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Flavor Chemistry of Cocoa and Cocoa Products—An Overview

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Abstract: Cocoa originates from beans of the cocoa tree (*Theobroma cacao* L.) and it is an important commodity in the world and the main ingredient in chocolate manufacture. Its value and quality are related to unique and complex flavors. Bulk cocoas (Forastero type) exhibit strong basic cocoa notes, whereas fine varieties (Criollo, Nacional) show aromatic, floral, or smoother flavor characteristics. About 600 various compounds (alcohols, carboxylic acids, aldehydes, ketones, esters, and pyrazines) have been identified as odor-active components. The specific cocoa aroma arises from complex biochemical and chemical reactions during the postharvest processing of raw beans, and from many influences of the cocoa genotype, chemical make-up of raw seeds, environmental conditions, farming practices, processing, and manufacturing stages. There has been much research on cocoa flavor components. However, the relationships between all chemical components that are likely to play a role in cocoa flavor, their sensory properties, and the sources and mechanisms of flavor formation are not fully understood. This paper provides an overview on cocoa flavor from a compositional and a sensory perspective. The nonvolatile and volatile chemical components of cocoa and chocolate flavor, and their sensory properties correlated to the main influences involved in flavor formation, are reviewed.

Keywords: chemistry, chocolate, cocoa, sensory quality

Introduction

Cocoa is an important agricultural commodity and the key raw material in chocolate manufacturing. It is derived from beans of the cacao tree (*Theobroma cacao* L.) belonging to the Malvaceae family (alternatively, Sterculiaceae; Alverson and others 1999). This is a small, evergreen tree, native to tropical regions of the Americas (Rusconi and Conti 2010). The fruits of the cacao tree are squash-like pods that grow proximal to the trunk and to thicker branches (McShea and others 2008; Fowler 2009), and each cocoa pod contains about 35 to 50 beans embedded in a mucilaginous pulp (Biehl and Ziegleder 2003a; McShea and others 2008). *T. cacao* is commercially cultivated in a climate belt within 20 degrees latitude of the equator (McShea and others 2008; Bernaert and others 2012).

Worldwide, the total production of cocoa beans exceeded 4 million tons in the 2011/2012 crop (Krähmer and others 2015). Most of the world's cocoa is produced in West Africa (70%) followed by Asia and Oceania (15.6%) and Latin America (14.1%; Giacometti and others 2015). World leaders in cocoa bean production are Ivory Coast, Ghana, Indonesia, Nigeria, Cameroon, Brazil, Ecuador, the Dominican Republic, and Malaysia, supplying 90% of the world production (Fowler 2009; Jahurul and others 2013). Most of the cocoa beans are produced in small or medium-sized farms; only 30% of the raw cocoa production originates from high-end farming (Bernaert and others 2012).

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The world demand for cocoa and chocolate has been increasing. The popularity of cocoa products is related to their unique sensory and pleasant melt-in-the-mouth properties (Fowler 2009; Torres-Moreno and others 2012). In addition, over the past few years a significant body evidence has been supporting the health benefits of cocoa and its role as a potential functional food (Rusconi and Conti 2010; Bernaert and others 2012). Flavor is one of the most significant consumer parameters and an essential attribute of cocoa quality. Raw beans are characterized by an unpleasant astringency and bitterness, and the specific cocoa and chocolate flavor profile is developed during the postharvest processing steps of beans that mainly include fermentation, drying, and roasting (Afoakwa and others 2008). Many factors are involved in these processes and they can impart an enormous variability to cocoa flavor and quality. There is a large volume of research reports on the identification of flavor components depending on cocoa types, processing methods, geographical location, and end-farming practices. To better understand the cocoa flavor and the manner of obtaining the products with desired flavor and quality, it is required to have knowledge of the relationships between all chemical components that are likely to play a role in cocoa flavor, their sensory properties, and the sources of flavor formation. A link between those players will aid in the development of flavor simulative models as suggested by Saltini and others (2013). This paper provides an overview of cocoa flavor from a compositional and a sensory perspective. The nonvolatile and volatile chemical components of the cocoa and chocolate flavor, and their sensory properties correlated to the main influences involved in flavor formation, are reviewed.

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Cocoa cultivars and flavor character

T. cacao has a significant genetic diversity and more than 14000 distinct varieties of the plant are known (McShea and others 2008). The main varieties of the species that are commercially exploited to make cocoa and chocolate are Forastero, Criollo, Trinitario, and Nacional (Giacometti and others 2015). They are distinguished by the morphological features of the fruit, geographic origin, and the flavor characteristics (Biehl and Ziegleder 2003a).

Forastero (*Theobroma cacao* L. ssp. *shaerocarpum* Cuat) is a productive and vigorous type cultivated since historic times. The beans are small and flat, with violet cotyledons (Wood and Lass 1988). It includes several subvarieties that are found in West Africa and South America (Wood and Lass 1988; Rusconi and Conti 2010). Amelonado is the most known subvariety of the Forastero type, being extensively cultivated in West Africa (except for Cameroon). It presents a large genetic variability and it is used for breeding in the main countries that produce cocoa (Jahurul and others 2013). The Forastero group shows strong basic chocolate flavor and it is classified as bulk, basic, or ordinary cocoa grade. Bulk cocoas represent over 90% of the world production of cocoa. They are used to manufacture cocoa mass, cocoa powder, cocoa butter, and milk/dark chocolate (Fowler 2009).

Criollo (*Theobroma cacao* L. ssp. *cacao* Cuat.) has been cultivated since prehistoric times in Central America, being the plant used by the Mayas (Wood and Lass 1988; Rusconi and Conti 2010). Nowadays, it is very rare. Criollo trees are found only in Central America, Venezuela, Madagascar, Sri Lanka, and Samoa. Their ripe pods are yellow or red, and the beans are large, rounded, with white-colored cotyledons (Fowler 2009; Jahurul and others 2013). This variety exhibits low resistance to pest damage and climatic changes, and it produces low yields (Ziegleder 1990; Jahurul and others 2013). Criollo cocoa is highly aromatic, and it develops mild, nutty, earthy, flowery, or tea-like flavors (Ziegleder 1990). Venezuela is the largest producer of Criollo cocoa (Jahurul and others 2013).

Trinitario is a hybrid of the Criollo and Forastero varieties. It has higher yields and it is less susceptible to diseases than the others (Jahurul and others 2013). Trinitario trees are found only in the cultivated state in the West Indies, South America, and Central America (Wood and Lass 1988). The variety presents strong basic chocolate characters and some wine-like flavor (Giacometti and others 2015).

The Nacional variety is grown only in Ecuador. It has large pale purple beans and it produces the Arriba flavor with aromatic, floral, spicy, and green notes (Afoakwa and others 2008). The Criollo, Trinitario, and Nacional types are classified as "fine" or flavor cocoas, being perceived as aromatic or smooth with fruity, raisin, floral, spicy, nutty, molasses, and caramel notes (Afoakwa and others 2008; Fowler 2009). They are mainly used to manufacture dark chocolate, and they represent 5% to 10% of the cocoa world market (Rusconi and Conti 2010).

Processing of cocoa beans

After harvesting, the cocoa beans undergo complex processing that alters their original chemical and physical properties in order to increase the seeds' palatability and to obtain chocolate flavors (McShea and others 2008; Aculey and others 2010). The primary processing include the fermentation and drying stages. The secondary processing converts cocoa beans into finished products, and it involves: roasting, alkalization, and conching (Nair Prabhakaran 2010; Figure 1).

Fermentation. It is a key stage in the processing of cocoa beans that determines the death of the beans, and it favors the removal of the pulp and the subsequent drying. The formation of flavor precursors, reduction in bitterness and astringency, as well as the development of color, are initiated during fermentation (Afoakwa and others 2008). The fermentation process starts with an anaerobic phase that takes place in the first 24 to 36 h after harvesting and opening the pods. In this phase, the beans and pulp are exposed to numerous microorganisms. The microbial ecology of cocoa fermentation involves various organisms: yeasts, bacteria (lactic acid and acetic acid bacteria, *Bacillus* species), and filamentous fungi. The major species involved in the control of fermentation are: *Hanseniaspora guilliermondii*, *Pichia kudriavzevii*, *Kluyveromyces marxianus* yeasts, *Lactobacillus plantarum*, *L. fermentum* lactic acid bacteria, and *Acetobacter pasteurianus* and *Gluconobacter frateurii* acetic acid bacteria (Ho and others 2014). The sugars (sucrose, glucose, and fructose) from the acidic pulp (pH below 4) undergo an alcoholic fermentation catalyzed by the yeasts, thus generating ethanol. Some yeasts initiate pectin degradation of the pulp cell walls that favor aeration (Afoakwa 2012; Nigam and Singh 2014). The microbial activity determines structural changes that contribute to the removal of the compartmentalization of enzymes and substrates, facilitating the movements of cell constituents (Afoakwa and others 2008).

After 48 to 96 h, the yeast activity is inhibited by aeration, alcohol concentration, and increased pH (as a result of citric acid depletion, which is used up by yeast metabolism) triggering the growth of lactic acid bacteria. The fermentation of pulp sugars creates lactic acid, acetic acid, ethanol, and carbon dioxide. Toward the end of the 2nd phase, lactic acid bacteria are replaced by acetic acid bacteria that are responsible for the oxidation of ethanol to acetic acid. All these processes that occur in this phase are exothermic, and they heat up the cocoa mass to 45 °C to 52 °C, being considered essential for flavor development. Acetic acid bacteria plays a key role in the formation of flavor precursors (Giacometti and others 2015). Some microbial metabolic end-products (acetic acid) diffuse into the bean and cause the death of cotyledons (Biehl and Ziegleder 2003b). As a consequence of the substrate's exhaustion, the production of acetic acid ceases, and the further oxidation of acetic acid leads to a slow increase in pulp pH up to about 5. The pH values of 3.8 and 5.8 are required for the optimal activity of endogenous proteases involved in the degradation of bean proteins and the generation of various flavor precursors (Afoakwa and others 2008; Ho and others 2014). The pH value of the cocoa pulp, the heat, and the high aeration in the cocoa mass toward the late stages of fermentation are often associated with a rise in the number of aerobic spore-forming bacteria. If the fermentation continues for too long, these bacteria and the growth of unwanted molds can cause the generation of some off-flavors (Schwan and Wheals 2004).

