2 Coffee Constituents

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2.1 INTRODUCTION

Coffee has been for decades the most commercialized food product and most widely consumed beverage in the world. Since the opening of the first coffee house in Mecca at the end of the fifteenth century, coffee consumption has greatly increased all around the world. In 2010, coffee production reached 8.1 million tons worldwide [1]. This represents more than 500 billion cups, with the United States, Brazil, Germany, Japan, and Italy being the major consumer countries. However, *per capita* consumption in North European countries such as Finland, Norway, Denmark, and Sweden may reach 8 kg/year, more than two times that of the United States or Brazil [2].

The reasons for this continuous increase in coffee consumption include improved cup quality through selection of varieties and breeding, better agricultural practices; the creation of specialty shops, and a change in coffee's image through the dissemination of information on the health benefits of long-term coffee consumption. Today, coffee is considered a functional food, primarily due to its high content of compounds that exert antioxidant and other beneficial biological properties. The characteristic flavor and richness of coffee aroma make it a unique beverage, with almost a thousand volatile compounds identified in roasted coffee [3].

The coffee tree belongs to the Rubiaceae family, genus *Coffea*. Although more than 80 coffee species have been identified worldwide [4], only two are economically important. *Coffea arabica,* also known as Arabica coffee, is responsible for approximately 70% of the global coffee market, and *Coffea canephora* or Robusta coffee (commercial name of one of the main *C. canephora* cultivars) accounts for the rest [1,5]. Arabica and Robusta coffees are different in many ways, including their ideal growing climates, physical aspects, chemical composition, and characteristics of the brew made with the ground roasted seeds. This chapter will focus on the chemical composition of these two coffee species, including the nonvolatile and volatile compounds important for flavor, quality, and health-promoting actions. To understand the chemical changes that occur during coffee production, we will briefly address the main technological processes green coffee seeds undergo before they are consumed as brewed coffee beverage.

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2.2 PRODUCTION OF COFFEE AND COFFEE-BASED BEVERAGES

The process of bringing the harvested coffee fruits to consumers as a beverage involves a series of steps. Greater control of each step of this process improves the ability to produce a good-quality coffee. After harvesting, fruits undergo primary processing to separate the seeds. Secondary processing such as decaffeination and steam treatment are performed before roasting. After roasting, the coffee is ground and packed or further processed to produce instant coffee.

2.2.1 Green coffee production

Good harvesting methods are important to produce good-quality coffee. Although the awareness of quality is important throughout the entire agricultural process, the degree of coffee fruit maturation and avoiding mold contamination during harvesting, drying, and storage of the seeds are especially critical. Mold contamination affects not only the aroma and flavour of the final beverage, but also its bioactivity, since undesirable mycotoxins and biogenic amines can affect human health [6–8].

Coffee fruits are typically harvested in one of three ways: picking, stripping, or mechanical harvest. In the first method, the ripe fruits, known as cherries, are picked one at a time. Because coffee fruits do not usually ripen simultaneously, this method is time-consuming and therefore expensive where the size of the workforce is not sufficient. However, picking tends to produce better-quality coffee seeds, in terms of both taste and health, than other methods. Manual stripping of the twigs collects immature, ripe, and overripe seeds along with leaves. Mechanical harvesting is performed by shaking the trees or by stripping the branches with an apparatus similar to a flexible comb. Stripping and mechanical harvesting yield defects derived from fruits in different degrees of maturation and fermented fruits. Extrinsic defects include stones, husks, and twigs that are mixed with the fruits during harvesting. Intrinsic defects, which are usually more relevant for cup quality and health, are defective seeds such as immature, black, sour, black-immature, bored or insect-damaged, and broken. Immature seeds, which originate mainly from unripe fruits, increase beverage astringency. Sour seeds can be due to lack of water during fruit development or abnormal fermentation of immature or mature seeds. Sour seeds may also precede the formation of black seeds, which usually originate from overripe cherries that fall to the ground by the action of rain or during harvest and contact with the soil promotes microbial fermentation. Black seeds can also originate from carbohydrate deficiency caused by poor agricultural practices or microbial fermentation of seeds while still on the tree or during postharvest processing. The silver skin of the black-immature seed is dark or black–green due to the action of high temperatures on the immature seed. Black-immature seeds can also be produced by inadequate drying of immature seeds. These black-immature seeds can be differentiated from ground-fermented black seeds by the shiny adherent silver skin on the seed surface. Black, black-immature, and immature seeds are considered serious defects because they dramatically affect cup quality [9].

After harvest, coffee fruits undergo pulp extraction to produce green coffee seeds. The most common methods of pulp extraction are known as wet and dry methods. With the dry processing method, seeds are exposed to the sun or air dryers until the moisture content is approximately 10%–12% [10]. If air dryers are not available, low rainfall during harvest is needed to ensure a good-quality coffee. After drying, the fruits are cleaned and dehulled, and then the dried skin and pulp are removed, leaving a mucilaginous material (silver skin)

adhering to the seed surface. To obtain a good-quality beverage, the seeds (two seeds per fruit) are mechanically and electronically sorted to separate defective seeds from the high-quality seeds. This method is commonly used in Brazil and Africa, where sun and space are abundant and fruits are often harvested by stripping.

The wet-processing technique is more sophisticated and generally produces a higher quality brew. Before dehulling and separating the seeds, cherry selection takes place in flotation tanks, followed by soaking and fermentation. During fermentation, during which enzymes may be added, the silver skin is removed and acidity increases; the pH may be reduced to 4.5 [11]. The seeds (parchment coffee) are then extensively washed, polished, and sun-dried and/or air-dried. Wet processing is frequently used where coffee is harvested by picking, such as Colombia, Asia, and Central America.

The primary difference between the processing methods is that with wet-processing seeds are separated from the pulp and skin before drying. An alternate method (natural processing) that combines aspects of both dry and wet methods has been developed in Brazil. This method consists of washing and selecting the seeds in flotation tanks without fermentation. Coffee seeds that undergo the natural process are often used in espresso coffee blends, as they tend to add more body to the beverage than wet processed because polysaccharides in the silver skin are not fermented, remaining on the seeds.

After the seeds are dried, coffee is sized, graded, and mechanically, manually, and/or electronically sorted to eliminate defective seeds. This process may be followed by ultraviolet (UV) excitation, which separates defective seeds produced during processing that are difficult to detect except by fluorescence [11,12]. The green coffee seeds are then ready for roasting. Alternatively, they may be decaffeinated, steam-treated, or stored before roasting.

2.2.2 Decaffeinated coffee production

The recent availability of good-quality decaffeinated coffee makes it a choice for coffee drinkers. A profile of decaffeinated coffee drinkers by Shlonsky et al. [13] revealed that decaffeinated coffee is generally consumed by people with health disorders or those in search of a healthier lifestyle; this population is characterized by a low rate of smoking, low alcohol consumption, and high consumption of health supplements. This health awareness appears to be responsible for the growth and expansion of the decaffeinated coffee market. For at least 8 years, decaffeinated coffee accounts for approximately 10% of coffee consumption [14–17].

Decaffeination is performed before roasting. The least costly caffeine extraction methods use an organic solvent (dichloromethane or ethyl acetate) and water/vapor before and after extraction to wash the seeds and open the pores. After caffeine removal, the seeds are dried until they reach moisture content similar to that prior to processing [18]. The caffeine extracted from the green seeds can be recovered and used for commercial products such as cola beverages and pharmaceutical drugs. Key flavor components can be lost during the decaffeination process [15], especially when using solvents that lack specificity (e.g., water). Devices have been created to return the aromatic fraction lost during caffeine extraction. Alternatively, coffee aroma may be added to the decaffeinated product.

There is a general concern about the amount of dichloromethane that remains in the coffee beverage after decaffeination. However, the boiling point of this solvent is 40°C, and coffee is exposed to temperatures around 70°C for solvent volatilization; therefore, green coffee seeds should not contain significant amounts of the solvent. In addition, roasting temperatures (usually 210°C–240°C) are high enough to promote volatilization of any

residual dichloromethane. The United States Food and Drug Administration (FDA) allows up to 10 mg residue/kg roasted coffee, and the European Union allows 3 mg/kg coffee. According to industry reports, the residual concentration of dichloromethane is usually 100 times lower than these limits [19].

The organic solvent methods were virtually the only available decaffeination methods until the last decade and are still often used in South and Central America. Because of health concerns, decaffeination methods using only water or supercritical carbon dioxide that is carbon dioxide held at high pressure (approximately 200 atm) and temperature above 31°C are the only methods used in many places, especially the United States and Europe. Although costly, decaffeination with supercritical carbon dioxide better preserves the original chemical composition of regular coffee, thereby maintaining its flavor [18,20].

2.2.3 Steam-treated and monsooned coffees

Coffee may be steam-treated before roasting to make coffee less irritating to the stomach [21]. This type of coffee is sometimes referred to as stomach-friendly. The reduced stomach irritation has been attributed to the reduction of chlorogenic acids' content during steam treatment [22].

Monsooned coffee is a specialty coffee in which dry green Arabica and Robusta seeds of good quality are naturally cured for 3–4 months by exposure to the moist monsoon winds prevailing in the west coast of Southern India (Malabar coast), especially in the regions of Mangalore and Tellicherry [23,24]. The coffee is loosely packed in gunny bags and stacked in piles with sufficient space between rows to allow air to circulate freely around the bags. The coffee cherries absorb moisture from the humid monsoon atmosphere, causing the seeds to swell, become light yellow, and acquire an intensely mellow but aggressively musty flavor. The coffee is bulked and repacked at frequent intervals or poured from one bag to another to prevent mold growth and ensure uniform monsooning. Only dry processed Arabica and Robusta seeds are used to prepare monsooned coffees. These seeds have good body, low acidity, and pleasant aroma and flavor in the cup. Monsooned coffees are exported from India to Europe, Asia, Africa, and North America [23,24].

2.2.4 Coffee roasting

The aroma of green coffee seeds is quite different from what we imagine when we hear the word coffee. It is only through roasting that the seeds gain the characteristic aroma and flavor of coffee. Although roasting appears to be simple in terms of processing conditions, the chemistry underlying this flavor development is highly complex and not completely understood. The high roasting temperatures cause a series of physical and chemical changes in the seeds. The specific roasting conditions strongly influence these changes and consequently affect the bioactivity and flavor of the beverage. The most common roasters available for home and industrial use are drum roasters, in which the seeds are in direct contact with fire and/or a hot surface. In newer fluid bed roasters, the seeds are in contact with hot air/gases. Fluid bed roasters are preferred for industrial use because they are faster, allow better control of air temperature and speed inside the roasting chamber, and produce a more homogeneous color than other roasters.

The temperatures used to roast the seeds depend on the roaster type, but the maximum temperatures used in industrial fluid bed roasters generally vary from 210°C to 240°C. In

the initial phase of roasting, free water evaporates. When the seed temperature reaches 130°C, sucrose caramelizes, and the seeds begin to brown and swell. Chemical changes in this initial phase are relatively small compared to those that occur at the end of the roasting process. At temperatures higher than 160°C, a series of exothermic and endothermic reactions take place; the seeds become light brown, their volume increases considerably, and aroma formation begins. The chemical reactions responsible for the aroma and flavor of roasted coffee are triggered at approximately 190°C. During the Maillard and Strecker reactions, which involve carbohydrates (reducing sugar), proteins, and other classes of compounds, low- and high-molecular-weight compounds such as melanoidins are simultaneously degraded and produced. During this process the light brown seeds can become almost black [4,25].

These reactions are interrupted at the desired point based on seed color or programmed time. The seeds are then rapidly cooled by water or air, which is preferred because water increases the risk of microbial growth. After roasting, the seeds are ground and marketed as ground roast coffee or used for instant coffee production.

Ground roast coffees are commercially available in different colors (roasting degrees) that vary from very light to very dark, according to national and individual preferences. In the United Kingdom and the United States, for example, light-medium to medium roasts are preferred, whereas dark roast coffee is more popular in some parts of Europe. Dark-medium to dark roasts are traditional in Brazil, although the consumption of medium-roast coffees has been increasing. Roasting degree standards for commercial and scientific purposes are subjective and may vary considerably. The loss of mass during roasting may be a useful parameter to evaluate roasting degree in small-scale production, but may be difficult to control in large-scale production. Visual inspection continues to be the most accepted method to determine the degree of roasting. To help develop standards for colorimetric assessment, color discs were created by the Specialty Coffee Association of America (SCAA). This *AGTRON/SCAA Roast Classification Colour Disc System* can be used to classify most commercially available coffees. The color palette is based on the linear progression of color development obtained under controlled roasting conditions.

In addition, roasting parameters such as the amount of coffee in the roaster, temperature, roasting time, and speed of hot air circulation (in the case of fluid and spouted bed roasters) used to reach a single roasting degree can vary considerably. The speed at which the seed reaches the desired color affects a number of physical–chemical and chemical parameters and therefore the flavor and bioactivity of the beverage. Thus, two samples of the same coffee roasted to the same degree may have distinct chemical compositions if roasted under different conditions. For example, coffees roasted at higher temperatures for a shorter time tend to exhibit higher acidity, more soluble solids, and a different volatile profile than those roasted for longer periods at lower temperatures [26].

2.2.5 Coffee brewing

Another variable that influences the brew's chemical composition is the brewing method. A common aspect to all brewing methods practiced worldwide is the use of hot water. Specialists agree that the water temperature should not exceed 90°C–95°C; however, it is not uncommon to see people boiling coffee for a few minutes before filtration. The proportion of coffee to water varies considerably in different countries and according to individual preferences, but is usually 8–20 g coffee/100 mL water. Extraction time also varies, and the

average size of the coffee particle (granulometry) ranges from pulverized to coarse according to the brewing method.

The most common brewing methods worldwide are simple percolation, boiled coffee, electric coffee maker, espresso machine, Italian coffee maker, and French press. In the first method, medium-ground coffee is evenly spread in a paper, cloth, or nylon filter set on a support, and hot water is poured over the coffee in a circular motion toward the center of the filter. For boiled or Turkish coffee, water is poured on finely ground or pulverized coffee in a pan and heated; when water begins to boil, the mixture is poured directly into the cup. In some places, fine- to medium-ground coffee is used, and the mixture is filtered. The electric coffee maker, also called electric drip brewer, uses fine- or medium-ground coffee placed on a filter paper as in the simple percolation method. The water compartment is filled with water, which is heated and percolated for approximately 2 minutes through coffee. To make espresso, coarse- or medium-ground coffee and water are placed in their respective compartments. The water is percolated through the coffee at approximately 90°C and 9 atm. To use an Italian coffee maker, also called Italian press or moka pot, water is deposited at the base of the kettle, which contains a pressure valve. When the kettle is heated, the water that is percolated through the medium ground coffee is continuously driven under pressure to the top compartment. With the French press, coarsely ground coffee and hot water are mixed together in a special brewer fitted with a plunger that has a mesh. After allowing the mixture to brew for a few minutes, the plunger is pressed to trap the coffee grounds at the bottom of the beaker, and the brew is poured into the cup.

2.2.6 Instant coffee production

Instant coffee production typically involves treating ground-roast coffee with hot water and high pressure to extract the water-soluble compounds. This soluble material is then cooled and sometimes centrifuged, concentrated by heating, and dried through freeze-drying to reduce moisture to approximately 5%. The spray-drying process uses high temperature under high pressure to volatilize the aqueous extract; hot air then dehydrates the small drops, which are powdered. The freeze-drying process uses very low temperatures to achieve sublimation of the frozen aqueous extract; the direct change from solid to gas gives the final product a higher quality than other methods. Alternatively, steam/water and/or oil may be used to rewet the surface of the instant coffee granules, followed by drying. This process is called agglomeration [27–29]. Manufacturers use different techniques to improve the appearance and taste of the final product. Although ground-roast coffee generally consists of Arabica species alone or a high percentage of Arabica, Robusta coffee is often used at a high percentage or alone in blends designated for instant coffee production, because Robusta seeds contain higher amounts of soluble solids, which increases yield.

2.3 NATURAL COFFEE CONSTITUENTS

The basic chemical composition of green coffee depends primarily on genetic aspects, especially species, and on physiologic aspects such as degree of maturation. The chemical composition of hybrid coffee seeds is similar to that of the parent species. For example, hybrids of *C. arabica* and *C. canephora* such as Timor hybrid and Catimor (cross between Timor hybrid with *C. arabica* cv. Caturra) tend to exhibit intermediate characteristics [30–32].

However, wild varieties with singular chemical compositions have been found. An example is the caffeine-free *C. arabica* variety that has recently been discovered [15,17]. When comparing the chemical compositions of different varieties, at least three consecutive crops of each variety should be evaluated to take into account natural metabolic fluctuations along the physiologic cycles.

In addition to these intrinsic factors, extrinsic factors such as soil composition, climate, agricultural practices, and storage conditions affect seed physiology and chemical composition, but to a lesser extent [33,34]. Therefore, these aspects should also be considered when comparing chemical compositions of coffees. The flavor of high-quality coffee can vary considerably among samples from the same species and variety grown in different regions. Climate and soil composition (including microbiota) are relevant because chemical compounds and minerals present in small amounts may produce considerable changes in the sensory attributes of the beverage.

