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Review

Key Aromatic Volatile Compounds from Roasted Cocoa Beans, Cocoa Liquor, and Chocolate

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Abstract: The characteristic aromas at each stage of chocolate processing change in quantity and quality depending on the cocoa variety, the chemical composition of the beans, the specific protein storage content, and the polysaccharides and polyphenols determining the type and quantity of the precursors formed during the fermentation and drying process, leading to the formation of specific chocolate aromas in the subsequent roasting and conching processes. Bean aroma is frequently profiled, identified, and semiquantified by headspace solid-phase microextraction combined with gas chromatography-mass spectrometry (HS-SPMEGC-MS) and by gas chromatography olfactometry (GC-O). In general, the flavors generated in chocolate processing include fruity, floral, chocolate, woody, caramel, earthy, and undesirable notes. Each processing stage contributes to or depletes the aroma compounds that may be desirable or undesirable, as discussed in this report.

Keywords: aroma profile; odor threshold; odor activity value; fermentation; cocoa liquor; chocolate

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1. Introduction

The volatile aromatic components of cocoa beans vary depending on whether the beans are dry, roasted, or processed in cocoa liquor or chocolate. Different concentrations of the most relevant volatile components in a particular variety (criollo, forastero, and trinitario) rely on geographical origin, the chemical composition of the beans, the postharvest techniques used, such as fermentation and drying, and industrial processes like roasting and conching [1]. Regarding the varieties and genotypes, criollo, trinitario, and national cocoa have higher fine aroma concentrations as fruit (fresh and ripe), floral, herbaceous, wood, nuts, and caramel notes [2–5]; forastero cocoa on the other hand (considered bulk grade), possess higher concentrations of the predominant aromas of malt, honey, roasted, caramel, cocoa, and chocolate [6], as well as low acidic and alcoholic notes [7]. Finally, the high yielding CCN51 cocoa genotype (Colección Castro Naranjal) grown in Ecuador (classified as bulk grade), has volatiles that exude a sweet and fruity or fresh character to the beans [5].

The unique aromatic profiles and diversity of aromas are linked to the number of components exhibited in the beans; the greater the number of components, the more complex the overall aroma of a specific cocoa sample. For instance, the aromatic profiles of criollo and trinitario cocoa from Mexico comprise 46 and 47 of the most relevant volatile components, respectively[8]. Likewise, 69 of the most important volatile components represent Ecuador's national cocoa [5], with 67 to 89% being aromatically desirable components, and the remaining fraction (11 to 33%) exudes unpleasant aromas that may originate during fermentation and drying [9–12]. The main cause of unpleasant aromas comes from excessive fermentation that originates compounds that impart smoky and ham aromas, which have significantly high concentrations that affect the final quality of the beans. The suggested limits for some unpalatable volatile components in fermented beans are 2 μg/kg for 3-ethylphenol (smoked) and 3-propylphenol (smoked, phenolic), 12.20 μg/kg for 4-methylphenol (fecal), 3-methylphenol (smoked) and 4-ethylphenol (smoked), and 70 μg/kg for 2-methoxyphenol [13]. If the drying process is performed slowly, there is a loss of volatile acids and water [14]. This is because the porosity of the husk enclosing the cotyledons increases [15], facilitating volatilization and implying that the dried beans are less acidic and have a higher pH, providing their aromatic potential [14]. As the moisture level decreases, the level of cocoa butter accumulates. Cocoa butter is the most abundant component in dried cocoa beans [16], accounting for half of the components. This butter fraction may include fatty acids (myristic, palmitic, palmitoleic, stearic, oleic, linoleic, and arachidic) and triacylglycerols (composed of about 42% of 1-palmitate-2-oleate-3-stearate triacylglycerol, 24% of 1,3-diestearate-2-oleate triacylglycerol, and 22% of 1,3-dipalmitate-2-oleate triacylglycerol), as the major components [17,18] have a serious impact on the perception of the chocolate flavor and aroma. The release of chocolate aromas during tasting is linked to lipophilic volatile compounds, mainly alkylpyrazines, which are considered to be more complex and more substituted with the alkyl group, therefore having higher lipophilicity [19]. The higher degree of saturation and hydrophobicity of cocoa butter may be associated with decreased pyrazine release [17].

Most aromatic volatile compounds are released during the roasting process. The number of volatile compounds varies with roasting intensity; therefore, a greater amount is formed when roasting at higher temperatures [20]. Several studies detail the diversity of these compounds within the temperature ranges of 95 to 160 °C [6,9,21,22]. The most volatile compounds found in roasted beans correspond to esters, followed by acids, alcohols, aldehydes, and ketones, which are characteristic of a given cocoa variety, and depending on the roasting time and temperature, other compounds, such as pyrroles, pyrazines, and Strecker aldehydes, are reduced or generated [6,9,21,22]. Higher roasting temperatures may generate unpleasant compounds with burnt or smoky notes and a dark brown color in processed chocolate. In the next process in which the roasted beans are ground, the cocoa liquor has particles measuring between 22–26.5 μm [9,23], driving the extra release of aroma compounds. Similarly, during conching, the removal of the remaining moisture and volatile unpleasant acetic acid compounds occurs, acquiring acceptable levels [24]. Prolonged conching (6–10 h at 80 °C) results in the loss of both undesirable volatile flavorings and other flavorings that provide desirable characteristic aromas like sweet, fruity, floral, and chocolate [25]. It has also been reported that the aromatic volatile compound profiles of dark chocolates are strongly affected by the brand-related formulation and processing conditions. In some cases, differences within the same brand have even been shown [26].

Chocolate production generally involves a combination of fermentation, drying, roasting, and conching processes [25]; thus, it is crucial to investigate how these processes impact the levels and types of compounds that determine the chocolate aroma and its origin. Therefore, this review provides information on more relevant volatile organic compounds arising from postharvest and bean manufacturing methods to their implication in the final quality of the chocolate. In addition, the active odor components in dried beans, roasted beans, liquor, and chocolate are identified and traced with emphasis on the most desirable compounds.