During the fermentation, complex biochemical reactions occur that generate such cocoa flavor precursors like reducing sugars and nitrogenous compounds. The concentrations and the ratio of flavor precursors at the end of the fermentation are crucial to the optimal development of flavor volatiles during bean roasting. During the anaerobic phase, sucrose is partially hydrolyzed to reducing sugars, proteins undergo proteolysis to peptides and amino acids, and polyphenols are hydrolyzed and oxidized. The aerobic phase is characterized by oxidative and condensation reactions such as oxidation of protein-polyphenol complexes and carbonyl-amino condensation that reduce astringency (Afoakwa and others 2008). Also, color changes occur during fermentation and they influence the final cocoa flavor (Afoakwa and others

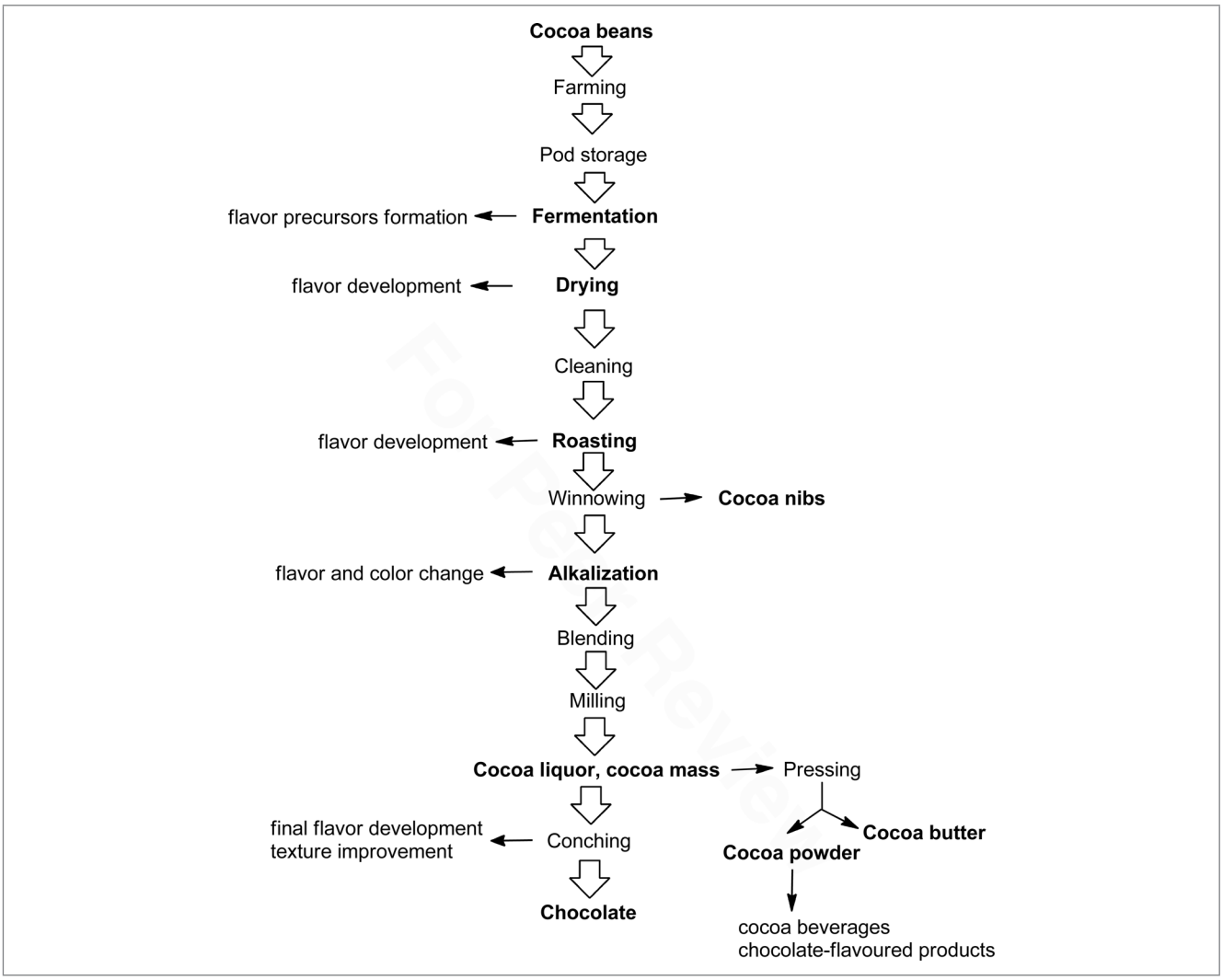

Figure 1–The processing of cocoa beans.

2008). Unfermented cocoa beans have a dark gray color and are more astringent, but fully fermented beans are brown due to enzymatic browning catalyzed by polyphenol oxidase (Caligiani and others 2007; Aculey and others 2010). Partially fermented beans are purple colored, and their flavor profile is bitter and harsh. Their subsequent processing leads to a loss of flavor in the final product (Caligiani and others 2007).

Many factors influence the fermentation, such as the method, duration, speed, pod storage, and cocoa bean genotype, leading to significant differences in the quality of cocoa (Afoakwa 2012; Afoakwa and others 2013). Platforms, heaps, baskets, trays, and boxes are among the most used fermentation methods (Guehi and others 2010b). The heap method offers a better quality of cocoa than the box method, due to a more uniform fermentation (Guehi and others 2010a). Moreover, the box method significantly affects pH value and tannins and sugars contents, and also the presence of purple beans. The platform method has a low fermentation rate and it can be applied adequately to Criollo beans, which require only a short fermentation (2 or 3 d) (Saltini and others 2013). In contrast, it cannot be applied to the Forastero cultivar, which requires a longer fermentation (5 to 8 d; Giacometti and others 2015). The length of fermentation has consequences on pH and temperature during the fermentation by affecting the enzymatic processes. As these conditions change at different stages of fermentation, aminopeptidases, invertase, and polyphenol oxidases are significantly inactivated, carboxypeptidase is partly inactivated, and endoprotease and glycosidases remain active. For instance, if the pH becomes too acidic too soon, some of these enzymes will be inactivated and there will be a significant reduction in flavor precursors (Camu and others 2008). The different genotypes of cocoa beans have different enzymatic activities. Hansen and others (2000) analyzed 10 genotypes of cocoa beans, demonstrating that there were significant differences in their enzyme activities. The most pronounced differences were found for the genotypes PA7 (high cocoa flavor), which had the highest endoprotease and aminopeptidase activities, and for UIT1 (low cocoa flavor), which had the lowest activity levels of endoprotease, aminopeptidase, and carboxypeptidase.

Overfermentation is characterized by an increased pH value, a characteristic hammy off-flavor, and a pronounced darkening or blackening of the beans (Biehl and Ziegleder 2003b; Nigam and Singh 2014). A period of 6 d of fermentation is considered as optimal for the production of flavor precursors (Giacometti and others 2015).

Drying. During the drying stage, the moisture content of the beans is reduced to an optimum of about 7% to 7.5% to prevent

overfermentation, mold contamination, and bean damage during storage. This phase also plays a significant role in reducing bitterness, astringency, and acidity, as well as in the characteristic flavor and brown color development (Afoakwa and others 2008; Merkus 2014). The oxidative processes started during fermentation continue during the drying stage. Polyphenol oxidases, still active, catalyze the transformation of polyphenols to quinones, which then undergo further condensation with free amino and sulfhydryl groups leading to brown polymers (Biehl and Ziegleder 2003a). Among the drying methods, sun drying is preferred because it gives a more pronounced chocolate flavor (Afoakwa and others 2008). Artificial drying can lead to off-flavors such as smoky, hammy, rubber, or gasoline notes (Bernaert and others 2012). Step-up-dried cocoa beans present the best flavor profile, with low sourness, bitterness, and astringency (Giacometti and others 2015). Well-dried, good-quality beans are recognized by their brown color, low astringency, and low bitterness, and also by the absence of off-flavors, such as smoky notes and excessive acidity (Afoakwa and others 2008). Incomplete drying and rain soaking produce high concentrations of strong-smelling carbonyl compounds and hammy off-flavors (Afoakwa and others 2008).

Roasting. After fermentation and drying, the processing continues with cleaning, blending, thermal pretreatment, winnowing, and roasting. During winnowing, the cotyledons, known as nibs, are separated from their shells, after breaking and subsequently sieving (Biehl and Ziegleder 2003b). Roasting of cocoa beans is the most important stage of bean processing. During roasting, the typical roasty and chocolate flavor and the specific texture of the beans are developed, undesired volatiles (acetic acid) are eliminated, and the moisture content is reduced to 1% to 2%; Nair Prabhakaran 2010; Giacometti and others 2015). Roasting affects the polyphenols' ability to interact with proteins, which causes a decline of astringency (Misnawi and others 2005; Ioannone and others 2015). Flavor precursors (free amino acids, oligopeptides, and reducing sugars) participate in nonenzymatic browning Maillard reactions (McShea and others 2008). The free amino groups of amino acids attack the reactive carbonyl groups of glucose and fructose (Jumnongpon and others 2012). In the 1st step, Schiff bases are obtained (glucosyl amines and fructosyl amines), and those undergo further tautomerization to 1,2 enaminols and the rearrangement to Amadori compounds (1-amino-1-deoxy-2-ketoses; Coultate 2009). In the next phase, under acidic conditions, these amino compounds are decomposed to 3-deoxyhexuloses that subsequently lose water to give hydroxymethylfurfurals and other furfural products. The basic or neutral pH yields 2,3-enediol and dehydroreductone intermediates, which lead to maltol, isomaltol, and α-dicarbonyl compounds. Further disintegration (dehydration, fragmentation, and transamination) of α-dicarbonyl compounds to smaller aldehydes and ketones is essential to cocoa flavor development. Another important route is the Strecker degradation, which leads either to volatile aldehydes, or to volatile pyrazines and other heterocyclic compounds (Afoakwa and others 2008; Coultate 2009). Complex reaction sequences starting from 2,3-enaminols and involving a 2nd amino acid in a Strecker degradation sequence generate pyrroles and pyridines that are believed to polymerize to brown melanoidin pigments (Coultate 2009).

Biogenic amines are an important class of compounds formed during roasting. They are not volatile, so they do not contribute to the flavor of cocoa and cocoa products, but they have important effects in the human organism after their consumption. Oracz and Nebesny (2014) identified several biogenic amines in roasted cocoa seeds and they observed that these compounds gave the highest concentrations when roasted at the highest temperature and in air with increased humidity. The most important biogenic amines studied were: 2-phenylethylamine, tyramine, tryptamine, serotonin, and dopamine. It is assumed that they are formed by decarboxylation of amino acids or by amination and transamination of the ketones and aldehydes produced during Strecker degradations. Roasting is frequently performed by 3 methods: whole bean roasting, nib roasting, and liquor roasting. In liquor roasting, a thermal pretreatment is first applied, then the shells are removed, and the nibs are transformed into liquor before roasting (Afoakwa and others 2008; Winkler 2014). The optimal roasting parameters depend on the raw material, the variety of cocoa, or type of the desired flavor (Ramli and others 2006). Generally, it is considered that a good flavor quality is positively correlated with a high degree of roasting only until it reaches an overroasting point (Saltini and others 2013). The roasting time ranges from 5 to 120 min (usually 10 to 35 min), and the roasting temperature ranges from 110 °C to 160 °C (usually 120 °C to 140 °C; Pätzold and Brückner 2006; Farah and others 2012; Kothe and others 2013; Ioannone and others 2015). Overroasting (over 160 °C) causes the development of burnt taste, as well as off-flavors.

Alkalization. Also known as "Dutching," alkalization consists of the treatment of cocoa mass, liquor, or powder with alkali. It can also be achieved prior to roasting (Nair Prabhakaran 2010). Nowadays, alkalization is carried out to improve the color and flavor of cocoa and to increase the dispersability of cocoa powder in beverages (Jolić and others 2011; Kothe and others 2013; Giacometti and others 2015). It reduces astringency by complex polymerization of polyphenols (Afoakwa and others 2008), it decreases bitterness, and it darkens cocoa products (Jolić and others 2011).

Conching. Conching is a mixing, multiday heat treatment step that contributes to the development of the final flavor and to the smooth texture of chocolate (Bolenz and others 2003; McShea and others 2008). The process improves the flavor profile, it reduces the concentration of free acids and other volatile byproducts of cocoa beans (Becket 2008; Giacometti and others 2015). Overall, off-flavors diminish after conching (Afoakwa and others 2008). It is usually performed by stirring at high temperatures (over 40 °C; Torres-Moreno and others 2012). Dark chocolate is typically conched at 70 °C or up to 82 °C (Afoakwa and others 2008).

Cocoa Flavor Chemistry

Nonvolatile components that contribute to cocoa flavor

Various chemical components from raw cocoa beans participate in the formation of specific cocoa flavors by changes occurring during processing. These components are alkaloids (methylxanthines), polyphenols, proteins, and carbohydrates (Figure 2). Cocoa genotype, cultivation conditions, and the environment are the primary factors that determine the variability of these components in the raw material.

