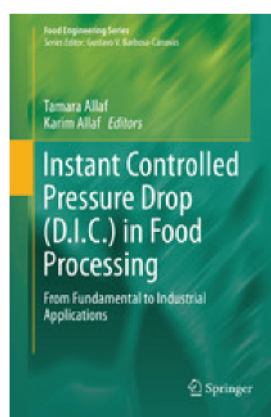
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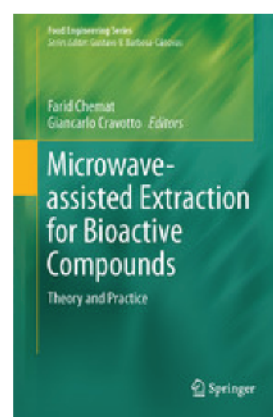
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Figure 1.2 Books devoted to green extraction of natural products.

Various industrial sectors, such as pharmaceutical, cosmetic, and food industries have increasing needs in natural products. They are obtained from plant or animal resources through extraction processes. In order to meet demand and fulfill regulations, the extraction processes are challenged to increase extraction efficiency (yield and selectivity toward compounds of interest), reduce or eliminate petrochemical solvents, together with moderate energy consumption. Within

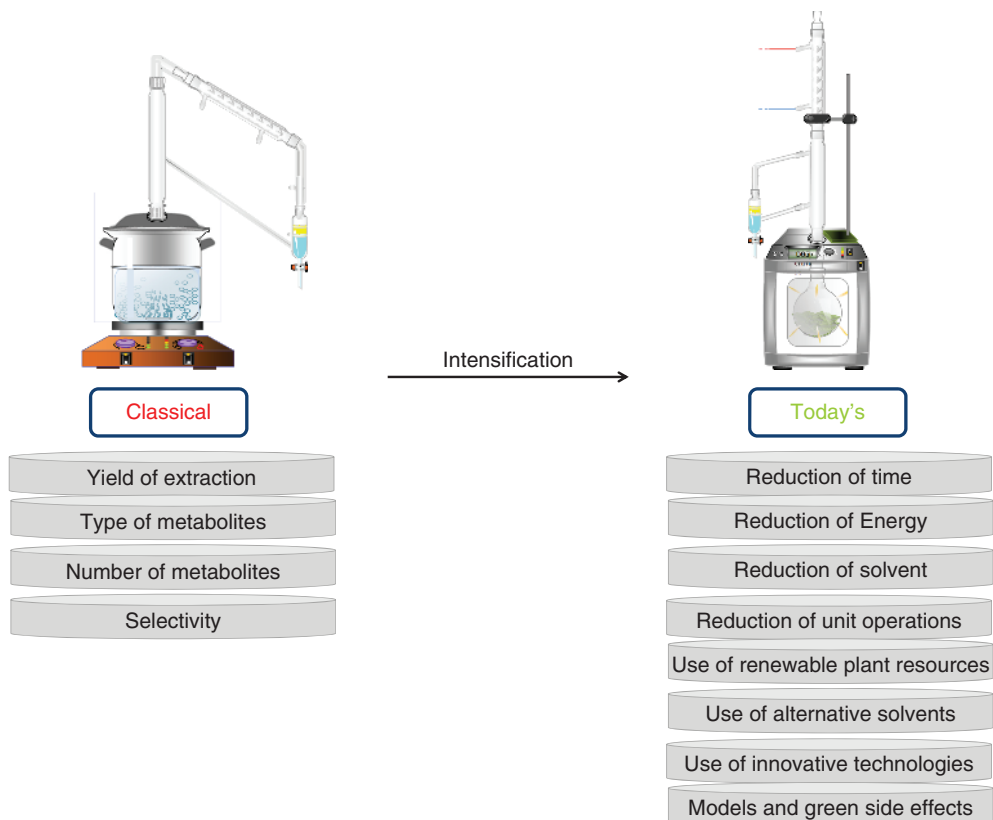


Figure 1.3 Extraction of natural products: evolution or revolution.

these constraints, green extraction has been recently introduced on the basis of green chemistry and green engineering further referring to modern “sustainable processes.” In relation to green extraction of natural products, its definition has been given by Chemat *et al.* [2]: “*Green Extraction is based on the discovery and design of extraction processes which will reduce energy consumption, allows use of alternative solvents and renewable natural products, and ensure a safe and high quality extract/product.*” The listing of the six principles of green extraction of natural products should be viewed for industry and scientists as a guideline to establish an innovative and green label, charter, and standard, and as a reflection to innovate not only in process but in all aspects of solid–liquid extraction. The principles have been identified and described not as rules but more as innovative examples to follow, discovered by scientists and successfully applied by industry.

- *Principle 1:* Innovation by selection and use of varieties of renewable plant resources
- *Principle 2:* Use of alternative solvents, principally water or bio-based solvents

- *Principle 3:* Reduction in energy consumption by energy recovery, using innovative technologies
- *Principle 4:* Manufacture of coproducts instead of waste to include the bio- and agro-refining industries
- *Principle 5:* Reduction in unit operations, favoring safe, robust, and controlled processes
- *Principle 6:* Aiming for a non-denatured and biodegradable extract without contaminants.

This chapter aims at specifying the notion of natural products and illustrating how natural products can be used as ingredients in different industrial sectors. For this, we limit ourselves to compounds obtained from the plant kingdom. The biomolecule composition of a vegetable cell is usually divided into two major groups: primary and secondary metabolites. The former refers to key compounds in plant metabolism, and the latter to compounds which are involved in specific functions in the plant. Croteau *et al.* [6] introduced the term *plant natural product* to designate secondary metabolites, due to their biological activity and role in plant ecology. The expression “*natural product*” is today broadly used to designate extracts obtained from plants containing specific compounds, possessing a technological, functional, or nutritional application [7, 8]. Using the example of the orange tree as a veritable biorefinery, a few valuable metabolites are evidenced. The chemical structure and diversity of the different classes of metabolites in the plant kingdom are then reviewed. Some applications of natural products as ingredients are ultimately presented.

This chapter also presents as ultimate examples, the successful application of green extraction of natural products in academia as a vector of green teaching and research and also in industry, presenting a continuous challenge for innovation and competitiveness.

1.2

Orange Fruit is not Limited to Produce Only Juice?

The pattern from metabolites to ingredients can be illustrated through the example of the orange tree. The major industrial use of oranges is juice production. However, the known diversity of the phytochemical composition of the fruit and the orange tree allows considering the tree as a biorefinery of natural products. The following section describes the by-product valorization of orange juice production along with some examples of the phytochemicals identified in the orange tree and fruit.

With a total production of 68 million tons in 2012 [9], orange is one of the major crops in the processing industry. Out of the total production, 95% is used to manufacture orange juice. Considering that 3 kg of oranges are necessary to produce 1 l of orange juice, there is a great potential for valorization of the by-products generated [10]. Further, not only the by-products from the fruits but also the whole

orange tree can be used for nutritional, pharmacological, or cosmetic purposes. In this section, the pathways for valorization of orange products are described. Different types of by-products can be generated from orange juice production: those from the orange tree and those from the orange itself. By-products generated by orange juice production are pulps, peels, and seeds [10, 11].

One of the main by-products obtained from the orange fruit is the pulp (42.5% w/w of orange waste) [9, 12]. Owing to their high fiber content, pulps are mainly used for livestock feeding. Moreover, inclusion of the pulp in cattle feed would support growth and lactation in a better way than would starch-rich supplements [13].

Accounting for 50% of the mass of the fruit, orange peels contribute to a high proportion of the by-products [11]. Peels can be processed by steam distillation [14] or by cold pressing [15] to extract essential oil. Essential oil is used in foods and beverages as a flavoring agent, in cosmetics, and in perfumes [16]. Peels also contain some valuable chemicals, for example, pectin [17], which used as a texturing agent and food stabilizer. Pectin can be incorporated in foods such as jam or jellies. Peels contain structural components such as cellulose [18] and hemicellulose. After biorefining, these molecules can be transformed into biofuels and biochemicals. The juice extracted from the peels is evaporated to give citrus molasses [19], marketed for livestock feeding [13], or is used in the fermentation process of alcohol. Further use of this by-product requires more refining as the phytochemicals in the juice contribute to a strong bitterness and dark color. As a refined product, orange peel syrup can be used as natural sweetener. Orange seeds represent a small part of the residue from orange juice production (from 0.5 to 5%). The main interest of the seeds is in their oil content (up to 40%). Extraction of orange seed oil has been performed by organic solvents [20, 21]. However, because the high oil content of the seeds, mechanical pressing could perhaps be used to recover oil.

Flowers, leaves, and the lignocellulosic biomass have found various applications. Flavoring substances such as essential oils, namely, Neroli and Petitgrain, are extracted from the flowers and leaves (less than 1% content [22]), respectively. Oils can be used in the cosmetics and food industries [23, 24]. It is noteworthy that the water distilled from flowers and leaves after the extraction of essential oils (orange blossom water and eau de brouts) can also be valorized in the same way as essential oils because of their aromatic characteristics. The lignocellulosic biomass issued from roots, twigs, and leaves can also be valorized by the production of biochemicals and/or biofuels [25, 26]. These inedible parts of the tree also contain some alkaloids, which may be used in the pharmaceutical industry.

The numerous uses of orange by-products are due to the phytochemical composition of the fruit and the tree. The following section examines the structure and localization of these natural products. The section focuses on the identification of the chemical structure of the phytochemicals of interest contained in the orange tree. Their localizations and structures are illustrated in Figures 1.4 and 1.5. The variety and structural complexity of these phytochemicals enables their

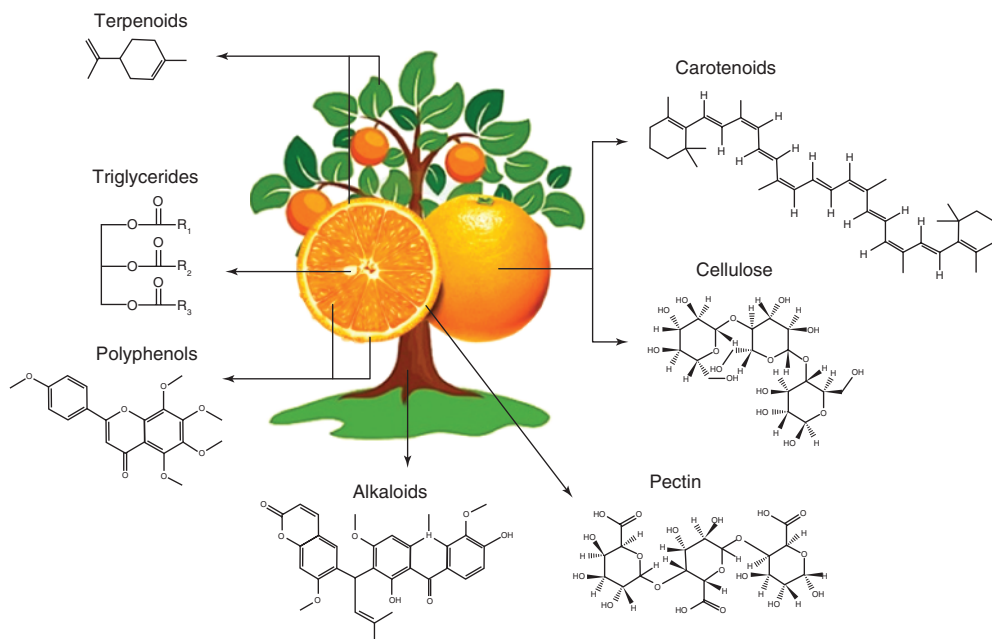


Figure 1.4 Localization and structure of phytochemicals contained in the orange tree and fruit.

classification according to their biochemical function into primary and secondary metabolites.

Two types of polymers issued from primary metabolites are constitutive of the by-products detailed in the previous section: cellulose and pectin. Cellulose, which is polymer of cellobiose, is a structural cell component [27] and, therefore, is present in the whole tree. Pectin would be located more specifically in the peel of the fruit. The lipidic fraction is mainly found in the seeds of oranges. Although seeds have a high oil content, the low amount of seeds in the orange fruit leads to an overall low lipidic fraction taking the whole tree into consideration.