As previously mentioned, *C. arabica* and *C. canephora* species differ in many ways. As suggested by the name, Robusta coffee trees are more robust; that is, they are stronger, more resistant to pests and disease, and less demanding than Arabica trees with regard to climate. Robusta coffee also contains higher amounts of antioxidant compounds and caffeine. Moreover, Robusta coffee contains more soluble solids; therefore, its inclusion in commercial blends used for instant coffee adds body to the beverage and increases yield. On the other hand, Arabica coffee provides superior cup quality and aroma compared with Robusta, which commonly possesses a more aggressive flavor and, in light roast coffee, has a flat popcorn-like aroma. For most consumers, some Arabica seeds are needed for a blend to seem like coffee. As a result, the value of Robusta seeds is approximately half that of Arabica [1]. However, it is worth mentioning that Robusta seeds that are carefully harvested and processed may produce a better cup than fermented, oxidized, or otherwise low-quality Arabica seeds.

Although most constituents of *C. arabica* are also present in *C. canephora*, their relative proportions can differ considerably. Additionally, *C. canephora* contains a few secondary metabolites (e.g., minor chlorogenic acids isomers and diterpenes) that are not present in *C. arabica*. For decades, hybridization efforts have attempted to combine the resistance of Robusta trees with the cup quality of Arabica seeds, but it is likely that the traits responsible for pest resistance in Robusta plants are partially responsible for the lower cup quality. For example, the higher chlorogenic acids content in Robusta coffee protect the plant against microorganisms, insects, and UV radiation [33]. Although low amounts of chlorogenic acids are important for flavor, high amounts may reduce cup quality, possibly due to the high levels of oxidation products generated before roasting [35]. In addition, differences in cell wall composition contribute to diverse chemical responses to roasting.

A description of the basic chemical composition of *C. arabica* and *C. canephora* seeds is given here.

2.3.1 Green coffee chemical composition

2.3.1.1 *Nonvolatile compounds in green coffee*

The nonvolatile fraction of green coffee is composed primarily of water, carbohydrates and fiber, proteins and free amino acids, lipids, minerals, organic acids, chlorogenic acids, trigonelline, and caffeine (Table 2.1). Of these compounds found in green coffee, chlorogenic acids, caffeine, trigonelline, soluble fiber, and diterpenes from the lipid fraction are most

Table 2.1 Chemical composition of green *Coffea arabica* and *Coffea canephora* seeds.

	Concentration[a] (g/100 g)	
Component	***Coffea arabica***	***Coffea canephora***
Carbohydrates/fiber		
Sucrose	6.0–9.0	0.9–4.0
Reducing sugars	0.1	0.4
Polysaccharides	34–44	48–55
Lignin	3.0	3.0
Pectin	2.0	2.0
Nitrogenous compounds		
Protein/peptides	10.0–11.0	11.0–15.0
Free amino acids	0.5	0.8–1.0
Caffeine	0.9–1.3	1.5–2.5
Trigonelline	0.6–2.0	0.6–0.7
Lipids		
Coffee oil (triglycerides with unsaponifiables, sterols/tocopherols)	15–17.0	7.0–10.0
Diterpenes (free and esterified)	0.5–1.2	0.2–0.8
Minerals	3.0–4.2	4.4–4.5
Acids and esters		
Chlorogenic acids	4.1–7.9	6.1–11.3
Aliphatic acids	1.0	1.0
Quinic acid	0.4	0.4

[a]Content varies according to cultivar, agricultural practices, climate, soil composition, and methods of analysis.
Source: Clarke and Macrae [28], Clifford [47], Trugo and Macrae [108], Trugo [46], Clarke [4], Kölling-Speer and Speer [97], Speer and Kölling-Speer [98], Farah et al. [18], Farah and Donangelo [33]), Holscher et al. [199], and Fischer et al. [198].

likely to be bioactive, and they may also be important contributors to the beverage flavor after roasting.

Minor phenolic compounds such as anthocyanins and lignans identified in green coffee seeds have been reported as residues of the fruits [33]. In addition, traces of theophylline and theobromine have been identified in the seeds and reported as caffeine metabolites [17].

The main bioactive compounds present in green coffee seeds are discussed in detail in the forthcoming sections.

Caffeine

Caffeine (Figure 2.1) is a methylxanthine with bitter characteristics; however, it is responsible for no more than 10% of the perceived bitterness of the coffee beverage [11]. This alkaloid is heat stable, and its concentration in *C. canephora* is approximately twice that found in *C. arabica* (Table 2.1).

Caffeine stimulates the central nervous system as an adenosine-receptor antagonist. Although caffeine is the most widely consumed and studied psychoactive substance in history, its effects on health are controversial [13]. While caffeine intake has been associated with

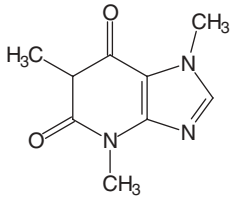

Figure 2.1 Chemical structure of caffeine.

high blood cholesterol, coronary diseases, and cancer, other studies suggest that its consumption may lower the incidence of suicide and hepatic cirrhosis [18]. Low to moderate caffeine intake is generally associated with increased alertness, learning capacity, exercise performance, and perhaps better mood, but high doses can produce negative effects in some sensitive individuals (e.g., anxiety, tachycardia, and insomnia) during its half-life, which is 2–6 hours after coffee intake [10,14,18,47]. Acute caffeine consumption had negative effects on glucose tolerance, glucose disposal, and insulin sensitivity in lean, obese, and type 2 diabetic rats and humans, but other compounds present in coffee can counteract this effect [38]. Acute caffeine intake also increases the urinary excretion of minerals such as calcium [39]. However, after long-term consumption, most of these acute effects tend to disappear because of metabolic adaptations in the body [40]. Caffeine metabolites, especially 1-methylxantine and 1-methylurate, have exhibited antioxidant activity *in vitro*, and the *in vivo* iron-reducing capacity of regular coffee is higher than that of decaffeinated coffee [41]. The antibacterial effect of regular coffee against cariogenic microorganisms was also higher than that of decaffeinated coffee [42].

Trigonelline

Trigonelline (Figure 2.2) is an alkaloid biologically derived from enzymatic methylation of nicotinic acid. It contributes to the bitterness of the brew and is a precursor for the formation of different classes of volatile compounds during roasting such as pyrroles and pyridines, some of which according to Flament [11] may confer an "objectionable flavor." The amount of trigonelline in *C. canephora* is approximately two-thirds that found in *C. arabica*.

Regarding potential bioactivity, trigonelline has inhibited the invasiveness of cancer cells *in vitro* [43]. In addition, this compound has been able to regenerate dendrites and axons in animal models, suggesting that it may improve memory [44]. More recently it has been considered a novel phytoestrogen [45]. Trigonelline demethylation during coffee roasting produces nicotinic acid, a B-complex vitamin also known as niacin [46].

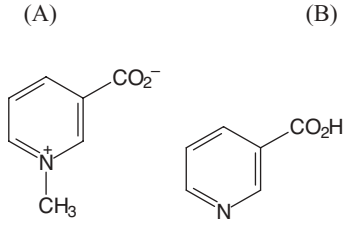

Figure 2.2 Chemical structures of (A) trigonelline and (B) nicotinic acid.

Chlorogenic acids

Chlorogenic acids (Figure 2.3) comprise a major class of phenolic compounds, which are derived primarily from esterification of *trans*-cinnamic acids (e.g., caffeic, ferulic, and *p*-coumaric) with (−)-quinic acid. They are subdivided according to the nature and number of cinnamic substituents and the esterification position in the cyclohexane ring of the quinic acid [47]. The esters are formed preferentially with the hydroxyl located at carbon 5 as well as those located at carbons 3 and 4. Less commonly, esters may be formed with the hydroxyl located at carbon 1. The main subclasses of chlorogenic acids in green coffee are caffeoylquinic

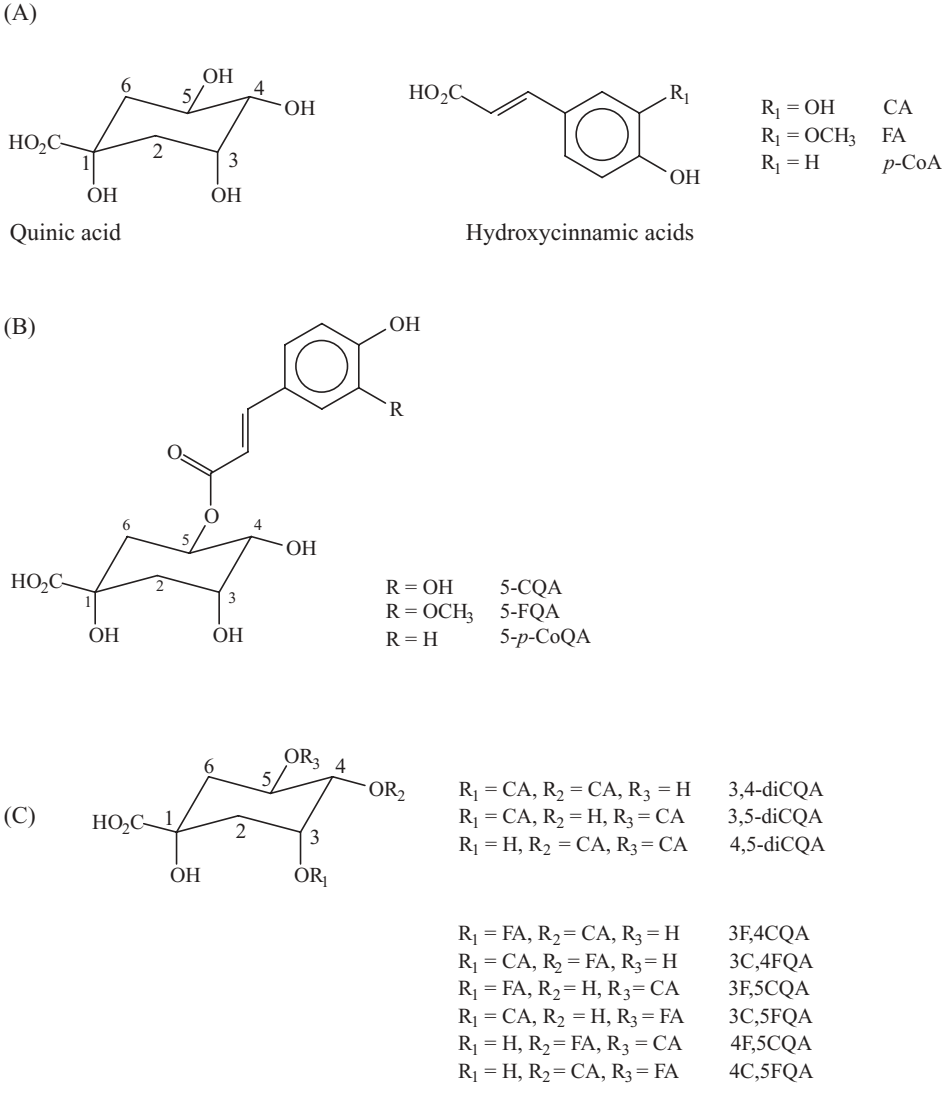

Figure 2.3 Chlorogenic acids and related compounds. (A) Basic compounds, (B) monoesters of quinic acid with hydroxycinnamic acids (example of 5-isomers), (C) di-esters of quinic acid with caffeic acid, and mixed esters. diCQA, dicaffeoylquinic acids; FQA, feruloylquinic acids; *p*-CoQA, *p*-coumaroylquinic acids.

acids, dicaffeoylquinic acids, feruloylquinic acids and, less abundantly, *p*-coumaroylquinic acids and caffeoyl-feruloylquinic acids. Each of these subclasses consists of at least three major positional isomers in addition to minor compounds, with the exception of the latter class, which contains six major isomers [48,49]. Among these classes, caffeoylquinic acids account for approximately 80% of the total chlorogenic acids content. In particular, 5-caffeoylquinic acid, the first of these compounds identified, accounts for almost 60% and is therefore the most studied isomer and the only one for which a commercial standard is available. For this reason, 5-caffeoylquinic acid is commonly called chlorogenic acid.

In the last decade, a series of minor chlorogenic acids and related compounds have been identified in green *C. arabica* and *C. canephora* seeds, including dicaffeoylquinic acids, acyl dicaffeoylquinic acids, dimethoxycinnamoylquinic acids, caffeoyl-dimethoxycinnamoylquinic acids, diferuloylquinic acids, and feruloyl-dimethoxycinnamoylquinic acids, sinapoylquinic acids, sinapoyl-caffeoylquinic acids, sinapoyl-feruloylquinic acids, feruloyl-sinapoylquinic acids, and new minor *p*-coumaric acid-containing compounds [50–53]. However, these minor compounds together are not responsible for even 1% of total chlorogenic acids.

Chlorogenic acids confer astringency, bitterness, and acidity to the coffee brew. Nevertheless, high amounts in green coffee, particularly caffeoylquinic and feruloylquinic acids, may produce undesirable flavor possibly due to oxidation and degradation products formed before roasting [35]. Chlorogenic acids are precursors of phenols and cathecols that may confer unpleasant sensory notes that are formed during roasting [46]. The content of chlorogenic acid in *C. canephora* is generally one and a half to two times higher than in *C. arabica,* but this concentration varies considerably in both species.

In the last few years, a series of epidemiologic and clinical studies have reported that coffee consumption, independent of caffeine intake, is associated with health benefits such lower risk of type 2 diabetes [54–59], Parkinson and Alzheimer diseases [60], and liver cancer [61,62]. *In vitro* and animal studies are the main source of data attributing these beneficial properties to antioxidant and other mechanisms involving chlorogenic acid compounds [38,63–73]. Additionally, because its high content of chlorogenic acids, studies performed in Denmark, the United States, Mediterranean countries, Japan, and Brazil have reported that coffee is the most important contributor to antioxidants intake in their diets [72,74–78].

Long before epidemiologic studies reported the relationship between coffee consumption and health, the antimutagenic property of chlorogenic acids and their metabolites has been demonstrated by a series of animal and *in vitro* studies [79–84]. Recent studies have confirmed these findings and elucidated several mechanisms of action including free radical scavenging, metal chelation, inactivation of reactive compounds, and metabolic pathway changes [85–89]. Pharmacologic properties attributed to caffeoylquinic and dicaffeoylquinic acids include antiviral activity against adenovirus and herpes virus [90], hepatoprotective activity in an experimental model of liver injury [91], and immunostimulatory activity [92]. Synthetic dicaffeoylquinic acid derivatives also inhibit HIV-1 replication in cells (93–96], which raises the possibility of novel coffee-based anti-HIV drugs. Because only a few chlorogenic acid compounds are commercially available or synthesized in laboratories, studies reporting the biological properties of feruloylquinic and coumaroylquinic acids are scarce.

Cafestol and kahweol

The coffee compounds cafestol and kahweol (Figure 2.4) are pentacyclic diterpene alcohols based on the kaurane skeleton. Methylated forms of cafestol and kahweol have been identified in Robusta seeds [97]. These bioactive compounds and their derivatives, which

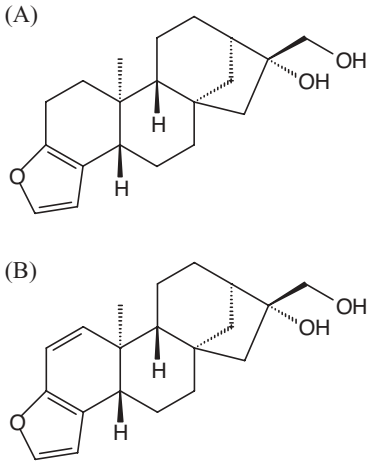

Figure 2.4 Chemical structures of the main coffee diterpenes. (A) Cafestol and (B) kahweol.

are mainly salts or esters of saturated fatty acids (predominant) and unsaturated fatty acids, represent approximately 20% of the lipid fraction of coffee [82,89,98]. Cafestol is the primary constituent of the unsaponifiable fraction of coffee oil, accounting for approximately 0.2%–0.6% of coffee weight. Kahweol is more sensitive to heat, oxygen, light, and acids and is therefore less abundant [11]. Higher levels of diterpenes are found in *C. arabica* than in *C. canephora*.

Coffee diterpenes have exhibited anticarcinogenic and hepatoprotective properties *in vitro* [82,89,99]. On the other hand, high consumption of these compounds has been associated with elevated homocysteine and low-density lipoprotein levels in human plasma, which may indirectly increase the risk of cardiovascular diseases [100]. Considerable amounts of these compounds are present primarily in unfiltered coffee since they are poorly soluble in water and are therefore trapped by paper filters.

Soluble dietary fiber

Soluble dietary fiber in coffee consists of high-molecular-weight polysaccharides that increase the brew's viscosity [101]. Galactomannans and type II arabinogalactans are the most important types of soluble fiber in coffee. Galactomannans are polymers of 1,4-linked mannans with a single galactose unit side chain at C_6, and type II arabinogalactans consist of a main chain of 1,3-linked galactose branched at C_6, with side chains containing arabinose and galactose residues [102]. *The hot* water-soluble green coffee type II arabinogalactans are highly branched and covalently linked to proteins in which 10% of the amino acid chains are 4-hydroxyproline residues. These polysaccharides are extremely complex. In addition to galactose and arabinose, they also contain raminose and glucuronic acid residues. Rhamnoarabinosyl and rhamnoarabinoarabinosyl side chains have recently been reported [103].