2. Extraction and Quantification of Volatile Cocoa Compounds

Several studies have been published on the aromatic components contained in cocoa beans, especially the forastero, national, criollo, and trinitario varieties. Forastero cocoa represents about 85% of world production [27]. It is characterized by producing seeds that, after being fermented, generate volatile floral and sweet aromas [28,29]. The national cocoa grown in Ecuador classified as "fine and aroma grade", produces seeds with fruity, green, and woody volatile aromas. This variety represents 63% of world production [28, 30]. On the other hand, Criollo cocoa represents only 5% [27] and its fermented beans are associated with floral, fruity, and woody, volatile aromas [28]. Finally, trinitario cocoa is a criollo hybrid variety and has strong basic chocolate characteristics together with fruity and floral aromas [28]. The characterization and comparison of the seeds in the different varieties are complex since the list of representative genotypes in all cocoa-producing countries is extensive [29,31–33], making the standardization of the preharvest, postharvest, and cocoa manufacturing processes derivatives since each variety has a unique flavor and aroma potential [34].

Cocoa aromas and flavors are detailed through several volatile aroma profiles and samples, such as fermented and roasted dry beans, cocoa liquor, and chocolate. Although there are several methods of characterization, the two most common are detailed. On the one hand, the analysis of volatile compounds is carried out by headspace solid-phase microextraction combined with gas chromatography-mass spectrometry (HS-SPMEGC-MS) [4,11,35,36]. The use of divinylbenzene carboxene polydimethylsiloxane (DVB/CAR/PDMS) fiber has been reported to define the organic volatiles in cocoa [8,12, 21,22,28,37–39]. Optimal fiber coating is related to the number of peaks in the chromatogram and generated intensity. When using CAR-PDMS, up to 100 peaks have been detected more intensely, while in PDMS-DVB, the number of peaks is less than 75 [40], implying that a higher number of volatiles are extracted from a specific cocoa matrix.

To cite an example, [28] identified a total of 121 volatile compounds in criollo, national and forastero cocoa, and 62 were positively identified, including nine organic acids, 12 alcohols, 14 aldehydes and ketones, five esters, 12 hydrocarbons, two amines, two furans, one sulfur, and five unspecified compounds. Likewise, [12] reported a total of 67 volatile components, including acids, alcohols, aldehydes, esters, ketones, pyrazines, furans, furanones, lactones, pyrans, pyrroles, and terpenes. The total volatiles concentration was higher in cocoa liquors than in chocolates.

In contrast, olfactometry, which collects data that are obtained from gas chromatography olfactometry (GC-O), helps to calculate to what extent a certain compound influences the overall aroma depending on its dilution factor (DF), [6,10,41–43]; it detects odors and describes them by means of an "aromagram"–a representation of the dilution factor logarithm versus retention time [10,42,43]. In order to illustrate this, [6] reported the dilution factors of roasted and unroasted forastero cocoa beans. In Table 1, 2- and 3-methylbutanoic acid (FD 8192; sweaty), acetic acid (FD 2048; acidic), and 3-hydroxy-4,5-dimethyl-2 (5H)-furanone (FD 1024; spiced) were detected at the highest dilutions, suggesting that these compounds are among those contributing to the aroma of unroasted cocoa beans.

Table 1. Dilution factors of active odor compounds from roasted and unroasted forastero cocoa beans.

No	Volatile	Odor	Dilution Factors (FD)	
			Unroasted	Roasted
1	4-hydroxy-2,5-dimethyl-3 (2H)-furanone	Like caramel	128	8192
1	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	Caramel-like	128	8192
2	2- and 3-Methylbutanale	Malty	64	4096
3	Phenylacetaldehyde	Honey-like	64	4096
4	2–and 3-Methylbutanoic acid	Rancid	8192	4096
5	2-Acetyl-1-pyrroline	Popcorn-like	32	1024
6	Acetic acid	Acetic	2048	1024
7	2-Methoxyphenol	Smoky	256	512
8	2-Phenylethanol	Flowery	256	512
9	2-Ethyl-3,5-dimethylpyrazine	Earthy	64	256
10	Linalool	Flowery	64	256
11	Methyl propanoic acid	Rancid	256	256

12	2-Methyl-3-(methyldithio)furan	Cooked meat-like	128	256
13	Ethyl methyl propanoate	Fruity	64	128
14	Ethyl 2-methylbutanoate	Fruity	256	128
15	Dimethyl trisulfide	Sulfur-like	64	128
16	2,3,5-Trimethylpyrazine	Earthy	32	128
17	Phenylethyl acetate	Flowery	64	128
18	δ-Decenolactone	Coconut-like	256	128
19	Phenylethyl acetic acid	Sweet	256	128
20	Ethyl 3-methylbutanoate	Fruity	32	64
21	2,3-Diethyl-5-methylpyrazine	Earthy	2	64
22	Butanoic acid	Rancid	128	64
23	δ-Octenolactone	Coconut-like	32	64
24	cis-Isoeugenol	Smoky	64	64
25	3-Hydroxy-4,5-dimethyl-2(5H)-furanone	Spiced	1024	-

Source: Key aroma compounds in fermented Forastero cocoa beans and changes induced by roasting [6].

Both techniques focus on cocoa matrix aroma profile characterization and the evaluation of the most relevant compounds. Volatile profiles can be estimated by the odor activity value (OAV) of a compound present in a specific cocoa matrix. The OAV is defined as the ratio between the compound concentration and the odor threshold value (OTV), for which the contribution to the overall aroma of the cocoa matrix is to be evaluated. The OTV found in the literature determined in a fatty medium (sunflower oil) is a reference for estimating the OTV in cocoa beans, for which the fat content is between 53.3 to 55 % in dry fermented beans. If the concentration of a volatile compound is higher than its respective odor threshold and its OAV > 1, this compound contributes to the overall aroma and flavor of the cocoa matrix. [9,12,44–46][9], determined that the concentration of 3-methylbutanal (provides malty, cocoa and chocolate notes) in cocoa liquor was 721.44 ng/g, much higher than its OUV: 5.4–80 ng/g. This compound clearly contributes to the overall aroma of cocoa liquor, with an OAV from 9.02 to 133.60. On the other hand, [12] reported OAV = 0.55 for linalool (floral notes) in the chocolate processed from cocoa liquor of the national variety; its concentration was 20.2 ng/g and its OTV = 37 ng/g, meaning this compound does not contribute to the final chocolate aroma. The volatile profiles of other matrixes studied are detailed in Tables 2–6.