Alkaloids. Raw cocoa beans contain methylxanthines (about 4%; Kadow and others 2013). Theobromine (3,7-dimethylxanthine) is the major alkaloid of cocoa (2% to 3%). Caffeine (1,3,7-trimethylxanthine) is found only in small amounts (0.2%), and theophylline as traces (Franco and others 2013). They all contribute to the typical bitter taste of cocoa and they are also involved in the palatability of food products containing them (Franco and others 2013). Along with polyphenols, methylxanthines are stored in polyphenolic cells in a single large vacuole

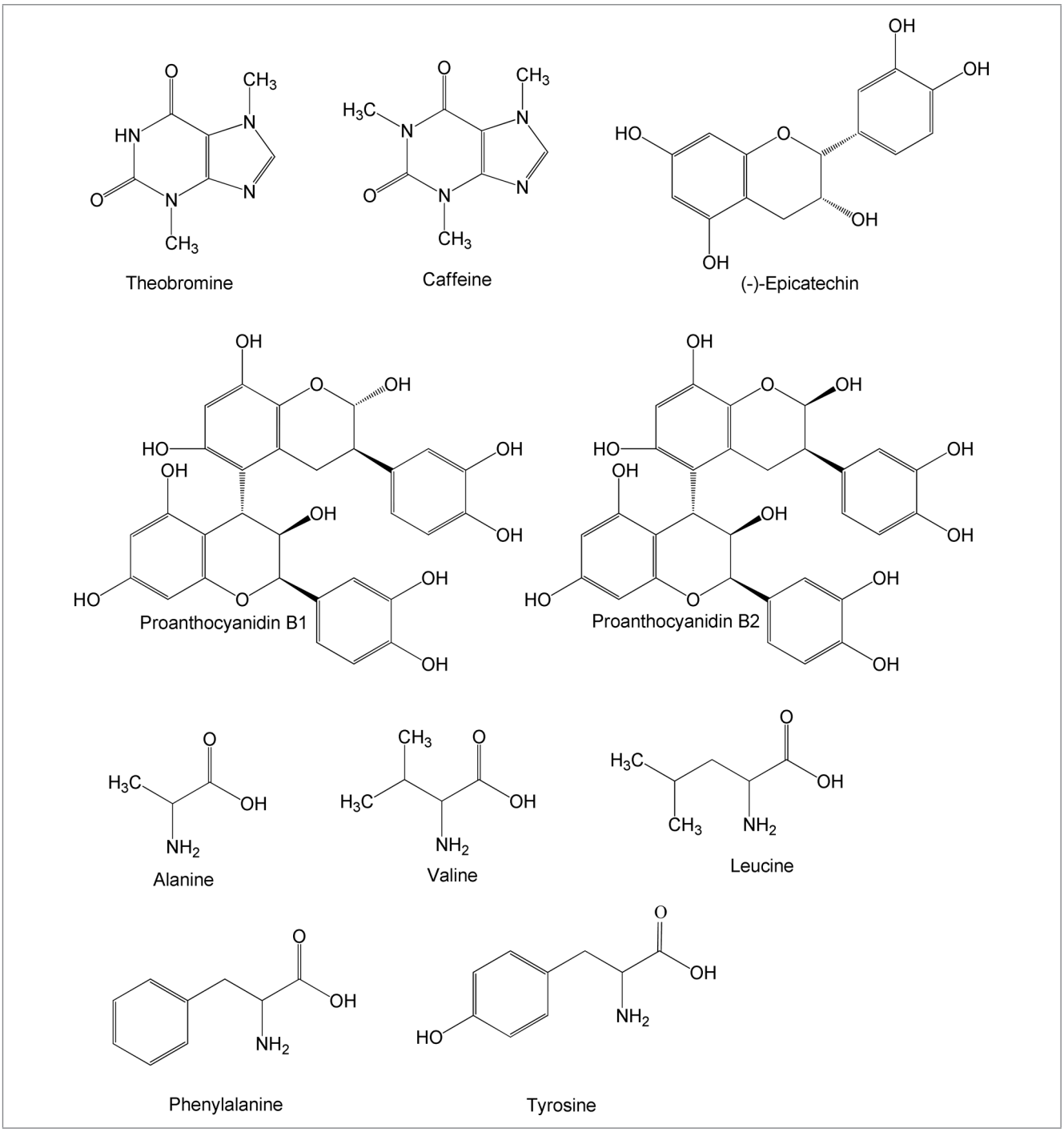

Figure 2–Nonvolatile contributors to cocoa flavor.

(Afoakwa and others 2008). The content of methylxanthines and the theobromine/caffeine ratio vary depending on the cocoa genotype. Unfermented West African (Forastero) cocoa contains 3.95% theobromine (in the dry fat-free material) and 0.192% caffeine (in the dry fat-free material). Fine American cocoas are richer in caffeine (0.30% to 0.60%, in fat-free dry nibs), while theobromine content ranges from 2.85% to 3.43% (in fat-free dry nibs; Afoakwa and others 2008). The content of methylxanthines decreases gradually after the first 72 h of fermentation, which leads to a reduction of bitterness. There is a reduction of about 30% of initial content of methylxanthines, but data from the literature vary. Ho and others (2014) reported that the loss of theobromine can range between 38% and 40%, while caffeine decreases more (50% to 54%). This significant loss can be explained by the diffusion of alkaloids from cotyledons (Nigam and Singh 2014). Also, caffeine and, to a lesser extent, theobromine can migrate into the fat of a cocoa kernel (Mattisek 1997). During roasting, theobromine and caffeine form adducts with diketopiperazines (1:2 mole ratio) that provide the specific bitterness of roasted beans (Biehl and Ziegleder 2003a). The alkalization process affects negatively the content of methylxanthines. The concentration of methylxanthines decreases as the degree of alkalization increases,

and the most dramatic decline was reported for theobromine (over 20%; Li and others 2012).

The methylxanthines are psychopharmacologically active molecules. The main pharmacological activities include: central nervous system stimulation, cardiovascular and metabolic effects, bronhodilatation, diuresis, gastric secretion stimulation, and, in high doses, the stimulation of skeletal muscles (Franco and others 2013). Methylxanthines, mainly caffeine, enhance physical and intellectual performance, mitigate fatigue, and cause a feeling of alertness (Aprotosoaie and Stănescu 2010). Theobromine has a weaker effect than caffeine, and, in the case of cocoa products, it is even lower or different (Franco and others 2013). Cocoa products represent only a small part of the human diet and the concentration of methylxanthine is low, so these products do not normally pose a risk to human health (Matissek 1997). Furthermore, theobromine appears to be even safer for humans than caffeine (Franco and others 2013). Dark chocolate contains 5 to 7 mg theobromine/g and 0.625 to 0.875 mg caffeine/g, while milk chocolate contains about 1 mg theobromine/g and 0.056 mg caffeine/g (Franco and others 2013).

Polyphenols. Cocoa seeds are a rich source of polyphenols (about 15% of dry bean weight) similar to wine, tea, or vegetables (Krähmer and others 2015). They are stored in the so-called polyphenolic cells, a type of parenchyma cells from cotyledons (Afoakwa and others 2008). These compounds confer astringent and bitter sensations and contribute significantly to the green and fruity flavors of cocoa liquors (Norr-Soffalina and others 2009). Also, polyphenols are responsible for positive health benefits associated with the consumption of cocoa. There are 3 main groups of polyphenols: catechins (flavan-3-ols), anthocyanins, and proanthocyanidins. Monomers account for 5% to 10% of the total cocoa polyphenols and polymers for ≥ 90% of the total cocoa polyphenols (Khan and Nicod 2012). The catechins (approximately 29% to 38% of the total polyphenols) are represented by (–)-epicatechin (up to 35% of the total polyphenols), (+)-catechin, (+)-gallocatechin, and (–)-epigallocatechin, while the anthocyanin fraction (approximately 4% of the total polyphenols) is formed by leucoanthocyanins L1, L2, L3, and L4, cyanidin-3-α-L-arabinoside, and cyanidin-3-β-D-galactoside. Proanthocyanidins (approximately 58% to 65% of the total polyphenols) contained in cocoa seeds are represented by dimers, trimers, or oligomers of flavan-3,4-diols linked by 4→8 or 4→6 bonds (Biehl and Ziegleder 2003a; Nazarrudin and others 2006; Niemanak and others 2006). The dimers to hexamers predominate, but polymers with 18 monomeric units can be found in cocoa products (Misnawi and others 2003; Rusconi and Conti 2010; Jolić and others 2011). The most important proanthocyanidins are B1, B2, B3, B4, B5, C1, and D (Andújar and others 2012). Other minor polyphenols include flavones (apigenin, luteolin, kaempferol, and their glycosides) and polyphenolic acids (caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, and syringic acid; Rusconi and Conti 2010). The content and composition of polyphenols vary strongly depending on several factors: genotype, origin, ripeness degree, and bean processing (Rusconi and Conti 2010; Kothe and others 2013). The Criollo cocoa bean type has a high content of procyanidins, while Trinitario and Forastero cultivars show lower procyanidin concentrations (Giacometti and others 2015). The anthocyanins provide the specific color to Forastero beans, but not in the Criollo cocoa type. The processing of beans can lead to a significant loss of flavanols (from 100% to 10% in chocolate; Rusconi and Conti 2010). During processing, polyphenols support complex biochemical reactions that are important to cocoa flavor and color formation. As a result of cell destruction during fermentation, polyphenols exude from storage cells, flavonoid glycosides are hydrolyzed, anthocyanins are converted to a colorless pseudo-base, catechins undergo nonenzymatic oligomerization, and proanthocyanidins are transferred into more complex forms (Biehl and Ziegleder 2003a). The fermentation processes can reduce more than 90% of the initial concentration of catechins (Kothe and others 2013). Also, after 4 d of fermentation, there is a significant loss of anthocyanins (over 90%; Misnawi and others 2003). However, the behavior of polyphenols may not be uniform during fermentation. Although the level of polyphenols usually decreases, there were reported cases in which their concentration did not change or even increased. This latter aspect may be a consequence of proanthocyanid in formation as a result of polymerization reactions (Rusconi and Conti 2010).

The microbial colonization, as well as duration of fermentation, can affect the flavanol content (McShea and others 2008). Yeasts, lactic acid bacteria, and acetic acid bacteria have a positive effect on the content of polyphenols, while aerobic spores and molds affect negatively (Giacometi and others 2015). A longer fermentation period is associated with a higher decrease in flavanol content (McShea and others 2008). During drying, the polyphenol content decreases significantly due to enzymatic browning and diffusion out of the beans; (–)-epicatechin content is significantly reduced after fermentation and drying (Ioannone and others 2015). Also, in dried and unfermented beans, the level of (–)-epicatechin decreases by about 50% (Giacometti and others 2015). It has also been suggested that the reduction of the polyphenol content during fermentation can be influenced by genetic peculiarities, such as anatomical features of the beans (Trognitz and others 2013).

Roasting dramatically affects the level and composition of polyphenols (Kothe and others 2013). Polyphenols are thermolabile molecular structures, and high temperatures and prolonged roasting cause a reduction of the content of total polyphenols. High temperatures are able to induce the epimerization of (–)-epicatechin (the major flavanol present in unroasted cocoa beans) to (–)-catechin, and (+)-catechin to (+)-epicatechin (Hurst and others 2011). It is assumed that the isomerization of catechins involves the ring opening of the oxygenated ring and subsequent reclosing to the corresponding enantiomers. The epicatechin/catechin ratio that was proposed as an indicator of cocoa processing decreases during roasting. This fact suggests a more rapid degradation of (–)-epicatechin than of catechin (Ioannone and others 2015). Proanthocyanidins also undergo epimerization reactions, but they are more complex processes, as the molecules of proanthocyanidins are dimers, trimers, or polymers (Kothe and others 2013). The content of proanthocyanidins with high molecular weight decreases at the start of the roasting, and then it increases. A possible explanation could be the polymerization of compounds with low molecular weight. It is considered that the roasting at low temperatures for a short time better preserves the content of polyphenols (Ioannone and others 2015). Temperatures below 140 °C are recommended. Alkalization causes a dramatic loss of polyphenols (64% loss of total phenolic content), and it alters the composition of polyphenols. Epicatechin and catechin show the highest loss (up to 98% and 80%, respectively). Also, quercetin shows a high reduction (86%; Giacometti and others 2015). A higher degree of alkalization leads to a more pronounced decrease of polyphenol content (Jolić and others 2011). These changes could be attributed to the reactions of oxidation and polymerization of polyphenols under alkaline conditions (Giacometti and others 2015).