In the last decade, these compounds have received special attention because they cannot be digested by humans; therefore, they reach the colon intact, potentially serving as substrates for beneficial colonic microbiota fermentation [104]. A high intake of dietary fiber is positively associated with several beneficial physiologic and metabolic effects such as lowering blood cholesterol and modulating the blood glucose and insulin responses. Fermentable polysaccharides are degraded by colonic microbiota to short-chain fatty acids

(e.g., acetate, propionate, and butyrate). This process lowers the colonic pH, impeding the growth of certain pathogenic species and supporting the growth of *Bifidobacterium* species and other beneficial lactic acid bacteria [104].

Additional natural coffee constituents that are common in other food products are water, carbohydrates, proteins, peptides and free amino acids, carboxylic acids, minerals, and lipids (e.g., triacylglycerols, sterols, tocopherols, and wax). These components are briefly addressed here.

Water

The water content of green seeds of *C. arabica* and *C. canephora* generally varies from approximately 8.5%–12% [25]. Above this level, the moisture is undesirable both for aroma/flavor quality and health effects, since it increases water activity and therefore the probability of microbial growth. On the other hand, low moisture produces cracks in the seeds and decreases their viability to germinate.

Carbohydrates

Carbohydrates are major constituents of coffee and may account for more than 50% of the dry weight. The poly-, oligo-, di-, and monosaccharides can be divided into reducing and nonreducing sugars [105]. Polysaccharides (soluble and insoluble) account for approximately 44% of dry matter in *C. arabica* and 47% in *C. canephora*. Sucrose is important for coffee flavor and quality; it accounts for up to 9% of *C. arabica* dry weight and approximately half of it in *C. canephora*. Small amounts of simple carbohydrates such as fructose, glucose, mannose, arabinose, and rhamnose and oligosaccharides such as raffinose and stachyose have been identified in green coffee [11,97]. Carbohydrates are precursors for the Maillard reaction (in the case of sucrose, after inversion) and caramelization, which are important for color and aroma development. They also contribute to the acidity of the brew after coffee roasting. A higher sucrose content is one of the reasons for the superior aroma and overall flavor of Arabica coffee. High-molecular-weight polysaccharides give body to the brew. Of the main polysaccharides in coffee, galactomannan and arabinogalactan are soluble but cellulose is not. As earlier described, brewed coffee contains a considerable amount of soluble fiber; due to their potential role as substrate for probiotic microorganisms in the human intestine, these polysaccharides are considered bioactive compounds.

Protein, peptides, and free amino acids

Protein, peptides, and free amino acids are vital for coffee flavor since they are needed for the Maillard reaction. They serve as precursors for the formation of volatile compounds such as furans, pyridines, pyrazines, pyrrols, aldehydes, and melanoidins. The melanoidins are responsible for coffee's color and to some extent, its antioxidant activity. The total nitrogenous compounds (excluding caffeine and trigonelline) account for 9%–16% of the green coffee chemical composition, with a slightly higher content in *C. canephora* than *C. arabica*. However, coffee is not a good nutritional source of protein because it lacks essential amino acids.

Minerals

Potassium accounts for approximately 40% of the mineral content of ground coffee (approximately 1–2 g/100 g green coffee). Phosphorus is another important mineral in coffee, accounting for 4% of its composition. The remaining mineral content consists of approximately 30 different elements, including sodium, magnesium, calcium, and sulfur [4,106]. Of

these elements, only the magnesium content appears to vary considerably between species (1–3 mg/100 g for *C. canephora* and 2.5–6 mg/100 g for *C. arabica*) [4]. Trace minerals in coffee include zinc, strontium, silicon, manganese, iron, cupper, barium, boron, and aluminum. The profile of trace minerals in coffee varies according to soil composition, which suggests that it may be possible to differentiate coffees grown in different types of soil by their mineral profile [107].

Lipids

Lipids are major components of coffee, and their total content varies considerably between *C. arabica* and *C. canephora* species. The lipid fraction of coffee is composed mainly of triacylglycerols (approximately 75%), free fatty acids (1%), sterols (2.2% unesterified and 3.2% esterified with fatty acids), and tocopherols (0.05%), which are typically found in edible vegetable oils. This fraction also contains diterpenes of the kaurene family (previously presented here) in proportions of up to 20% of the total lipid fraction [97,108,109]. Other recently identified components are coffeadiol and arabiol I, which have structures similar to the diterpenes cafestol and kahweol, respectively, but with different substitutions in the furan ring [98].

The total lipid content in Arabica seeds (approximately 14 g/100 g dry matter) is approximately two times that of Robusta seeds [110]. Fatty acids in coffee are found primarily in combined forms; most are esterified with glycerol in the triacylglycerol fraction, 20% esterified with diterpenes, and a small proportion in sterol esters. Most fatty acids in coffee are unsaturated. Linoleic acid (18:2(*n*-6)), oleic acid (18:1(*n*-9)), and linolenic acid (18:3(*n*-3)) account for approximately 43%–54%, 7%–14%, and 1%–2.6% of the triacylglycerol fraction, respectively, and approximately 46%, 11%, and 1% of the free fatty-acid fraction, respectively [97,109,111,112]. Fatty acids are not only important for health, but their integrity is important to keep coffee fresh and avoid the staleness caused by hydrolysis and oxidation of triacylglycerols [113].

The major categories of sterols in coffee are 4-desmethylsterols (accounting for approximately 93% of total sterols), 4-methylsterols (2%), and 4,4-dimethyl-sterols (5%). Sitosterol belongs to the first category and accounts for up to 54% of the sterol fraction; stigmasterol and campesterol each account for approximately 20% [11,98].

The average amount of tocopherols in coffee has been reported as 11.9 mg/100 g green coffee [114], but varies considerably depending on the methodology used. The α, β, and γ forms of tocopherols are present in coffee. The analytical separation of the β and γ forms is difficult, but a few studies have reported the predominance of β-tocopherol, followed by α and γ. Folstar et al. [115] found concentrations of 8.9–18.8 mg α-tocopherol and 25–53 mg β- + γ-tocopherol/100 g coffee oil. Ogawa et al. [114] reported the maximum total tocopherol content as 15.7 mg/100 g green coffee, with α-tocopherol accounting for 2.3–4.5 mg and β-tocopherol accounting for 3.2–11.4 mg/100 g green coffee; γ-tocopherol was not detected, possibly because of separation difficulties.

Although most lipids are located in the endosperm of green coffee seeds, the coffee wax is located in the outer layer. This fraction accounts for 0.2%–0.3% of the coffee seed's weight. The main components of coffee wax are carboxylic acid-5-hydroxytryptamides, which are amides of serotonin and fatty acids of varying chain lengths [98].

2.3.1.2 *Volatile compounds in green coffee*

The poor volatile fraction of unroasted coffee seeds gives them a weak but characteristic aroma. Approximately 100 different volatile compounds have been identified in green coffee

seeds [11]. The most abundant classes of volatile compounds are alcohols, esters, hydrocarbons, and aldehydes. Ketones, pyrazines, furans, and sulfur compounds have also been identified [9,11].

The maturation stage of the coffee fruits is important for the volatile composition of green coffee. The volatile composition of coffee berries was studied by Ortiz et al. [116], who observed that, as with the seeds, the volatile composition of coffee berries is dominated by high levels of alcohols, mainly ethanol, in all stages of ripeness. Overripe coffee berries, which produce black defective seeds, exhibited high concentrations of volatile compounds dominated by esters, followed by alcohols, ketones, and aldehydes, with very low levels of monoterpenes. Toci and Farah [9] identified potential markers for green defective coffee seeds generated from fruits at different maturation stages. This subject will be explored later in this chapter.

2.3.2 Changes in coffee chemical composition during roasting

2.3.2.1 *Nonvolatile components in roasted coffee*

The seed composition dramatically changes during roasting as a consequence of pyrolysis, caramelization, and Maillard reactions. Some compounds are destroyed and others are formed, including bioactive compounds and substances of high and medium volatility, which are important for the aroma and flavor of the brew. The final composition of roasted coffee varies according to the raw material, roasting degree, and other roasting variables such as roaster type and the time, temperature, and air-flow speed in the roasting chamber.

The moisture content of roasted coffee (1.5%–5%) is much lower than that of green coffee, and varies depending on the roasting degree [25,108].

A portion of the coffee protein is degraded, and free amino acids and peptides are consumed by Strecker reactions. Some of the amino acids react with reducing sugars to form (via Maillard reaction) low-molecular-weight compounds and melanoidins that incorporate into their structures other components, such as chlorogenic acids, galactomannans, and arabinogalactan-proteins [117]. Melanoidin polymers, which exhibit variable composition and molecular mass, are responsible for the brown color of roasted coffee and approximately 25% of its dry matter [118]. Different studies suggest that melanoidins are partially responsible for the antioxidant, antibacterial, and metal-chelating properties of coffee beverages and therefore may be considered bioactive compounds [118–121]. However, their physiologic relevance in humans is unknown.

Sucrose is consumed by caramelization and Maillard reactions (after inversion). Soluble fiber is partially degraded and incorporated into melanoidins. The brew acidity may increase as levels of aliphatic acids (formic, acetic, glycolic, and lactic) rise through degradation of sucrose, polysaccharides, and other compounds [28,122], especially during short high-temperature roasting [26].

Because of thermal instability, chlorogenic acids undergo many changes during roasting, namely, isomerization, epimerization, lactonization, degradation to low-molecular-weight compounds (including phenols and cathecols; Figure 2.5), and incorporation into melanoidins, contributing to color and flavor development. Their degradation follows first-order Arrhenius-compliant kinetics; however, distinct models should be used for *C. arabica* and *C. canephora* samples [123]. Depending on the roasting degree, the total chlorogenic acids content is reduced to less than 1% of the original content. Chlorogenic acid contents in commercial roasted coffees may vary from 0.5–6 g/100 g, dry weight, depending on the type

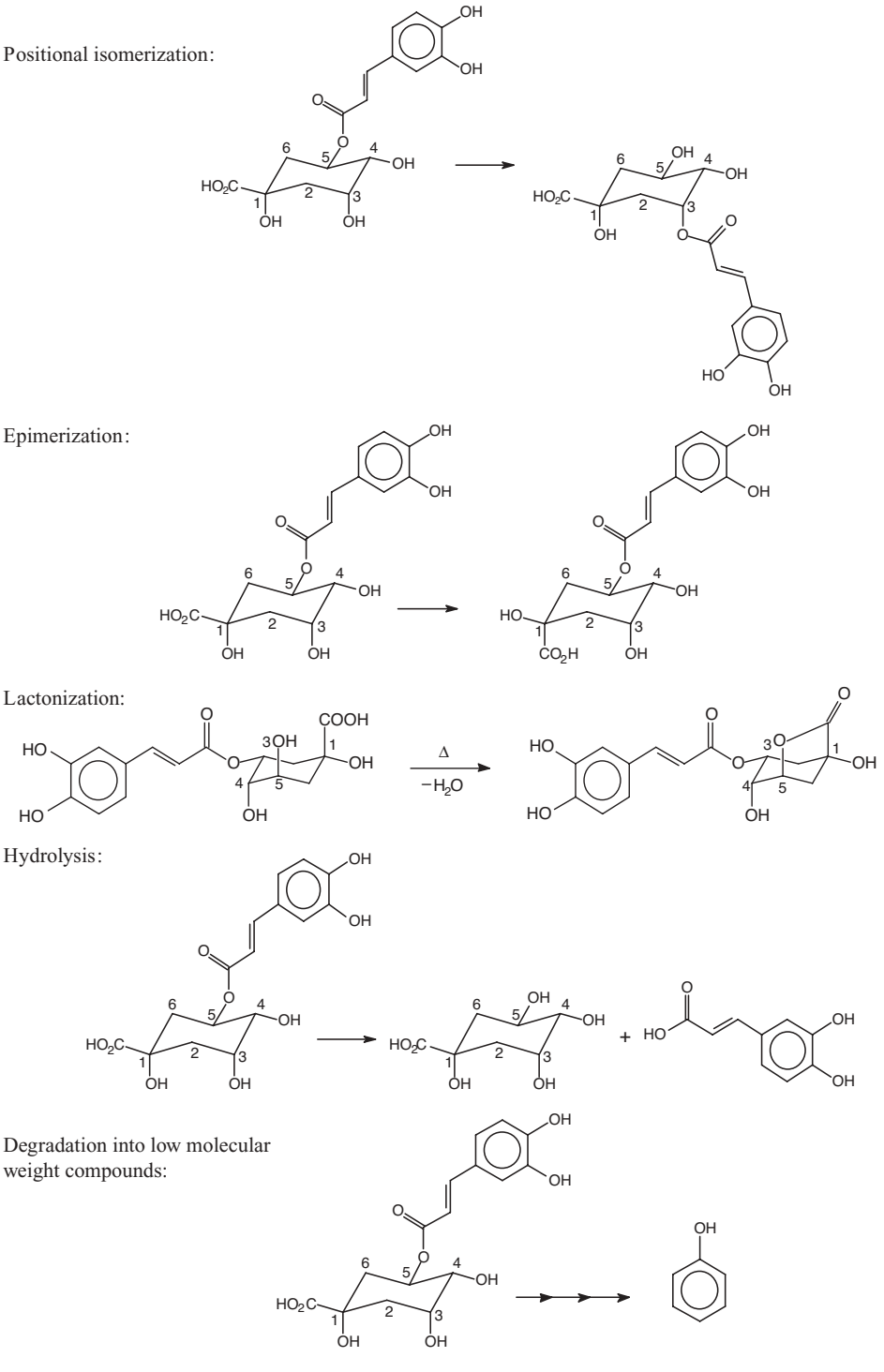

Figure 2.5 Examples of changes in chlorogenic acids during roasting.

of processing, blend, roasting degree, roasting method, and analytical conditions [124,125]. Fast high-temperature roasting (230°C) reduces the loss of these compounds [26,33,49].

Chlorogenic acid lactones or quinides are formed by less than 10% of chlorogenic acids in green coffee through loss of a water molecule from the quinic acid moiety and formation of an intramolecular ester bond. The main chlorogenic acid lactones formed in coffee are the 1,5-γ-lactones (Figure 2.5). The formation of minor δ-lactones has also been observed [126]. Chlorogenic acid lactones contribute considerably to the bitterness of the coffee beverage, an important aspect of quality. These lactones have also received attention because of their potential effects on brain function independent of the pharmacologic effects of caffeine. They exhibit opiate receptor binding activity with characteristics similar to those of opiate antagonists [127] and can reverse morphine-induced analgesia in mice [128]. However, their relatively weak *in vitro* affinity to opioid receptors suggest that acute pharmacologic effects are unlikely with normal coffee consumption. Further, the bioavailability of these compounds is unknown. In a study by Farah et al. [129], part of the lactone administrated via gavage to rats was recovered in the form of chlorogenic acid. This finding was confirmed by an ex vivo study in which a percentage of the major 1,5-caffeoylquinic acid lactones was converted into caffeoylquinic acids after contact with the alkaline pH of human digestive fluids (unpublished). Therefore, it is likely that a large proportion of these lactones consumed in the brew return to their respective chlorogenic acid forms during digestion, indirectly increasing the total chlorogenic acids intake. In fact, chlorogenic acid lactones were shown to exert blood glucose-normalizing effects in rats [130], and these effects were later observed for the chlorogenic acids themselves [38].

Caffeine is not significantly altered during coffee roasting, but small losses may occur due to sublimation. However, an increase in caffeine content may be observed due to the loss of other compounds.

Roasting degrades trigonelline, producing a variety of compounds including nicotinic acid (3%) and volatile compounds such as pyrrols (3%), pyridines (46%), pyrazines, and methyl nicotinate [11,108]. Nicotinic acid, also called niacin, vitamin B_3, or vitamin PP, is formed via trigonelline demethylation (Figure 2.2) [108]. In humans, nicotinic acid participates as a coenzyme in various metabolic processes, and its deficiency causes pellagra, a disease characterized by skin lesions. Although niacin production increases as roasting progresses, a 100-mL cup of a medium roast coffee can supply approximately 20% of the daily dietary reference intake (DRI) recommendation [131]. Fast roasting tends to produce coffees with higher trigonelline content than slow roasting.

The lipid fraction including triacylglycerols and sterols is relatively heat stable. Although diterpenes are more sensitive to heat, reasonable amounts (0.2–0.9 g/100 g dry weight) may still be found in roasted coffee, especially in *C. arabica*. Tocopherol content also decreases during roasting. Depending on the degree of roasting, α-tocopherol, β-tocopherol, and total tocopherols may be reduced 79%–100%, 84%–100%, and 83%–99%, respectively [98].

Table 2.2 shows the typical chemical composition of medium roast *C. arabica* and *C. canephora* seeds.

2.3.2.2 *Volatile compounds in roasted coffee*

It is only during roasting that the complex aroma of coffee is formed by pyrolysis, Strecker degradation, and Maillard reaction. The variety and concentrations of volatile compounds in roasted coffee depend on the composition of nonvolatile compounds in the raw seeds and on roasting conditions. Therefore, factors such as genetics, soil, agricultural practices,

Table 2.2 Chemical composition of roasted *Coffea arabica* and *Coffea canephora* seeds.