3. Flavor Precursors in Cocoa Beans

Aromas and flavors are generated during fermentation. This is a postharvest process carried out by various micro-organisms (yeasts, lactic acid bacteria, and acetic acid bacteria) generating flavor and aroma precursors, like the reducing sugars (glucose and fructose) formed by the action of cotyledon invertase and free amino acids release by carboxypeptidase (optimum pH 5.6) and aspartic protease (optimum pH 3.5), for which, later, during the drying and roasting of the beans, is combined in Maillard reactions to produce a high potential of aromas of different chemical classes [5,47–52]. According to the cut test method, the cotyledons of well-fermented and dry beans from a set of 100 have at least 60% brown beans and a smaller percentage of slaty and defective beans [49,50]. For example, [21] reported that the percentage of forastero brown beans ranged from 75.33 to 84%. Further, the fermentation index (FI) estimates the fermentation quality and provides a fermented bean final state analytical evaluation. According to [53], well-fermented beans have a (FI) ≥ 1. [21] reported a FI from 1.04 to 1.22 in preconditioned and fermented beans. Likewise, [54] reported values from 0.37 to 1.05 in beans fermented from 0 to 6 days, respectively. The FI correlates with the amount of reducing sugars, free amino acids, pH, and cocoa bean cotyledon color [55]. Therefore, the evaluation of precursors formed during fermentation is crucial to determine the quality of cocoa since well-fermented beans have a higher amount of precursors [56].

Regarding sugar reduction, a 78% and 93.5% increase at the end of fermentation was reported for trinitario and forastero cocoa beans, respectively [5,21]. Similarly, [16] reported a 60% to 70% increase for a forastero cocoa hybrid in Ghana. The difference is due to the postharvest process in which the cob is stored in order to reduce the moisture and the amount of pulp adhering to the beans; this method reduces the concentration of fermentable sugars and, therefore, the acidity at the end of fermentation. Additionally, the increment in reducing sugars is related to the method and fermentation duration, where the greatest amount of fructose is produced with respect to glucose [5,21,47]. A fructose-to-glucose ratio of 2:1 is expected when using the heap and tray fermentation method, 4:1 when the pods have been preconditioned and fermented in heap [5,57], and 4:1 when the pods have been preconditioned and fermented in heap [21]. This ratio gives an indication of fermentation quality in terms of the reducing sugars in cocoa varieties [58].

Likewise, the proteolysis of cocoa globulin (similar to vicilin) (storage protein of 566 amino acids) leads to the breakdown and abundance of free amino acids from 300 to 800 di- and tripeptide units, highlighting acidic, hydrophobic, basic, etc. [59,60]. [57] reported that hydrophobic amino acids comprised 68–73% of the total amount of amino acids, with leucine, phenylalanine, and alanine being the most abundant in dried forastero cocoa beans. Similarly, [56] indicated that leucine, phenylalanine, valine, alanine, and isoleucine were the predominant hydrophobic amino acids in dried criollo cocoa beans, comprising 56 to 70% of the total amino acids, while [61] quantified 50% of leucine and phenylalanine in fermented and dried forastero cocoa beans. When roasting the beans, this hydrophobic amino acid fraction partially descends through Maillard reactions, increasing the pyrazine levels [52]. In beans, these hydrophobic amino acid fractions partially decrease via Maillard reactions, increasing the pyrazine levels [62]. Therefore, the level of pyrazines can be used to evaluate the efficiency of roasting during this stage because of its pronounced influence on the cocoa's final aroma[56].

The abundance of peptides depends on geographical origin. [63], and [64], reported that high levels of oligopeptides and amino acids are found in cocoa bean samples from Central America, Caribbean Islands, Mexico, Santo Domingo, and Peru, together with samples from Papua New Guinea and the criollo variety from Mexico; in contrast, the samples from Flores, Sulawesi, Malaysia, and Ivory Coast showed low peptide concentrations and free amino acids. The difference in peptide concentration in relation to the origin of the cocoa is due to the nitrogen fertilization of the plants and mainly the pH found in the soil [65]. Furthermore, the season in which the pods are harvested particularly affects the pH and nitrogen concentration of the beans. [66] reported that the pods harvested in summer had beans with a lower pH than those harvested in winter; likewise, the beans harvested in the dry season had a higher concentration of nitrogen than the ripe beans during the rainy season.

3.1. Volatile Compounds Generated from Precursors

Table 2 indicates the number of compounds in the different chemical groups and cocoa matrixes. It is noted that the availability of the compounds increases as the beans are turned into chocolate. Overall, acids, alcohol, aldehydes, aldehydes, ketones, and esters are present throughout the processing chain. The compounds found represent sour, vinegar, rancid, sweaty, fruity, floral, soapy, creamy, green leafy, herbal, and spicy notes. Obviously, unfermented beans do not have pyrazines, furans, furanones, pyrans, pyrones, or pyrroles as they are more common in well-fermented roasted beans, except for the lower concentrations of pyrazines that are found in dry beans, a metabolic product of *B. subtilis* or *Bacillus megaterium*, which are present at the end of cocoa fermentation [5,38,67]. These compounds have cocoa, chocolate, walnut, popcorn, coconut, and candies notes, although they also have fruity notes like furans (furaneol) [45].

Dried beans have organic acids that volatilize during roasting [39]. It has been reported that 2- and 3-methylbutanoic acid and acetic acid have been found to be most relevant in dried forastero cocoa beans. During roasting, the acid level decreases significantly depending on the roasting temperature. Thus, at 135 °C and 160 °C, a significant increase in acids is observed, while at lower temperatures, the increase is negligible. The 2- and 3-methylbutanoic acids persist at the end of roasting at a temperature of 95 °C and their concentration reaches 17300 ng/g ([6]. This concentration exceeds the odor threshold of 203 and 11 ng/g, respectively, indicating that these acids greatly affect the final chocolate aroma. Likewise, [44] reported that only acetic acid and isovaleric acid are of an active odor in bitter chocolate samples, especially from beans roasted at 100 °C to 140 °C and in concentration rises from 171.26 to 381.4 ng/g. Figure 1 illustrates the compound percentage generated from foreign cocoa beans from Africa, America, and Southeast Asia when roasted at 140 °C for 30 min. There is a rise in aldehydes, phenols, pyrazines, furans, pyrans, and pyrroles at the end of roasting. A reduction in undesirable compounds, like acids and alcohols, is clearly evident. However, not all acids change at the end of the process.