Proteins. The cotyledons of ripe cocoa beans contain between 10% and 16% (dry weight) proteins: an albumin fraction (52%) and a globulin fraction (43%) represented by a vicilin (7S)-class globulin (Voigt and others 1993; Biehl and Ziegleder 2003a). The globulin protein consists of 3 subunits of 47, 31, and 15 kDa, respectively, which are derived from a common 66-kDa precursor (Kratzer and others 2009). The protein content of Forastero beans is higher than that of Criollo beans (Biehl and Ziegleder 2003a). Only vicilin (7S)-class globulin is degraded during the fermentation stage (Voigt and others 1994). The enzymatic proteolysis of globulin, under the combined action of cocoa aspartic endoprotease and serine carboxyexopeptidase, yields cocoa-specific flavor precursors like oligopeptides and free amino acids. The activities of both proteases are pH-dependent. A pK near to the 3.8 value, the activity of aspartic endopeptidase is optimal, and more hydrophobic oligopeptides are generated. At a pH close to 5.8, serine carboxyexopeptidase cleaves globulin to more hydrophilic oligopeptides and hydrophobic amino acids (leucine, tyrosine, valine, phenylalanine, and alanine; Biehl and Ziegleder 2003a; Afoakwa and others 2008). Hydrophobic peptides and free amino acids participate in Maillard reactions during roasting, and they yield characteristic cocoa flavor components; the amount of amino acids is reduced between 24% and 72% (Misnawi and others 2004). Some residual proteins participate in phenol–protein interactions, and their concentrations decrease (Afoakwa and others 2008).

Carbohydrates. Raw cocoa beans contain about 2% to 4% (dry weight) free sugars (fructose, glucose, sucrose, galactose, sorbose, xylose, arabinose, mannitol, and inositol) and around 12% (dry weight) polyssacharides (starch, pectins, cellulose, pentosans, and mucilage). In unfermented beans, sucrose represents about 90% of total sugars. The mucilaginous sweet pulp of ripe seeds contains variable amounts of sugars (hexoses and sucrose) and polysaccharides (pectins, hemicelluloses, and cellulose; Biehl and Ziegleder 2003a). During fermentation, sucrose is converted into reducing sugars (fructose and glucose) due to invertase activity (Biehl and Ziegleder 2003a; Afoakwa and others 2008). The Criollo cultivar has a high concentration of reducing sugars, while the Nacional variety shows very low levels of reducing sugars after fermentation (Giacometti and others 2015). Reducing sugars are critically involved in the development of typical chocolate flavor through Maillard reactions with amino acids during roasting (Ho and others 2014). Up to 90% of glucose and fructose are consumed during roasting (Biehl and Ziegleder 2003a).

Volatile components that contribute to cocoa flavor

Cocoa volatiles are derived from aroma precursors generated during fermentation and bean drying. The typical chocolate flavor is obtained during the roasting stage through Maillard reactions and the Strecker degradation of flavor precursors and their intermediates (Afoakwa and others 2008). About 600 volatiles have been identified in cocoa flavor (Ziegleder 2009). Table 1 summarizes the compounds thought to be main contributors to cocoa flavor. They include several chemical classes such as aldehydes, ketones, esters, alcohols, pyrazines, quinoxalines, furans, pyrones, lactones, pyrroles, and diketopiperazines (Figure 3). Different cocoa types may exhibit various and specific flavors since the concentration and sensory character of these compounds vary significantly.

Alcohols. These compounds occur during fermentation as a result of microbial activity. Also, they may result from heat degradation of amino acids. During drying and roasting, the concentration of alcohols decreases through chemical degradation or volatilization. High temperatures (160 °C to 170 °C) and the prolonged heat duration promote the loss of alcohols (Ramli and others 2006). Alcohols confer a fruity, green, floral aroma. High alcohol contents are desirable in order to obtain cocoa products with flowery and candy notes (Rodriguez-Campos and others 2012). 2-Heptanol imparts a fruity, herbaceous, flowery, and spicy aroma. Linalool and 2-phenylethanol are major alcohols in roasted nibs (Jinap and others 1998). Also, 2-phenylethanol is the most odor-active compound in dried and fermented cocoas (Rodriguez-Campos and others 2012). Flavor-grade cocoas from South America (Ecuador and Venezuela) and Trinidad contain important concentrations of linalool and other terpenoids (1.6 to 3.8 mg/kg), which confer a flowery, leafy, and tea-like aroma. On the other hand, the level of linalool is very low in basic cocoas from West Africa (0 to 0.5 mg/kg) or Malaysia (0 to 0.2 mg/kg; Ziegleder 1990; Biehl and Ziegleder 2003a). Basic cocoas from Ghana have a medium content of linalool (0.2 to 0.8 mg/kg; Ziegleder 1990). During roasting, the linalool content slightly decreases, but the difference between flavor and basic grade cocoas remains. The ratio of linalool/benzaldehyde may be used as a flavor index. A value higher than 0.3 indicates typical fine-grade cocoas (Ziegleder 1990).

Aldehydes and ketones. The carbonylic compounds of aldehyde type are crucial for the development of good cocoa flavor. A high concentration of aldehydes as well as of ketones is favorable for cocoa quality (Rodriguez-Campos and others 2012). Usually, they are formed by Strecker degradation of free amino acids during roasting. However, low concentrations of aldehydes may arise even during fermentation and drying. 2-Methylbutanal and 3-methylbutanal arising during fermentation produce malty and chocolate notes in unroasted and roasted cocoa (Rodriguez-Campos and others 2012). Desirable aldehydes and ketones are obtained during the stage of fermentation of 6 to 8 d and drying at 70 °C (Rodriguez-Campos and others 2012). High temperatures and a longer roasting decrease the content of aldehydes. Aldehydes are not only flavor components but also important reactants involved in the formation of heterocyclic compounds (pyrazines; Ziegleder 2009). They generate, *via* aldol condensation, phenylalk-2-enals with a typical flowery note fairly reminiscent of cocoa/chocolate (Biehl and Ziegleder 2003a). 5-Methyl-2-phenyl-2-hexenal exhibits a deep bitter cocoa note (Ramli and others 2006). Among the ketones, acetophenone determines sweet, floral notes, and acetoin appears to be a precursor of tetramethylpyrazine, an important odor-active component of cocoa flavor (Rodriguez-Campos and others 2012).

Esters. Esters are the second most important class of volatiles after pyrazines. Ethyl-, methylesters, and acetates predominate (Ramli and others 2006; Rodriguez-Campos and others 2011). They confer a fruity flavor and are the typical aroma components (mainly acetates) in unroasted cocoas that arise from amino acids (Biehl and Ziegleder 2003a). 2-Phenylethylacetate has flowery and honey notes and it is mainly responsible for the characteristic aroma of Asian cocoa liquor (Jinap and others 1998). It has been found in unroasted and roasted cocoa, and it can also results from yeast metabolism. Also, ethyl-2-methylbutanoate is an important flavor generated during fermentation (Afoakwa and others 2008). The formation of amyl acetates during fermentation must be avoided. They are considered as indicators of flavor defects (Rodriguez-Campos and others 2012). A summary of main aroma-damaging compounds to the cocoa flavor is provided in Table 2. High levels of 2-phenylethylacetate and low

Table 1–Main cocoa on-odor volatile compounds identified in cocoa flavor.

Compound	Odor quality	Sensory perception	Reference
Alcohols and phenols			
1-Propanol	Sweet, candy	Sweet chocolate	Rodriguez-Campos and others (2012)
2-Methyl-1-butanol	Fruity, grape	Fruity	Ramos and others (2014)
2,3-Butanediol	Natural odor of cocoa butter	Sweet chocolate	Ramos and others (2014)
2-Pentanol	Green, mild green	Vegetal	Rodriguez-Campos and others (2011)
1-Hexanol	Fruity, green	Fruity, herbal	Bonvechi (2005)
2-Hexanol	Fruity, green	Fruity, herbal	Bonvechi (2005)
Trans-3-hexen-1-ol	Grassy, green	Vegetal	Ramos and others (2014)
2-Heptanol	Citrusy	Fruity	Rodriguez-Campos and others (2012)
1-Phenylethanol	Honey, floral	Floral	Rodriguez-Campos and others (2012)
2-Phenylethanol	Honey, floral	Floral	Rodriguez-Campos and others (2012)
Benzyl alcohol	Sweet, floral	Floral	Rodriguez-Campos and others (2012)
Aldehydes and ketones			
2-Phenyl acetaldehyde	Honey, floral	Floral	Rodriguez-Campos and others (2011)
2-Methylpropanal	Chocolate	Sweet chocolate	Rodriguez-Campos and others (2011)
2-Phenylpropanal	Floral	Floral	Bonvechi (2005)
2-Methylbutanal	Chocolate	Sweet chocolate	Rodriguez-Campos and others (2012)
3-Methylbutanal	Chocolate	Sweet chocolate	Rodriguez-Campos and others (2012)
2-Phenyl-2-butenal	Sweet	Sweet chocolate	Rodriguez-Campos and others (2012)
4-Methyl-2-phenyl-2-pentenal	Cocoa	Sweet chocolate	Bonvechi (2005)
n-Hexanal	Green	Herbal	Afoakwa (2012)
5-Methyl-2-phenyl-2-hexenal	Cocoa	Sweet chocolate	Bonvechi (2005)
2-Nonenal	Green	Herbal	Afoakwa (2012)
Vanillin	Chocolate, sweet, vanilla	Sweet chocolate	Bonvechi (2005)
2-Pentanone	Fruity	Fruity	Rodriguez-Campos and others (2011)
2-Heptanone	Fruity, floral	Fruity, floral	Rodriguez-Campos and others (2012)
Acetophenone	Floral	Floral	Rodriguez-Campos and others (2012)
2-Hydroxy acetophenone	Heavy floral, herbaceous	Floral, herbal	Bonvechi (2005)
4-Methyl acetophenone	Fruity, floral	Fruity, floral	Bonvechi (2005)
Acids			
2-Methylpropionic acid	Floral	Floral	Krings and others (2006)
3-Phenylpropionic acid	Sweet, rose	Floral	Bonvechi (2005)
Cinnamic acid	Honey, floral	Floral	Bonvechi (2005)
Esters			
Ethyl acetate	Pineapple	Fruity	Rodriguez-Campos and others (2012)
Isobutyl acetate	Fruity	Fruity	Rodriguez-Campos and others (2012)
Isoamyl acetate	Fruity, banana	Fruity	Ramos and others (2014)
Benzyl acetate	Floral, jasmine	Floral	Rodriguez-Campos and others (2012)
Methylphenyl acetate	Sweet, honey, jasmine	Floral	Bonvechi (2005)
Ethylphenyl acetate	Fruity, sweet	Floral	Rodriguez-Campos and others (2012)
2-Phenylethyl acetate	Honey, floral	Floral	Rodriguez-Campos and others (2012)
Ethyl butyrate	Pineapple	Fruity	Ramos and others (2014)
Ethyl lactate	Fruity	Fruity	Rodriguez-Campos and others (2012)
Diethyl succinate	Pleasant aroma	Floral	Ramos and others (2014)
Ethyl 2-methylbutanoate	Fruity	Fruity	Rodriguez-Campos and others (2012)
Ethyl 3-methylbutanoate	Fruity	Fruity	Rodriguez-Campos and others (2012)
Ethyl valerate	Fruity, apple	Fruity	Bonvechi (2005)
Ethyl hexanoate	Fruity	Fruity	Rodriguez-Campos and others (2012)
Ethyl octanoate	Fruity, floral	Fruity	Rodriguez-Campos and others (2012)
Ethyl decanoate	Pear, grape	Fruity	Rodriguez-Campos and others (2012)
Ethyl laurate	Fruity, floral	Fruity, floral	Bonvechi (2005)
Isoamyl benzoate	Balsam, sweet	Floral	Rodriguez-Campos and others (2012)
Methyl salicylate	Bitter-almond	Nutty	Bonvechi (2005)
Methyl cinnamate	Balsamic, strawberry	Fruity	Bonvechi (2005)
Ethyl cinnamate	Sweet, cinnamon-like	Sweet chocolate	Rodriguez-Campos and others (2012)
Amines, amides, nitriles, purines			
Benzonitrile	Almond	Nutty	Bonvechi (2005)
N-(2-phenethyl) formamide	Essences	Floral	Bonvechi (2005)
Lactones			
δ-Octenolactone	Coconut	Nutty	Afoakwa (2012)
γ-Decalactone	Peach	Fruity	Afoakwa (2012)
Terpenoids			
Geraniol	Floral, rose, fruity	Floral, fruity	Bonvechi (2005)
Geranyl acetate	Rose, lavender	Floral	Bonvechi (2005)
α-Terpenyl formate	Herbaceous, citrus	Herbal, fruity	Bonvechi (2005)
Linalool (cis-pyranoid)	Floral, green	Floral, herbal	Bonvechi (2005)
Linalool (trans-pyranoid)	Floral	Floral	Bonvechi (2005)
Linalool oxide (cis-furanoid)	Nutty	Nutty	Bonvechi (2005)
Linalool oxide (trans-furanoid)	Floral, citrus	Fruity, floral	Bonvechi (2005)
Furans, furanones, pyrans, pyrones			
2-Furfural	Almond	Nutty	Bonvechi (2005)