	Concentration[a] (g/100 g)	
Compounds	***Coffea arabica***	***Coffea canephora***
Carbohydrates/fiber		
Sucrose	4.2-tr	1.6-tr
Reducing sugars	0.3	0.3
Polysaccharides (arabinogalactan, mannan, and glucan)	31–33	37
Lignin	3.0	3.0
Pectins	2.0	2.0
Nitrogenous compounds		
Protein	7.5–10	7.5–10
Free amino acids	ND	ND
Caffeine	1.1–1.3	2.4–2.5
Trigonelline	1.2–0.2	0.7–0.3
Nicotinic acid	0.016–0.026	0.014–0.025
Lipids		
Coffee oil (triglycerides with unsaponifiables)	17.0	11.0
Diterpene esters	0.9	0.2
Minerals	4.5	4.7
Acids and esters		
Chlorogenic acids	1.9–2.5	3.3–3.8
Aliphatic acids	1.6	1.6
Quinic acid	0.8	1.0
Melanoidins	25	25

[a]Content varies according to cultivar, agricultural practices, climate, soil composition, methods of analysis, and roasting degree.
Source: Clarke and Macrae [28], Clifford [47], Trugo and Macrae [108], Trugo [46], Clarke [4], Kölling-Speer and Speer [97], Speer and Kölling-Speer [98], Farah et al. [18], Farah and Donangelo [33], Holscher et al. [199], and Fischer et al. [198].
ND, not detected.

climate, and degree of maturation influence the final composition of the volatile fraction of roasted coffee.

More than 950 compounds have been identified after roasting in different types of coffee, depending on their origin, degree of roasting, and analytical methods used [3]. The classes of volatile compounds typically found in roasted coffee are furans and pyrans, pyrazines, pyrroles, ketones and phenols, hydrocarbons, alcohols, aldehydes, acids and anhydrides, esters, lactones, thiophenes, oxazoles, thiazoles, pyridines, amines, and various sulfur and nitrogen compounds [9,11,106,132]. It is difficult to determine all the reactions that generate these volatile compounds, since a number of them may be produced by more than one route. Generally, carbohydrates (including soluble polysaccharides) produce furans, aldehydes, ketones, and phenols; proteins, peptide, and amino acids produce ketones, pyrrols, and pyrazines; lipids are responsible for only small amounts of aldehydes and ketones given their resistance to changes during the roasting process; chlorogenic acids produce phenolic volatile compounds (e.g., catechols, pyrogallol, and phenol); and trigonelline produces pyrroles, pyridines, and pyrazines. Almost all thiophenes, oxazoles, and thiazoles are formed during roasting, since they are not usually detected in green coffee.

The roasting degree and roasting parameters affect the volatile composition of coffee. The effect of roasting degree is readily apparent because the aroma of a light roasted coffee

differs considerably from that of a dark roasted coffee. The formation of volatile compounds depends on the stability of their precursors and location within the seed. In addition, different volatile profiles have been observed in coffee samples roasted under different conditions to achieve the same roasting degree [133]. Compounds that may be affected by roasting conditions include pyridine, 2-methylpyrazin, furfural, furfuryl formate, 2-furanomethanol acetate, 5-methyl-furancarbaldehyde, 1-(2-furanylmethyl)-1H-pyrrol, 1-(1H-pyrrol-2-yl)-ethanone, 2-methoxyphenol, and 4-ethyl-2-metoxyphenol.

It is worth noting that compounds of the same class may impact coffee aroma differently, and thus the concentrations of weak compounds will not necessarily matter. Table 2.3 shows a few volatile odorants that have been reported to be important for coffee aroma.

The seeds of *C. arabica* and *C. canephora* species have different nonvolatile compositions; therefore, it is not surprising that they also exhibit different profiles of volatile compounds, which results in markedly different aromas. Blank et al. [132] reported that caramel-like and "sweet-roasty" attributes predominate in *C. arabica,* whereas spicy and "earthy-roasty" attributes prevail in *C. canephora* species. Differences in the volatile profiles that contribute to these dominant notes are the predominance of 3-mercapto-3-methylbutylformate; sotolon; abhexon; 2-methyl-3-furanthiol, phenylacetaldehyde; 3,4-dimethyl-2-cyclopentenol-l-one; 2-/3-methylbutanoic acid; and linalool in Arabica coffee whereas in Robusta, 2,3-diethyl-5-methylpyrazine; 4-ethylguaiacol; and 3-methyl-2-buten-l-thio are more abundant.

The quality of the unroasted seeds used in a blend is critical for the final flavor in the cup. In a comparison of coffee samples containing variable amounts of defective seeds, Toci and Farah [133] observed that low-quality seeds contained a greater number of specific volatile compounds and a higher total concentration of volatile compounds than high-quality seeds. Further, high-quality and low-quality seeds exhibited distinct behaviors during roasting (slower or faster roasting) under different time and temperature parameters. Compounds affected by the quality of raw seeds and roasting conditions include 2-methylpyrazine, 2,3,5-trimethylpyrazine, 1H-pyrrole, and 2-furfurylmethanol. The initial nonvolatile and volatile compositions of seeds from fruits at different maturation stages may be one reason for the different responses to roasting. In addition, cell walls of defective and healthy seeds contain different amounts of cellulose, lignin, and hemicellulose, which affect heat transfer from outside to inside, accelerating or retarding the roasting process.

Toci and Farah [9] identified potential markers for roasted coffee seeds from fruits at different maturation stages. Since defective seeds are considered to be incidental in coffee, this topic is discussed in Section "Incidental Coffee Constituents."

2.3.3 Changes in coffee chemical composition during special coffee processing

Processing coffee seeds produces numerous chemical changes. High temperatures affect thermolabile compounds, whereas processing with water and vapor can remove water-soluble compounds (e.g., polysaccharides and oligosaccharides) and increase the water content of the seeds.

In decaffeinated coffees, caffeine content is usually reduced from 1–2 g/100 g to 0.020.3 g/100 g [10,18,134]. The loss of other coffee components depends on the decaffeination method. Whereas water tends to remove many soluble components, the affinity of the various organic solvents used tends to be more specific. Analyses of decaffeinated seeds have shown that their chemical composition changes considerably during the process. However, the

Table 2.3 Impact compounds in coffee aroma.

Compound	Sensory note
2,3-Butanedione	Buttery
2,3-Pentanedione	
1-Octen-3-one	Mushroom
2-Hydroxy-3-methyl-2-ciclopentene-1-one	Sweet/caramel
Propanal	
2-Methylpropanal	
3-Methylpropanal	
2-Methylbutanal	Buttery
4-Methylbutanal	Buttery
Hexanal	
(*E*)-2-Nonenal	Buttery
Methional	Baked potato
Methanethiol	
4-Methyl-2-buteno-1-thiol	Tobacco/roasted
2-Methyl-4-furanthiol	Meat
5-Dimethyl-trisulfide	Sulphur
2-Furfurylthiol	Roasted
2-Furanmethanthiol	Smoke/roasted
2-(Methylthiol)propanal	Soy sauce
2-(Methylthio-methyl)furan	Tobacco/roasted
3,5-Dihydro-4(2*H*)-thiophenone	Tobacco/roasted
2-Acetyl-2-tyazoline	Roasted
4-Methylbutanoic	Sweet/acidic
β-Damascene	Cooked apple/fruity/sweet
4-Hydroxy-2,5-dimethyl-4(2*H*)-furanone (furaneol)	Caramel/sweet
2-Ethyl-furaneol	Caramel
4-Hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon)	Spicy
5-Ethyl-4-hydroxy-4-methyl-2(5*H*)-furanone (abexona)	Spicy
2-Ethyl-4-hydroxy-5-methyl-4(5*H*)-furanone	Sweet/caramel caramelDoce/caramelado
2-Methoxyphenol	Phenolic/burnt
4-Methoxyphenol	Phenolic
4-Ethyl-2-methoxyphenol	Phenolic
4-Vinyl-2-methoxyphenol	Clove
4-Ethenyl-2-methoxyphenol	Phenolic
3-Methylindole	Coconut
Vanilline	Vanilla
2,3-Dimethylpyrazine	Nutty/roasted
2,5-Dimethylpyrazine	Nutty/roasted
2-Ethylpyrazine	
2-Ethyl-6-methylpyrazine	
2,3-Diethyl-5-methylpyrazine	Nutty/roasted
2-Ethyl-3,5-dimethylpyrazine	Earthy/nutty/roasted
3-Ethyl-2,5-dimethylpyrazine	Earthy
3-Isopropyl-2-methoxypyrazine	Earthy
3-Isobutyl-2-methoxypyrazine	Earthy
2-Ethenyl-3,5-dimethylpyrazine	Earthy
2-Ethenyl-3-ethyl-5-methylpyrazine	Earthy
6,7-Dihydro-5*H*-ciclopentapyrazine	Nutty/roasted
6,7-Dihydro-5-methyl-5*H*-ciclopentapyrazine	Nutty/roasted
3-Mercapto-3-methylbutyl formate	Cat/green/blueberry
3-Mercapto-3-methylbutanol	Nutty/roasted

Source: Czerny et al. [200], Maeztu et al. (202), Sanz et al. (203), Akiyama et al. (204), and Toci [107].

changes and losses of all components in the matrix are difficult to determine with precision and are therefore expressed as relative gains and losses. For example, loss of chlorogenic acids during decaffeination with dichloromethane have been reported as 16% for Arabica and 11% for Robusta coffees [10], whereas a 1.5% gain of chlorogenic acids has been observed in Arabica green coffee seeds after water decaffeination [18]. On the other hand, a study using a different water decaffeination method reported an average 20% loss of chlorogenic acids in Arabica samples [20]. Supercritical carbon dioxide appears to be more selective, removing caffeine while sparing compounds responsible for coffee aroma and bioactivity such as chlorogenic acids. A relative average gain of 1.2% in chlorogenic acids content of green Arabica samples has been reported with the supercritical carbon dioxide method [20]. Although the relative increase in chlorogenic acids in water-decaffeinated coffees may be due to lixiviation of highly soluble compounds, the supercritical carbon dioxide method appears to reduce chlorogenic acid losses by maintaining the integrity of the seeds. This integrity will surely be reflected later in the cup.

The chemical composition of instant coffee reflects blend composition, roasting degree, and the method used for extraction and concentration of the brew. Concentration with excessive heat can increase the loss of thermolabile compounds, whereas caffeine content in instant coffee (2.5–5 g/100 g) depends primarily on blend composition and extraction method, as this compound is quite resistant to heat. The goal of manufacturers is to create an instant coffee beverage with a chemical composition similar to that of ground roast coffee brew. Instant coffee should contain (per 100 g dry weight) polysaccharides (50–60 g), protein (12.6–21 g), lipids (0.2–1.6 g), minerals (8.8–10 g), and oligosaccharides (5.2–7.4 g) [134,135]. The moisture content should be up to 5%, but commonly ranges from 2.7%–3.5% [136,137].

With monsooned and steam-treated coffees, differences in the volatile compositions are mainly due to the partial hydrolysis of chlorogenic acids and loss or changes of low-molecular-weight compounds in the humidity. Variyar et al. [23] assessed raw nonmonsooned coffee seeds and reported that monsooned Arabica coffees contained lower concentrations of methoxypyrazines and higher concentrations of 4-vinylguaiacol and isoeugenol, which accounts for the dominant spicy note of monsooned coffee. According to the authors, these phenolic compounds exist partly as glycosides and are released from the bound precursors during monsooning.

Steaming coffee beans before roasting eliminates stomach-unfriendly substances, including the chlorogenic acids. In addition, individual free diterpenes such as cafestol, kahweol, dehydrokahweol, and dehydrocafestol can be reduced depending on the steaming parameters [40,138,139].

2.3.4 Chemical composition of coffee brew

Factors that affect the brew's composition include the ground roast coffee composition, grid, brewing method, proportion of coffee to water, hardness and temperature of water, length of time coffee is in contact with water, and the filter material. The amount of soluble solids in the brewed coffee varies from 2 to 6 g/100-mL cup [140].

Preparing a filtered brew from ground and roast coffee, involves extracting water-soluble compounds; most of the lipophilic fraction remains in the filter with the solid materials. Espresso is prepared by a special brewing technique in which a small amount of hot water

is percolated through ground coffee under high pressure for a short time. In this case, the composition and quality of the brew also depends on the water pressure.

Usually, extraction of water-soluble components including chlorogenic acids, caffeine, nicotinic acid, soluble melanoidins, and hydrophilic volatile compounds is greater at higher temperatures and pressures [108]. Although the lipid fraction is not water-soluble, part of the amount remaining in the seeds after roasting is extracted due to the high temperature of the water and is present in the brew as an emulsion. However, oil particles are likely to be retained in filters made of paper or similar types of materials. The high pressure used to make espresso and absence of a filter made of paper or another lipophilic material to retain the lipids facilitates their extraction into the brew. Thus, unfiltered coffee and espresso coffee brews contain higher amounts of these compounds, including bioactive diterpenes and sterols.

Approximately 80%–100% of chlorogenic acids, the components primarily responsible for the functional properties of coffee, are extracted in home coffee brewing [47], resulting in 35–100 mg chlorogenic acids/100-mL cup of Arabica coffee and 35–175 mg/100-mL cup of Robusta coffee [141]. However, maintaining coffee brews at a high temperature reduces chlorogenic acids and lactones concentrations [142,143]. Despite their thermolability, chlorogenic acids are still present in relatively high amounts in light- to dark-medium-roasted coffees compared with most food sources [47,144]. Coffee abstainers typically ingest less than 100-mg chlorogenic acids/day, whereas modest and heavy coffee drinkers ingest 0.1–2 g [47,141,145]. In addition to free chlorogenic acids and those incorporated by melanoidins, Díaz-Rubio and Saura-Calixto [102] reported that 8.7–10.5 mg chlorogenic acids and their derivatives are associated with soluble fiber in 100 mL brewed coffee.

Coffee's acidity is due to organic acids such as acetic, formic, malic, citric, and lactic acids, as well as chlorogenic and quinic acids. The pH varies from approximately 5.2 in a brew made from light roast to 5.8 [139].

Approximately 0.3–0.7 g potassium may be found in 100 mL brew at 8%–20% (coffee/water) [106,140], and low amounts of sodium (approximately 3 mg/100 mL brew) were found in a brew prepared at 20% (w/v) [106]. The antidemineralization effect of *C. canephora* reported by Antonio et al. [106] has been attributed, in part, to phosphorus (50 mg/100 mL brew). Costa and Farah [146] observed that the total mineral extraction achieved by espresso machines and electric coffee makers was higher than that of all other percolation methods.

As previously mentioned, galactomannans and type II arabinogalactans are the predominant polysaccharides that pass into the brew. After roasting the coffee seeds and brewing, these polysaccharides represent approximately 15%–25% of the dry matter [117,147]. According to Petraco [147], a typical amount of soluble fiber in espresso coffee is 800 mg/100 mL, and a regular percolation method produces approximately 200 mg/100 mL. Similarly, Díaz-Rubio and Saura-Calixto [102] reported 470–750 mg soluble fiber in 100 mL brewed coffees.

Caffeine, trigonelline, and nicotinic acid are also soluble in hot water. Typical amounts in brewed coffee prepared with medium roasted coffee vary from 50–100 mg caffeine, 40–50 mg trigonelline, and approximately 10 mg nicotinic acid. Trigonelline tends to be completely degraded in dark roasts, whereas nicotinic acid content increases [52].

As previously mentioned, most of the lipid fraction is retained in the remaining solid material after brewing. Triacylglycerols account for approximately 75% (w/w) of total coffee lipids in freshly brewed coffee, whereas free fatty acids account for only approximately 1% [46]. Because espresso machines use pressure to extract the brew and do not use paper filter, more of the lipids, including diterpenes, can be found in brewed espresso compared with regular percolation methods.

Figure 2.6 Typical chemical composition per 100 mL of coffee brew from medium roasted coffee. Brew composition vary according to blend, roasting degree, grid, and method of preparation. (*Sources:* Clifford [201], Clark [4], Farah and Donangelo [33], Petracco [120], Perrone et al. [55], Dias Rubio and Saura-Calixto [100], and Farah (unpublished results).)

Figure 2.6 summarizes the concentrations of the main nonvolatile components in brews made from medium roasted coffee seeds.

2.4 INCIDENTAL COFFEE CONSTITUENTS

2.4.1 Incidental nonvolatile compounds in coffee

Green coffee can contain minor constituents that may be undesirable both for flavor and bioactivity of the brew. Most of these compounds are microbial byproducts that occur due to inappropriate harvesting, weather conditions during primary processing, or improper storage. Examples of such incidental compounds are ochratoxin A (OTA) and specific biogenic amines. Other minor undesirable compounds, especially in terms of health concerns, are acrylamide and polycyclic aromatic hydrocarbons (PAHs) formed by high roasting temperatures. In addition, β-carbolines harman and norharman are formed during coffee roasting. Although studies are inconclusive regarding the health effects of these β-carbolines, they appear to be beneficial. A brief description for each of these incidental coffee constituents is found in the sections discussed here.