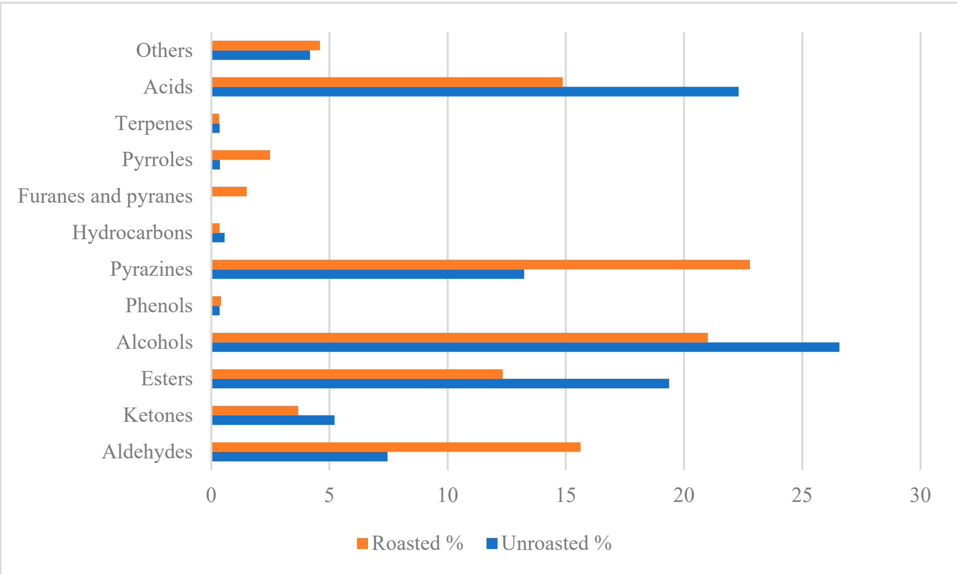


Figure 1. Relative percentage of volatile compounds before and after roasting. Source: taken and adapted from [22].

Cocoa liquor is the product obtained from fermented, dried, roasted, and ground cocoa beans. The concentration of total aromatic volatiles in cocoa liquor is higher than in chocolates. [12]reported that cocoa liquor had four to seven times more volatiles than the processed national and forastero variety chocolates than cocoa liquor, respectively. These included acids, alcohols, aldehydes, esters, ketones, pyrazines, lactones, and terpenes. Similarly, [9] highlighted acids, alcohols, esters, terpenes and terpenoids, aldehydes, ketones, pyrazines, furans, furanones, pyrans, pyrones, and pyrroles in the cocoa liquor of the forastero variety. Conversely, in the criollo cocoa variety, [68] reported that the main components were alcohol, esters, aldehydes, ketones, hydrocarbons, nitrogen and oxygen heterocycles, nitriles, and sulfides. Many of the compounds are similar in each variety, and what differentiates them is the contribution of each of them to the overall aroma of a particular variety. Hence the need to know the aromatic profiles of the varieties.

Finally, processed chocolates have a wide variety of compounds. Table 2 shows that 85 compounds present in chocolate are subdivided into several chemical groups, including acids, alcohols, aldehydes, ketones, esters, and pyrazines as the major compounds. Pyrazines (16 compounds) are most abundant in chocolates and impart their malty, roasted cocoa, nutty, almond, and hazelnut notes. In contrast, Table 2 also indicates the presence of ethers (carbitol, 2-(2-butoxyethoxy) ethanol furfural), which are only present in unroasted trinitario cocoa beans and do not contribute to the processed chocolate [7].

Table 2. Number of common volatile compounds in the cocoa bean processing chain.

* Variety	** Matrix	Volatile Compounds													Total Volatiles	Reference
		Acids	Alcohols	Aldehydes	Ketones	Esters	Ethers	Pyrazines	Lactones	Furans	Furanones	Pyrans, Pyrodons and Pyrroles	Terpenes and terpenoids	Other		
CR	CL	2	4	4	2	7		7					6	2	34	[2]
TR	DB	3	10	5	6	7		4	1				5		41	[4]
NC, CCN51	RB	4	10	7	10	11		9		4	4	3	6	2	70	[5]
FR	DB	6	3	4	1	3		5	2	1	2		1	3	31	[6]
CR, FR, TR	UDB	4	12	4	4	7	2		2	5			9	3	52	[7]
CR	DB	6	7	10	5	11		5		1			4	1	50	[8]
	CH	6	1	9	7	11		8	1	1	2		1	2	49	[10]
	CH	4	4	7	6	3		6		2		1	2		35	[11]
NC, FR	CL	3	11	8	7	9		12	1	1	2	4	8	1	67	[12]
FR	DB	10	9	3	5	11		1							39	[14]
	RB			9	8			10	1	3	2	1		5	39	[21]
FR	CL	3	7	7	10	8		15		4	3	10	6	7	80	[23]
CR, FR, NC	DB	9	12	14		5				2			12	6	60	[28]
	CH	11	14	13	11	8		16	2	5			3	2	85	[37]
FR	DB	11	12	3	5	20		4						3	58	[39]
FR	CH	4	4	4	3	3		5		1	1	1	6	1	33	[44]
FR	CL	2	9	7	5	9		9		2			6	3	52	[57]

* Cocoa varieties: CR: Criollo, FR: Forastero, TR: Trinitario, NC: Nacional, CCN51: Colección Castro Naranjal. ** Cocoa matrix, UDB: unfermented dried beans, DB: dried beans, RB: roasted beans, CL: cocoa liqueur, CH: dark chocolate.

3.2. Specific Volatile Compounds

3.2.1. Fermented and Dried Beans

Table 3 indicates the contribution of the individual compounds to the overall aroma of the beans; their OAV > 1. 20 and 36% out of the compounds impart undesirable aromas. Acetic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, and 2-methylpropanoic acid were common in both studies. They differ by approximately 55 times for acetic acid and 48 times for 3-methylbutanoic acid due to the fermentation method used (wood box vs. pile fermentation), days fermented (5 d vs. 3 d with prefermentation), and the origin of the beans (Costa Rica vs. Ecuador). These compounds are present in the over-fermented beans from leucine metabolism; such acids are undesirable in any cocoa matrix, imparting vinegar, pungent, sweaty, and rancid flavors [5,42]; it was reported in separate and complementary studies that box fermentation offers less aeration compared to the heap method. It was expected that the acetic acid concentration in the heap method would be higher, as aeration drives the emergence of acetic acid bacteria; however, the box method offers a higher concentration. The prefermentation of the beans helped to reduce their moisture and sugar content, thus decreasing the acid content at the end of fermentation. Prefermentation was absent in the box fermentation study. 2-methoxyphenol and 3-ethylphenol are also compounds that impart an unpleasant smoky flavor and odor and have been reported as a crucial marker of this aroma. In this study, these compounds are very significant, presenting concentrations of 221,00 and 7,66 μg/kg and an OAV of 122,8 and 3,5, respectively. [13] proposed the maximum tolerable concentration based on the rounded threshold value of 2 μg/kg for 3-ethylphenol. Sometimes, the aroma imparted by 2-methoxyphenol goes unnoticed as it is masked by the more odorous compounds in cocoa. [13] reported a concentration of 70 μg/kg for 2-methoxyphenol, and the reason for this depends on compound concentration and the authentic cocoa odors contributing to the pleasant aroma of cocoa masking the foul odor.