Three common classes of secondary metabolites are identified in the composition of the orange tree: terpenoids, alkaloids, and polyphenols. Terpenoids are constitutive of essential oils and those found in oranges (Neroli and Petitgrain oils) have different terpenic profiles. Neroli oil is composed of linalool, limonene, beta-pinene, *trans*-beta-ocimene, linalyl acetate, and terpineol, while Petitgrain oil is mostly composed of linalyl acetate and linalool [23, 24, 28]. Orange peels also contain a terpenic fraction, which is exclusively composed of limonene. The high concentration of limonoids were identified at first in the fruits of the the Rutaceae family (e.g., *Citrus*), however those compounds were further identified in other plants [29, 30]. Carotenoids are another terpenoid class identified in the orange tree: β-cryptoxanthin, β-carotene, lycopene, and isomers of violaxanthin were detected, more specifically, in the fruit [31]. Alkaloids are located in the

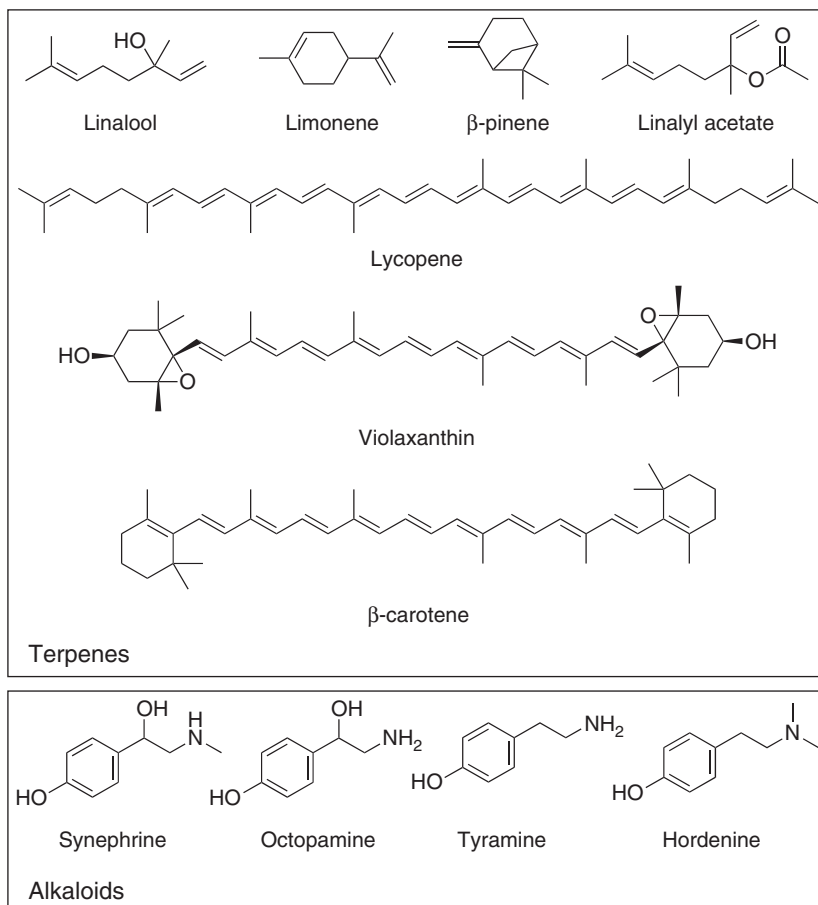


Figure 1.5 Structure of secondary metabolites (terpenes and alkaloids) contained in the orange tree.

leaves and the roots of the orange tree [32]. Owing to their biological activity, these compounds are of interest in pharmaceutical applications. Synephrine, which is an adrenergic and used for weight management, has been identified in the orange tree [33]. It is mainly isolated from citrus species and commonly found in juices. Although synephrine can be synthesized, the increasing demand for natural extract makes this alkaloid an important extraction target. Other alkaloids commonly reported from *Citrus aurantium* are octopamin, tyramine, *N*-methyltyramine, and hordenine [34]. Polyphenols are the third class of secondary metabolites found in the orange tree. Phenolic compounds identified in the orange fruit (located more specifically in peel and juice) are essentially flavanone glycosides such as nariginin, hesperidin, narirutin, and neohesperidin [35]. These polyphenols are specific of the *Citrus* family, and are of increasing

interest because of their antioxidant potential. In a less specific occurrence, lignin, a structural component mostly found in the membranes of vegetable cells, is found in the whole orange tree.

This introductory example of the orange tree, through the valorization of by-products generated from orange juice production, allows us to distinguish different categories of molecules considered as natural products. The following section summarizes the categories of metabolites that can be identified in vegetables, aiming at a classification of natural products.

1.3

Chemistry of Natural Products

The term *natural product* designates any substance or chemical produced by nature. Considering vegetables, this wide definition can be restricted to any chemical vegetable component or phytochemical. In biochemistry, phytochemicals are commonly classified into two groups according to their function: primary and secondary metabolites. Primary metabolites include any compound essential to basic plant metabolism and thus survival. On the other hand, secondary metabolites are involved in ecological functions such as attractants for pollinators, in the protection against herbivorous and microbial infections, and in allelopathy (which refers to a positive or negative effect, e.g., the growth of one plant into another induced by phytochemicals). In this section, the well-known primary metabolites are briefly reviewed, focusing on secondary metabolites.

1.3.1

Primary Metabolites

1.3.1.1 Glucides

Glucides are metabolites mainly used for the storage and transport of energy and are located in every organ of a plant [27]. In cells, glucides are stored as starch which is a polymer of glucose. Glucides are also metabolic precursors of secondary metabolites and are constitutive of cell membranes. For instance cellulose, a macromolecule composed of a repetition of glucose moieties, is one of the main components of vegetable cell membranes. It is one of the most abundant compounds on the Earth.

Owing to their numerous functions in basic plant metabolism, glucides constitute a wide class of metabolites. The classification, chemistry, and structure of glucides are described in many biochemistry reference books [36, 37], therefore, their diversity is not reviewed in this section, but examples are given of glucides having industrial applications. Glucides are divided into two classes: oses and osides, which are polymers of oses or a combination of oses with a non-glucidic molecule, respectively [38].

Oses classically processed in industry are fructose, glucose, and oses derivatives such as polyols. One of the most-extracted oside is saccharose (e.g., isolated

from sugar beet and sugar cane) [38]. The main polysaccharides which can be extracted from vegetables are fructosanes such as insulin, starch, cellulose, fibers, mucilages, and pectins [38]. These macromolecules can be used as such for food or nonfood application, or can also be chemically transformed to produce chemicals or ingredients (e.g., starch is the starting point of modified starches, dextrans, glucose syrups, glucose, and sorbitol). Fibers are a group mainly composed of cellulose, hemicellulose, pectins, and lignins; because of their indigestibility, these compounds qualified as “dietary fibers” [38]. Mucilages are highly hydrophilic colloids and can be used for their texturing properties (e.g., in pharmaceutical applications). Pectins are mostly located in fruits (apples, citrus fruits) and are structural macromolecules that constitute cell walls. They can be used for their jellifying properties [38].

1.3.1.2 Lipids

Similar to glucides, lipids have an energy storage function [27], and are the precursors of numerous metabolites. Lipids are a group of heterogeneous compounds (fatty acids, glycerolipids, cerides, etc.). In plants, lipids are stored in oleosomes as oils, which are mainly composed of triacylglycerols (esters of glycerol and fatty acids) [27]. Some lipid structures are illustrated in Figure 1.6.

Fatty acids are a large group composed of linear saturated and unsaturated hydrocarbonated chains with a carboxylic acid function. Commonly found saturated fatty acids range from butyric (C4) to arachidic fatty acids (C20) [39]. Longer chains exist and are synthesized through the same metabolic pathway by elongation, but are less common in the plant kingdom. Unsaturated fatty acids are classically produced by the addition of double bonds to the corresponding saturated fatty acids [8]. The number of unsaturations and their position along the carbon chain are used to classify unsaturated fatty acids. Palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), and arachidonic (C20:4) fatty acids are among the commonly found unsaturated fatty acids [27].

Through the combination of fatty acids with primary metabolites present in plants (e.g., glycerol), complex compounds are formed (mono-, di-, and triglycerides), such as glycolipids, glyceroglycolipids, phospholipids and phosphosphingolipids (based on sphingosine structure) [40], or aromatic polyketides such as anthraquinones [41] and naphthoquinones [42].

1.3.1.3 Amino Acids and Proteins

Amino acids are of great importance in metabolism, as they are the building blocks of enzymes, peptides, and structural proteins. Amino acids are also precursors of numerous secondary metabolites (alkaloids, betalains, some polyphenols, etc.) [38]. Proteins play a major role in human and animal nutrition. However, the applications as the final product or extract composed specifically of amino acids, proteins, and enzymes are quite limited, and therefore are not developed in this section.

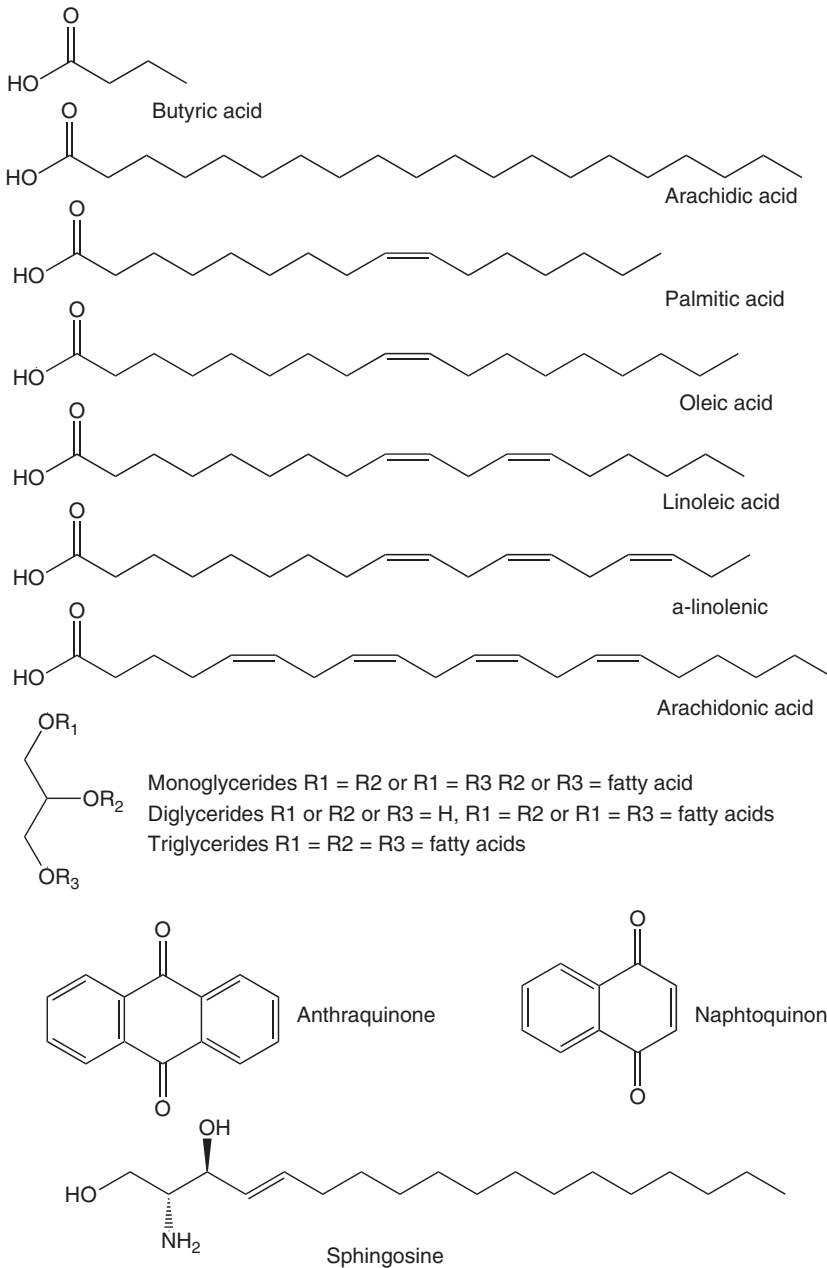


Figure 1.6 Chemical structure of some lipids.