2.4.1.1 *Ochratoxin A*

OTA (Figure 2.7) is a mycotoxin produced by several *Aspergillus* and *Penicillium* species in semitropical and temperate climates; *A. westerdijkiae*, formerly included in the *A. ochraceus*

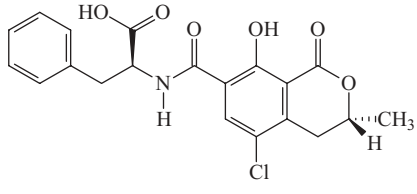

Figure 2.7 Chemical structure of ochratoxin A.

species, is its main producer. OTA has a dihydroisocoumarin moiety linked through its 7-carboxy group to L-phenylalanine by a peptide bond. This is a potent nephrotoxin and hepatotoxin that exerts teratogenic, mutagenic, carcinogenic, and immunosuppressive effects even at trace levels [6,7,148]. Consumption of OTA-contaminated foods is associated with Balkan endemic nephropathy, a disease characterized by severe kidney damage. In 1993, the International Agency for Research on Cancer classified OTA as possibly carcinogenic for humans (i.e., Group 2B carcinogen) [148,149]. This toxin is generally stable at normal cooking temperatures but is not resistant to the high temperatures of coffee roasting. Therefore, medium to dark-roasted coffees do not usually contain OTA unless the initial concentrations in the seeds were extremely high. OTA destruction in coffee is time- and temperature-dependent and follows first-order reaction kinetics in compliance with the Arrhenius equation, similar to chlorogenic acids [7]. However, because diverse roasting methods and degrees are used around the world, OTA is still detected in some coffees. In fact, because of the high coffee consumption in Europe, coffee accounts for approximately 7% of the total intake of OTA [150]. Miraglia and Brera [151] estimated that coffee accounts for 9% of human OTA intake after cereals (44%) and wine (10%) [121,152]. Romani et al. [153] analyzed 162 samples of green coffee seeds from various countries and reported that 106 were positive for OTA, with concentrations up to 48 μg/kg [148]. Furthermore, almost 100% of the OTA in roasted coffee passes into the brew using the most commonly employed brewing methods [154,155]. The World Health Organization has set a provisional tolerable daily intake for OTA of 14 ng/kg body weight [156]. The Scientific Committee on Food of the European Commission suggested an even lower level of 5 ng/kg body weight/day [157].

2.4.1.2 *Biogenic amines*

Biogenic amines are aliphatic, alicyclic, or heterocyclic organic bases of low molecular weight that participate in the regular metabolic processes of plants, microorganisms, and animals. They are produced by decarboxylation of specific amino acids, and most are named after their precursors. Examples are histamine (from histidine), tyramine (from tyrosine), and tryptamine (from tryptophan). Cadaverine and putrescine, however, were named after the decomposed food material in which they were first identified, and spermine and spermidine were named after the seminal fluids from which they were first isolated [158,159].

High levels of biogenic amines in meat and fish indicate spoilage. In coffee, biogenic amines originate from the action of microbial decarboxylases on amino acids during fermentative processes, suggesting inappropriate storage, or low-quality defective fermented seeds [160]. In addition, decarboxylation of amino acids or hydrolysis of conjugated amines may occur during roasting, increasing the content of free biogenic amines.

The profile and levels of biogenic amines reported for green and roasted coffees vary significantly in the literature. This is probably due to differences in analytical methods, sources and microbiological quality of the samples, and roasting methods. Generally, the predominant biogenic amines in coffee (in order of abundance) are putrescine, spermidine, and spermine (Figure 2.8). The latter two compounds may originate from the former. Minor amines identified in coffee are serotonin, agmatine, cadaverine, and tyramine. The total biogenic amine content in green Arabica and Robusta coffees varies from 3.0 to 12.5 mg/100 g. In roasted coffees, the amine content varies from 0.46 mg/100 g to undetectable amounts [8,161–164]. Amine contents of the brew and instant coffee were reported to be intermediate between green and ground roast coffee [158].

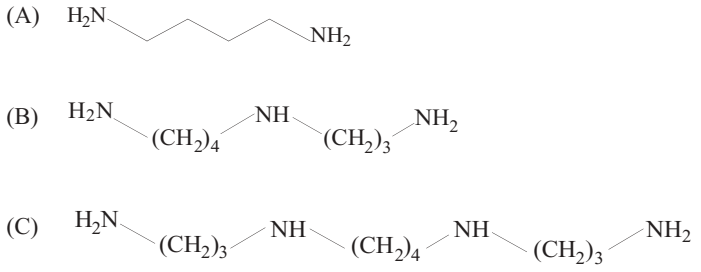

Figure 2.8 Chemical structure of selected biogenic amines in coffee. (A) Putrescine, (B) spermidine, and (C) spermine.

The biological effects of biogenic amines are diverse and not completely understood. Histidine and tyramine are the most toxic (especially histidine) and are associated with increase in blood pressure and strong headache (especially tyramine); however, the physiologic effects of coffee's predominant biogenic amines are not clear [165]. Putrescine, cadaverine, and tyramine are toxic only in large doses. These compounds exhibit a fairly low acute oral toxicity in rats (2000 mg/kg body weight) [166], whereas spermine and spermidine were slightly more toxic, with acute oral toxicity of 600 mg/kg weight. However, the toxicity of these compounds in humans is not well understood. Consumption of putrescine and cadaverine appears to increase the uptake and transport of histamine by inhibiting the enzymes that degrade it in the intestine. Individual sensitivity to these compounds varies considerably and causes different responses [165].

2.4.1.3 *β-carbolines*

Norharman and harman (Figure 2.9) are two heterocyclic β-carboline alkaloids found in many thermally processed foods. They are formed during coffee roasting through a Pictet–Spengler condensation of indolethylamines (as L-tryptophan) and carbonylic compounds (e.g., acetaldehyde or formaldehyde) followed by oxidation and decarboxylation. Their formation is dependent on temperature and roasting time [167,168]. As with biogenic amines, the human body naturally produces some β-carbolines, which frequently act as monoamine oxidase inhibitors to regulate neurotransmitters [169]. However, most β-carbolines produced in the body are different from exogenous β-carbolines, and research results are inconclusive as to regular intake of exogenous carbolines such as norharman and harman is beneficial or detrimental to health. Herraiz and Chaparro [169] reported that β-carbolines isolated from ready-to-drink coffee were competitive and reversible inhibitors of monoamine oxidase. Inhibition of monoamine oxidase enzymes by coffee may play a role in its neuroactive effects,

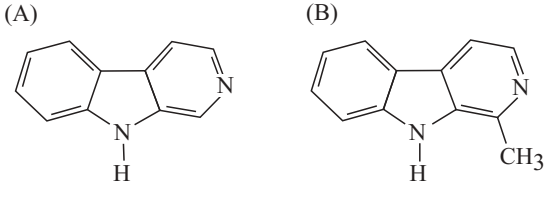

Figure 2.9 Chemical structure of coffee β-carbolines. (A) Harman and (B) norharman.

including protection against Parkinson disease. Other effects of harman include antidepressant and antianxiety actions in rats; vasorelaxant and anti-HIV-1 activity by inhibition of H9 lymphocyte cells replication; possible beneficial effects on mononeuropathic pain in rats and naloxone-precipitated withdrawal syndrome in morphine-dependent rats; and stimulation of insulin secretion from isolated human islets of Langerhans. On the other hand, undesirable effects such as comutagenicity, *in vivo* genotoxicity, and involvement in addictions have also been suggested [148,167,168,170].

Typical β-carbolines concentrations in coffee brews range from 4 to 20 μg/100 mL; thus, coffee is the most important exogenous source of harman and norharman other than cigarette smoking [169,171]. The norharman content in the brew is usually higher than that of harman, and both depend on the coffee species and method of preparation. Roasting degree does not appear to strongly influence the final β-carboline content [139]. Alves et al. [170] detected approximately 14 μg norharman and 5 μg harman/100 mL regular Arabica espresso coffee brew. In brews prepared with commercial blends (containing both Arabica and Robusta species), the contents were higher, ranging from 14 to 34 μg/100 mL for norharman and 5 to 15 μg/100 mL for harman. Italian coffee prepared with a moka pot and espresso coffees contained more β-carbolines than filtered and press pot coffees.

2.4.1.4 *Acrylamide*

Although the mechanism of acrylamide (Figure 2.10) formation in cooked foods is not completely understood, the generation of high amounts of acrylamide by a sugar-asparagine adduct, *N*-glycosyl-asparagine, suggests the early Maillard reaction as a major source of acrylamide [172]. Other possible routes include decarboxylation of asparagine to 3-aminopropionamide upon heating in the absence of reducing sugars [173] and the Strecker reaction of asparagine with the Strecker aldehyde as the direct intermediate [174]. Acrylamide is carcinogenic to laboratory rodents and is described by the International Agency for Research of Cancer as a probable carcinogen to humans [175]. Exposure to acrylamide may damage nervous system in humans and animals [176,177].

The contribution of coffee to the dietary daily intake of acrylamide is high in countries with a high coffee consumption. Granby and Fagt [178] estimated that dietary intake of acrylamide from coffee in Denmark averages 10 μg/day for men and 9 μg/day for women aged 35–45 years. According to studies of the effect of nutritional habits on acrylamide intake, coffee contributes to approximately 20% of the total acrylamide exposure in Denmark, 30% in Norway and Sweden, and 36% in Switzerland. These findings indicate that coffee is a significant contributor to acrylamide intake, especially in North European countries where per capita coffee consumption is the highest in the world [148,179].

The acrylamide content of coffee depends on the blend and roasting degree. It tends to be higher in *C. canephora* than *C. arabica* and reaches maximum values at light roast, decreasing with longer roasting. This finding is consistent with the hypothesis of formation during the early Maillard reaction. Granby and Fagt [178] reported 1 μg acrylamide/100 g medium roasted coffee and 0.5 μg/100 g dark roasted coffee. Alves et al. [180] reported a

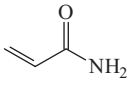

Figure 2.10 Chemical structure of acrylamide.

25% decrease in acrylamide in espresso prepared with medium and dark roasted coffees. A recent study showed that the acrylamide content in coffee after slow roasting (i.e., low-temperature roasting for a long period of time) is lower than that of coffee roasted at high temperatures for a short period of time [181]. Similar acrylamide levels were reported for instant and ground coffees (approximately 29 μg/100 g) [182,183]. Water effectively extracts acrylamide into the brew. Alves et al. [180] reported extraction ranging from 80% to 99% in espresso coffee, with an average content of 3.9 μg/100 mL (prepared from Arabica coffee) and 7.7 μg/100 mL (prepared from Robusta coffee). Espressos prepared from commercial blends contain average acrylamide levels of 4.2 μg/100 mL. Granby and Fagt [178] found similar acrylamide concentrations in a comparison of 25 coffee samples brewed with electric coffee makers (0.8 μg/100 mL) or French press (0.9 μg/100 mL). Prepared instant coffees contained similar amounts.

2.4.1.5 *Polycyclic aromatic hydrocarbons*

PAHs (Figure 2.11) are a large group of potentially carcinogenic organic compounds with two or more fused aromatic rings that are formed at very high roasting temperatures. The most studied of these compounds is benzo[*a*]pyrene, which has the highest carcinogenic potential. Although chlorogenic acids from coffee were reported to inhibit both the formation of benzo[*a*]pyrene and the mutagenicity of its carcinogenic metabolites [79,184], the formation of phenanthrene, anthracene, and benzo[*a*]anthracene in coffee beans was observed at temperatures above 220°C, whereas formation of pyrene and chrysene required 260°C [148].

The concentrations of PAHs depend primarily on the roasting degree, but unlike acrylamide they tend to increase as roasting progresses and are more commonly detected in very dark roasts [22,127,185]. Other factors influence PAH levels in coffee, such as contamination of raw materials and roasting method. In a study comparing brews prepared from different types of raw materials, Kayali-Sayadi et al. [186] found the highest concentration of PAH (2.9 ng/L) in the brew prepared from *torrefacto* seeds (roasted in the presence of sugar). In addition, slow roasting tends to yield lower PAH concentrations as it does with acrylamide [181].

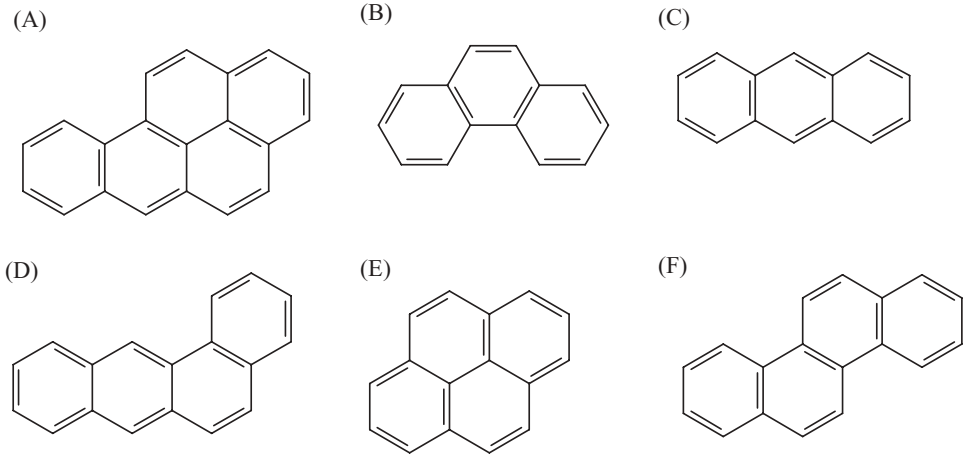

Figure 2.11 Chemical structure of polycyclic aromatic hydrocarbons identified in coffee. (A) Benzo[*a*]pyrene, (B) phenanthrene, (C) anthracene, (D) benzo[*a*]anthracene, (E) pyrene, and (F) chrysene.

Fortunately, the medium to low solubility (<35%) of PAHs results in beverage concentrations lower than those found in the roasted and ground seeds [22,148,185]. For example, Hietaniemi et al. [187] reported high PAH levels (approximately 100–200 μg/kg) in a roasted and ground coffee sample, but these compounds could not be detected in the prepared beverage [148]. However, those who consume coffee prepared with methods that extract these compounds more efficiently (unfiltered or espresso coffee) should be aware of the possibility of higher PAH levels.

2.4.1.6 *Pesticide residues*

Pesticides comprise a large number of chemicals that belong to different chemical groups. Many pesticides or their metabolites are toxic to humans; therefore their residues are undesirable in foods. Statutory maximum residue levels for pesticides in food and water have been defined in most countries to protect consumers and regulate pesticide levels in the environment. The FDA, which monitors the presence and amounts of pesticide residues in the United States, conducted a survey in the 1990s in which 60 green coffee samples were collected from 21 major exporter countries. These samples were analyzed for a variety of pesticide chemicals such as organochlorine/organophosphorus, *N*-methyl carbamate, and benomyl group residues; 7% of the samples were contaminated with chlorpyrifos and pirimiphos-methyl [148]. Cetinkaya et al. [188] analyzed 19 coffee samples originating from 11 countries. Besides two samples in which no residues were detected, pesticides found in the green coffee seeds were at levels considerably lower than those permitted in Germany. The residues were reduced to insignificant amounts during the roasting process, with degradation rates of 85%–100%. On the other hand, green, roasted, and instant coffee samples from plants treated with five foliar applications of the insecticide imidacloprid were found to contain residues of this substance [126,189].

These findings suggest that the residual amount of pesticides in coffee after roasting is probably not a cause for concern and depends on factors such as the stability of the active substance to high temperatures and its solubility, roasting conditions, and coffee species. These factors, especially thermolability, should be considered when choosing a pesticide for use on coffee trees.

2.4.2 Incidental volatile constituents in coffee

Incidental volatile compounds in coffee are those not typically found in high-quality green or roasted seeds. They may be added to green or roasted coffee to provide different sensory notes (e.g., vanilla or almond flavor) or present in low-quality defective coffee seeds such as sour (oxidized) and black (overmature) seeds. Those detected in defective seeds are often the result of microbial fermentation, infestation of insects such as the coffee berry borer (*Hypothenemus hampei*), or contact with jute bags and similar material during storage. It is therefore important to store coffee in dry, ventilated, insect-free places that are located away from odors that may interfere with the natural coffee aroma, unless such interference is desired as in the case of monsooned coffees that are deliberately exposed to the monsoon winds to acquire a musty flavor.