Table 3 shows the difference between the two studies on fruit aromas. The highest amount of these aromas is present in the study of [5], who used beans of the Ecuadorian trinitario variety in their study. In contrast, [42] proposed a single relevant fruit aroma, as is the case for ethyl phenylacetate. In both studies, the same variety (trinitario) was analyzed; however, there were large differences in the amount of its volatile compounds. The difference may be due to geographical origin, postharvest techniques, the chemical composition of the beans, and the method used for their quantification [22]. Fruity volatile compounds, such as isoamyl acetate, 2-nonanone, 2-heptanol, 2-heptanone, 2-pentyl acetate, and ethyl, in descending order of their OAV, contribute to the overall aroma of the dried beans. These fruity aroma compounds are most relevant in the trinitario cocoa variety from Ecuador, known for aromatic cocoa. The esters synthesized during fermentation are dependent on environmental precursors, as well as on aeration and the presence of alcohol, such as ethanol. For example, the production of isoamyl acetate by yeasts (*Pichia fermentants*) uses isoamyl alcohol as a precursor [69]. According to Table 2, this compound is also odor active (OAV = 4), and its concentration exceeds its OTV; therefore, it remains in the aromatic profile and serves as a precursor for other compounds. 2-pentyl acetate (OAV = 5.47) and ethyl acetate (OAV = 1) can act as the precursors of secondary alcohols, such as 2-pentanol and 2-heptanol [70]. The latter contributes its relevant citrus aroma (OAV = 11.08) to Ecuadorian trinitario cocoa beans.

Floral aromas in both studies include, in descending order, linalool, 2-phenylethanol, 2-phenylacetaldehyde, 2-phenylethyl acetate, acetophenone, and 2-methyl-3-buten-2-ol. [71] proposed linalool as a grade indicator (fine or basic grade) in some varieties. A linalool/benzaldehyde ratio of greater than 0.3 indicates fine-grade cocoa. Our review showed linalool and benzaldehyde OAVs of 17.38 and 35.15, respectively, with both compounds aromatically active at a 0.3 ratio. [20] reported a 0.56-to-0.89 linalool/benzaldehyde ratio in roasted Criollo cocoa beans. As linalool is a biosynthesis product, its creation depends on plant varieties, growing, and fermentation conditions. During the roasting process, the linalool content decreases slightly due to volatility, but the relative difference between basic and fine-grade cocoa remains [71].

Table 3. Odor activity values of key aroma compounds in fermented dried beans.

Reference	Variety	Method	Matrix	Volatile	OTV (µg/kg)	OAV	Odor Description
[5]	Trinitario (Sacha Gold)	HS-SPME GC–MS	Dried beans	Isoamyl alcohol	100	4	Banana, fruity, fermented, cognac
				Isoamyl acetate	9.6	91.46	Banana, fruity
				2-heptanone	300–98000	5.79	Fruity, coconut, floral, cheesy
				2-heptanol	263	11.08	Citrus, fruity
				2-nonanone	100	31.14	Fruity, fresh, sweet
				Ethyl acetate	940–22000	1	Pineapple, fruity, sweet, grape
				2-pentyl acetate	13–27000	5.47	Fruity, orange, tropical
				2-Phenylacetaldehyde	22–154	10.82	Floral, honey
				2-phenylethyl acetate	137–233	2.42	Floral, honey
				2-phenylethanol	211	13.12	Floral, honey
				Acetophenone	5629	1	Floral
				Linalool	37	17.38	Floral, pink, sweet, green, citrus
				2-methyl-3-buten-2-ol	480	1	Herbal, earthy

				2-octanol	100	1	Spicy, green, woody, earthy
				2-methylbutanal	2.2–152	85.37	Chocolate
				3-methylbutanal	5.4–80	37.39	Chocolate
				Benzaldehyde	60	35.15	Sweet, bitter almond, cherry, woody
				Trimethylpyrazine	290	1.32	Cocoa, roasted nuts
				Tetramethylpyrazine	38000	1	Chocolate, cocoa, coffee
				2,3-butanedione	3–10	217.53	Buttery
				Acetic acid	124	154.77	Sour vinegar
				2-methylpropanoic acid	190–755	4.87	Rancid butter
				3-methylbutanoic acid	22	131.78	Rancid sweat
				Ethanol	3×10^4–6×10^5	1	Alcoholic
				2-methyl-1-propanol	1000	1	Wine, ethereal
[42]	Trinitario	GC–OAEDA-AIDS	Dried beans	Acetic acid	124	8467.7	bitter, vinegar
				2-methylbutanoic acid	203	99.0	spicy, sweaty
				3-methylbutanoic acid	11	6590.9	spicy, sweaty
				2-phenylethanol	211	8.5	flowery
				3-methylbutanal	5.4	115.2	of malt
				2-methylbutanal	2.2	320.5	of malt
				Fethyl enilacetate	300	1,0	flowery, fruity
				Ethyl 3-methylbutanoate	0.98	2,8	tasty
				2-phenylethyl acetate	0.137	9489.05	Nuts
				Ethyl 2-methylbutanoate	0.37	59.7	tasty
				4-hydroxy-2,5-dimethyl-3 (2H)-furanone	27	1.0	like caramel
				2-Methoxyphenol	1.8	122.8	Smoked
				3-Ethylphenol	2.2	3.5	phenolic, animalic
				2-ethyl-3,5-dimethylpyrazine	2.2	18.0	earthy
				2-ethyl-3,6-dimethylpyrazine	57	0.01	earthy
				dimethyl trisulfide	0.03	83.3	Cabbage

OTV: Odor threshold values. OAV: Odor activity values.

Schluter et al. (2020) [42] reported a high OAV with a prevalence of chocolate and malt flavors, likely related to the high concentrations of isoleucine, leucine, and phenylalanine during fermentation. They attributed such aromas to Strecker aldehydes, such as 2- and 3-methylbutanal (chocolate, malt flavor) and phenylacetaldehyde (honey flavor), respectively. On the contrary, 2-phenylethyl acetate, trimethylpyrazine, ethyl acetate, acetophenone, 2-methyl-3-buten-2-ol, 2-octanol, tetramethylpyrazine, ethanol, and 2-methyl-1-propanol show a low OAV, which, individually, would not contribute to their respective aromas, but, as a whole, would present a range of odors from wine to floral and honey.