1.3.2

Secondary Metabolites

Secondary metabolites are involved in the development of specific characteristics of the plant such as color and flavor (e.g., flowers), therefore, playing a role in the ecological functions of a vegetable [6]. Three types of phytochemicals are classified as secondary metabolites: terpenoids, alkaloids, and phenylpropanoids with allied phenolic compounds [6]. Since secondary metabolites are involved in specific functions, their number and diversity will depend on the plant being considered.

1.3.2.1 Terpenoids

Terpenoids cover a great range of diverse compounds: up to 25 000 terpenoids have been identified [6]. Because of their flavoring properties, terpenoids are mainly known as major components of essential oils [43]. Isoprenoids are the building blocks of the terpenoid family and are synthesized through either the mevalonate pathway or the deoxyxylulose pathway [44]. Terpenoids are classified according to the number of isoprene units. The main classes are listed in Table 1.1, with some structures represented in Figure 1.7.

The tetraterpene carotenoid family contains the carotenoid and the xanthophyll family, which are major pigmentation components and which have also been found to possess nutraceutical properties. Modifications of these terpenoid classes lead to specific subclasses of compounds found to be bioactive principals of certain plant varieties. Some of the terpenoids of interest are listed in the following. Ginkgolids such as Ginkgolide B are highly modified diterpenes from the exclusive *Ginkgo biloba* species [45]. Steviosides are steviolglucosides (tricyclic diterpene), which are of high interest in the sweetener industry [46]. Limonoids like limonin, are degraded triterpenoids called tetranortriterpenoids due to the removal of four carbons from a side chain [30]. Quassinoids such as bruceantin, are another example of triterpenoids, which have lost 10 carbons and are not to be confused with diterpene structures [47]. Steroids are tetracyclic-modified triterpenoids, which represent a class of bioactive compounds. Their typical structure is based on the cholesterol structure and the main sterols in plants are phytosterols such as stigmasterol, sitosterol, fucosterol, ergosterol, and campesterol [48].

Table 1.1 Classification and examples of terpenoids according to the number of isoprene units.

Category	Example of terpenoid groups
Monoterpene (C10)	Linalool (acyclic), limonene (cyclic), pinene (bicyclic)
Sesquiterpene (C15)	Farnesol (acyclic), bisabolene (cyclic), α -eudesmol (polycyclic)
Diterpenes (C20)	Phytol (acyclic), retinol (cyclic), taxadiene (polycyclic)
Sesterpene (C25)	Geranylarnesol
Triterpene (C30)	Sapogenin (polycyclic)
Tetraterpene (C40)	β -carotene (red pigment), xanthophyll (yellow pigment)

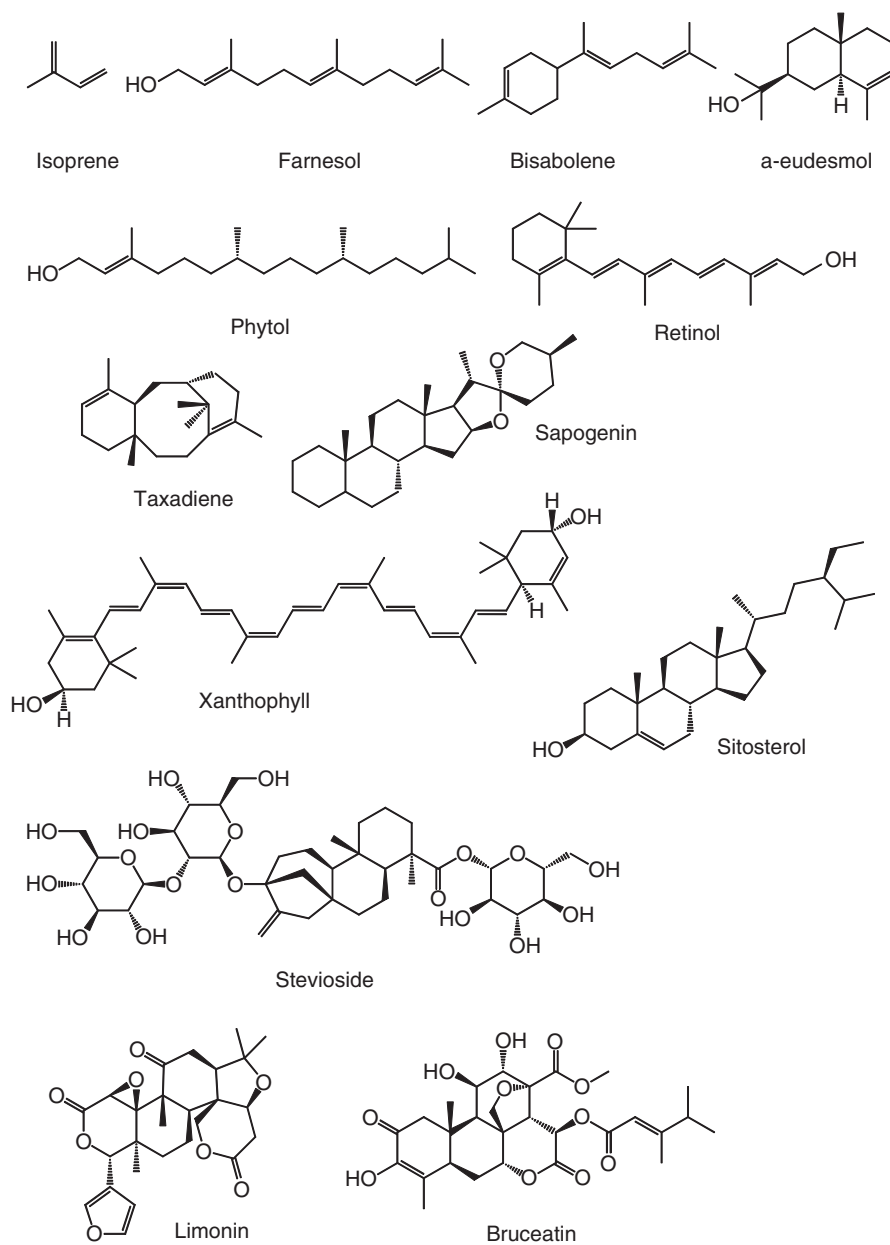


Figure 1.7 Examples and chemical structure of some terpenes.

1.3.2.2 Alkaloids

In general, alkaloids are defined as nitrogen-containing compounds. They possess strong pharmacological effects which results in a close monitoring of edible products that may contain such compounds [49]. Alkaloids are biosynthesized starting from various amino acids and it is easier to classify them regarding to their precursor, rather than based on their structure [8]. Examples of alkaloid chemical structures are indicated in Figure 1.8.

Alkaloids originating from L-glutamate transformed into L-ornithine contain the pyrrolidine ring system as well as the tropane ring system, which is found in cocaine [39], atropine [41], or scopolamine [49]. The pyrrolizidine ring system is another structure derived from the L-arginine pathway. The second group of alkaloids derives from the L-lysine amino acid which forms the piperidine core structure. One of the best-known alkaloids based on this structure is the piperine found in black pepper [50]. But L-lysine is also precursor to the quinolizidine structure [51], which is not found in common fruits and vegetables but can potentially exist in more specific ethnic vegetables at some trace levels. The same goes for alkaloids derived from the indolizidine structure [52]. Pyridine alkaloids, as the vitamin B family, are more widespread in the human diet (e.g., niacin) [53], contain such a core structure and originate, from the amino acid aspartic acid. Alkaloids obtained from tryptophane can be built with the indole core structure (tryptamine [54], harmine [55], serotonin [56]), and more rarely, as pyrroloindole alkaloids or derived into ionic compounds like betalains [57], for example, betanin. The biosynthetic pathway of betalains, however, suggests that its synthesis starting from a tyrosine and not from tryptophan.

Anthranilic acid, as intermediates to tryptophane, can also lead to alkaloids found in nature (quinazoline, quinoline, and acridine) [58] but none of importance in fruits and vegetables. The same goes for the imidazole-structured alkaloids, which are derived from histidine [59]. The last alkaloid family we report are the purine-derived alkaloids, which are extremely well known for their occurrence and wide consumption [60]. They are mostly represented by caffeine, theobromine, and theophylline. All other alkaloids are generally combinations of the pure alkaloids described here with combination into other families such as terpenoid alkaloids (taxol) [2] or steroidal terpenoids (tomatidine) [61].

1.3.2.3 Polyphenols

Polyphenols are well known as nutrients from nature, because of their ubiquitous occurrence; and their pharmacological significance makes them major research subjects in the nutraceutical sciences [62–66]. As they have been reviewed thoroughly by different authors, we briefly recall the main classes of polyphenols.

Phenolic acids constitute a wide class of polyphenols. The simplest phenolic acids contain a carboxylic acid function with one or more hydroxyl substitutions on the benzene ring (Figure 1.9). The most common phenolic acids are gallic acid, used as is or as an ester (galloyl), chlorogenic acids such as 4-chlorogenic acid, and feruloylquinic acids such as 4 feruloyl-D-quinic acid, which originate from *trans*-cinnamic acid [67]. Other commonly found phenolic acids are differently

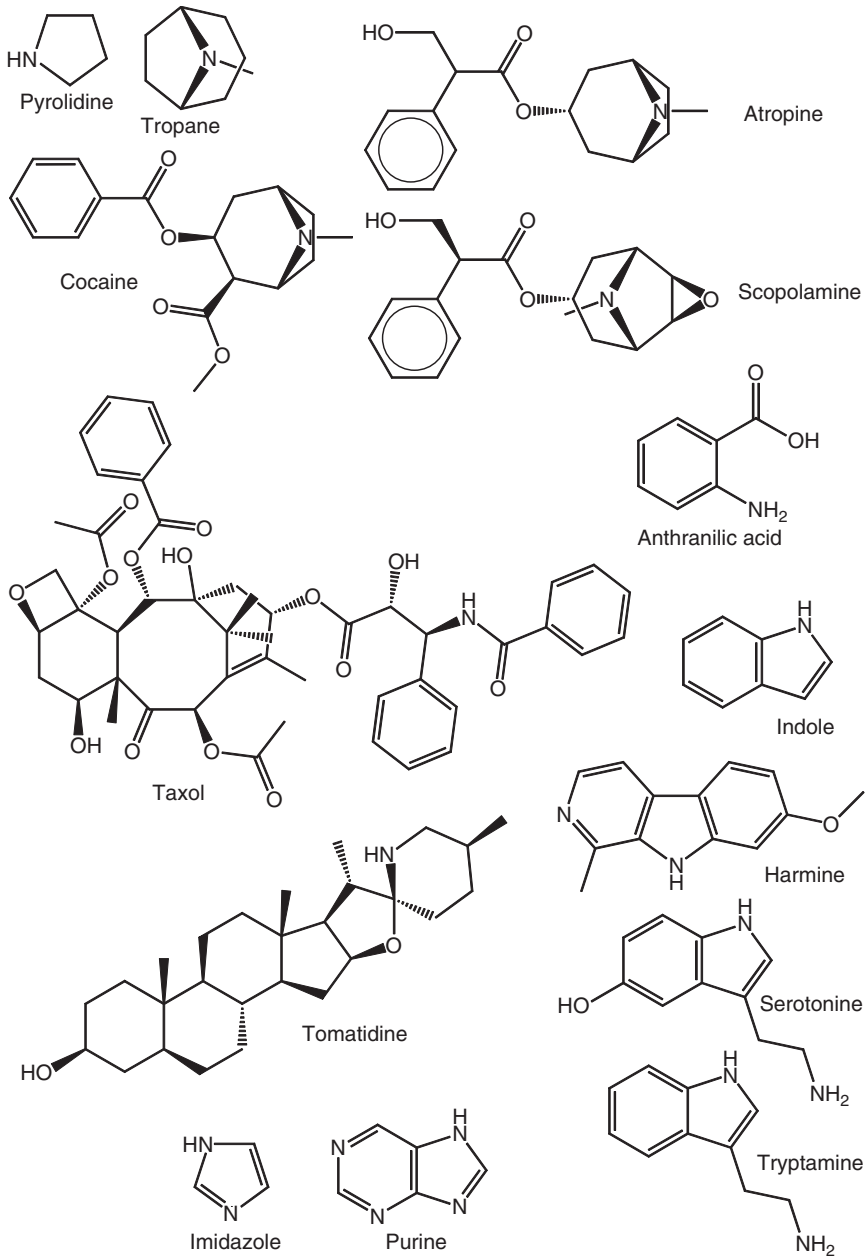


Figure 1.8 Examples of alkaloid structures and molecules.