(Continued)

Table 1–Continued

Compound	Odor quality	Sensory perception	Reference
5-Methyl-2-furfural	Sweet, caramel	Sweet chocolate	Bonvechi (2005)
2-Furfuryl acetate	Fruity, banana	Fruity	Ramos and others (2014)
2-Acetylfuran	Sweet, balsamic, slightly coffee	Sweet chocolate	Bonvechi (2005)
2-Acetyl-5-methylfuran	Strong nutty	Nutty	Bonvechi (2005)
2-Furfuryl propionate	Spicy, floral	Floral	Bonvechi (2005)
5-(1-Hydrohyethyl)-2-furanone	Red fruit, jam, green notes	Fruity, herbal	Krings and others (2006)
Dihydro-3-hydroxy-4,4-dimethyl-2-furanone	Coconut	Nutty	Krings and others (2006)
4-Hydroxy-2,5-dimethyl-3-furanone (furaneol)	Fruity, strawberry, hot sugar	Fruity, nutty	Bonvechi (2005)
3-Hydroxy-2-methyl-4-pyrone (maltol)	Roasted nuts	Nutty	Bonvechi (2005)
5,6-Dihydro-6-pentyl-2-pyrone	Coconut	Nutty	Krings and others (2006)
Pyrroles			
Pyrrole	Nutty	Nutty	Bonvechi (2005)
2-Acetylpyrrole	Chocolate, hazelnut	Sweet chocolate	Rodriguez-Campos and others (2012)
Pyrrole-2-carboxaldehyde	Nutty	Nutty	Krings and others (2006)
Pyrazines			
2-Methylpyrazine	Nutty, chocolate, cocoa, roasted-nuts	Sweet chocolate, nutty	Bonvechi (2005)
2-Ethylpyrazine	Peanut butter, musty nutty	Nutty	Bonvechi (2005)
2,5-Dimethylpyrazine	Cocoa, rusted nuts	Sweet chocolate, nutty	Bonvechi (2005)
2,6-Dimethylpyrazine	Nutty, coffee, green	Nutty, herbal	Bonvechi (2005)
2-Ethyl-5-methylpyrazine	Nutty, raw potato	Nutty, hernal	Bonvechi (2005)
2,3-Diethylpyrazine	Nutty, hazelnut, cereal	Nutty	Bonvechi (2005)
2,3-Dimethylpyrazine	Caramel, cocoa	Sweet chocolate	Bonvechi (2005)
2,3,5-Trimethylpyrazine	Cocoa, rusted nuts, peanut	Sweet chocolate, nutty	Bonvechi (2005)
2,3,5,6-Tetramethylpyrazine	Chocolate, cocoa, coffee	Sweet chocolate	Bonvechi (2005)
2,3,5-Trimethyl-6-ethylpyrazine	Candy, sweet	Sweet chocolate	Rodriguez-Campos and others (2012)

concentrations of 3-methyl-1-butanol acetate are important for an aromatic quality of cocoa (Rodriguez-Campos and others 2012). High temperatures during roasting negatively affect the content of esthers (Ramli and others 2006).

Pyrazines. These compounds are the main class of heterocyclic volatiles and the key odor components in cocoa aroma. They display nutty, earthy, roasty, and green aromas (Semmelroch and Grosch 1996; Wagner and others 1999; Czerny and Grosch 2000; Czerny and others 2008). About 80 pyrazines contribute to the overall cocoa flavor (Afoakwa and others 2008). They are alkylpyrazines with different substituents (methyl-, ethyl-, propyl-, furyl-, vinyl-, and methoxy; Ziegleder 2009), and tetramethylpyrazine and trimethylpyrazine are the most important. These pyrazines exhibit nutty, grassy, and persistent cocoa notes, and tetramethylpyrazine has cocoa flavor enhancer properties (Ramli and others 2006). It has been reported that the tetramethylpyrazine constitutes about 90% of the total pyrazines (Rodriguez-Campos and others 2012). Well-fermented cocoa from Ghana has higher levels of pyrazines (698 μg/100 g) than Mexican cocoas (142 μg/100 g; Afoakwa and others 2008). Generally, the Criollo cultivar shows high levels of pyrazines while Nacional/Arriba cocoa has the lowest concentrations of pyrazines (Afoakwa and others 2008; Giacometti and others 2015). Most of the pyrazines originate from α-aminoketones by Strecker degradation and Maillard reactions during roasting (Rodriguez-Campos and others 2012). Temperature and duration of thermal reactions are critical factors that influence the concentration of pyrazines. Tetramethylpyrazine reaches high concentrations (7 mg/kg) at medium roasting conditions (Ziegleder 2009). The concentration ratio of tetramethylpyrazine (TMP)/trimethylpyrazine (TrMP) has been proposed as an indicator of roasting degree. For a normal degree of roasting, the ratio TMP/TrMP ranges from 1.5 to 2.5, and for an overroasting degree, the value is below 1 (Ziegleder 2009). Tetramethylpyrazine could occur during fermentation as a metabolic product of *Bacillus subtilis* (Ramli and others 2006). However, the fermentation of cocoa beans in the absence of yeasts and their subsequent roasting leads to less pyrazines and a less chocolaty character of the final flavor (Ho and others 2014). Also, pyrazines may arise during the drying process via Maillard reactions initiated by a drop in moisture content and temperatures of 30 °C to 50 °C (Puziah and others 1999).

Acids. During fermentation the concentration of organic acids increases as a result of sugar metabolism. Acetic acid with sour and vinegar-like aroma is considered the highest odor-active compound in fermented and unroasted beans. Besides acetic acid, other short-chain carboxylic acids (isobutyric, isovaleric, and propionic) predominate in fermented cocoa beans. They produce off-odor notes (rancid, butter, and hammy) and they are eliminated during the roasting and conching stages. A prolonged fermentation (over 6 d) increases the level of organic acids and their off-flavor notes (Rodriguez-Campos and others 2012). Drying reduces the content of volatile fatty acids such as acetic, propionic, butyric, and isobutyric acids (Paramo and others 2010), and 70% of acetic acid is removed during roasting (Rodriguez-Campos and others 2012).

Phenols (phenol, 2-methoxyphenol) are compounds with aroma-damaging properties, producing smoky and undesirable notes. They arise during drying or storage by contamination from burning wood or charcoal smoke. The roasting of cocoa beans at 110 °C to 140 °C for 5 to 30 min increases the level of phenols. A high-quality cocoa should be mostly free of them (Jinap and others 1998).

Other components. Furanones and pyrones are generated during drying and roasting via degradation of monosaccharides. Moderate temperatures and relative high humidities favor their formation (Ziegleder 2009; Rodriguez-Campos and others 2012). Roasting at 130 °C for 20 min is the optimal condition for the production of pyrones and furanones (Ziegleder 1991). The most important compounds are furaneol [4-hydroxy-2,5-dimethyl-3(2H)furanone], hydroxymaltol (3,5-dydroxy-6-methyl-4-pyrone), dyhydroxymaltol, and cyclotene (2-hydroxy-3-methyl-2-cyclopentene-1-one). They confer pleasant caramel notes and enhance flavor impression. The alkalization process destroys those compounds (Rodriguez-Campos and others

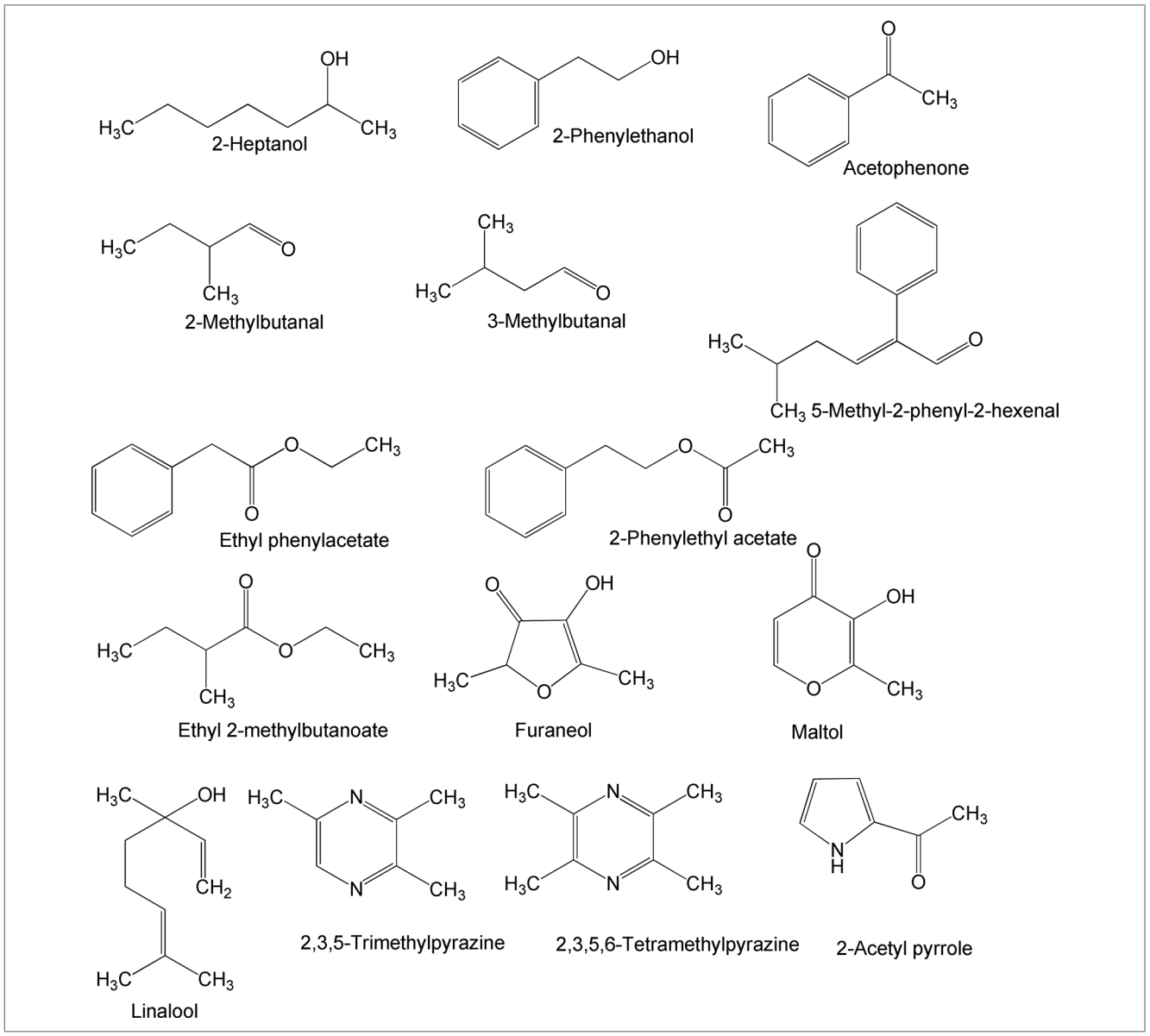

Figure 3–Chemical structures of main aroma-active volatiles of cocoa flavor.