A number of scientists have investigated the compounds responsible for unpleasant off-flavors in coffee. In seeds exhibiting the Rio off-flavor, common in humid Brazilian plantations, 2-methyl-isobutanol, 2,4,6-tricloroanisol, geosmin and the pyrazines 2-methoxy-3-isopropylpyrazine and 2-metyoxy-3-isobutylpyrazine have been identified [190–192]. In a

study of healthy defective green coffee seeds from various Brazilian producers, Toci et al. [9,193] identified the following compounds only in defective seeds: hexanoic acid; 2,3,5-trimethylpyrazine; 2,3,5,6-tetramethylpyrazine; benzaldehyde; butyrolactone (sour seeds); 2-methylpyrazine (black-immature seeds); 2-furylmethanol acetate (black-immature seeds); 2-pentyl-furane; 2-octenal; and 3-octen-2-one. In roasted coffees, compounds unique to defective seeds were pyrazine; 2,3,5-trimethyl-6-ethyl-pyrazine (immature seeds and others); 3,5-dimethyl-2-butylpyrazine; isoamyl-6-methylpyrazine (sour); 3-methyl-2-butylpyrazine (sour); 2,3 butanediol *meso;* 4-ethylguaiacol; isopropyl *p*-cresol sulfide; 3-methylpiperidine (black); 2-pentyl-piperidine (black); 2-pentyl-furan (black); 3,7-dimethyl-1,6-octadienol (β-linalool) (sour); and 3-ethyl-2-methyl-1,3-hexadiene. Pyrazines are common in defective seeds and are known to produce objectionable flavors [11].

Several of these compounds associated with off-flavors in coffee are typical of fermentation and oxidation processes. For example, butyrolactones are produced by mold; 2-pentyl-furan is generated during fermentative processes by bacteria from the genus *Bacillus*; aldehydes and ketones are produced during fermentative and oxidative processes; some volatile acids are produced by fermentation; and some hydrocarbons such as 3-ethyl-2-methyl-1,3-hexadiene derive from lipid oxidation. Some of these compounds can be used as markers for specific defective seeds to monitor the quality of green and roasted coffees. It is worth noting that some volatile compounds produce a pleasant aroma at low concentrations but are intolerable at high concentrations. Toci and Farah [107] observed that this was the case with a few compounds in defective seeds, which suggests that this is a complex issue and deserves thorough investigation.

Another incidental volatile compound in coffee is furan, a heterocyclic organic compound consisting of a five-membered aromatic ring with four carbon atoms and one oxygen atom. Furan is thought to be a human carcinogen based on evidence of malignant tumor formation in multiple tissues in several species of experimental animals. A current hypothesis for the mechanism of furan-induced carcinogenesis is metabolic activation of cytochrome P450 to a reactive and cytotoxic intermediate that stimulates cell replication, increasing the likelihood of tumor induction [194]. The postulated reactive metabolite is *cis*-2-butene-1,4-dial, which was recently characterized as a furan metabolite by Chen et al. [194].

Furan can be formed by pyrolysis of amino acids, simple carbohydrates, and fatty acids during coffee roasting. Model systems using ^{13}C-labeled sugars and amino acids show that certain amino acids (e.g., serine and cysteine) degrade to produce acetaldehyde and glycolaldehyde that can undergo aldol condensation to produce furan after cyclization and dehydration steps. Other amino acids (e.g., aspartic acid, threonine, and α-alanine) produce only acetaldehyde and thus need sugars as a source of glycolaldehyde to generate furan. Monosaccharides are also known to undergo degradation to produce both acetaldehyde and glycolaldehyde; however, ^{13}C-labeling studies have revealed that degradation of hexoses primarily results in aldotetrose derivatives to produce the parent furan. In addition, 4-hydroxy-2-butenal, a decomposition product of lipid peroxidation, was proposed as a precursor of furan originating from polyunsaturated fatty acids [195].

Arisseto et al. [196] reported furan contents of 91–585 μg/100 g roasted coffee, with higher contents in *C. canephora* species and dark roasted coffees. Content in brewed coffees varies from less than 1 to 28.8 μg/100 g. Altaki et al. [197] evaluated furan in brews prepared from regular, decaffeinated, instant coffee, and commercial packed capsules. The effect of roasting conditions (temperature and time) on furan formation was also studied. Results showed that low temperature and long roasting time (140°C and 20 minutes) decreased the final furan content, whereas regular high temperatures (200°C–220°C for 10–15 minutes) resulted in high concentrations, similar to acrylamide and PAH. Furan concentrations in regular brews

prepared with an espresso machine were 4.3–14.6 μg/100 mL, higher than those obtained with a home drip coffee maker (2–7.8 μg/100 mL), whereas decaffeinated coffee brews from a home drip coffee maker showed a furan concentration of 1.4–6.5 μg/100 mL, similar to those obtained from regular coffee. Relatively low concentrations of this compound (1.2–3.5 μg/100 mL) were found in instant coffee brews, whereas commercial packed coffee capsules showed the highest concentrations (11.7–24.4 μg/100 mL) because these sealed packages prevent the release of furan molecules after the coffee is roasted and ground. The daily intake of furan through coffee consumption in Barcelona (Spain) was estimated as 0.03–0.38 μg/kg body weight, which is less than the maximum acceptable level for furan intake (2 μg/ kg body weight).

2.5 CONCLUDING REMARKS

Coffee contains a number of compounds that contribute to the flavor and bioactivity of the brew. Complex reactions take place during roasting at high temperatures and modify considerably coffee's chemical composition, with some beneficial compounds degraded and some created. A small amount of harmful compounds is also created during roasting; however, the beneficial compounds appear to predominate. To obtain a functional, healthy coffee, it is important to consider every aspect of coffee production, starting with high-quality seeds roasted to light-medium to dark-medium color degree, preferably at low to medium temperatures. Medium-roast coffees contain relatively high amounts of antioxidant compounds compared with other food products, a considerable amount of niacin, low acrylamide content, and typically no PAHs. Decaffeinated coffee is indicated for individuals sensitive to caffeine's effects and those who wish to use coffee as an additional tool to reduce the risk of type 2 diabetes.

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REFERENCES

1. International Coffee Organization (ICO). Statistics. Breakdown of exports of green Arabica and green Robusta of countries exporting significant volumes of both types of coffee June 2009, January 2011. www.ico.org (accessed January 21, 2011).
2. World Resource Institute (WRI). Countries by coffee consumption *per capita*. 2010. http://earthtrends.wri.org/ (accessed February 2011).
3. Yeretzian, C., Jordan, A., Lindinger, W. Analysing the headspace of coffee by proton-transfer-reaction mass-spectrometry. *Int. J. Mass Spectr.* 2003, **223–224**, 115–139.
4. Clarke, R. J. Coffee: green coffee/roast and ground. In: *Encyclopedia of Food Science and Nutrition*, 2nd edition, Caballero, B., Trugo, L. C., Finglas, P., eds. Oxford: Academic Press; 2003, Vol. 3.
5. ABIC, 2011. Brazilian Association of Coffee Industry (Technical information).
6. Taniwaki, M. H., Pitt, J. I., Teixeira, A. A., Iamanaka, B. T. The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. *Int. J. Food. Microbiol.* 2003, **82**, 173–179.
7. Ferraz, M. B. M., Farah, A., Iamanaka, B. T., Perrone, D., Copetti, M. V., Marques, V. X., Vitali A. A., Taniwaki, M. H. Kinetics of ochratoxin A destruction during coffee roasting. *Food Control*. 2010, **21**, 872–877.

8. Cirilo, M. P. G., Coelho A. F. S., Araújo C. M., Gonçalves F. R. B., Nogueira F. D., Glória M. B. A. Profile and levels of bioactive amines in green and roasted coffee. *Food Chem*. 2003, **82**, 397–402.
9. Toci, A. T., Farah, A. Volatile compounds as potential defective coffee seeds' markers. *Food Chem*. 2008, **108**, 1133–1141.
10. Toci, A. T., Farah, A., Trugo, L. C. Efeito do processo de descafeinação com diclorometano sobre a composição química dos cafés Arábica e Robusta antes e após a torração. *Química Nova*. 2006, **29**, 965–971.
11. Flament, I., Gautschi, F., Winter, M., Willhalm, B., Stoll, M. Les composants furanniques de l'arôme café: quelques aspects chimiques et spectroscopiques. Proc. 3rd Coll. Int. Coffee Sci. ASIC, 197–215. 1968. Paris.
12. Bee, S., Brando, C. H. J., Brumen, G., Carvalhaes, N., Kolling-Speer, I., Speer, K., Liverani, F. S., Teixeira, A. A., Teixeira, R., Thomaziello, R. A., Viani, R., Vitzthum, O. G. The Raw Seed. In: *Espresso Coffee, the Science of Quality.* Illy A., Viani R., eds. Italy: Elsevier Academic Press; 2004, pp. 87–178.
13. Shlonsky, A. K., Klatsky, A., Armstrong, A. Traits of persons who drink decaffeinated coffee. *Ann. Epidemiol*. 2003, **13**, 273–279.
14. Ogita, S., Uefugi, H., Yamaguchi, Y., Koizumi, N., Sano, H. Producing decaffeinated coffee plants. *Nature* 2003, **423**, 823.
15. Silvarola, M. B., Mazzafera, P., Fazuoli, L. C. A naturally decaffeinated arabica coffee. *Nature* 2004, **249**, 826.
16. National Coffee Association (NCA). U.S. Coffee Consumption. http://www.ncausa.org. (accessed March 2011).
17. Mazzafera, P., Baumann, T. W., Shimizu, M. M. Decaf and the steeplechase towards decaffito the coffee from caffeine-free Arabica plants. *Tropical Plant Biol*. 2009, **2**, 63–76.
18. Farah, A., de Paulis, T., Trugo, L. C., Martin, P. R. Chlorogenic acids and lactones in regular and water-decaffeinated arabica coffee. *J. Agric. Food Chem.* 2006, **54**, 374–381.
19. Food and Drug Administration. Code of Federal Regulations. Title 21, Vol. 3, Revised April 1, 2006. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=173.255 (accessed September 19, 2007).
20. Farah, A., Perrone, D., Fernandes, J., Silanes, J. Chorogenic acids and lactones in coffees decaffeinated by water and supercritical CO_2 and roasted in a pilot plant scale fluidized bed roaster. Proc. 23rd Int. Conf. Coffee Sci. 2010. ASIC, 367–372. 2010., Bali, Indonesia.
21. Steinhart, H., Luger, A. An analytical Distinction between untreated and steam-treated roasted coffee. Proc. 17th Int. Sci. Coll. Coffee (Nairobi). ASIC, 155–160. 1997. Paris.
22. Maier, H. G. Status of research in the field of non-volatile coffee components. Proc. 15th Coll. Sci. Int. Café, ASIC, 567–576. 1994. Montpellier, Paris.
23. Variyar, P. S., Ahmad, R., Bhat, R., Niyas, Z., Sharma, A. Flavouring components of raw monsooned Arabica coffee and their changes during radiation processing. *J. Agric. Food Chem.* 2003, **51**, 7945–7950.
24. Coffee Board of India. http://www.indiacoffee.org/default.htm. (accessed December 3, 2007).
25. Farah, A. Distribuição nos grãos, influência sobre a qualidade da bebida e biodisponibilidade dos ácidos clorogênicos do café. Instituto de Química, Universidade Federal do Rio de Janeiro, RJ, Brasil, Doctorate Thesis, 2004.
26. Toci, A. T., Silva, C. M., Fernandes, F., Farah, A. Effect of the fluid speed changes on the chemical composition of coffee samples roasted in an industrial semi-fluidized bed roaster. Proc. 23rd Int. Conf. Coffee Sci. ASIC, 500–503. 2009. Trieste, Italy.
27. Adams M. R., Dougan J. Waste products In: *Coffee*. Clarke, R. J., Macrae, R., eds. New York: Elsevier Science; Vol. 2 *Technology*, 1987.
28. Clarke, R. In: *Coffee*, 1st edition, Clarke, R. J., Macrae, R., eds. Essex, UK: Elsevier Applied Science Publishers; 1985, Vol. 1: Chemistry, p. 115.
29. ABICS, 2011. Brazilian Association of Instant Coffee (Technical Information).
30. Clifford, M. N., Ramirez-Martinez, J. R. Phenols and caffeine in wet-processed coffee seeds and coffee pulp, *Food Chem.* 1991, **40**, 35–42.
31. Clifford, M. N., Kazi, T. The influence of coffee seed maturity on the content of chlorogenic acids, caffeine and trigonelline. *Food Chem.* 1987, **26**, 59–69.
32. Duarte, G., Pereira, A., Marques, V., Farah, A. Comparison of chlorogenic acids contents in *Coffea arabica*, *Coffea canephora* and hybrids resistant to *Meloidogyne exigua*. Proc. 22rd Int. Conf. Coffee Sci. ASIC, 508–512. 2009. Trieste, Italy.

33. Farah, A., Donangelo, C. M. Phenolic compounds in coffee. *Braz. J. Plant Physiol.* 2006, **18**, 23–36.
34. Perrone, D., Neves, Y. P., Brandão, J. M., Martinez, H. E. P., Farah, A. Influence of zinc fertilization on chlorogenic acids and antioxidant activity of coffee seeds. 22nd Int. Conf. Coffee Sci, 2008. ASIC, 220–223. 2009. Campinas, SP, Brazil.
35. Farah, A., Monteiro, M., Calado, V., Trugo, L. C. Correlation between the chemical attributes of coffee and cup quality. *Food Chem.* 2006b, **98**, 373–380.
36. Viani, R. The composition of coffee. In: *Caffeine, Coffee and Health*. New York: Haven; 1993.
37. Nehlig, A. Exploring biotechnology. *Chemtech*. 1999, **29**, 30–35.
38. Shearer, J., Sellars, E., Farah, A., Graham, T. E., Wasserman, D. H. Effects of chronic coffee consumption on glucose kinetics in the conscious rat. *Can. J. Phys. Pharm.* 2007, **85**, 823–830.
39. Ribeiro-Alves, M., Trugo, L. C., Donangelo, C. Use of oral contraceptives blunts the calciuric effect of caffeine in young adult women. *J. Nutr.* 2003, **133**, 393–398.
40. Demirbag, D., Ozdemir, F., Ture, M. Effects of coffee consumption and smoking habit on bone mineral density, *Rheumatol. Int.* 2006, **26**, 530–535.
41. Lee, C. Antioxidant ability of caffeine and its metabolites based on the study of oxygen radical absorbing capacity and inhibition of LDL peroxidation. *Clin. Chim. Acta.* 2000, **295**, 141–154.
42. Antonio, A. G., Moraes, R. S., Perrone, D., Maia, L. C., Santos, K. R. N., Iório, N. L. P., Farah, A. Species, roasting degree and decaffeination influence the antibacterial activity of coffee against *Streptococcus mutans*. *Food Chem.* 2010, **118**, 782–788.
43. Hirakawa, N., Okauchi, R., Miura, Y., Yagasaki, K. Anti-invasive activity of niacin and trigonelline against cancer cells. *Biosci. Biotechnol. Biochem.* 2005, **69**, 653–658.
44. Tohda, C., Kuboyama, T., Komatsu, K. Search for natural products related to regeneration of the neuronal network. *Neurosignals* 2005, **14**, 34–45.
45. Allred, K. F., Yackley, K. M., Vanamala, J., Allred, C. D. Trigonelline is a novel phytoestrogen in coffee seeds. *J. Nutr.* 2009, **139**, 1833–1838.
46. Trugo, L. C. Coffee Analysis. In: *Encyclopedia of Food Science and Nutrition*, 2nd edition, Caballero, B., Trugo, L. C., Finglas, P. M., eds. Oxford, UK: Oxford Academic Press; 2003, Vol. 2, p. 498.
47. Clifford, M. N. Chlorogenic acids and other cinnamates—nature, occurrence, dietary burden, absorption and metabolism, *J. Sci. Food Agric.* 2000, **80**, 1033–1043.
48. Clifford, M. N., Johnston, K. L., Knight, S., Kuhnert, N. The characterisation by LC-MS^n of coffee seed caffeoylferuloylquinic acids. *J. Agric. Food Chem.* 2003, **51**, 2900–2911.
49. Farah, A., de Paulis, T., Trugo, L. C., Martin, P. R. Effect of roasting on the formation of chlorogenic acid lactones. *J. Agric. Food Chem.* 2005, **53**, 1505–1513.
50. Clifford, M. N., Knight, S., Kuhnert, N. Discriminating between the six isomers of dicaffeoylquinic acid by LC-MS^n. *J. Agric. Food Chem.* 2005, **53**, 3821–3832.
51. Clifford, M. N., Marks, S., Knight, S., Kuhnert, N. Characterization by LC-MS^n of four new classes of *p*-coumaric acid-containing diacyl chlorogenic acids in green coffee seeds. *J. Agric. Food Chem.* 2006, **54**, 4095–4101.
52. Perrone, D., Donangelo, C. M., Farah, A. Fast simultaneous analysis of caffeine, trigonelline, nicotinic acid and sucrose in coffee by liquid chromatography-mass spectrometry. *Food Chem.* 2008, **110**, 1030–1035.
53. Jaiswal, R., Patras, M. A., Eravuchira, P., Kuhnert, J. Profile and characterization of the chlorogenic acids in green Robusta coffee seeds by LC-MS^n: identification of seven new classes of compounds. *J. Agric. Food Chem.* 2010, **58**, 8722–8737.
54. Agardh, E. E., Carlsson, S., Ahlbom, A., Efendic, S., Grill, V., Hammar, N., Hilding, A., Ostenson, C. G. Coffee consumption, type 2 diabetes and impaired glucose tolerance in Swedish men and women. *J. Intern. Med.* 2004, **255**, 645–652.
55. Salazar-Martinez, E., Willett, W. C., Ascherio, A., Manson, J. E., Leitzmann, M. F., Stampfer, M. J., Hu, F. B. Coffee consumption and risk for type 2 diabetes mellitus. *Ann. Intern. Med.* 2004, **140**, 1–8.
56. Rosengreen, A., Doterval, A., Wilhelmsen, L., Thele, D., Johanssens, S. Coffee and incidence of diabetes in Swedish women: a perspective 18-year follow-up study. *Intern. Med.* 2004, **255**, 89–95.
57. Soriguer, F., Rojo-Martinez, G., Antonio, I. E. Coffee consumption and type 2 diabetes mellitus. *Ann. Intern. Med.* 2004, **17**, 321–323; author reply 3–4.
58. Van Dam, R. M., Willett, W. C., Manson, J. E., Hu, F. B. Coffee, caffeine, and risk of type 2 diabetes: a prospective cohort study in younger and middle-aged U.S. women. *Diabetes Care.* 2006, **29**, 398–403.