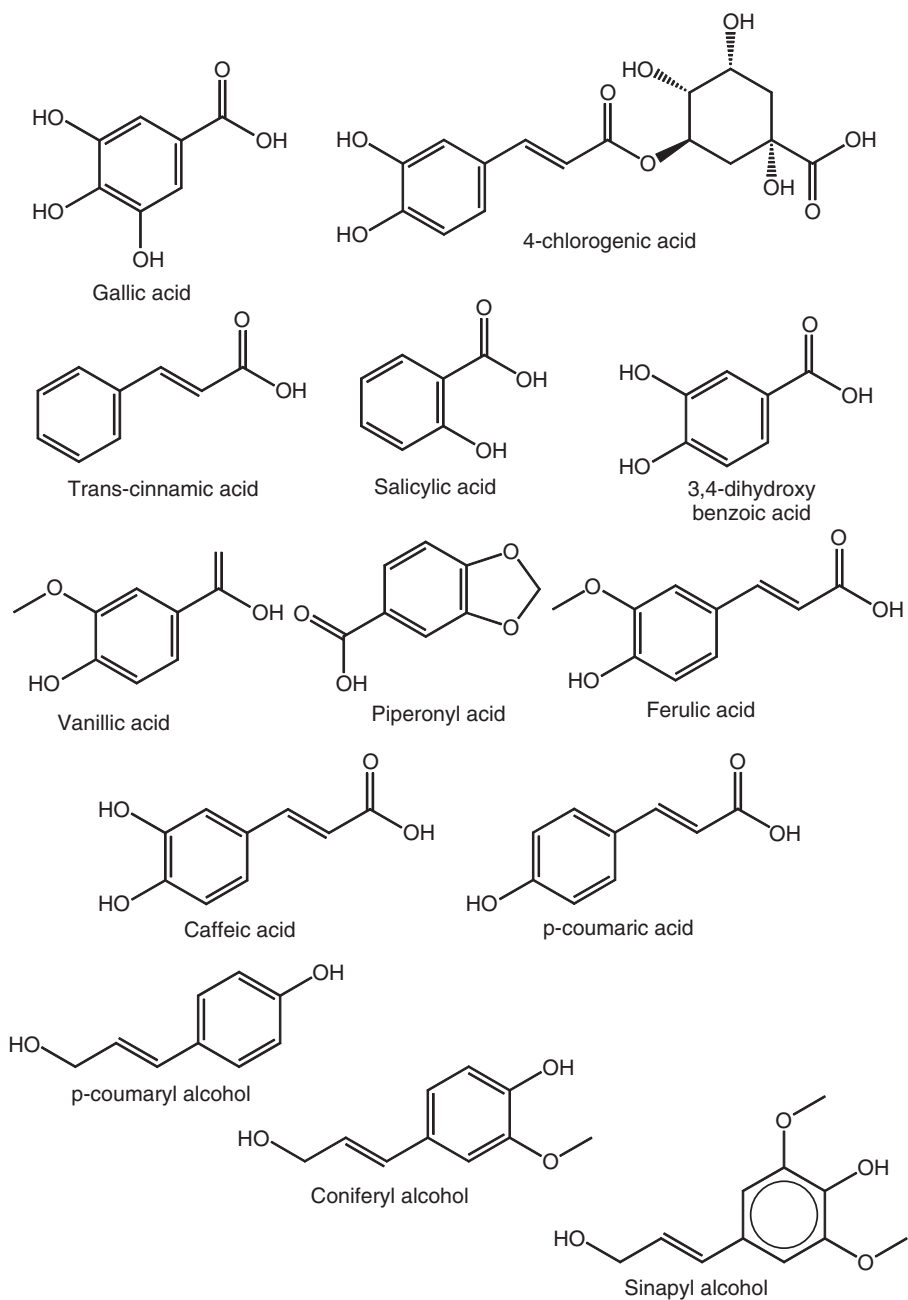


Figure 1.9 Examples of polyphenol structures (phenolic acids).

substituted ones, including hydroxybenzoic acids such as salicylic acid [68], hydroxycinnamic acids such as 3-coumaric acid [69], protocatechuic acids such as 3,4-dihydroxybenzoic acid, vanillic and piperonylic acids [70], and cinnamic derivatives such as, ferulic, caffeic, and *p*-coumaric acids [62]. Owing to their early occurrence in the main pathways for the biosynthesis of phytochemicals, they are quite ubiquitous and well known either as stand-alone compounds or as reagents in the food industry and in nutraceutical supplements [71].

More complex structures based on phenolic acids possess longer chain substitution, extending the range of phenolic acids (Figure 1.10). These compounds are hydrolyzable tannins, which are gallic acid esters with a glucose central structure. Tannic acid [72] is a good example of highly substituted hydrolyzable tannin, yielding 10 gallic acid moieties per glucose moiety. Their chemical composition and geometry make hydrolyzable tannins class an extremely diverse family.

Apart from the hydrolyzable tannins, the largest family of polyphenols is the flavonoids [73–75]. They are characterized as containing a benzopyrone moiety as central core structure. The family is subdivided into the following subfamilies: coumarins, flavones, isoflavones, neoflavones, and all subsequent derivatizations of those core structures. All of them have been isolated from nutritionally relevant sources. Flavones contain the following groups of compounds: flavonol, flavanol, flavanone, and flavanonol [76–78]. Flavonol dimers or trimers also lead to the proanthocyanidin group [79]. And finally, the very specific group of the anthocyanins (as the aglycone core structure of the anthocyanidins) is an ionic version of the flavones with the chemical specificity of the flavyllium core structure [62, 71]. Other compounds that are part of this greater family are the xanthenes [80, 81] and the stilbenes [82], which are present in specific genus and families of edible plants.

Apart from the mentioned polyphenol classes, structural polymerized polyphenols are constitutive of cells. Lignin is an important part of the secondary cell wall for plants and accounts for 30% of all nonfossil carbon fuel on earth [83, 84]. It is found at a higher concentration in wood and wooden structures, but as structural cell component is also located in leaves, flowers, and fruits. Lignin is a macromolecule composed of three monolignol molecules, namely, *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, all of which are methoxylated to different degrees [83].

1.4

From Metabolites to Ingredients

Utilization of plants either for medicinal, industrial, or food purposes is related to their biomolecular composition. While primary metabolites are ubiquitous to the plant kingdom, by having functions essential to plant metabolism and constituting the basis of human and animal nutrition, secondary metabolites occur at much lower concentrations levels and, because of their biological function, can be specific of a plant specie. Secondary metabolites, however, benefit from

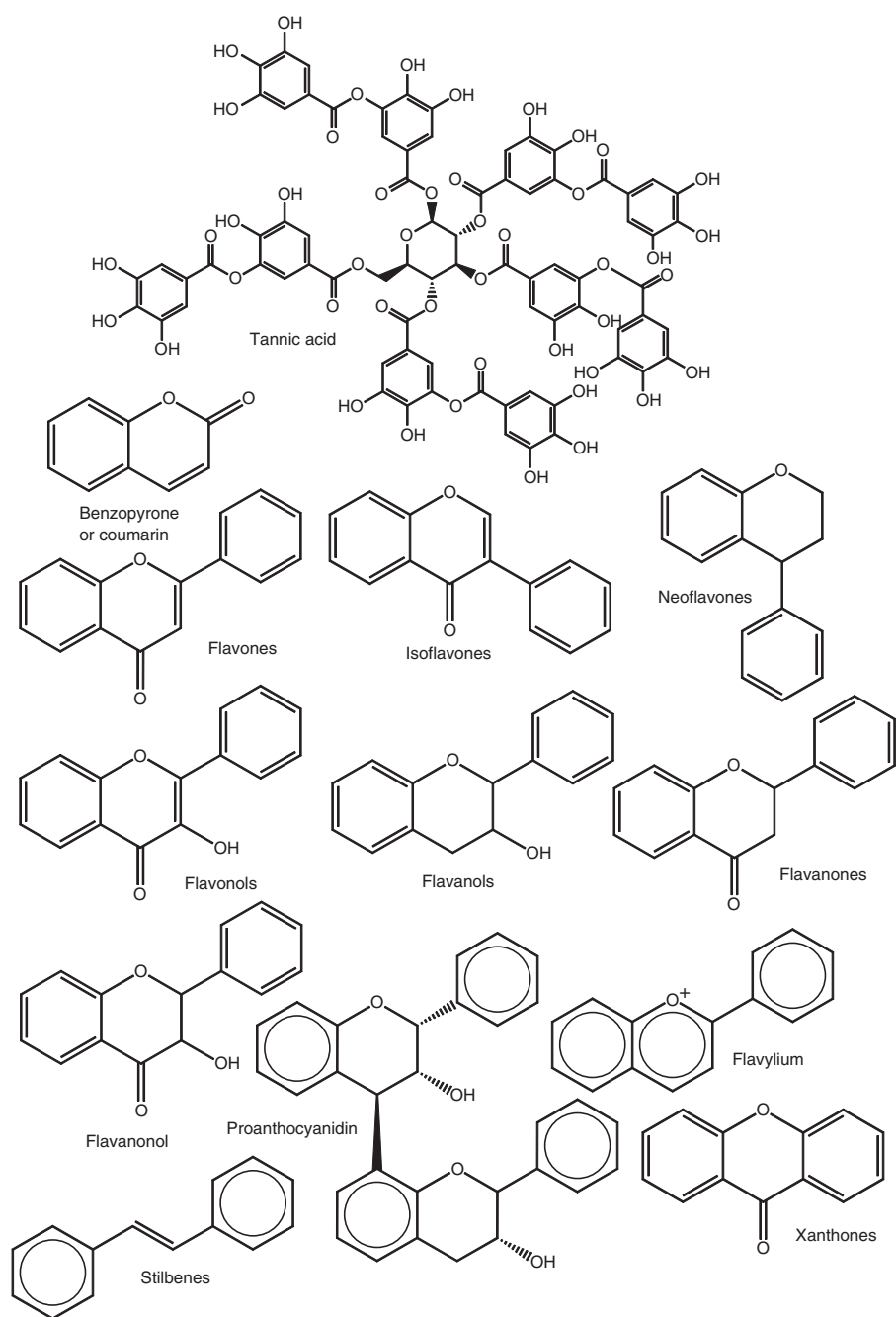


Figure 1.10 Examples of polyphenol structures (flavonoids).

a strong interest as some compounds affect biological systems [85]. This characteristic enables to qualify such compounds as bioactive [85]. Bernhoft [86] proposed a definition of bioactive compounds: secondary plant metabolites eliciting pharmaceutical or toxicological effects on humans and animals. Owing to their reactivity and chemistry, bioactives are used in numerous applications. The following section details the use of some secondary metabolites as ingredients in the pharmaceutical, nutritional, and food sectors. The uses of bioactive compounds can be classified according to their technological role: coloring agents, functional food and nutraceuticals, preserving agents, flavors, fragrances, and edible oils [7]. Some bioactive applications examples are presented in Table 1.2.

The natural coloring agent category refers to dyes, pigments, or any substance that produces color [7]. Natural colors found in nature range over yellow, orange, red, blue, and green. The bioactive compounds responsible for the production of such colors differ according to their chemical structure, physical properties (e.g., solubility), and localization in the plant (flowers, leaves, berries, etc.) [7]. All compounds have in common a chromophore (molecule-specific structure) that absorbs light in the wavelength range of the visible region known as *pigment* [100]. Some natural colorants such as chlorophylls contain metallic ions (e.g., Mg, Fe, Zn), responsible for the overall color of the complex [101]. Considering the color diversity, different major groups of chemicals were identified as responsible for coloring: carotenoids (terpens), chlorophylls, anthocyanins (polyphenols), betalains (alkaloids), and curcuminoids [100]. These groups of molecules are commercially available natural pigments and are used as alternative to synthetic pigments. However, natural colorants are less stable than the synthetic ones as regards to physical (light, temperature, and oxygen), chemical (alkalis, acids, oxidizing, and reducing agents), and biological (enzymes, microorganisms) factors [100, 101].