2012). 2-Acetyl-1-pyrrole is produced during drying and roasting *via* Maillard reactions and Strecker degradation starting from the amino acid proline. It confers caramel, chocolate, and roasty desirable notes (Rodriguez-Campos and others 2012).

Health Effects of Cocoa and the Influence of Bean Processing

Due to high polyphenol levels, cocoa has attracted increased attention from nutritional and pharmacological viewpoints. It shows promising antioxidant, cardioprotective, neuroprotective, and chemopreventive potential (Andújar and others 2012).

Antioxidant potential

Cocoa polyphenols have strong antioxidant properties. The antioxidant capacity of cocoa beans per serving is higher than that of red wine or black or green tea (Jolić and others 2011), and the content of total polyphenols and flavonoids is about 611 mg gallic acid equivalents and 564 mg epicatechin equivalents, respectively (Jahurul and others 2013). *In vitro* experimental studies have proved that cocoa polyphenols scavenge reactive species, such as: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid; ABTS), and superoxide radicals, hypochlorite and peroxynitrite anions, inhibit lipid peroxidation, and chelate free pro-oxidant metal ions (Fe^{2+}, Cu^{+}; Andújar and others 2012).

Purified cocoa flavanols and cocoa inhibit ultraviolet C-induced oxidative DNA damage, being as effective as glutathione, α-tocopherol, and vitamin C. A decrease of DNA and glutathione oxidation has been reported in rats with chronic supplementation of diet with 2% cocoa powder. In humans, flavanol-rich cocoa products increase the total plasma antioxidant capacity, and dose-dependently reduce the concentrations of plasma thiobarbituric acid reactive substances (Keen and others 2005). In a randomized crossover study in obese adults at risk for insulin resistance, Stote and others (2012) have showed that the consumption of cocoa beverages providing an average dose of 400 mg flavanols per day

Table 2–Main off-odor volatile compounds in cocoa flavor.

Compound	Odor quality	Sensory perception	Reference
Alcohols and phenols			
2-Methyl-1-propanol	Wine-like	Undesirable	Rodriguez-Campos and others (2012)
1,2-Propanediol	Odorless	Repulsive	Ramos and others (2014)
3-Methyl-1-butanol	Malty	Undesirable	Rodriguez-Campos and others (2011)
2-Butanol	Medicinal	Undesirable	Bonvechi (2005)
1-Pentanol	Pungent	Bitter pungent	Bonvechi (2005)
1-Heptanol	Wizened	Repulsive	Bonvechi (2005)
1-Octanol	Fatty, waxy	Undesirable	Bonvechi (2005)
Phenol	Smoky	Repulsive	Rodriguez-Campos and others (2012)
2-Methylphenol	Musty, phenolic	Repulsive	Bonvechi (2005)
3-Methylphenol	Medicinal, woody	Undesirable	Bonvechi (2005)
4-Methylphenol	Medicinal, heavy	Undesirable	Bonvechi (2005)
2-Methoxyphenol	Smoky	Repulsive	Rodriguez-Campos and others (2012)
Aldehydes and ketones			
Acetaldehyde	Pungent	Bitter pungent	Ramos and others (2014)
n-Pentanal	Pungent	Bitter pungent	Rodriquez-Campos and others (2011)
2-Octenal	Fatty, waxy	Undesirable	Afoakwa (2012)
Benzaldehyde	Bitter	Bitter pungent	Bonvechi (2005)
2-Hydroxybenzaldehyde	Pungent	Bitter pungent	Bonvechi (2005)
Acetoin	Buttery, creamy	Undesirable	Rodriquez-Campos and others (2012)
2,3-Butanedione	Buttery	Undesirable	Rodriguez-Campos and others (2012)
2,3-Pentanedione	Bitter	Bitter pungent	Bonvechi (2005)
Benzylideneacetone	Pungent	Bitter pungent	Bonvechi (2005)
Acids			
Acetic acid	Sour, vinegar	Repulsive	Rodriguez-Campos and others (2011)
Propionic acid	Pungent	Bitter pungent	Rodriguez-Campos and others (2011)
Butyric acid	Rancid, cheese	Repulsive	Rodriguez-Campos and others (2011)
2-Methylbutyric acid	Sweaty	Repulsive	Krings and others (2006)
3-Methylbutyric acid	Sweaty	Repulsive	Krings and others (2006)
Isobutyric acid	Rancid butter	Repulsive	Rodriguez-Campos and others (2012)
Isovaleric acid	Sweat, rancid	Repulsive	Rodriguez-Campos and others (2012)
Hexanoic acid	Sweat, pungent	Repulsive	Rodriguez-Campos and others (2012)
Heptanoic acid	Rancid, sour	Repulsive	Rodriguez-Campos and others (2012)
Octanoic acid	Sweaty, fatty	Repulsive	Rodriguez-Campos and others (2012)
Nonanoic acid	Fatty	Repulsive	Rodriguez-Campos and others (2012)
Decanoic acid	Rancid, fatty	Repulsive	Rodriguez-Campos and others (2012)
Dodecanoic acid	Metal	Undesirable	Rodriguez-Campos and others (2012)
Benzoic acid	Urine-like	Repulsive	Bonvechi (2005)
Phenylacetic acid	Sweat	Repulsive	Krings and others (2006)
Esters			
Ethyl heptanoate	Wine-like, brandy	Undesirable	Bonvechi (2005)
Methyl myristate	Fatty	Undesirable	Bonvechi (2005)
Ethyl myristate	Waxy, soapy	Undesirable	Bonvechi (2005)
Ethyl palmitate	Waxy	Undesirable	Rodriguez-Campos and others (2012)
Methyl stearate	Oily	Undesirable	Bonvechi (2005)
Ethyl stearate	Waxy	Undesirable	Bonvechi (2005)
Ethyl benzoate	Fatty	Undesirable	Bonvechi (2005)
Amines, amides, nitriles, purines			
2-Phenyl acetamide	Phenolic odor	Repulsive	Bonvechi (2005)
Sulfur compounds			
Benzenethiol	Penetrating	Repulsive	Bonvechi (2005)
Trithio acetone	Sulfurous, earthy, wizened	Undesirable	Bonvechi (2005)
Dimethyldisulfide	Sulfurous	Undesirable	Bonvechi (2005)
2-Methyl-3-(methyldithio)furan	Cooked meat-like	Repulsive	Afoakwa (2012)
Furans, furanones, pyrans, pyrones			
2-Furfuryl alcohol	Faint burning	Repulsive	Ramos and others (2014)
5-Hydroxymethyl-2-furfural	Fatty, musty, waxy	Undesirable	Bonvechi (2005)
2-furoic acid	Odorless	Repulsive	Bonvechi (2005)
3,5-Dihydroxy-6-methyl-4-pyrone	Roasted	Undesirable	Bonvechi (2005)
2,3-Dihydro-3,5-dihydroxy-6-methyl-4-pyrone	Roasted	Undesirable	Bonvechi (2005)
5,6-Dihydro-4-methyl-2-pyrone	Slightly sour	Repulsive	Krings and others (2006)
Pyridines			
3-Hydroxy-2-methylpyridine	Wizened	Repulsive	Bonvechi (2005)
3-Hydroxy-6-methylpyridine	Wizened	Repulsive	Bonvechi (2005)
Pyrazines			
2-Ethyl-3,5-dimethylpyrazine	Earthy, roasty	Undesirable	Afoakwa (2012)
2,3-Diethyl-5-methylpyrazine	Earthy, roasty	Undesirable	Afoakwa (2012)

for 5 d, lowers the levels of total 8-isoprostanes, markers of *in vivo* lipid peroxidation, and the plasma concentration of C-reactive protein, a biomarker of inflammation. Conversely, other authors did not detect changes of biomarkers of oxidative stress in healthy volunteers (Arranz and others 2013). It is possible that the beneficial effects of cocoa to be more visible in conditions of pronounced oxidative stress or redox homeostasis disturbances such as: aging, smoking, nutritional deficiency, and pathological status (Allgrove and Davison 2014). In this respect, an extensive research in different population subjects is needed to understand better the effects of cocoa consumption on biomarkers of oxidative stress. A critical issue of antioxidant studies is also the fact that *in vitro* antioxidant capacity may not be directly correlated to *in vivo* antioxidant effects. There are many factors (the absorption and bioavailability of cocoa polyphenols, presence of other components affecting the redox status of the organism, and food matrix) that contribute to the overall postconsumption antioxidant effects (Allgrove and Davison 2014).

Cardioprotection

Many epidemiological studies have shown that polyphenol-rich cocoa and dark chocolate intake is correlated with a reduction in the occurrence of stroke, atherosclerosis, coronary artery disease, heart failure, and cardiovascular disease–related mortality (Crozier and Hurst 2014). As a result of an observational study, Buijsse and others (2006) have reported that cocoa intake is associated with a 45% to 50% lower risk of cardiovascular death in prospective analysis. The cardioprotective properties of cocoa are attributed to an improvement in antioxidant status and endothelial function, modulation of the blood pressure, metabolic and anti-inflammatory effects, and inhibition of the platelet activation and aggregation (McShea and others 2008; Lippi and others 2009; Kothe and others 2013).

Effects on blood pressure

Several studies conducted on spontaneously hypertensive rats (SHR) have demonstrated that single oral administration of cocoa powder with high content of procyanidins decreases blood pressure, whereas long-term soluble cocoa fiber intake reduces the development of hypertension in SHR (Cienfuegos-Jovellanos and others 2009; Sánchez and others 2010). Various epidemiological studies as well as a number of short-term randomized interventional trials (intervention duration varies from 2 to 18 wk, and daily intake of flavanols ranges from 30 to 1000 mg) have reported that cocoa/dark chocolate consumption has antihypertensive effects, lowering systolic, diastolic, and mean blood pressure in normotensive subjects, and also in prehypertensive and hypertensive patients with and without glucose intolerance (Arranz and others 2013; Grassi and Ferri 2014). The consumption of cocoa-based beverages with high flavanol concentrations attenuates the blood pressure response to exercise. A meta-analysis of 10 randomized controlled trials has concluded that the short-term consumption of polyphenol-rich chocolate and cocoa products is associated with statistically significant reductions of systolic (−4.5 mm Hg) and diastolic (−2.5 mm Hg) blood pressures (Desch and others 2010). In hypertensive patients, such values may be considered of clinical relevance in the context in which it is estimated that a reduction of 5 mm Hg in systolic blood pressure has been shown to decrease the risk of cardiovascular diseases by approximately 20% over 5 y (Grassi and Ferri 2014). The effects of cocoa on blood pressure may be more pronounced in younger subjects due to their high vascular reactivity to physiological stimuli (Arranz and others 2013).