59. Bravi, F., Bosetti, C., Tavani, A., Bagmardi, V., Gallees, S., Negri, E., Fauschi, S., La Vecchia, C. Coffee drinking and hepatocellular carcinoma risk: a meta-analysis. *Hepatology*, 2007, **46**, 430–435.
60. Lindsay, J., Laurin, D., Verreault, R., Hebert, R., Helliwell, B., Hill, G. B., McDowell, I. Risk factors for Alzheimer's disease: a prospective analysis from the Canadian study of health and aging. *Am. J. Epidemiol*. 2002, **156**, 445–453.
61. Larsson, S. C., Wolk, A. Coffee consumption and risk of liver cancer: a meta-analysis. *Gastroenterology*, 2007, **132**, 1740–1745.
62. Ranheim, T., Halvorsen, B. Coffee consumption and human health—beneficial or detrimental? Mechanisms for effects of coffee consumption on different risk factors for cardiovascular disease and type 2 diabetes mellitus. *Mol. Nutr. Food Res*. 2005, **49**, 274–284.
63. Hemmerle, H., Burger, H. J., Bellow, P., Schubert, G., Rippel, R., Schindler, P.W., Paulus, E., Herling, A. Chlorogenic acid and synthetic chlorogenic acid derivatives: novel inhibitors of hepatic glucose-6-phosphate translocase. *J. Med. Chem*. 1997, **40**, 137–145.
64. Herling, A. W., Burger, H. J., Schwab, D., Hemmerle, H., Below, P., Schubert, G. Pharmacodynamic profile of a novel inhibitor of the hepatic glucose-6-phosphatase system, *Am. J. Physiol*. 1998, **274**, G1087–G1093.
65. Aruoma O. I. Antioxidant actions of plant foods: use of oxidative DNA damage as a tool for studying antioxidant efficacy. *Free Radic. Res*. 1999, **30**, 419–427.
66. Andrade-Cetto, A., Wiedenfeld, H. Hypoglycemic effect of *Cecropia obtusifolia* on streptozotocin diabetic rats. *J. Ethnopharmacol*. 2001, **78**, 145–149.
67. Natella, F., Scaccini, C. Does Coffee Drinking Influence Plasma antioxidant activity? Proc. 19th Int. Conf. Coffee Sci., ASIC, 2001. Trieste, Italy.
68. Natella, F., Nardini, M., Giannetti, I., Dattilo, C., Scaccini, C. Coffee drinking influences plasma antioxidant capacity in humans. *J. Agric. Food Chem*. 2002, **50**, 6211–6216.
69. Gerin, I., Van Schaftingen, E. Evidence for glucose-6-phosfate transport in rat liver microsomes. *FEBS Lett*. 2002, **517**, 257–260.
70. Johnston, K. L., Clifford, M., Morgan, L. M. Coffee acutely modifies gastrointestinal hormone secretion and glucose tolerance in humans: glycemic effects of chlorogenic acid and caffeine, *Am. J. Clin. Nutr*. 2003, **78**, 728–733.
71. Herrera-Arellano, A., Aguilar-Santamaria, L., Garcia-Hernandez, B., Nicasio-Torres, P., Tortoriello J. Clinical trial of Cecropia obtusifolia and Marrubium vulgare leaf extracts on blood glucose and serum lipids in type 2 diabetics. *Phytomedicine*, 2004, **11**, 561–566.
72. Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., Brighenti, F. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays. *J. Nutr*. 2003, **133**, 2812–2819.
73. Arion, W. J., Canfield, W. K., Ramos, F. C., Schindler, P. W., Burger, H. J., Hemmerle, H., Schubert, G., Below, P., Herling, A. W. Chlorogenic acid and hydroxynitrobenzaldehyde: new inhibitors of hepatic glucose 6-phosphatase. *Arch. Biochem. Biophys*. 1997, **339**, 315–322.
74. Svilaas, A., Sakhi, A. K., Andersen, L. F., Svilaas, T., Strom, E. C., Jacobs, D. R., Jr., Ose, L., Blomhoffr, R. Intakes of antioxidants in coffee, wine and vegetables are correlated with plasma carotenoids in humans. *J. Nutr*. 2004, **134**, 562–567.
75. Vinson, J. Polyphenols: total amounts of foods and beverages and US per capita consumption. The 230th ACS National Meeting, 2005. Washington, DC.
76. Saura-Calixto, F., Goni, I. Antioxidant capacity of the Spanish Mediterranean diet. *Food Chem*. 2006, **94**, 442–447.
77. Fukushima, Y., Ohie, T., Yonekawa, Y. Coffee and green tea as a large source of antioxidant polyphenols in the japanese population. *J. Agric. Food Chem*. 2009, **57**, 1253–1259.
78. Torres, T., Farah, A. Coffee is the most important contributor to the antioxidant capacity in Brazilians' diet. *FASEB J*. 2010, **24**, 919.
79. Wattenberg, L. W., Coccia, J. B., Lam, L.K. Inhibitory effects of phenolic compounds on benzo[a]pyrene-induced neoplasia. *Cancer Res*. 1980, **40**, 2820–2823.
80. Wood, A. W., Huang, M. T., Chang, R. L., Newmark, H. L., Lehr, R. E., Yagi, H., Sayer, J. M., Jerina, D. M., Conney, A. H. Inhibition of the mutagenicity of bay–region diol epoxides of polycyclic aromatic hydrocarbons by naturally occurring plant phenol: exceptional activity of ellagic acid. *Proc. Natl. Acad. Sci. U S A*. 1982, **79**, 5513–5517.
81. Stich, H. F., Rosin, M. P., Bryson, L. Inhibition of mutagenicity of a model nitrosation reaction by naturally occurring phenolics, coffee and tea. *Mutat. Res*. 1982, **95**, 119–128.

82. Wattenberg, L. W. Inhibition of neoplasia by minor dietary constituents. *Cancer Res.* 1983, **43**, 2448s–2453s.
83. Mori, H., Tanaka, T., Shima, H., Kuniasu, T., Takahashi, M. Inhibitory effect of chlorogenic acid on methylazoxymethanol acetate-induced carcinogenesis in large intestine and liver of hamsters. *Cancer Lett*. 1986, **30**, 49–54.
84. Namiki, M. Antioxidants/antimutagens in food. *Crit. Rev. Food Sci. Nutr.* 1990, **29**, 273–300.
85. Mori, H., Sugie, S., Tanaka, T., Makita, H., Yoshimi, N. Suppressive effects of natural antioxidants on carcinogenesis in digestive organs. *Environm. Mutagen. Res. Commun.* 1996, **18**, 73–77.
86. Pannala, A., Razaq, R., Halliwell, B., Singh, S., Rice Evans, C. Inhibition of peroxynitrite dependent tyrosine nitration by hydroxycinnamates: nitration or electron donation? *Free Rad. Biol. Med.* 1998, **24**, 594–606.
87. Lo, H. H., Chung, J. G. The effects of plant phenolics, caffeic acid, chlorogenic acid and ferulic acid on arylamine N-acetyltransferase activities in human gastrointestinal microflora. *Anticancer Res.* 1999, **19**, 133–140.
88. Kasai, H., Fukada, S., Yamaizumi, Z., Sugie, S., Mori H. Action of chlorogenic acid in vegetables and fruits as an inhibitor of 8-hydroxydeoxyguanosine formation *in vitro* and in rat carcinogenesis model. *Food Chem. Toxicol.* 2000, **38**, 467–471.
89. Cavin, C., Holzhaeuser, D., Scharf, G., Constable, A., Huber, W. W., Schilter, B. Cafestol and kahweol, two coffee specific diterpenes with anticarcinogenic activity. *Food Chem. Toxicol.* 2002, **40**, 1155–1163.
90. Chiang, L. C., Chiang, W., Chang, M. Y., Ng, L. T., Lin, C. C. Antiviral activity of *Plantago major* extracts and related compounds *in vitro*, *Antiviral Res.* 2002, **55**, 53–62.
91. Basnet, P., Matsushige, K., Hase, K., Kadota, S., Namba, T. Four di-*0*-caffeoyl quinic acid derivatives from propolis. Potent Hepatoprotective activity in experimental liver injury models. *Biol. Pharm. Bull.* 1996, **19**, 1479–1484.
92. Tatefuji, T., Izumi, N., Ohta, T., Arai, S., Ikeda, M., Kurimoto, M. Isolation and identification of compounds from Brazilian propolis which enhance macrophage spreading and mobility. *Biol. Pharm. Bull.* 1996, **19**, 966–970.
93. Robinson, W. E., Jr., Cordeiro, M., Abdel-Malek, S., Jia, Q., Chow, S. A., Reinecke, M. G., Mitchell, W. M. Dicaffeoylquinic acid inhibitors of human immunodeficiency virus integrase: inhibition of the core catalytic domain. *Mol. Pharmacol.* 1996, **50**, 846–855.
94. Robinson, W. E., Jr., Reinecke, M. G., Abdel-Malek, S., Jia, Q., Chow, S. A. Inhibitors of HIV-1 replication that inhibit HIV integrase. *Proc. Natl. Acad. Sci. U S A* 1996, **93**, 6326–6331.
95. McDougall, B., King, P. J., Wu, B. W., Hostomsky, Z., Reinecke, M. G., Robinson, W. E., Jr. Dicaffeoylquinic and dicaffeoyltartaric acids are selective inhibitors of human immunodeficiency virus type 1 integrase. *Antimicrob. Agents Chemother.* 1998, **42**, 140–146.
96. Kyng, P. J., Ma, G., Miao, W., Jia, Q., McDougall, B. R., Reinecele, M. G., Cornel, C., Kuan, J., Kim, T., Robinson, W. E., Jr. Structure-activity relationships: analogues of the dicaffeoylquinic and dicaffeoyltartaric acids as potent inhibitors of human immunodeficiency virus type 1 integrase and replication. *J. Med. Chem.* 1999, **42**, 497–509.
97. Kölling-Speer, L., Speer, K. The Raw Seed composition. In: *Espresso Coffee, the Science of Quality.* Illy, A., Viani, R., eds. Italy: Elsevier Academic Press; 2005, pp. 148–178.
98. Speer, K., Kölling-Speer, I. The lipid fraction of the coffee bean. *Braz. J. Plant Physiol.* 2006, **18**, 201–216.
99. Lee, K. J., Choi, J. H., Jeong, H. G. Hepatoprotective and antioxidant effects of the coffee diterpenes kahweol and Cafestol on carbon tetrachloride induced liver damage in mice. *Food Chem. Toxic.* 2007, **45**, 2118–2125.
100. Olthof, M. R., Hollman, P. C., Zock, P. L., Katan, M. B. Consumption of high disease of chlorogenic acid present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. *Am. J. Clin. Nutr.* 2001, **73**, 532–538.
101. Nunes, F. M., Coimbra, M. A. Chemical characterization of the high molecular weight material extracted with hot water from green and roasted Arabica coffee. *J. Agric. Food Chem.* 2001, **49**, 1773–1782.
102. Díaz-Rubio, M. E., Saura-Calixto, F. Dietary fiber in brewed coffee. *J. Agric. Food Chem.* 2007, **55**, 1999–2003.
103. Nunes, F. M., Reis, A., Silva, A. M. S., Rosário, M., Domingues, M., Coimbra, M. A. Rhamnoarabinosyl and rhamnoarabinoarabinosyl side chains as structural features of coffee arabinogalactans. *Phytochemistry*. 2008, **69**, 1573–1585.

104. Gntechwitz, D., Reichardt, N., Blaut, M., Steinhart, H., Bunzel, M. Dietary fiber from coffee beverage: degradation by human fecal microbiota. *J. Agric. Food Chem.* 2007, **55**, 6989–6996.
105. Trugo, L. C. Carbohydrates. In: *Coffee*, 1st edition, Clarke R. J., Macrae, R., eds. Essex: Elsevier Applied Science Publishers; 1985, Vol 1: Chemistry, p. 83.
106. Antonio, A. G., Iório, N. L. P., Pierro, V. S. S., Candreva, M. S., Farah, A., dos Santos K. R. N., Maia, L. C. Inhibitory properties of *Coffea canephora* extract against oral bacteria and its effect on demineralisation of deciduous teeth. *Arch. Oral Biol.* 2011, **56**(6), 556–564.
107. Costa, L. L., Toci, A. T., Silveira, C. L. P., Herszkowicz, N., M., Pinto, A., Farah, A. Discrimination of Brazilian *C. Canephora* by location using mineral composition. Proc. 23rd Int. Conf. Coffee Sci. ASIC, 2010. Bali, Indonesia.
108. Trugo, L. C., Macrae R. A study of the effect of roasting on the chlorogenic acid composition of coffee using HPLC. *Food Chem.* 1984, **15**, 219–227.
109. Folstar, P. Lipids. In: *Coffee*, Clarke, R. J., Macrae, R., eds. London: Elsevier Applied Science; 1985, Vol. 1: Chemistry, pp. 203–222.
110. Stephanucci, A., Clinton, W. P., Hamel, M. *Kirk-Othmer Encyclo. Chem. Technol.* New York: John Wiley & Sons; 1979, **6**, 511–512.
111. Nikolova-Damyanova, B., Velikova, R., Jham, G. N. Lipid classes, fatty acid composition and triacylglycerol molecular species in crude coffee beans harvested in Brazil. *Food Res. Int.* 1998, **31**, 479–486.
112. Lercker, G., Caboni, M. F., Bertacco, G., Turchetto, E., Lucci, A., Bortolomeazzi, R., Frega, N., Bocci, F. Coffee lipid fraction I. Influence of roasting and decaffeination. *Industrie Alimentari* 1996, **35**, 1057–1065.
113. Toci, A. T., Neto, V. J. F. M., Torres, A. G., Calado, V., Farah, A. Tryacylglicerols changes during the storage of roasted coffee. Proc. 22nd Int. Conf. Coffee Sci. ASIC. 504–507. 2008. Campinas, SP, Brazil.
114. Ogawa, A., Kamiya, C., Iida, Y. Contents of tocopherols in coffee beans,coffee infusions and instant coffee. *Nippon Shokuhin Kogyo Gakkaishi*. 1989, **36**, 490–494.
115. Folstar, P., Van der Plas, H. C., Pilnik, W., De Heusk J. G. Tocopherols in the unsaponifiable matter of coffee bean oil. *J. Agric. Food Chem.* 1977, **25**, 283–285.
116. Ortiz, A., Veja, F. E., Posada, F. Volatile composition of coffee berries at different stages of ripeness and their possible attraction to the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae). *J. Agric. Food Chem.* 2004, **52**, 5914–5918.
117. Bekedam, E. K., Loots, M. J., Schols, H. A., Van Boekel, M. A. J. S., Smit, G. Roasting effects on formation mechanisms of coffee brew melanoidins. *J. Agric. Food Chem.* 2008, **56**, 7138–7145.
118. Nicoli, M. C., Anese, M., Manzocco, L., Lerici, C. R. Antioxidant properties of coffee brews in relation to the roasting degree, *Lebensm. Wissens. Tech.* 1997, **30**, 292–297.
119. Daglia, M., Cuzzoni, M. T., Dacarro C. Antibacterial activity of coffee. *J. Agric. Food Chem.* 1994, **42**, 2270–2272.
120. Homma, S., Murata, M. Characterization of metal-chelating compounds in instant coffee. In: *Sixième Colloque Scientifique International sur le Café*. Kyoto, Japan: Association Scientifique International du Café; 1995, pp. 183–191.
121. Daglia, M., Papetti, A., Gregotti, C., Bertè, F., Gazzani, G. *In vitro* antioxidant and *ex vivo* protective activities of green and roasted coffee. *J. Agric. Food Chem.* 2000, **48**, 1449–1454.
122. Ginz, M., Balzer, H. H., Bradbury, A. G. W., Maier, H. G. Formation of aliphatic acids by carbohydrate degradation during roasting of coffee. *Eur. Food Res. Technol.* 2000, **211**, 404–410.
123. Perrone, D., Donangelo, R., Donangelo, C. M., Farah, A. Modeling weight loss and chlorogenic acid content in coffee during roasting. *J. Agric. Food Chem.* 2010, **58**, 12238–12243.
124. Duarte, G., Farah, A. Chlorogenic acids and lactones in Brazilian commercial coffee. Proc. 22nd Int. Conf. Coffee Sci. ASIC/Prospero, 224–227. 2009. Trieste, Italy.
125. Farah, A., Donangelo, C. M. Phenolic compounds in coffee. *Braz. J. Plant Physiol.* 2006, **18**, 26–36.
126. Sholz, B. M., Maier, H. G., Isomers of quinic acid and quinides in roasted coffee. *Z. Lebensm. Unters. Forsch.* 1990, **190**, 132–134.
127. de Paulis, T, Martin, P. R. *Coffee Tea, Chocolate and Brain*, Nehligh, A. ed. London: Taylor and Francis Book; 2003.
128. de Paulis, T., Commers, P., Farah, A., Zhao, J., McDonald, M. P., Galici, R., Martin, P. R. 4-Caffeoyl-1,5-quinide in roasted coffee inhibits [3H] naloxone binding and reverses antinociceptive effects of morphine in mice. *Psychopharmacol.*, 2004, **176**, 146–153.