Flavors and fragrances may mostly be attributed to essential oils contained in aromatic plants. In industry, essential oils are formulated to produce flavor and fragrances for a wide range of end uses such as in soaps, cosmetics, perfumes, baked foods, ice creams, aerosols, syrups, and pharmaceutical preparations [102]. Essential oils are characterized by a strong odor, and appear as volatile and clear liquids. As essential oils usually have a lower density than water, they are commonly extracted by hydro or steam distillation [16]. The concentration of essential oils in plants varies between 0.01 and 15% (w/w) [7], depending on the type of plant and the part of the plant considered. Different plant organs can be used to extract essential oils: seeds (anise [103]), barks (cinnamon [104]), woods (rosewood [105]), rhizomes (ginger [106]), leaves (rosemary [107]), flowers (lavender [108]), peels (orange [87]), and roots (valerian [109]). From a chemical point of view, essential oils are a complex mixture of compounds. Terpenes are the most common compounds found in essential oils and more specifically the monoterpene subclass (90% of identified terpenoids [7]). Aromatic and aliphatic compounds of the shikimate, polyketide, and alkaloid groups are also constitutive of essential oils (for example, aldehydes, alcohols, phenols, methoxy derivatives, and methylene dioxy compounds [7, 110]).

Table 1.2 Applications in the food, nutrition, and cosmetics industries of some secondary metabolites.

Metabolites	Vegetable source	Extract	Examples of applications	References
Terpenoids				
<i>Monoterpenoids</i>				
Limonene, linalool	Genus <i>Citrus</i>	Liquid, volatile extracts	Major components of essential oils issued from the different vegetable sources, applications in cosmetics (perfumes, deodorizers, body care products) and food (flavoring) industries	[87] [88] [89] [90] [91]
Geraniol	<i>Melissa officinalis</i> L.			
Citronellol	<i>Petalagonium graveolens</i>			
Myrcene	Hops (<i>Humulus lupulus</i> L.)			
Menthol	Cornmint, peppermint			
Tetraterpenoids				
<i>Carotenoids</i>				
Bixin	<i>Bixa orellana</i> L.	Anatto: bixin-rich oil extracts, norbixin-rich extract water soluble	Yellow-red pigments, coloring agent E106b (butter, margarine, cheese, cereals, fish products)	[92]
Norbixin		Paprika: oleoresin, powder	Orange-red pigments, coloring and flavoring agent E160c	[93]
Ketocarotenoids	Red peppers (<i>Capsicum annum</i> L.)		<ul style="list-style-type: none"> • Food industry (soups, sausages, cheese, snacks, salad dressing) • Nutraceutical and pharmaceutical industries 	
Capsanthin				
Capsorubin				
Zeaxanthin				
Violaxanthin				
Antheraxanthin				

Crocin	<i>Crocus sativus</i>	Saffron	Orange-red pigments, coloring and flavoring agent (beverages, bakery, and confectionary products, curry products, soups)	[94]
Picrocrocin				
Safranal				
β -Carotene	Carrots (<i>Daucus carota</i> L.) Palm fruit	β -Carotene-rich oil-soluble extract	Food pigment, nutritional/pharmaceutical use (e.g., pro-vitamin A)	[95]
Polyphenols				
<i>Flavonoids</i>				
Anthocyanins	Grape extracts (<i>Vitis vinifera</i> L.)	Powder	From blue to red food pigments E163 (soft drinks, sugar confectionery, dairy products, fruit preservatives (jams, canned fruit)	[96]
Isoflavones	Soybeans	Powder	Use as antioxidants and nutritional applications	[97]
Lignans	Flaxseeds (<i>Linum usitatissimum</i> L.)	Powder	Nutritional applications	[98]
Alkaloids				
Caffeine	Coffee beans	Powder	Food and beverage ingredient, pharmaceutical applications	[99]

While all food products and food ingredient play an essential role in nutrition and sensory characteristics, some food component may provide an extra functionality, conferring to the overall product health benefits or desirable physiological effects beyond basic nutrition [111]. These functional foods are obtained by enrichment in a given bioactive, which is used as an ingredient able to promote or provide a beneficial effect on health such as acting on cardiovascular diseases, cholesterol reduction, degenerative diseases, among numerous potential health applications [7]. In functional foods, nutraceuticals may be distinguished by a concentration of the bioactive compounds considered as a health-promoting compound. These compounds may also be concentrated, serving as nutritional supplements, known as nutraceuticals [112]. In plants, the bioactives found in functional foods are mainly used for their different properties: polyunsaturated fatty acids and phytosterols may also be encountered. While all of these bioactive compounds are used for their specific health benefits (fibers, organic acids, polyunsaturated fatty acids [113], phytosterols [114], and organosulfides [115]), some may also be used additionally for a technological property: antioxidants (phenolics, tocopherols [116, 117]) and colorants (anthocyanins, betalains [118, 119]).

Edible fats and oils are respectively solid and liquid materials composed predominantly of glyceryl esters of fatty acids (90–95%), traces of non-glycerol-based lipids are also identified (phytosterols, tocopherols, etc.) [120, 121]. Fats and oils have been much used in food industry. Some of the major applications include nutrition (more specifically regarding to essential fatty acids), cooking, spread products (ingredients in bakery products and butters, respectively), shortening, and biodiesel feedstock [122, 123]. The nonfood applications of fats and oils include ingredients for formulation of soaps, detergents, paints, varnishes, plastics, and lubricants, among others [124].

This section reviewed how natural products contained in plants can be used as ingredients. Far from being exhaustive, it can be identified that a wide range of metabolites can be used in various industrial sectors: mainly in food as complements or as additives (aromas, flavors, colorants) but also in many other industries such as the pharmaceutical and agricultural industries, or in medicinal extracts or feedstuff. The introductive example of the orange tree biorefinery underlines the huge potential of valorization of process by-products. By generalizing biorefining, the agricultural industry would gain much benefit by commercializing their unused metabolites left in the waste biomass.

1.5

Green Extraction from Research to Teaching

Education plays an important role in materializing the concepts and principles of green chemistry and engineering [125]. Teaching green chemistry serves several key functions. First, it provides fundamental knowledge related to new chemical products and processes, data that are necessary to develop cleaner technologies [126]. Moreover, these new products and processes developed in an academic

setting can, in some cases, have direct applications to industry. Finally, academia serves as the primary means to educate students about the need to design green chemistry technologies, and provides them with the tools to do so.

For example, in Avignon University, to illustrate an application of green chemistry in teaching laboratories, we used a new green procedure employing ultrasound energy and microwave energy as the energy source to teach fundamental extraction concepts.

As an example, we have developed a new green procedure, using microwave energy as energy source, to teach the fundamental concepts of extraction of essential oils (Figure 1.11). Essential oils are volatile secondary metabolites that plants produce for their own needs other than nutrition. They are widely used



Figure 1.11 Practical work on green and conventional extraction techniques for Master students at Avignon University.

in foods, cosmetics, and pharmaceuticals [127]. In general they are complex mixtures of organic compounds that give characteristic odor and flavor to the plants. The objective of this teaching was to offer students the opportunity to compare the potential of this green technique for extraction of essential oil with a traditional hydrodistillation method (used in all the teaching laboratories all over the world) and to appreciate the benefits of using greener chemical methods. Green microwave extraction procedure is regarded as a greener method of heating because extraction times tend to be shorter than those required using the conventional procedure.

Microwave energy is well known to have a significant effect on the rate of various processes in the chemical and food industry [128]. Microwave extraction is an area of research which has an impact in several fields of modern chemistry. All the reported applications have shown that microwave-assisted extraction is an alternative to conventional techniques for such matrices. The advantages of using microwave energy, which is a noncontact heat source, for the extraction of essential oils from plant materials, include more effective heating, faster energy transfer, reduced thermal gradients, selective heating, reduced equipment size, faster response to process heating control, faster start-up, increased production, and elimination of process steps. Extraction processes performed under the action of microwave radiation are believed to be affected in part by polarization and volumetric and selective heating.

Microwave energy is a key enabling technology in achieving the objective of sustainable and green chemistry for research, teaching, and commercial applications. It has been shown that solvent-free conditions are especially suited to microwave-assisted organic synthesis, as reactions can be run safely under atmospheric pressure in the presence of significant amounts of products. When coupled to microwave radiation, solvent-free techniques have proved to be of special efficiency as green and economic procedures. Major improvements and simplifications over conventional methods originate from their rapidity, their enhancement in yields, and product purities. Microwave hydrodiffusion and gravity (MHG) of essential oil has been compared with the conventional hydrodistillation method, for the extraction of essential oil from fresh grapefruit peel. The method offers important advantages over traditional alternatives, namely, shorter extraction times, better yields, lower costs, greener features (as there is no residue generation and no water or solvent is used), and greener production (reduced CO₂ is rejected in the atmosphere). MHG is a laboratory exercise which easily teaches fundamental sustainable chemical lessons and successfully incorporates green chemistry into the teaching laboratory in both developed and developing nations.

At the same time, the idea has also been to develop a portable microwave-assisted extraction (PMAE) [129, 130] that can be used directly in the crop or the forest; this procedure is also appropriate for the teaching laboratory because it does not require any special microwave equipment.

In recent years, portable sample preparation apparatus have been developed, which can help with in-field extraction, sample preparation, analysis, and data

evaluation. A portable instrument is defined as “easily” movable, convenient for carrying, and capable of being transferred or adapted in altered circumstances [131]. Miniaturization (downscaling) is an important factor in modern society reflected strongly in science and technology [132] including medicine, chemistry, environment, food safety [133], and so on. Miniaturization of a procedure can be achieved simply by reducing the dimensions of the systems used in earlier approaches or by developing completely new setups or techniques. When compared to conventional systems, miniature systems can perform similar methods with remarkably reduced consumption of plant matrix and solvents, size and power requirement, system cost, faster analysis time, and massively parallel analysis capability. It is an attractive method for application requiring on-field rapid assay [134, 135]. Given its reduced size, this microwave technique requires a small amount of sample in a small glassware system which is designed as a miniature alembic to be placed inside a microwave oven. In this way, the PMAE apparatus can provide information about the actual state of the fresh samples and their essential oil content. This procedure is also appropriate for the teaching laboratory, does not require any special microwave equipment. The experiment developed, in addition to providing fast information on real samples, allows the students to learn extraction, chromatographic, and spectroscopic analysis skills. It is a visual example of rapid, sustainable, and green extraction of natural products. It is suitable of achieving success if commercially introduced, and involves green chemical processing with microwave energy (Figure 1.12).

Extraction of natural products has a very long history and has been associated with commerce since ancient times. Archeological findings indicated that extraction processes were performed in Mesopotamia in 3500 BC [136]. During the same period, trading activities and exchange of goods were intensifying. The first solvents that were used were with water using hydrodistillation in Syria [136] or fermented beverage such as beer and wine in Ancient Egypt [137].

Nowadays, the extraction of natural products takes an important place in manufacturing processes of ingredients derived from natural products. Growing numbers of finished products formula contain botanical ingredients for their functional properties (coloring, antioxidants, flavor, and biological activity). The largest market that uses botanical extract are the pharmaceutical segment and food, followed by personal care. Botanical ingredients are a growing business with very dynamic markets such personal care with approximately 10% of estimated growth (especially cosmetics), foods (driven by the nutraceutical sector with approximately 5% growth in 2012), and the pharmaceutical area (2%).

The growth of the botanical extract industry is linked to several drivers including

- request for naturally derived components by the consumers;
- a need for standardization and stability of the ingredients when compared to raw botanicals.



Figure 1.12 Introduction to green extraction for primary schools at Avignon University.

A current trend in the different markets of food, personal care, and nutrition and health is the replacement of synthetic ingredients by naturally derived ingredients. This trend has been driven by consumer request for more natural products. The industry of natural products has been successful in providing natural solutions as replacement to food dyes, food antioxidants, texturizers, aromas, active compounds, and so on. It is interesting to note that the natural labeling does not provide clear indication or evidence about the environmental impact of the manufacturing process applied to the botanical raw material, nor does it indicate the type of process and level of processing behind a botanical ingredient. Furthermore, it does not allow the consumer to distinguish between hand-made unstandardized products and industrially made standardized products.

Green extraction is a research thematic that encompasses a comprehensive strategy based on the discovery and the design of extraction processes in order to reduce energy consumption and promote the use of alternative solvents and renewable raw material. Green extraction has been investigated mainly at laboratory scale by several research teams mostly in Europe. Green extraction represents a good opportunity to rationalize and formalize eco-friendly developmental and industrial practices across the industries.