Figure 2 illustrates the key aroma profile in both cocoa samples. The aroma profile of the trinitario Sacha Gold variety (Figure 2b) is broader and has a distinct fine diversity of aromas representing processed chocolate. Figure 2a, on the other hand, presents a limited aroma profile that is not appreciated by the chocolate industry. In both studies, a total of four pyrazines are observed, including 2-ethyl-3,5 and 3,6-dimethylpyrazine, trimethylpyrazine, and tetramethylpyrazine, for which the OAVs are less than 18. During fermentation, both trimethyl and tetramethylpyrazines appeared; however, they represent only 0.6% of the total volatiles [72]. During grain roasting, the number and concentration of pyrazines notably rose: 2,5-dimethylpyrazine (DMP), 2,6-DMP, 2-ethylpyrazine, 2,3-DMP, 2,3,4-trimethylpyrazine (TrMP) and 2,3,5,6-tetramethylpyrazine (TMP) [62], evidenced in the following section.

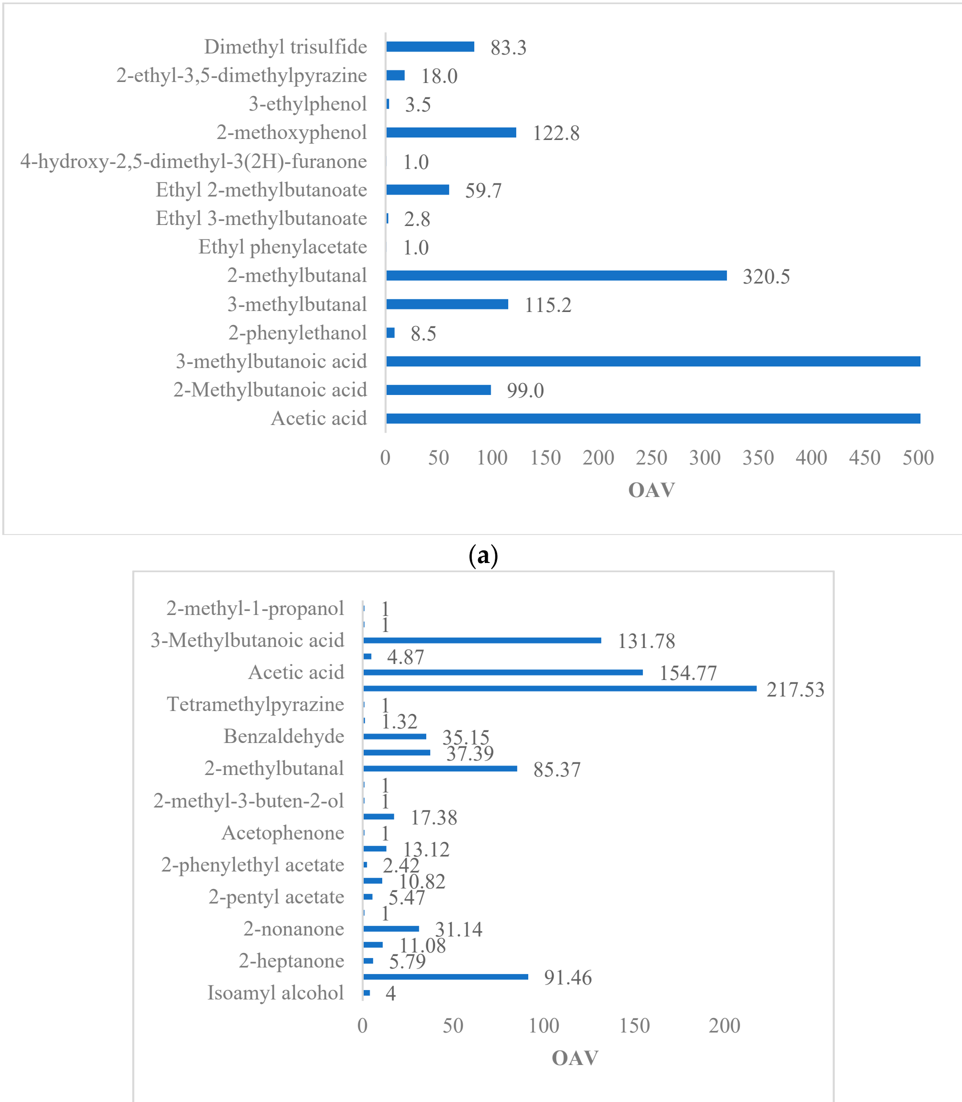
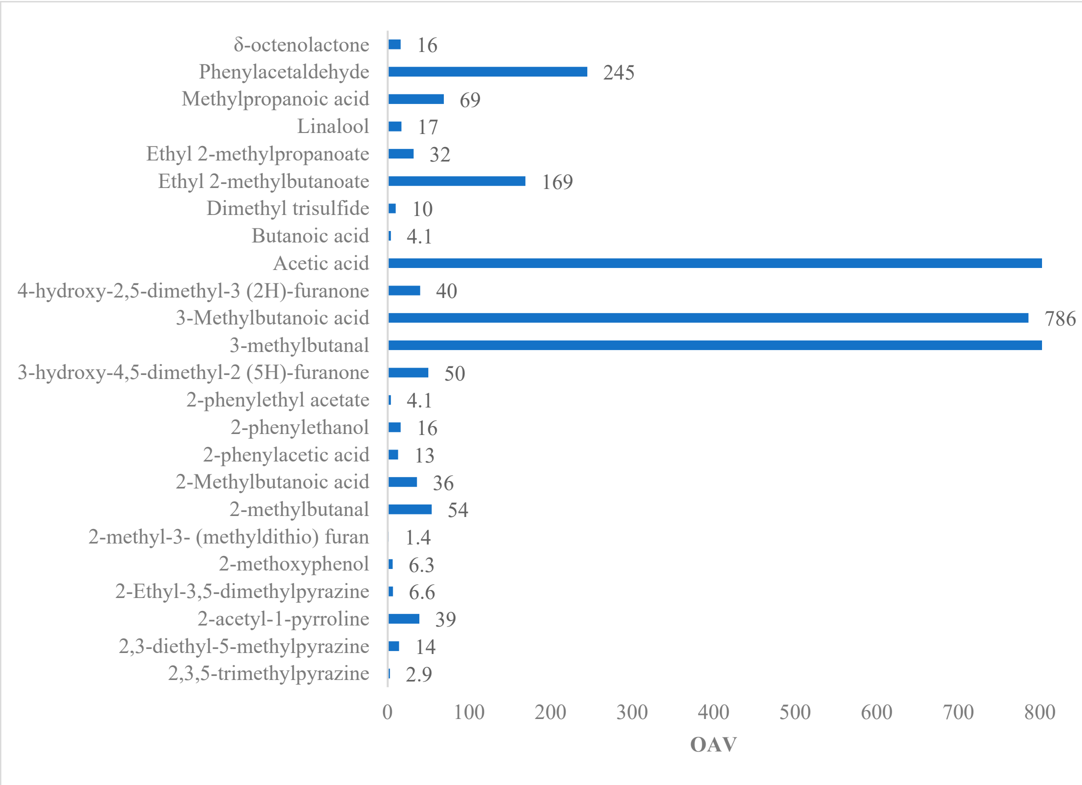