The main mechanism that explains the antihypertensive effects of cocoa is the improvement in nitric oxide (NO) bioavailability, a molecule which plays a crucial role in the maintenance of vascular homeostasis. Using a model of rabbit aortic rings, Karim and others (2000) showed for the 1st time that cocoa procyanidins could stimulate endothelial-dependent vasodilation mediated through activation of NO synthase (NOS) and subsequent NO production. NO is an endogenous molecule that is synthesized by endothelial NOS (eNOS) from L-arginine, in the presence of tetrahydrobiopterin as cofactor. Once released from the endothelium, NO diffuses to vascular smooth muscle cells and leads to augmentation of intracellular cyclic guanosine monophosphate levels, and elicits relaxation of the vessel. It also prevents other processes involved in atherosclerosis, such as leukocyte–endothelial interactions, smooth muscle cell proliferation, and platelet adhesion and aggregation (Grassi and Ferri 2014). Moreover, cocoa intake causes a reduction of vascular arginase activity in human endothelial cells, increasing the local levels of L-arginine which is then transformed into NO by the eNOS pathway (Corti and others 2009). Cocoa polyphenols can also enhance NO production *via* insulin- and oxidant-mediated cell signaling mechanisms (Lippi and others 2009). The binding of insulin to its endothelial receptors activates PI3K/AKT pathway which in turn induces an increase in gene expression and activation of eNOS (Vicent and others 2003). The antioxidant properties of cocoa polyphenols and the improvement of cellular redox status can contribute to the increase of NO bioavailability (Lippi and others 2009). Another mechanism of the antihypertensive activity of cocoa polyphenols involves the renin-angiotensin-aldosterone system (Sudano and others 2012). *Ex vivo* studies have shown that 100 microM of dimer or hexamer procyanidins from cocoa as well as rich-procyanidin chocolate (634 microM catechin equivalents) inhibit angiotensin-converting enzyme that catalyzes the conversion of the decapeptide angiotensin I to the octapeptide angiotensin II, a potent vasopressor (Grassi and Ferri 2012).

Despite the fact that many scientific reports have demonstrated the efficacy of cocoa polyphenols in reducing blood pressure elucidating some of the mechanisms involved in this effect, their clinical applicability as antihypertensive agents is far from being clarified. The available data present some critical points: trials with a small number of subjects, the different quality of trials assessment, wide range of polyphenols and/or chocolate doses, and a significant statistical heterogeneity across studies (Grassi and Ferri 2014). More long-term, large, randomized, controlled, cross-over, multidose trials are needed in order to confirm the antihypertensive effects of cocoa and their clinical applicability (Lippi and others 2009; Arranz and others 2013). The flavanol content and composition of the cocoa products must be carefully specified as well as the concentrations of other components such as fats, sugars, and milk proteins (Lippi and others 2009).

Effects on the endothelial function

Endothelial dysfunction is directly involved in the early stages of atherosclerosis and in other cardiovascular diseases or diabetes (Allgrove and Davison 2014). It is characterized by the inability to regulate vascular tone and alterations in the endothelium-derived regulatory mediators (Keen and others 2005). Cocoa consumption improves the flow-mediated dilatation (FMD) of the brachial artery, a clinical marker of endothelial function (Crozier and Hurst 2014). The ingestion of cocoa flavanols leads to a dose-dependent

increase in FMD; a dose of 616 mg flavanols causes half-maximal FMD response (Allgrove and Davison 2014). The beneficial effects on the endothelial function are induced by the increase in NO generation which may mediate the dietary polyphenol-induced activation of the nuclear factor erythroid 2-related factor (Nrf2), a transcription factor, which in turn triggers the antioxidant response element-driven transcription of phase II detoxifying and antioxidant defense enzymes in vascular cells (Mann and others 2007). Moreover, flavanols ameliorate the endothelial dysfunction by modulating the endothelial cell eicosanoid system pathway, and lowering the oxidative damage (Keen and others 2005; Lippi and others 2009). They inhibit 5-lipoxygenase, decrease the level of the vasoconstrictor leukotrienes, and increase the level of vasodilating prostacyclins (Lippi and others 2009). Based on the results of many randomized double-blind placebo-controlled trials, European Food Safety Authority (EFSA 2012) concluded that cocoa polyphenols contribute to normal blood flow by maintaining endothelium-dependent vasodilation and, in order to obtain the claimed effects, 200 mg of cocoa polyphenols (provided by 2.5 g of rich-polyphenol cocoa powder or 10 g of rich-polyphenol dark chocolate) should be consumed daily by general population, in the context of a balanced diet.

Metabolic effects

The oxidation of low-density lipoproteins (LDL) is considered a key step in the development of atherosclerosis, because oxidized LDL become more susceptible to be uptaken by the arterial wall-resident macrophages. Animal studies showed that the administration of polyphenol-rich fractions derived from cocoa powder increases the resistance of LDL to oxidation and suppresses the formation of the atherosclerotic plaques in the hypercholesterolemic rabbits (Kurosawa and others 2005). Studies in healthy human subjects have also shown that daily consumption of cocoa powder ($\geq$13 g/d) reduces LDL-cholesterol levels and LDL's susceptibility to oxidation and increases HDL-cholesterol levels (Allgrove and Davison 2014; Grassi and Ferri 2014). Also, cocoa consumption ameliorates the lipid profile in hypercholesterolemic subjects, and dark chocolate intake reduces the serum LDL-cholesterol in hypertensive patients (Arranz and others 2013). Despite its high fat content, cocoa itself does not seem to have undesired effects on lipid metabolism, but the fact that many chocolate products contain milk or processed fats suggest that these additives could have unfavorable consequences on the lipid profile (Grassi and Ferri 2014). The effects of cocoa on the LDL oxidation have been attributed to the free radicals scavenging properties, metal ion chelating abilities, or changes in the LDL surface that promote a reduction in the LDL oxidative susceptibility (Allgrove and Davison 2014). HDL-cholesterol enhancing effects of the cocoa flavanols may be a consequence of the increase in the expression of several targets which are involved in the regulation of lipid metabolism (sterol regulatory element binding proteins), cholesterol homeostasis, HDL-metabolism (ATP binding cassette transporter A1-ABCA1), and exchange of cholesterol between cells and LDL (scavenger receptor B type I, SR-BI; Sarría and others 2015). There are also studies reporting a neutral effect of cocoa consumption on the serum total cholesterol and LDL-cholesterol in healthy subjects while others have concluded that the intake of dark chocolate or cocoa products for a period of 2 to 12 wk does not produce statistically significant effects on HDL and triglycerides. The differences in the pathophysiological state of study subjects, concentration of polyphenols, duration of the study, and formulation of cocoa products could explain these divergences. Further long-term studies are needed to assess the optimum amount of cocoa to improve the lipid profile (Arranz and others 2013).

Several studies have shown that augmented NO production following cocoa and chocolate consumption could reduce the insulin resistance (a well-known risk factor for cardiovascular disease), by increasing the muscle blood flow, uptake, and oxidation of glucose (Crozier and Hurst 2014; Grassi and Ferri 2014). The consumption of flavanol-rich cocoa or dark chocolate is associated with the decrease of insulin resistance in healthy and hypertensive subjects with or without impaired glucose tolerance. Also, the intake of flavanol-rich cocoa induces more pronounced effects in overweight and obese subjects in comparison to low-flavanol content cocoa (Arranz and others 2013).

Recently, cocoa polyphenols have been reported to exert beneficial effects in the obesity-mediated metabolic diseases (cardiovascular diseases, dyslipidemia, type 2 diabetes mellitus, and metabolic syndrome) *via* molecular mechanisms, which involve several targets, such as: peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), adiponectin gene, and uncoupling proteins. They may prevent visceral fat deposition, reduce insulin resistance, stimulate lipolysis by PPARs-regulated and insulin-dependent GLUT4 genes activation, and increase the expression of adiponectin. PPARs are ligand-activated transcription factors that are involved in the regulation of inflammation, lipid, and glucose metabolism. Adiponectin is an adipocyte protein hormone that exerts anti-atherogenic, anti-inflammatory, and insulin-enhancing effects. In addition, cocoa polyphenols may inhibit LXRs-regulated lipogenesis genes thereby decreasing fatty acids, triglyceride, and cholesterol synthesis. LXRs are transcriptional factors which play a pivotal role as regulators of lipid homeostasis in mammals (Ali and others 2014).

Anti-inflamatory effects

Chronic inflammation is strongly involved in atherogenesis. *In vitro* studies have shown that cocoa polyphenols may modulate cytokines involved in the inflammatory response. The moderate consumption of cocoa products is associated with an anti-inflammatory effect: thus, the subjects who consumed 20 g of dark chocolate every 3 d had lower serum C-reactive protein levels than the nonconsumers or high consumers (Allgrove and Davison 2014). Several mechanisms have been proposed for the anti-inflamatory effects of cocoa polyphenols: inhibition of mitogen-activation of T cells, and polyclonal activation of B cells, reduction of the secretion of the proinflammatory cytokines such as interleukin-1 (IL-1) and IL-2, increase of the production of the anti-inflammatory cytokine IL-4, beneficial modulation of tumor necrosis factor-α (TNF-α) and transforming growth factor-β, and inhibition of 15-lipoxygenase activity. Catechins can suppress the endothelial production of IL-8, a cytokine which plays an important role in the onset and development of atherosclerosis. Cocoa B-type procyanidins and catechins can inhibit the nuclear factor kappa-B (NF-kB)-driven gene transcription. Besides the regulation of the expression of the genes involved in inflammation, cell proliferation, and survival, NF-kB regulates gene transcription of cytokines implicated in the atherosclerosis progression (Keen and others 2005; Lippi and others 2009).

Antiplatelet activity

Platelet dysfunction plays an important role in the development of atherosclerotic diseases, such as acute myocardial infarct, stroke, and peripheral arterial occlusion. *In vitro* and *in vivo*

studies have demonstrated that cocoa polyphenols possess antithrombotic effects, decreasing platelet activation, aggregation, and adhesion. The consumption of flavanol-rich cocoa beverages is associated with a significant antiplatelet activity which can be achieved with doses of 600 and 900 mg of flavanols. The acute intake of procyanidin-rich chocolate (148 mg procyanidins) significantly lowers the levels of leukotrienes and increases the levels of prostacyclin as compared to the consumption of low content-procyanidin chocolate (3.3 mg; Schramm and others 2001). These effects could be explained by the modulation of eicosanoid metabolism within platelets, and changes in plasma leukotriene–prostacyclin ratio, membrane fluidity, membrane receptor function, and intracellular signaling. The ability of cocoa polyphenols to reduce the ADP-induced expression of the activated conformation of glycoprotein IIb/IIIa surface proteins might also be involved (Lippi and others 2009; Andújar and others 2012; Grassi and Ferri 2014). The latter are platelet receptors that bind adhesive proteins and promote aggregation (Scarborough and others 1999). Besides, the antiplatelet effects may be mediated through antioxidant activity and NO-related pathways (Keen and others 2005).

Chemoprevention

The chemopreventive potential of cocoa, cocoa-derived products, and cocoa polyphenols has been investigated in many *in vitro* and *in vivo* studies. Cell culture studies focused on the evaluation of the anticarcinogenic properties and elucidation of the molecular mechanisms, whereas the *in vivo* cancer preventive effects were demonstrated in various animal models of carcinogenesis or human interventional or epidemiological trials (Martin and others 2013). Cell cultures studies were conducted on different cancer cell lines, showing that cocoa extracts and cocoa polyphenols possess antioxidant, anti-inflammatory, pro-apoptotic, antiproliferative, anti-angiogenetic, and antimetastatic effects.

DNA lesions produced by free radicals, such as reactive oxygen species and reactive nitrogen species can be prevented by cocoa polyphenols through: direct radical-scavenging effects, chelation of pro-oxidant divalent cations, modulation of the enzymes related to oxidative stress (glutathione peroxidase, glutathione-S-transferase, glutathione reductase, catalase, superoxide dismutase, eNOS, lipoxygenase, and xanthine oxidase), or nonenzymatic defensive systems (glutathione and ascorbic acid), alteration of the procarcinogenic metabolism by inhibiting the phase I drug-metabolizing enzymes (cytochrome P450) or stimulating the phase II conjugating processes (glucuronidation, sulfation, acetylation, and methylation; Martin and others 2013).