Several industrials have shown interest in the principles of green extraction. Some of these principles are already in line with industrial practices and

compatible with the primary interests of the botanical extraction industry. Some of these principles areas follows:

- *The use of alternative solvents.* The spectrum of solvents that are currently used in the industry varies according to the nature of the botanical raw material and targeted compounds, availability of the solvent, and its cost. The regulatory framework also defines what solvent can or cannot be used for a specific application. Almost all the solvents currently used are derived from crude oil, with the exception of water and ethanol produced by fermentation. Lipophilic solvents such as hexane are among the primary targets in the quest for alternative solvents. The use of alternative solvents also requires the involvement of solvent manufacturers in term of solvent cost reduction and registration. Some solvents already used do not need replacement solutions. For example, alcohol from fermentation is used as an extraction solvent and it meets the definition of a green solvent. Water is also the obvious choice of green solvent for polar compounds. Water is already largely used for manufacturing processes in many applications in food, personal care, or pharmaceutical ingredients. Although ecofriendly, water extraction processes require optimization on energy uses and recovery of intermediate polarity compounds (flavonoids, phenolic compounds, alkaloids) that are often the targeted compounds. Water is also a solvent that promotes hydrolysis and enzymatic activity during the manufacturing process.
- *Use of renewable raw material for botanical extracts.* Agricultural by-products are already used as starting raw material of botanical extractions (e.g., grape seeds and grape marc and ginseng fibers) by several industries. The use of agricultural by-products provides additional revenue to the primary producers (growers) and globally improves the whole agricultural value chain. The current trends in by-product valorization are to increase the value added by the process derived from the by-product and to move from low-value valorization (organic fertilizers, pellets for feedstock) to high-value specialty ingredients (functional ingredients, health ingredients).
- *Energy consumption by energy recovery and reduction of unit operations.* The natural products industry is a very competitive environment and to survive, the botanical extract manufacturers are already using optimized processes. The R&D, industrial, and cost control departments are constantly looking for more effective and cost-efficient ways to extract botanicals. Most of the industry members are aware that they need to reduce the carbon footprint of their processes. Therefore, the green extraction principles of reduction in energy consumption by energy recovery and reduction of unit operations and favoring safe robust and controlled processes are already existing objectives for industries especially on large volume items and/or commodity products. The arrival of new extraction technologies at production scale (pressurized liquid extraction, microwave-assisted extraction, etc.) will certainly benefit those industries in the quest for more efficient extraction process on a long-term basis.

Even though the concept of green extraction meets the demand of the final consumer in terms of a greener product, education needs to be imparted in order to explain what the benefits are for the final consumer. Consumers are not always familiarized with what is an extract or that solvents are used in manufacturing botanical ingredients. This will require popularization by the scientific community and industry members and avoid the shortcuts of “green washing.”

How does green extraction applies to the botanical extraction industry and what remains to be done? We can take the antioxidant rosemary extraction processes as an example. Rosemary is a symbol of the success of a safe and efficient antioxidant botanical solution that has replaced partially synthetic preservatives in various food matrices (especially meat and beverages). Initially, rosemary extracts were declared as flavoring ingredients, in 1997, when Naturex, Raps, and Robertet decided to clarify the situation and invited all the rosemary extract producers to join forces in order to register rosemary as a food antioxidant within the European legislation. In 2010, rosemary extracts were classified as food additives by the European Commission and assigned the number E392 (Commission Directives 2010/67/EU and 2010/69/EU repealed in 2013 by EU regulation 231/2012 and 1333/2008). “Antioxidant: extracts of rosemary” are to be produced with one of the four extraction processes described in the regulation, by means of solvent extraction (ethanol, acetone, or ethanol followed by hexane) or supercritical carbon dioxide extraction, paying respect to purity criteria. The volume of rosemary extracted by Naturex are significant, it currently represents about 50% of the rosemary collected in Morocco.

1.5.1

Principle: Innovation by Selection of Varieties and Use of Renewable Plant Resources

The starting point of the industrial extraction process is the selection of the feedstock biomass that will have the highest concentration in targeted compounds or at the least the more cost-effective per kilogram of pure compounds. Naturex has been using rosemary leaves from Morocco and other locations for 20 years, and has maintained a long history of quantification of carnosic acid/rosmarinic acid content material harvested in different locations and at different time of years. Some specific varieties growing in the Atlas Mountains are especially high in carnosic acid content. Carnosic acid concentrations decreased at high temperatures and low precipitation rates [138] and acid levels decreased in parallel with dropping relative water content in the plant [139]. Rosemary is a perennial plant that supports very well the trimming of the aerial part. This wild harvesting is sustainable and renewable and is contributing significantly to Moroccan economy.

1.5.2

Principle: Use of Alternative Solvents and Agro Solvent

Several extraction processes are derived from rosemary leaves. Polar compounds such as rosmarinic acid are extracted with aqueous solvents and also methanol.

Lipophilic compounds such as diterpenes phenolic acids are extracted with acetone or ethanol solvent. Several versions of lipophilic extracts (containing carnosic acid) are obtained after a multistep process, starting with ethanolic extraction and followed by several steps of purification using various pH-differential precipitations, and solubilization [140]. The organic versions use ethanol derived from fermentation.

1.5.3

Principle: Production of Coproducts Instead of Waste to Include Biorefinery

The rosemary leaves used in most of our rosemary processes are hydrodistilled first and the obtained essential oil is further refined for fragrance and flavor applications. We note that this operation is performed in order to increase value chain but also for technological reasons. Most customers request deodorized and discolored versions of rosemary extracts in order to reduce organoleptic and color impact on their recipes and the current purity criteria of the antioxidant E392 includes a ratio between antioxidant compounds and volatiles compounds. Rosemary leaves contains several others valuable fractions: ursolic acid, rosmarinic acid, carnosic acid and derivatives, flavonoids, and lignocellulosic fibers. More work is needed, because although several fractions are already valorized (carnosic, ursolic, and rosmarinic acids), some fractions are not (fibers, waxes, etc.). Rosemary is a good model for applied research for a complete and comprehensive biorefinery process.

1.5.4

Principle: Prioritizing a Non-denatured and Biodegradable Extract without Contaminant

Once the product has been purified and dried, the native purified extract is analyzed according to a specification sheet that ensures that it meets the acceptance criteria. The conformity to the specifications is also matched with the target process yield (both mass yield and active yield). Naturex has been developing an extensive portfolio of testing methods for both rosmarinic and carnosic acids; these methods have been recognized as market standards and are used worldwide. Internal specification such as the ratio between carnosol and carnosic acid allows to ensure that carnosic acid has not been degraded in carnosol during the process.

1.6

Conclusions and Perspective

Green extraction is a good idea and comprehensive strategy that promotes energy consumption reduction and process efficiency, and minimizes environmental impact of manufacturing processes of botanical extracts. Green extraction will

benefit from industrial involvement and exposure to industrial constraints. Industry members will benefit, as well, from green extraction with new technologies and new research tools to develop new products and to optimize existing products. The research work remaining is important and will require synergies and open communications between academic and private sectors in order to transform these principles into an industrial use and bring eco-extracts to a large number of consumers.

References

- Armenta, S., Garrigues, S., and De La Guardia, M. (2008) Green analytical chemistry. *TrAC, Trends Anal. Chem.*, **27**, 497–511.
- Chemat, F., Vian, M.A., and Cravotto, G. (2012) Green extraction of natural products: concept and principles. *Int. J. Mol. Sci.*, **13**, 8615–8627.
- Anastas, P.T. and Warner, J.C. (1998) *Green Chemistry: Theory and Practice*, Oxford University Press, New York, 135 p.
- Li, X. and Franke, A.A. (2009) Fast HPLC-ECD analysis of ascorbic acid, dehydroascorbic acid and uric acid. *J. Chromatogr. B*, **877**, 853–856.
- Warner, M.G., Succaw, G.L., and Hutchison, J.E. (2001) Solventless syntheses of mesotetraphenylporphyrin: new experiments for a greener organic chemistry laboratory curriculum. *Green Chem.*, **3**, 267–270.
- Croteau, R., Kutchan, T., and Lewis, N. (2000) in *Biochemistry and Molecular Biology of Plants* (eds B. Buchanan, W. Gruissen, and R. Jones), American Society of Plant Physiologists, Rockville, MD, pp. 1250–1319.
- Cavalcanti, R.N., Forster-Carneiro, T., Gomes, M.T.M.S., Rostagnon, M.A., Prado, J.M., and Meireles, M.A.A. (2013) in *Natural Product Extraction: Principles and Applications*, Green Chemistry, Vol. 21 (eds M.A. Rostagnon, J.M. Prado, and G.A. Kraus), Royal Society of Chemistry, Dorchester, pp. 1–46.
- Dewick, P.M. (2002) *Medicinal Natural Products: A Biosynthetic Approach*, 2nd edn, John Wiley & Sons, Ltd, 520 p.
- FAOSTATS <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor> (accessed 14 March 2014).
- Rezzadori, K., Benedetti, S., and Amante, E.R. (2012) Proposals for the residues recovery: orange waste as raw material for new products. *Food Bioprod. Process.*, **90**, 606–614.
- Kesterson, J.W. and Braddock, R.J. (1976) *By-Products and Specialty Products of Florida Citrus*, Bulletin 784, Agricultural Experiment Stations, University of Florida.
- United States Agricultural Research Service (1956) *Chemistry and Technology of Citrus, Citrus Products and By-Products*, U.S. Department of Agriculture.
- Bampidis, V. and Robinson, P. (2006) Citrus by-products as ruminant feeds: a review. *Anim. Feed Sci. Technol.*, **128**, 175–217.
- Ferhat, M.A., Meklati, B.Y., Smadja, J., and Chemat, F. (2006) An improved microwave Clevenger apparatus for distillation of essential oils from orange peel. *J. Chromatogr. A*, **1112**, 121–126.
- Minh Tu, N.T., Thanh, L.X., Une, A., Ukeda, H., and Sawamura, M. (2002) Volatile constituents of Vietnamese pummelo, orange, tangerine and lime peel oils. *Flavour Fragrance J.*, **17**, 169–174.
- Chemat, F. and Sawamura, M. (2010) in *Citrus Essential Oils* (ed M. Sawamura), John Wiley & Sons, Inc., Hoboken, NJ, pp. 9–36.
- Masmoudi, M., Besbes, S., Chaabouni, M., Robert, C., Paquot, M., Blecker, C., and Attia, H. (2008) Optimization of pectin extraction from lemon by-product with acidified date juice