Figure 2. Profile of key volatile compounds present in fermented dry beans determined by (**a**) [42] trinitario cocoa beans and (**b**) [5] trinitario Sacha Gold from Ecuador.

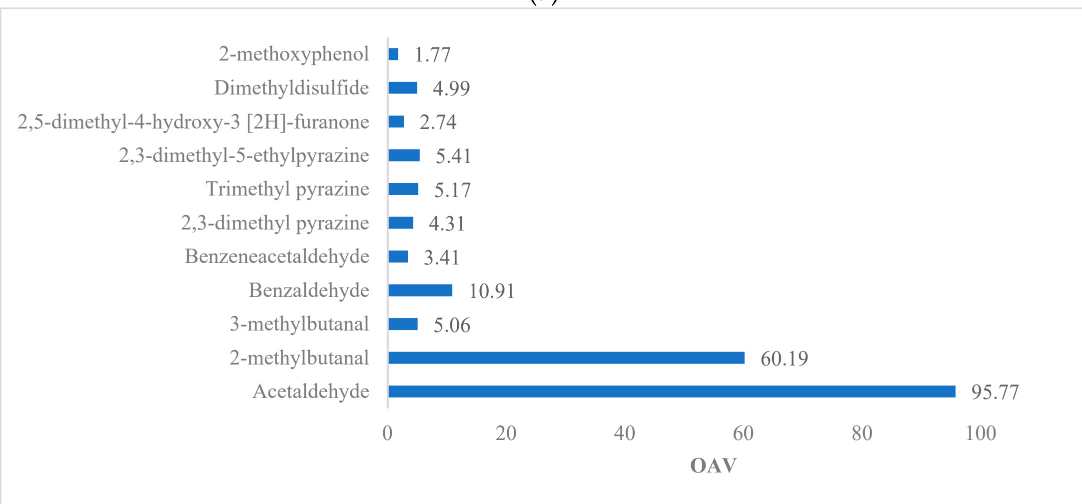
3.2.2. Roasted Beans

Roasting the beans boosts the intensity of some of the volatile compounds present in dried beans, generating the appearance of new compounds. [6] reported 31 compounds in forastero cocoa beans roasted at 95 °C, 71 compounds in beans (of the same variety) roasted at 140 °C, while [9] reported 78 compounds in forastero cocoa beans roasted at 160 °C. They found 34 compounds that had increased in their concentration during roasting, nine compounds that had increased in concentration up to 140 °C and had then decreased or were undetectable; A total of 26 new compounds were added, and the concentration of nine compounds decreased significantly. After roasting, the distribution of the different classes of compounds changed, increasing in pyrazines (22.79%) and aldehydes (15.62%) and forming new compounds from Maillard reactions, such as pyrroles, furans, and pyrans [22]. Some pyrrole derivatives are formed at moderate roasting temperatures and relatively high humidity [40]. These compounds come from sugar precursor degradation in cocoa and decrease during roasting. [73] reported that these volatiles are a useful indicator for which their level can be used to monitor the early stages of roasting.

The presence of undesirable compounds, Figure 3, in both studies represents 42% and 27% of the total aroma profile, indicating the presence of acidic, musty/earthy, burnt, and smoky flavors [74]. Its high intensity and overall contribution are reflected in the OAVs ranging from 1.77 for 2-methoxyphenol to 4920 for acetic acid. The concentration of acetic acid is more than half in the unroasted beans when roasted at 130 °C for 30 mins. Roasting at higher temperatures or for a longer period does not significantly affect the acetic acid concentration. On the contrary, the concentration is higher when roasted at 160 °C for 30 min [75]. Other compounds, such as sulfur derivatives, are also present in cocoa beans roasted at temperatures >160 °C. These compounds generate unpleasant smoky, onion, cabbage, and gasoline notes that persist in the final chocolates, albeit in lower concentrations [39,44].



(**a**)



(**b**)

Figure 3. Volatile compounds profile present in the roasted beans determined by (**a**) [6] forastero roasted cocoa beans, and (**b**) [21] forastero roasted cocoa beans.

A percentage greater than 50% of the compounds in Table 4 indicates a contribution of desirable aromas, such as fruity, floral, cocoa, and chocolate, including the Strecker aldehydes (2- and 3-methylbutanal, phenylacetaldehyde) present in dry beans in smaller quantities. 3-methylbutanal increases its contribution during roasting, especially at 95 and 110 °C, while at 125 °C, there is a return to the initial values. This is due to the prevalence of the volatilization phenomenon compared to that of generation [76]. Furthermore, the concentration of benzaldehyde increases during fermentation [5]and during roasting, making it susceptible to losses at temperatures above 125 °C [76]. Throughout the processing of the beans, the concentration of this compound decreases. In dry beans, the concentration is 1.08 μg/g, and its OAV is 18.08, whereas, in roasted beans, it drops to 1.01 μg/g and presents OAV = 16.90 [9,21].

Table 4. Odor activity values of key aroma compounds in roasted beans.