129. Farah, A., Shearer, J., de Paulis, T. Diferuloylquinide is converted to diferuloylquinic acid and other phenolic compounds during digestion and/or metabolism in rats. *FASEB J*. 2008, **22**, 889–913.
130. Shearer, J., Farah, A., de Paulis, T., Bracy, D. P., Pencek, R. R., Graham, T. E., Wasserman, D. H. Quinides of roasted coffee enhance insulin action in conscious rats. *J. Nutr.* 2003, **133**, 3529–3532.
131. *Dietary Reference Intake*. Institute of Medicine. National Academies Press: Washington, DC, 2000.
132. Blank, I., Sen, A., Grosh, W. Aroma impact compounds of Arabica amd robusta coffee. Qualitative and quantitative investigations. *Proc. 14th Coll. ASIC*, 117–129. 1991. San Francisco.
133. Toci, A., Farah, A. New volatile compounds as Brazilian defective coffee beans' markers. 23rd International Conference on Coffee Science. ASIC/Prospero, 263–269. 2009. Trieste, Italy.
134. Smith, A. W. Introduction. In: *Coffee*. Clark R. J., Macrae, R., eds. London: Elsevier Applied Science; 1985, pp. 31–33.
135. USDA National Nutrient Database for Standard Reference. 2010, NDB No: 14218. http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl. (accessed November 2010).
136. Alves, R. M. V., Bordin, R. M. Estimativa de vida de café solúvel por modelo matemático. *Cien. Tecn. Aliment*. 1998, **18**, 19–24.
137. Alves, R. M. V., Milanez, C. R., Padula, M. Embalagens alternativas para café solúvel. *Cienc. Tecn. Aliment*. 2000, **20**, 204–211.
138. Speer, K., Kurt, A. Effects of steam treatment on diterpenes. 19th Int. Coll. Chem. Coff. ASIC, 2001. Paris.
139. Kurt, A., Speer, K. A new component in the lipid fraction of coffee. *Proc. Euro. Food Chem*. 1999, **3**, 882–336.
140. Petraco, M. The cup. In: *Espresso Coffee: The Science of Quality*, 2nd edition, Illy, A., Viani, R., eds. Italy: Elsevier Academic Press; 2005, pp. 290–298.
141. Clifford, M. N. The nature of chlorogenic acids. Are they advantageous compounds in coffee? Proc. 17th Int. Sci. Coll. Coffee (Nairobi). ASIC, 79–91, 1997. Paris.
142. Bennat, C., Engelhardt, U. H., Kiehne, A., Wirries, F., Maier, H. G. HPLC analysis of chlorogenic acid lactones in roasted coffee. *Z. Lebensm. Unters. Forsch*. 1994, **199**, 17–21.
143. Schrader, K., Kiehne, A., Engelhardt, U. H., Maier, H. G. Determination of chlorogenic acids with lactones in roasted coffee. *J. Sci. Food Agric*. 1996, **71**, 392–398.
144. Farah, A., Neves, D. F., Trugo, L. C., Rosenthal, A., Della Modesta, R. C. Compostos fenólicos em café torrado. In: *Annals of the II Simpósio de PNP and D Embrapa Café*. Embrapa, ed. Vitória, ES, Brazil, 2001; pp. 1144–1149.
145. Del Castillo, M. D., Ames, J. M., Gordon, M. Effect of roasting on the antioxidant activity of coffee brews. *J. Agric. Food Chem*. 2002, **50**, 3698–3703.
146. Costa, L. L., Donangelo, C. M., Silveira, C. L. P., Farah, A. Mineral Content in Brazilian Arabica coffee brewed by different methods. 23rd Int. Conf. Coffee Sci. ASIC/Prospero, 2010. Trieste, Italy.
147. Petraco, M. Physico-chemical and structural characteristics of 'espresso' coffee brew. Proc. 13th ASIC Coll, 246–261. 2001.
148. Perrone, D., Farah, A. Application of mass spectrometry on the analysis of coffee components. In: *Handbook on Mass Spectrometry: Instrumentation, Data and Analysis, and Applications*. J.K. Lang, ed. New York: Nova Science Publishers; 2009, pp. 465–498.
149. Romani, S., Pinnavaia, G. G., Rosa, M. D. Influence of roasting levels on ochratoxin A content in coffee. *J. Agric. Food. Chem*. 2003, **51**, 5168–5171.
150. World Health Organization. Evaluation of Certain Mycotoxins: Ochratoxin A. In: *Food Additives Series 47*. FAO Food and Nutrition. 2001, Paper 74.
151. Miraglia, M., Brera, C. SCOOP task 3.2.7.Assessment of dietary intake of ochratoxin A by the population of EU member states. 2002. http://europa.eu.int/comm/food/fs/scoop/3.2.7_en.pdf (accessed September, 2005).
152. Petraco, M. The cup. In: *Espresso Coffee: The Science of Quality*, 2nd edition, Illy, A., Viani, R., eds. Italy: Elsevier Academic Press; 2005, pp. 290–298.
153. Romani, S., Sacchetti, G., Chaves López, C., Pinnavaia, G. G., Dalla Rosa, M. Screening on the occurrence of ochratoxin A in green coffee seeds of different origins and types. *J. Agric. Food Chem*. 2000, **48**, 3616–3619.
154. Van der Stegen, G. H. D., Jörissen, U., Pittet, A., Saccon, M., Steiner, W., Vincenti, M., Winkler, M., Zapp, J., Schlatter, C. Influence of roasting levels on ochratoxin A content in coffee. *Food Addit. Contam*. 1997, **14**, 211–216.
155. Studer-Rohr, I., Dietrich, D. R., Schlatter, J., Schlatter, C. The occurrence of ochratoxin A in coffee. *Food Chem. Toxicol*. 1995, **33**, 341–355.

156. World Health Organization. Evaluation of Certain Food Additives and Contaminants. In *37th Report of the Joint FAO/WHO Expert Committee on Food Additives*. WHO Technical Report Series No. 859, WHO: Geneva, Switzerland, 1995.
157. European Commission. Scientific Committee on Food: Opinion on Ochratoxin A, CS/CNTM/MYC/14 final, Annex II to Document XXIV/2210/98, Brussels, 1998.
158. Silveira, T. M. L., Tavares, E., Glória, M. B. A. Profile and levels of bioactive amines in instant coffee. *J. Food. Compos. Anal.* 2007, **20**, 451–457.
159. Lima, A. S., Glória, M. B. A. Aminas Bioativas em alimentos. *Boletim SBCTA*, 1999, **33**, 70–79.
160. Oliveira, S. D., Franca, A. S., Glória, M. B. A., Borges, M. L. A. The effect of roasting on the presence of bioactive amines in coffees of different qualities. *Food Chem.* 2005, **90**, 287–291.
161. Amorim, H. V., Basso, L. C., Crocomo, O. J., Teixeira, A. A. Polyamines in green and roasted coffee. *J. Agric. Food Chem.* 1977, **25**, 957–958.
162. Casal, S., Mendes, E., Alvez, R. M., Alves R. C., Oliveira, M. B. P. P., Ferreira, M. A. Free and conjugated biogenic amines in green and roasted coffee beans. *J. Agric. Food Chem.* 2004, **52**, 6188–6192.
163. Casal, S., Mendes, E., Oliveira, M. B. P. P., Ferreira, M. A. Roast effects on coffee free and conjugated polyamines. *J. Environ. Agric. Food. Chem.* 2005, **4**, 1063–1068.
164. Vanconcelos, A. L. S., Franca, A. S., Glória, M. B. A., Mendonça, J. C. F. A comparative study of chemical attributes and levels of amines in defective green and roasted coffee beans. *Food Chem.* 2007, **101**, 26–32.
165. Lawley, R., Curtis L., Davis, J. *Biogenic Amines. The Food Safety Hazard Guidebook*. Cambridge, UK: RSC Publishing, pp. 279.
166. Till, H. P., Falke, H. E., Prinsen, M. K., Willems, M. I. Acute and subacute toxicity of tyramine, spermidine, spermine, putrescine and cadaverine in rats. *Food Chem. Toxicol.* 1997, **35**, 337–348.
167. Herraiz, T. Tetrahydro-β-carboline-3-carboxylic acid compounds in fish and meat: possible precursors of co-mutagenic *â*-carbolines norharman and harman in cooked foods. *Food Addit. Contam.* 2000, **17**, 859–866.
168. Herraiz, T. Relative exposure to β-carbolines norharman and harman from foods and tobacco smoke. *Food Addit. Contam.* 2004, **21**, 1041–1050.
169. Herraiz, T., Chaparro, C. Human monoamine oxidase enzyme inhibition by coffee and β-carbolines norharman and harman isolated from coffee. *Life Sci.* 2006, **78**, 795–802.
170. Alves, R. C., Casal, S., Oliveira, B. P. P. Factors influencing the Norharman and Harman contents in espresso coffee. *J. Agric. Food Chem.* 2007, **55**, 1832–1838.
171. Herraiz, T. Identification and occurrence of the bioactive *â*-carboline norharman and harman in coffee brews. *Food Addit. Contam.* 2002, **19**, 748–754.
172. Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, A. P., Robert, M. C., Riediker, S. Acrylamide from Maillard reaction products. *Nature.* 2002, **419**, 449.
173. Zyzak, D. V., Sanders, R. A., Stojanovic, M., Tallmadge, D. H., Eberhart, B. L., Ewald, D. K., Gruber, D. C., Morsch, T. R., Strothers, M. A., Rizzi, G. P., Villagran, M. D. Acrylamide formation mechanism in heated foods. *J. Agric. Food Chem.* 2003, **51**, 4782–4787.
174. Mottram, D. S., Wedzicha, B. L., Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature* 2002, **419**, 448–449.
175. International Agency for Cancer Research. *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*. Lyon, France: IARC; 1993, Vol. 56, pp. 163–242.
176. Tilson, H. A. The neurotoxicity of acrylamide: an overview. *Neurobehav. Toxicol. Teratol.* 1981, **3**, 445–461.
177. Lopachin, R. M., Lehning, E. J. Acrylamide-induced distal axon degeneration: a proposed mechanism of action. *Neurotox.* 1994, **15**, 247–259.
178. Granby, K., Fagt, S. Analysis of acrylamide in coffee and dietary exposure. *Analytica Chimica Acta*. 2004, **52**, 177–182.
179. Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., Tornqvist, M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* 2002, **50**, 4998–5006.
180. Alves, R. C., Soares, C., Casal, S., Fernandes, J. O., Oliveira, M. B. P. P. Acrylamide in espresso coffee: influence of species, roast degree and brew length. *Food Chem.* 2010, **119**, 929–934.
181. Soares, C., Farah, A., Fernandes, F., Fernandes, J. O. Influence of the roasting conditions on the formation of acrylamide in Brazilian coffee: preliminary results. Proc. 22nd Int. Conf. Coffee Sci. ASIC, 239–241. 2009. Campinas, SP, Brazil.

182. Soares, C., Cunha, S., Fernandes, J. Determination of acrylamide in coffee and coffee products by GC-MS using an improved SPE clean-up. *Food Addit. Contam.* 2006, **23**, 1276–1282.
183. Dybing, E., Farmer, P. B., Andersen, M., Fennell, T. R., Lalljle, S. P. D., Muller, D. J. G., Olin, S., Peterson, B. J., Schlatter, J., Scholz, G., Scimeca, J. A., Slimani, N., Tornqvist, M., Tuijtelaars, S., Verger, P. Human exposure and internal dose assessments of acrylamide in food. *Food Chem. Toxicol.* 2005, **43**, 365–410.
184. Huang, M. T., Chang, R. L., Wood, A. W., Newmark, H. L., Sayer, J. M., Yagi, H., Jerina, D. M., Conney, A. H. Inhibition of the mutagenicity of bay-region diol-epoxides of polycyclic aromatic hydrocarbons by phenolic plant flavonoids. *Carcinogenesis.* 1985, **6**, 237–242.
185. Lintas, C., De Matthaeis, M. C., Merli, F. Smoked, cooked and toasted food products. *Food Cosmet. Toxicol.* 1979, **17**, 324–328.
186. Kayali-Sayadi, M., N. Rubio-Barroso, S., Cuesta-Jimenez, M. P., Polo-Diez, L. M. A new method for the determination of selected PAHs in coffee brew samples by HPLC with fluorimetric detection and solid-phase extraction. *J. Liquid Chromatogr. Relat. Technol.* 1999, **22**, 615–627.
187. Hietaniemi, V., Ovaskainen, M. L., Hallikainen, A. PAH compounds and their intake from foodstuffs on the market. National Food Administration, 1999, Research Notes 6.
188. Cetinkaya, M., von Düszeln, J., Thiemann, W., Silwar, R. Organochlorine pesticide residues in raw and roasted coffee and their degradation during the roasting process. *Z. Lebensm. Unters Forsch.* 1984, **179**, 5–8.
189. Jacobs, R. M., Yess, N. J. Survey of imported green coffee beans for pesticide residues. *Food Addit. Contamin.* 1993, **127**, 575–577.
190. Spadone, J. C., Takeoka, G., Liardon, R. Analytical investigation of Rio off-flavor in green Coffee. *J. Agric. Food Chem.* 1990, **38**, 227–236.
191. Cantergiani, E., Brevard, H., Amado, R., Krebs, Y., Feria-Morales, A., Yeretzian, C. Characterisation of mouldy/earthy defect in green Mexican coffee. Proc. 18th Int. Coll. Chem. Coffee. 43–49. 1999.
192. Bortoli, G., Fabian, M. Survey of the presence in green coffee of substances associated with important off-flavors, and their correlation with ochratoxin A contamination. Proc. 19th Int. Coll. Chem. Coff. 2001. Trieste, Italy.
193. Toci, A. T., Benedetti, M., Farah, A. New volatile compounds as Brazilian defective coffee seeds markers. 22nd Int. Conf. Coffee Sci. ASIC/Prospero, 263–269. 2009. Trieste, Italy.
194. Chen L., Hecht, S. S., Peterson, L. A. Identification of cis-2-Butene-1,4-dial as a microsomal metabolite of furan. *Chem. Res. Toxicol.* 1995, **8**, 903–906.
195. Locas, C. P., Yaylayan, V. A. Origin and mechanistic pathways of formation of the parent furan-a food toxicant. *J. Agric. Food Chem.* 2004, **52**, 6830–6836.
196. Arisseto, A. P., Vicente, E., Ueno, M. S., Tfouni, S. A., Toledo, M. C. Furan levels in coffee as influenced by species, roast degree, and brewing procedures. *J. Agric. Food Chem.* 2011, **59**, 3118–3124.
197. Altaki, M. S., Galceran, M. T. Occurrence of furan in coffee from Spanish market: contribution of brewing and roasting. *Food Chem.* 2011, **126**, 1527–1532.
198. Fischer, M., Reimann, S., Trovato, V., Redgwell, R. J. Polysaccharides of green Arabica and Robusta coffee beans. *Carbohyd. Res.* 2001, **330**, 93–101.
199. Holscher, W., Vitzthum, O. G., Steinhart, H. Identification and sensorial evaluation of aroma-impact-compounds in roasted Colombian coffee. *Cafe, Cacao, The.* 1990, **34**, 205–212.
200. Czerny, M., Grosch, W. Potent odorants of raw arabica coffee and their changes during roasting. *J. Agric. Food Chem.* 2000, **48**, 868–872.
201. Clifford, M. N. Chlorogenic acids and other cinnamates—nature, *occurrence and dietary burden. J. Sci. Food Agric.* 1999, **79**, 362–372.
202. Maetzu, L., Sanz, C., Andueza, S., Paz de Peña, M., Bello, J., Cid, C. Characterization of espresso coffee aroma by static headspace GC-MS and sensory flavor profile. *J. Agric. Food Chem.* 2001, **49**, 5449–5444.
203. Sanz, C., Czerny, M., Cid, C., Schieberle, P. Comparison of potent odorants in a filtered coffee brew and in an instant coffee beverage by aroma extract dilution analysis (AEDA). *Eur. Food Res. Technol.* 2002, **214**, 299–302.
204. Akiyama, M., Murakami, K., Ikeda, M., Iwatsuki, K., Kobuco, S., Wada A., Tokuno, K., Onishi, M., Iwabuchi, H., Tanaka, K. Characterization of flavor compounds released during grinding of roasted robusta coffee beans. *Food Sci. Technol. Res.* 2005, **11**(3), 298–307.