- using response surface methodology. *Carbohydr. Polym.*, **74**, 185–192.
18. Bicu, I. and Mustata, F. (2011) Cellulose extraction from orange peel using sulfite digestion reagents. *Bioresour. Technol.*, **102**, 10013–10019.
 19. Grohmann, K., Manthey, J.A., Cameron, R.G., and Buslig, B.S. (1999) Purification of Citrus peel juice and molasses. *J. Agric. Food Chem.*, **47**, 4859–4867.
 20. Okoye, C., Ibetu, C., and Ihedioha, J. (2011) Preliminary studies on the characterization of orange seed and pawpaw seed oils. *Am. J. Food Technol.*, **6**, 422–426.
 21. Aranha, C.P.M. and Jorge, N. (2011) Physico-chemical characterization of seed oils extracted from oranges (*Citrus sinensis*). *Food Sci. Technol. Res.*, **19**, 409–415.
 22. Smadja, J. (2009) in *Essential Oils and Aromas Green Extraction and Applications* (ed F. Chemat), Har Krishan Bhalla & Sons, Dehradun, pp. 122–146.
 23. Dugo, G., Bonaccorsi, I., Sciarrone, D., Costa, R., Dugo, P., Monde, L., Santi, L., and Fakhry, H.A. (2011) Characterization of oils from the fruits, leaves and flowers of the bitter orange tree. *J. Essent. Oil Res.*, **23**, 45–59.
 24. Bonaccorsi, I., Sciarrone, D., Schipilliti, L., Trozzi, A., Fakhry, H.A., and Dugo, G. (2011) Composition of egyptian Neroli oil. *Nat. Prod. Commun.*, **6**, 1009–1014.
 25. Demura, T. and Ye, Z.-H. (2010) Regulation of plant biomass production. *Curr. Opin. Plant Biol.*, **13**, 298–303.
 26. Kumar, R., Singh, S., and Singh, O.V. (2008) Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives. *J. Ind. Microbiol. Biotechnol.*, **35**, 377–391.
 27. Guignard, J.L. (1996) *Biochimie végétale*, Dunod, Paris, 274 p.
 28. Bousbia, N., Vian, M., Ferhat, M., Meklati, B., and Chemat, F. (2009) A new process for extraction of essential oil from Citrus peels: microwave hydrodiffusion and gravity. *J. Food Eng.*, **90**, 409–413.
 29. Manners, G.D. (2007) Citrus limonoids: analysis, bioactivity, and biomedical prospects. *J. Agric. Food Chem.*, **55**, 8285–8294.
 30. Roy, A. and Saraf, S. (2006) Limonoids: overview of significant bioactive triterpenes distributed in plants kingdom. *Biol. Pharm. Bull.*, **29**, 191–201.
 31. Fanciullino, A.-L., Dhuique-Mayer, C., Luro, F., Casanova, J., Morillon, R., and Ollitrault, P. (2006) Carotenoid diversity in cultivated citrus is highly influenced by genetic factors. *J. Agric. Food Chem.*, **54**, 4397–4406.
 32. Pellati, F. and Benvenuti, S. (2007) Fast high-performance liquid chromatography analysis of phenethylamine alkaloids in Citrus natural products on a pentafluorophenylpropyl stationary phase. *J. Chromatogr. A*, **1165**, 58–66.
 33. Putzbach, K., Rimmer, C., Sharpless, K., Wise, S., and Sander, L. (2007) Determination of bitter orange alkaloids in dietary supplement Standard Reference Materials by liquid chromatography with atmospheric-pressure ionization mass spectrometry. *Anal. Bioanal. Chem.*, **389**, 197–205.
 34. Pellati, F. and Benvenuti, S. (2007) Chromatographic and electrophoretic methods for the analysis of phenethylamine alkaloids in *Citrus aurantium*. *J. Chromatogr. A*, **1161**, 71–88.
 35. Khan, M.K., Zill, E.H., and Dangles, O. (2014) A comprehensive review on flavanones, the major citrus polyphenols. *J. Food Compos. Anal.*, **33**, 85–104.
 36. Harper, A.H., Murray, E.G.D., Mayes, P.A., and Rodwell, V.W. (2002) *Biochimie de Harper*, 25th edn, De Boeck, Presses de l'Université de Laval, 933 p.
 37. Voet, D., Voet, J.G., Rousseau, G., and Domenjoud, L. (2005) *Biochimie*, De Boeck Supérieur, 1600 p.
 38. Bruneton, J. (1987) *Éléments de phytochimie et de pharmacognosie*, Technique et Documentation Lavoisier, 585 p.
 39. Hounsome, N., Hounsome, B., Tomos, D., and Edwards-Jones, G. (2008) Plant metabolites and nutritional quality of vegetables. *J. Food Sci.*, **73**, R48–R65.
 40. Sugawara, T. and Miyazawa, T. (1999) Separation and determination of glycolipids from edible plant sources by

- high-performance liquid chromatography and evaporative light-scattering detection. *Lipids*, **34**, 1231–1237.
41. He, X.-G. (2000) On-line identification of phytochemical constituents in botanical extracts by combined high-performance liquid chromatographic–diode array detection–mass spectrometric techniques. *J. Chromatogr. A*, **880**, 203–232.
 42. Dzoyem, J., Kechia, F., and Kuete, V. (2011) Phytotoxic, antifungal activities and acute toxicity studies of the crude extract and compounds from *Diospyros canaliculata*. *Nat. Prod. Res.*, **25**, 741–749.
 43. Meyer-Warnod, B. (1984) Natural essential oils: extraction processes and application to some major oils. *Perfum. Flavor.*, **9**, 93–104.
 44. Dewick, P.M. (2002) The biosynthesis of C5–C25 terpenoid compounds. *Nat. Prod. Rep.*, **19**, 181–222.
 45. Acton, Q.A. (2012) *Diterpenes – Advances in Research and Application: 2012 Edition*, Scholarly Editions, Atlanta, 109 p.
 46. Wölwer-Rieck, U. (2012) The leaves of *Stevia rebaudiana* (Bertoni), their constituents and the analyses thereof: a review. *J. Agric. Food Chem.*, **60**, 886–895.
 47. Houël, E., Bertani, S., Bourdy, G., Deharo, E., Jullian, V., Valentin, A., Chevalley, S., and Stien, D. (2009) Quassinoid constituents of *Quassia amara* L. leaf herbal tea. Impact on its antimalarial activity and cytotoxicity. *J. Ethnopharmacol.*, **126**, 114–118.
 48. Ghanbari, R., Anwar, F., Alkharfy, K.M., Gilani, A.-H., and Saari, N. (2012) Valuable nutrients and functional bioactives in different parts of olive (*Olea europaea* L.) – A review. *Int. J. Mol. Sci.*, **13**, 3291–3340.
 49. Cordell, G.A. (2013) Fifty years of alkaloid biosynthesis in Phytochemistry. *Phytochemistry*, **91**, 29–51.
 50. Srinivasan, K. (2007) Black Pepper and its pungent principle-piperine: a review of diverse physiological effects. *Crit. Rev. Food Sci. Nutr.*, **47**, 735–748.
 51. Bunsupa, S., Yamazaki, M., and Saito, K. (2012) Quinolizidine alkaloid biosynthesis: recent advances and future prospects. *Front. Plant Sci.*, **3**, article 239, 1–7.
 52. Michael, J.P. (2008) Indolizidine and quinolizidine alkaloids. *Nat. Prod. Rep.*, **25**, 139–165.
 53. Dembitsky, V., Poovarodom, S., Leontowicz, H., Leontowicz, M., Vearasilp, S., Trakhtenberg, S., and Gorinstein, S. (2011) The multiple nutrition properties of some exotic fruits: biological activity and active metabolites. *Food Res. Int.*, **44**, 1671–1701.
 54. Servillo, L., Giovane, A., Balestrieri, M.L., Casale, R., Cautela, D., and Castaldo, D. (2013) Citrus genus plants contain N-methylated tryptamine derivatives and their 5-Hydroxylated forms. *J. Agric. Food Chem.*, **61**, 5156–5162.
 55. Dhawan, K., Dhawan, S., and Sharma, A. (2004) Passiflora: a review update. *J. Ethnopharmacol.*, **94**, 1–23.
 56. Badria, F.A. (2002) Melatonin, serotonin, and tryptamine in some Egyptian food and medicinal plants. *J. Med. Food*, **5**, 153–157.
 57. Stintzing, F. and Carle, R. (2007) Beta-lains – emerging prospects for food scientists. *Trends Food Sci. Technol.*, **18**, 514–525.
 58. D'Yakonov, A.L. and Telezhenetskaya, M.V. (1997) Quinazoline alkaloids in nature. *Chem. Nat. Compd.*, **33**, 221–267.
 59. Jin, Z. (2011) Muscarine, imidazole, oxazole, and thiazole alkaloids. *Nat. Prod. Rep.*, **28**, 1143–1191.
 60. Ashihara, H., Sano, H., and Crozier, A. (2008) Caffeine and related purine alkaloids: biosynthesis, catabolism, function and genetic engineering. *Phytochemistry*, **69**, 841–856.
 61. Andersson, C. (1999) *Glycoalkaloids in Tomatoes, Eggplants, Pepper and Two Solanum Species Growing Wild in the Nordic Countries*, Nordisk Ministerråd and Nordisk Råd.

62. Scalbert, A. and Williamson, G. (2000) Dietary intake and bioavailability of polyphenols. *J. Nutr.*, **130**, 2073S–2085S.
63. Gorinstein, S., Zemser, M., Haruenkit, R., Chuthakorn, R., Grauer, F., Martin-Belloso, O., and Trakhtenberg, S. (1999) Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmon. *J. Nutr. Biochem.*, **10**, 367–371.
64. Duthie, G., Duthie, S., and Kyle, J. (2000) Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutr. Res. Rev.*, **13**, 79–106.
65. Saleh, Z., Stanley, R., and Nigam, M. (2006) Extraction of polyphenolics from apple juice by foam fractionation. *Int. J. Food Eng.*, **2**, article 2, 1–15.
66. Llorach, R., Martínez-Sánchez, A., Tomás-Barberán, F., Gil, M., and Ferreres, F. (2008) Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. *Food Chem.*, **108**, 1028–1038.
67. Soong, Y. and Barlow, P. (2006) Quantification of gallic acid and ellagic acid from longan (*Dimocarpus longan* Lour.) seed and mango (*Mangifera indica* L.) kernel and their effects on antioxidant activity. *Food Chem.*, **97**, 524–530.
68. Szajdek, A. and Borowska, E. (2008) Bioactive compounds and health-promoting properties of berry fruits: a review. *Plant Foods Hum. Nutr.*, **63**, 147–156.
69. Rice-Evans, C., Miller, J., and Paganga, G. (1997) Antioxidant properties of phenolic compounds. *Trends Plant Sci.*, **2**, 152–159.
70. Haminiuk, C., Maciel, G., Plata Oviedo, M., and Peralta, R. (2012) Phenolic compounds in fruits—an overview. *Int. J. Food Sci. Technol.*, **47**, 2023–2044.
71. Crozier, A., Jaganath, I., and Clifford, M. (2009) Dietary phenolics: chemistry, bioavailability and effects on health. *Nat. Prod. Rep.*, **26**, 1001–1043.
72. Santos-Buelga, C. and Scalbert, A. (2000) Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutrition and health. *J. Sci. Food Agric.*, **80**, 1094–1117.
73. Kuhnau, J. (1976) The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet.*, **24**, 117–191.
74. Cook, N. and Samman, S. (1996) Flavonoids – Chemistry, metabolism, cardioprotective effects, and dietary sources. *J. Nutr. Biochem.*, **7**, 66–76.
75. Ross, J. and Kasum, C. (2002) Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu. Rev. Nutr.*, **22**, 19–34.
76. Schieber, A., Berardini, N., and Carle, R. (2003) Identification of flavonol and xanthone glycosides from mango (*Mangifera indica* L. Cv. “Tommy Atkins”) peels by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J. Agric. Food Chem.*, **51**, 5006–5011.
77. Hoffmann-Ribani, R., Huber, L., and Rodriguez-Amaya, D. (2009) Flavonols in fresh and processed Brazilian fruits. *J. Food Compos. Anal.*, **22**, 263–268.
78. de Almeida, A., Miranda, M., Simoni, I., Wigg, M., Lagrota, M., and Costa, S. (1998) Flavonol monoglycosides isolated from the antiviral fractions of *Persea americana* (Lauraceae) leaf infusion. *Phytother. Res.*, **12**, 562–567.
79. Dixon, R. and Xie, D. (2005) Proanthocyanidins—a final frontier in flavonoid research? *New Phytol.*, **165**, 9–28.
80. Suksamrarn, S., Suwannapoch, N., Ratananukul, P., Aroonlerk, N., and Suksamrarn, A. (2002) Xanthones from the green fruit hulls of *Garcinia mangostana*. *J. Nat. Prod.*, **65**, 761–763.
81. Balunas, M.J., Su, B., Brueggemeier, R.W., and Kinghorn, A.D. (2008) Xanthones from the botanical dietary supplement mangosteen (*Garcinia mangostana*) with aromatase inhibitory activity. *J. Nat. Prod.*, **71**, 1161–1166.
82. Boonlaksiri, C., Oonanant, W., Kongsaree, P., Kittakoop, P., Tanticharoen, M., and Thebtaranonth, Y. (2000) An antimalarial stilbene from *Artocarpus integer*. *Phytochemistry*, **54**, 415–417.