Cocoa phenolic extracts reduce the activity of many inflammatory mediators associated with cell proliferation, angiogenesis, and metastasis, such as: cyclooxygenase 2, inducible NOS, prostaglandin E2, IL1, IL6, IL8, and TNF-α. TNF-α-induced activation of protein kinase B (AKT), mitogen-activated protein kinase 1 (MEK1), phosphatidylinositol-3-kinase (PI3K), and TNF-α-induced translation of NF-κB were inhibited in cell culture assays (Lee and others 2006; Kim and others 2010; Rodríguez-Ramiro and others 2013). Cocoa polyphenols were also found to be involved in the suppression of cell proliferation, by blocking the cell cycle at G1/S or G2/M phases, modulating the activities of some cell-cycle regulatory proteins, and inhibiting type II topoisomerases (Carnésecchi and others 2002; Kozikowski and others 2003; Lanoue and others 2009).

Some studies have found that cocoa procyanidin B2 and epicatechin exert pro-apoptotic properties, by decreasing the expression of the anti-apoptotic proteins BCL-X_L and BCL-2 and inhibiting peroxide-induced caspase-3 activation (Spencer and others 2001; Mackenzie and others 2008). The anti-angiogenic effects of cocoa polyphenols were found to be owed to the suppression of TNF-α-induced upregulation of vascular endothelial growth factor, whereas the antimetastatic activity was achieved through the inhibition of H_2O_2-induced phosphorylation and internalization of connexin 3, stimulation of extracellular-activated kinase (ERK), and downregulation of metalloprotease 9 (Günther and others 2007; Kim and others 2010; Lee and others 2010).

Cancer prevention by cocoa polyphenols has been investigated in different animal models of carcinogenesis, including: mammary, pancreatic (Yamagashi and others 2002), lung, thyroid (Yamagashi and others 2003), prostate (Bisson and others 2008), leukemia (Papiez and others 2011), hepatic (Granado-Serrano and others 2009), and colorectal cancers (Weyant and others 2001). However, the extrapolation of the results obtained in animal studies to humans is extremely difficult, so many human interventional and epidemiological trials were designed. Overall, some of these studies have found an inverse correlation between cocoa polyphenol intake and some forms of cancer, whereas others have failed in showing a beneficial effect of cocoa consumption in the occurrence of cancer (Arts and others 2002; Rouillier and others 2005).

Neuroprotection

Experimental data suggest that cocoa polyphenols have neuroprotective, neuromodulatory, and neurorescue activities that are beneficial against aging and neurodegenerative diseases. They cross the blood-brain barrier, increase the cerebral blood flow and stimulate the brain perfusion, reduce the cerebral edema, promote the neuronal survival and synaptic plasticity, and improve the neuronal function and different kinds of visual and cognitive tasks (McShea and others 2008; Nehling 2013).

Cocoa polyphenols are believed to directly interact with cellular and molecular signaling cascades, primarily with MEK, ERK, and PI3K pathways, especially in the brain regions associated with learning and memory. Moreover, as a result of increased NO production with vasodilating and anti-inflammatory effects, they stimulate angiogenesis and central and peripheral blood flow (Sokolov and others 2013). It seems that NO is also responsible for α-secretase upregulation and β-secretase suppression, leading to a decreased formation of β-amyloid peptide (Aβ) in culture human neuroblastoma cells (McCarthy 2006).

Several human studies have shown that dietary flavanol-rich cocoa consumption was correlated with an increase in the cerebral blood flow velocity (Sorond and others 2008), improvement of the cognition, learning, memory, mood (Letenneur and others 2007), and vision (Huber and others 2006), amelioration of the cognitive decline due to aging or Alzheimer's disease (Letenneur and others 2007; Desideri and others 2012), and reduction in the incidence of stroke (Buijsse and others 2010).

Single acute appropriate dose or subchronic administration of cocoa flavanols determines immediate effects on behavior and cognition, while chronic administration causes long-lasting effects. Although there is a relatively large body of data that supports the long-lasting neuroprotective effects of cocoa, the immediate effects on the cognitive and affective functions, executive control, and behavior are rather unknown. Also, it is unclear the manner in which flavanol-rich cocoa influences brain networks involved in neurocognitive processes. Future studies should clarify several issues related to the onset and duration of consumption, appropriate

dose of flavanols, sex differences in both long-term and immediate effects, mechanisms of immediate neurocognitive and behavioral effects of cocoa, influence of food matrix, and interactions of polyphenols with other constituents of cocoa that contribute to the overall neurobiological activity. Clinical trials should include a larger number of subjects, and the design of study should take into consideration the reduction of the reward outcome commonly associated with the chocolate intake (Sokolov 2013).

Other possible beneficial effects of flavanol-rich cocoa include immune function modulation, skin health/photoprotective properties (Bernaert and others 2012; Visioli and others 2012; Jahurul and others 2013; Kothe and others 2013), and anticariogenic activity (Ferrazzano and others 2009).

As already shown, the polyphenols are the main contributors to the health benefits of cocoa/dark chocolate. The processing of raw cocoa beans and the chocolate manufacture influence the content and the polyphenol profile. About 70% to 90% of the phenolic compounds may be lost during these processes (Zzaman and others 2014; Bellesia and Tagliazucchi 2014). Heat-based steps, mainly roasting, and alkalization produce the most pronounced effects, affecting the content, composition, and bioavailability of polyphenols. Higher temperatures and/or longer processing times, and an alkaline pH decrease the content of polyphenols, mainly flavanol monomers and small procyanidin oligomers, which are the most readily absorbed and bioavailable flavanols (Stahl and others 2009; Zzaman and others 2014). Also, the roasting treatment results in about 50% loss of clovamide, a caffeoylated aminoacid from cocoa beans, with significant antioxidant activity (Arlorio and others 2008). The loss and the structural changes of the polyphenolic compounds are generally correlated with a reduction in free-scavenging activity and antioxidant properties of cocoa-processed products as many authors have already reported (Suma and others 2006; Arlorio and others 2008; Jolić and others 2011). However, Bordiga and others (2015) have noticed that the roasting process did not influence significantly antioxidant activity of cocoa, although the content of polyphenols decreased. The authors suggested that the formation of melanoidins during roasting may be responsible for this effect. Melanoidins are high molecular weight compounds that have been recently considered as biologically active molecules with antioxidant, anticancer, antihypertensive, and prebiotic properties (Bellesia and Tagliazucchi 2014). The addition of some ingredients (sucrose and lecithin) and conching step during chocolate manufacture cause a dilution effect of the cocoa polyphenols and their antioxidant properties (Bordiga and others 2015).

The achievement of a balance between flavor and health effects remains a challenge due to the huge impact of processing on the cocoa bioactive components and flavor acceptability of the consumers. It has been suggested that the "Omics" technologies (transcriptomics, proteomics, metabolomics, and glycomics) could be used in monitoring cocoa processing in order to obtain products with a high bioactive content and appropriate flavor (Giacometti and others 2015).

Flavor of Chocolate

Chocolate is considered a luxury food and one of the most valued flavors worldwide (Liu and others 2015). It is consumed most frequently for its pleasant, stimulant, and euphorizing effects (Torres-Moreno and others 2012). The essential ingredients of chocolate are alkalized cocoa mass, cocoa powder or cocoa butter, sugars, lecithin, and, in the case of milk chocolate, milk powder or crumbs **(**Merkus 2014). All these materials are mixed and refined, and the obtained powdered chocolate mass is then conched. Chocolate contains not less than 350 g/kg of dry cocoa solids and not less than 140 g/kg of dry nonfat cocoa solids. Chocolates with a low cocoa content cause a melting and creamy mouthfeel, while those with a higher cocoa content produce dry, mealy, and sticky sensations (Saltini and others 2013). Dark chocolate may contain 600 g/kg cocoa liquor (Torres-Moreno and others 2012), and products with a very high content of cocoa mass (more than 35%) are considered the richest in polyphenols (Rusconi and Conti 2010). The popularity of chocolate is mainly related to its sensory properties. These are greatly influenced by cocoa solids aroma and by the manufacturing process. Dark chocolate is characterized by a praline, chocolate flavor with malty, nutty, and caramel notes, while the typical flavor of milk chocolate is sweet, milky, and honey-like with coconut notes (Liu and others 2015). Many studies have investigated the flavor composition of cocoa and chocolate products. The key odor-active compounds are primarily pyrazines, aldehydes, esters, alcohols, acids, and hydrocarbons (Afoakwa and others 2008). 2-Methylpropanal (malty, dark chocolate), 3-methylbutanal (malty, dark chocolate), phenylacetaldehyde (rosy), 2-ethyl-3,5-dimethylpyrazine (popcorn-like, potato-like), tetramethylpyrazine (milk coffee-mocha roasted, nutty), trimethylpyrazine (earthy, nutty), 2-acetyl-1-pyrroline (popcorn-like), 3-methylbutanoic acid (cheesy, sweaty), 3,5 (or 2)-diethyl-2 (or 5)-methylpyrazine (cocoa, chocolate, rum, roasted), 2-methylbutanal (chocolate), and furaneol (caramel-like) are considered the main contributors to the overall flavor profile of cocoa products, and the first 8 compounds listed have the greatest impact in chocolate aroma (Afoakwa and others 2008; Liu and others 2015). In a recent study, Liu and others (2015) have identified 32 major odor-active compounds in dark and milk chocolate. Dark chocolate contains higher levels of pyrazines (4254 mg/g; tetramethylpyrazine), Strecker aldehydes (10036 mg/g; 3-methylbutanal, 2-methylpropanal), pyrroles, alcohols (phenylethanol), and carboxylic acids (3-methylbutanoic acid). Lactones (decalactone), esters (phenylethylacetate), long-chain aldehydes (heptanal, octanal), ketones (2-nonanone), and sulfur compounds (dimethyl disulfide, trimethyl trisulfide) predominate in milk chocolate. Also, Counet and others (2002) have reported as major and specific nitrogen heterocyclies of dark chocolate: 2,3-dimethylpyrazine, trimethylpyrazine, teramethylpyrazine, 3 (or 2), 5-dimethyl-2 (or 3)-ethylpyrazine), 3,5 (or 6)-diethyl-2-methylpyrazine), furfurylpyrrole, and acetylpyrrole, all with praline, nutty, and coffee notes. Tetramethylpyrazine was also found to be the most abundant pyrazine (>6 ppm) in dark chocolate (Counet 2002; Afoakwa and others 2008). In another study, Schnermann and Schieberle (1997) have indicated the key aroma components in milk chocolate as follows: 3-methylbutanal, 2-ethyl-3,5-dimethylpyrazine (potato chip-like), 1-octen-3-one (mushroom-like), 2-ethyl-3,6-dimethylpyrazine (nutty, earthy), 2,3-diethyl-5-methylpyrazine (potato chip-like), 2-nonenal (green, tallowy), (Z)-2-methyl-3-(methyldithio)furan (cooked meat-like), (E,E)-2-nonadienal (fatty), (E,E)-2,4-decadienal (fatty, waxy), and R-δ-decalactone (peach-like, milky). Most of them originate in roasted cocoa beans. It appears that δ-decalactone is derived from the milk powder used in the manufacturing process (Liu and others 2015).

Conclusions

Cocoa flavor is unique, complex, and fascinating. There is no single key component that determines the final flavor character. Both nonvolatile and volatile chemical components contribute to the cocoa aroma. Many chemical, biological, and physical factors

influence the formation and the development of flavor. The formation of flavor precursors during fermentation is critical for the development of the final flavor. As compared to other processing stages, the fermentation is performed at the originating farms that provide a very heterogeneous material, and flavor potential can be weakened there. The introduction of starter cultures for the fermentation, as has been suggested, could optimize and enhance the flavor potential. The improvement of flavor requires the control of cocoa bean flavor potential and an optimized sensory strategy. Without good-quality sensory information, knowledge on the chemical composition of cocoa flavor alone could not explain the contribution of individual components to overall flavor character. A comprehensive link between the components of cocoa flavor, sensory properties, and human acceptability, and also the processes involved in flavor generation, will aid the implementation of a traceability system, a challenging issue for quality assurance teams.

Author Contributions

A.C. Aprotosoaie designated the format, searched and evaluated sources, and drafted the manuscript. S.V. Luca searched the literature and drafted the manuscript. A. Miron evaluated the literature and drafted the manuscript.

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