83. Boerjan, W., Ralph, J., and Baucher, M. (2003) Lignin biosynthesis. *Annu. Rev. Plant Biol.*, **54**, 519–546.
84. Pfaltzgraff, L.A., De Bruyn, M., Cooper, E.C., Budarin, V., and Clark, J.H. (2013) Food waste biomass: a resource for high-value chemicals. *Green Chem.*, **15**, 307–314.
85. Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H.A., Ghafoor, K., Norulaini, N.A.N., and Omar, A.K.M. (2013) Techniques for extraction of bioactive compounds from plant materials: a review. *J. Food Eng.*, **117**, 426–436.
86. Bernhoft, A. (2010) A brief review on bioactive compounds in plants. Proceedings from a Symposium held at The Norwegian Academy of Science and Letters, Oslo, Norway.
87. Allaf, T., Tomao, V., Besombes, C., and Chemat, F. (2013) Thermal and mechanical intensification of essential oil extraction from orange peel via instant autovaporization. *Chem. Eng. Process.: Process Intensif.*, **72**, 24–30.
88. Saeb, K. and Gholamrezaee, S. (2012) Variation of essential oil composition of *Melissa officinalis* L. leaves during different stages of plant growth. *Asian Pac. J. Trop. Biomed.*, **2**, S547–S549.
89. Boukhatem, M.N., Kameli, A., and Saidi, F. (2013) Essential oil of Algerian rose-scented geranium (*Pelargonium graveolens*): chemical composition and antimicrobial activity against food spoilage pathogens. *Food Control*, **34**, 208–213.
90. Bernotiene, G., Niviskiene, O., Butkiene, R., and Mockute, D. (2004) Chemical composition of essential oils of hops (*Humulus lupulus* L.) growing wild in Aukštaitija. *Chemija*, **15**, 31–36.
91. Charles, D.J., Joly, R.J., and Simon, J.E. (1990) Effects of osmotic stress on the essential oil content and composition of peppermint. *Phytochemistry*, **29**, 2837–2840.
92. Scotter, M. (2009) The chemistry and analysis of annatto food colouring: a review. *Food Addit. Contam., Part A*, **26**, 1123–1145.
93. Giuffrida, D., Dugo, P., Torre, G., Bignardi, C., Cavazza, A., Corradini, C., and Dugo, G. (2013) Characterization of 12 Capsicum varieties by evaluation of their carotenoid profile and pungency determination. *Food Chem.*, **140**, 794–802.
94. Cossignani, L., Urbani, E., Simonetti, M., Maurizi, A., Chiesi, C., and Blasi, F. (2014) Characterisation of secondary metabolites in saffron from central Italy (Casia, Umbria). *Food Chem.*, **143**, 446–451.
95. Ahamad, M.N., Saleemullah, M., Shah, H.U., Khalil, I.A., and Saljoqi, A.U.R. (2007) Determination of beta carotene content in fresh vegetables using high performance liquid chromatography. *Sarhad J. Agric.*, **23**, 767–770.
96. Bridle, P. and Timberlake, C.F. (1997) Anthocyanins as natural food colours – selected aspects. *Food Chem.*, **58**, 103–109.
97. Setchell, K.D. and Cassidy, A. (1999) Dietary isoflavones: biological effects and relevance to human health. *J. Nutr.*, **129**, 758S–767S.
98. Saleem, M., Kim, H.J., Ali, M.S., and Lee, Y.S. (2005) An update on bioactive plant lignans. *Nat. Prod. Rep.*, **22**, 696–716.
99. Riddell, L.J., Sayompark, D., Penny, O., and Keast Russell, S.J. (2012) in *Caffeine: Chemistry, Analysis, Function and Effects* (ed V.R. Preedy), The Royal Society of Chemistry, Cambridge, pp. 22–38.
100. Delgado-Vargas, F., Jiménez, A.R., and Paredes-López, O. (2000) Natural pigments: carotenoids, anthocyanins, and betalains — characteristics, biosynthesis, processing, and stability. *Crit. Rev. Food Sci. Nutr.*, **40**, 173–289.
101. Wilksa-Jeszka, J. (2007) in *Chemical and Functional Properties of Food Components* (ed Z.E. Sikorski), CRC Press, Boca Raton, FL, pp. 245–274.
102. Brud, W.S. (2009) in *Handbook of Essential Oils* (eds K.H.C. Baser and G. Buchbauer), CRC Press, Boca Raton, FL, pp. 843–853.
103. Rodrigues, V.M., Rosa, P.T.V., Marques, M.O.M., Petenate, A.J., and Meireles, L.P.

- M.A.A. (2003) Supercritical extraction of essential oil from aniseed (*Pimpinella anisum* L.) using CO₂: solubility, kinetics, and composition data. *J. Agric. Food Chem.*, **51**, 1518–1523.
104. Li, Y.-Q., Kong, D.-X., and Wu, H. (2013) Analysis and evaluation of essential oil components of cinnamon barks using GC–MS and FTIR spectroscopy. *Ind. Crops Prod.*, **41**, 269–278.
105. Almeida, M.R., Fidelis, C.H.V., Barata, L.E.S., and Poppi, R.J. (2013) Classification of Amazonian rosewood essential oil by Raman spectroscopy and PLS-DA with reliability estimation. *Talanta*, **117**, 305–311.
106. Roy, B.C., Goto, M., and Hirose, T. (1996) Extraction of ginger oil with supercritical carbon dioxide: experiments and modeling. *Ind. Eng. Chem. Res.*, **35**, 607–612.
107. Allaf, T., Tomao, V., Ruiz, K., Bachari, K., ElMaataoui, M., and Chemat, F. (2013) Deodorization by instant controlled pressure drop autovaporization of rosemary leaves prior to solvent extraction of antioxidants. *LWT-Food Sci. Technol.*, **51**, 111–119.
108. Chemat, F., Lucchesi, M.E., Smadja, J., Favretto, L., Colnaghi, G., and Visinoni, F. (2006) Microwave accelerated steam distillation of essential oil from lavender: a rapid, clean and environmentally friendly approach. *Anal. Chim. Acta*, **555**, 157–160.
109. Safaralie, A., Fatemi, S., and Salimi, A. (2010) Experimental design on supercritical extraction of essential oil from valerian roots and study of optimal conditions. *Food Bioprod. Process.*, **88**, 312–318.
110. Charles, S. (2009) in *Handbook of Essential Oils* (eds K.H.C. Baser and G. Buchbauer), CRC Press, Boca Raton, FL, pp. 121–150.
111. Hasler, C.M., Bloch, A.S., Thomson, C.A., Enrione, E., and Manning, C. (2004) ADA reports, position of the American Dietetic Association: functional foods. *J. Am. Diet. Assoc.*, **104**, 814–826.
112. Herrero, M., Cifuentes, A., and Ibañez, E. (2006) Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae: a review. *Food Chem.*, **98**, 136–148.
113. Kolanowski, W. and Laufenberg, G. (2006) Enrichment of food products with polyunsaturated fatty acids by fish oil addition. *Eur. Food Res. Technol.*, **222**, 472–477.
114. Jones, P.J. and Abumweis, S.S. (2009) Phytosterols as functional food ingredients: linkages to cardiovascular disease and cancer. *Curr. Opin. Clin. Nutr. Metab. Care*, **12**, 147–151.
115. Santhosha, S.G., Jamuna, P., and Prabhavathi, S.N. (2013) Bioactive components of garlic and their physiological role in health maintenance: a review. *Food Biosci.*, **3**, 59–74.
116. Anna-Maija, L., Vieno, P., and Afaf, K.-E. (2002) in *Functional Foods* (eds G. Mazza, M. Le Maguer, and J. Shi), CRC Press, Boca Raton, FL, pp. 1–38.
117. Shahidi, F. (2005) Nutraceuticals and functional foods in health promotion and disease prevention, in *Proceedings of WOCMAP III*, Vol. 6: Traditional Medicine and Nutraceuticals, Acta Horticulturae 680, International Society for Horticultural Science, Belgium.
118. Stintzing, F.C. and Carle, R. (2004) Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends Food Sci. Technol.*, **15**, 19–38.
119. Dillard, C. (2000) Phytochemicals: nutraceuticals and human health. *J. Sci. Food Agric.*, **80**, 1744–1756.
120. Xenakis, A., Papadimitriou, V., and Sotiroudis, T. (2010) Colloidal structures in natural oils. *Curr. Opin. Colloid Interface Sci.*, **15**, 55–60.
121. Ollé, M. (2002) Analyse des corps gras, Techniques de l'ingénieur, dossier P3325, p. 15.
122. Gui, M.M., Lee, K.T., and Bhatia, S. (2008) Feasibility of edible oil vs. non-edible oil vs. waste edible oil as biodiesel feedstock. *Energy*, **33**, 1646–1653.
123. Gunstone, F.D. (2002) *Vegetable Oils in Food Technology. Composition, Properties and Uses*, Blackwell Publishing Ltd, Oxford, p. 352.

124. O'Brien, R.D. (2003) *Fats and Oils: Formulating and Processing for Applications*, 2nd edn, CRC Press, Boca Raton, FL, pp. 234–291.
125. Peñas, F.J., Barona, A., Elias, A., and Olazar, M. (2006) Implementation of industrial health and safety in chemical engineering teaching laboratories. *J. Chem. Health Saf.*, **13**, 19–23.
126. Armenta, S., Garrigues, S., and De La Guardia, M. (2008) On-line vapour-phase generation combined with Fourier transform infrared spectrometry. *TrAC, Trends Anal. Chem.*, **27**, 15–23.
127. Arous, K., Uquiche, E., and Del Valle, J.M. (2009) Matrix effects in supercritical CO₂ extraction of essential oils from plant material. *J. Food Eng.*, **92**, 438–447.
128. Wiesbrock, F. and Schubert, U.S. (2006) Microwaves in chemistry: the success story goes on. *Chim. Oggi – Chem. Today*, **24**, 30–34.
129. Chemat, F., Perino-Issartier, S., Petitcolas, E., and Fernandez, X. (2012) “in situ” extraction of essential oils by use of Dean-Stark glassware and a Vigreux column inside a microwave oven: a procedure for teaching green analytical chemistry. *Anal. Bioanal. Chem.*, **404**, 679–682.
130. Perino, S., Petitcolas, E., De La Guardia, M., and Chemat, F. (2013) Portable microwave assisted extraction. An original concept for green analytical chemistry. *J. Chromatogr. A*, **1315**, 200–203.
131. McMahon, G. (2007) *Analytical Instrumentation: A Guide to Laboratory, Portable and Miniaturized Instruments*, John Wiley & Sons, Ltd, Chichester, 318 p.
132. Contreras, J.A., Murray, J.A., Tolley, S.E., Oliphant, J.L., Tolley, H.D., Lammert, S.A., Lee, E.D., Later, D.W., and Lee, M.L. (2008) Hand-portable Gas Chromatograph-Toroidal Ion Trap Mass Spectrometer (GC-TMS) for detection of hazardous compounds. *J. Am. Soc. Mass Spectrom.*, **19**, 1425–1434.
133. Kim, S.J., Gobi, K.V., Harada, R., Shankaran, D.R., and Miura, N. (2006) Miniaturized portable surface plasmon resonance immunosensor applicable for on-site detection of low-molecular-weight analytes. *Sens. Actuators B*, **115**, 349–356.
134. Ryvolová, M., Macka, M., and Preisler, J. (2010) Portable capillary-based (non-chip) capillary electrophoresis. *TrAC, Trends Anal. Chem.*, **29**, 339–353.
135. Sui, X., Liu, T., Ma, C., Yang, L., Zu, Y., Zhang, L., and Wang, H. (2012) Microwave irradiation to pretreat rosemary (*Rosmarinus officinalis* L.) for maintaining antioxidant content during storage and to extract essential oil simultaneously. *Food Chem.*, **131**, 1399–1405.
136. Levey, M. (1955) Evidences of ancient distillation, sublimation and extraction in Mesopotamia. *Centaurus*, **4**, 23–33.
137. Bart, H.-J. (2011) Extraction of natural products from plants – An introduction, in *Industrial Scale Natural Products Extraction* (eds H.-J. Bart and S. Pilz), Wiley-VCH Verlag GmbH, Weinheim.
138. Luis, J.C. and Johnson, C.B. (2005) Seasonal variations of rosmarinic and carnosic acids in rosemary extracts. Analysis of their in vitro antiradical activity. *Span. J. Agric. Res.*, **3**, 106–112.
139. Munné-Bosch, S. and Alegre, L. (2000) Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. *Planta*, **210**, 925–931.
140. Naturex (1999) High purity carnosic acid from rosemary and sage extracts by pH-controlled precipitation. US Patent US 5859293 A.