Reference	Variety	Method	Matrix	Volatile	OTV (ng/g)	OAV	Odor Description
[6]	Forastero	SAFE-AEDA	Roasted beans	2. 3. 5-trimethylpyrazine	290	2.9	Earthy
				2. 3-diethyl-5-methylpyrazine	0.5	14	Roasted potato
				2-acetyl-1-pyrroline	0.1	39	Like popcorn
				2-ethyl-3. 5-Dimethylpyrazine	2.2	6.6	Chocolate, sweet
				2-Methoxyphenol	16	6.3	Smoked
				2-methyl-3- (methyldithio) furan	0.4	1.4	Similar to cooked meat
				2-methylbutanal	140	54	Malt
				2-methylbutanoic acid	203	36	Rancid
				2-phenylacetic acid	360	13	Floral smell, nasty geranium
				2-phenylethanol	211	16	Flowery
				2-phenylethyl acetate	233	4.1	Floral, honey
				3-hydroxy-4. 5-dimethyl-2 (5H)-furanone	0.2	50	Spicy
				3-methylbutanal	13	2030	Malt
				3-methylbutanoic acid	22	786	Rancid
				4-hydroxy-2. 5-dimethyl-3 (2H)-furanone	25	40	Like to caramel
				Acetic acid	124	4920	Sour, vinegar
				Butanoic acid	135	4.1	Rancid
				Dimethyl trisulfide	2.5	10	Sulfuric
				Ethyl 2-methylbutanoate	0.26	169	Tasty
				Ethyl 2-methylpropanoate	1.24	32	Tasty
				Linalool	37	17	Flowery
				Methylpropanoic acid	190	69	Rancid
				Phenylacetaldehyde	22	245	Like honey
				δ-octenolactone	4730	16	Like coconut
[21]	Forastero			Acetaldehyde	0.22	96.78	Sour, fruity

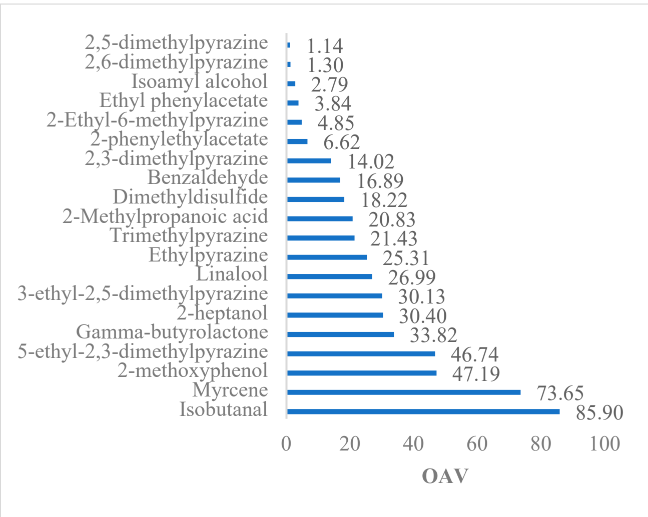
HS–SPME–GC–MS	Roasted beans	2-methylbutanal	2.2–152	53.36	Chocolate
		3-methylbutanal	5.9–80	5.35–79.32	Chocolate
		Benzaldehyde	60	18.08	Sweet almond, bitter, cherry
		Benzeneacetaldehyde	22–154	2.41	Honey, sweet, pink, floral
		2. 3-Dimethyl pyrazine	123	1.45	Caramel, cocoa
		Trimethylpyrazine	290	1.64	Cocoa, toasted walnuts, peanuts
		2. 3-dimethyl-5-ethylpyrazine	60	2.95	Popcorn, burnt cocoa, toasted
		2. 5-dimethyl-4-hydroxy-3 [2H]-furanone	1.6–50	1.15–36.06	-
		Dimethyl disulfide	12	3.68	Sulfur-like, cabbage, onion
		2-Methoxyphenol	10–70	6.81	Smoked, repulsive

Other compounds that turn up after roasting are 4-hydroxy-2,5-dimethyl-3 (2H)-furanone, 2-acetyl-1-pyrroline, 2,3-dimethyl-5-ethylpyrazine, trimethylpyrazine, and 2,3-dimethylpyrazine. 2-acetyl-1-pyrroline, which is typically associated with roasting (Parker, 2015), exudes a popcorn and cracker-like aroma, having a low threshold in oil 0,1 ng/g and a concentration above OTV = 39 ng/g. Therefore, the contribution to the overall aroma of the roasted beans is significant (Table 3). Likewise, the pyrazines generated during roasting at temperatures above 100 °C exude notes of cocoa, baked potato, and chocolate. [45] reported that simple unsubstituted or monosubstituted pyrazines have a roasted, biscuit aroma and relatively high aroma thresholds, but as the substitution increases, the odor threshold decreases. It is evident from Table 3 that some of the pyrazines have low OTVs: 2,3-diethyl-5-methylpyrazine and 2-ethyl-3,5-dimethylpyrazines, and high OTVs: 2,3,5-trimethylpyrazine and 2,3-dimethyl-5-ethylpyrazine. These compounds exude baked potato, chocolate, earthy, popcorn, and cocoa aromas, respectively. However, their OTVs are inversely related to their OAVs. There are a few fruity and floral aromas, as shown in Table 3, including linalool, 2-phenylethanol, and 2-phenylethyl acetate, in descending order of their aromatic contribution. This deficiency in fruity aromas is common in the forastero variety [28]. Finally, the 59 volatile compounds present in the roasted beans include alkyl pyrazines and aldehydes from reactions between oligopeptides derived from vicilin class globulin (7S) and reducing sugars of the cocoa beans [36]. The author reported eight unidentified volatile compounds likely to expel specific cocoa notes.

3.2.3. Cocoa Liquor

The aroma of cocoa liquor is an important characteristic affecting its quality; therefore, the affecting factors are the origin of the cocoa bean and the postharvest processing (fermentation and drying), roasting, and storage [19]. The origin of the cocoa bean is vital in determining the aroma of all its cocoa products and is related to genetic and environmental factors. In this regard, only a few aromas are characteristic of cocoa liquor from different origins, including 3-methylbutanal (malty), linalool (floral), β-phenylethyl alcohol (pink), benzaldehyde (almond-like), benzeneacetaldehyde (pink), β-phenylethyl acetate (fruity), 3-methylbutyl benzoate (fruity), 2,5-6-dimethylpyrazine (potato-like), ethylpyrazine (popcorn), trimethylpyrazine (roasted), 3-ethyl-2,5-dimethylpyrazine (roasted), tetramethylpyrazine (nutty), 3,5-diethyl-2-methylpyrazine (cocoa), furfural (potato), acetic acid (acid), 3-methylbutanoic acid (stench), and dimethyltrisulfide (onion), which are considered the key active aroma compounds contributing to the overall cocoa liquor odor of the forastero variety. However, the intensity of each aroma among the different cocoa liquors is distinct [77]. Likewise, [78] reported that the specific aroma of bulk cocoa liquor included sweet, nutty, caramel, and chocolate notes associated with trimethylpyrazine, tetramethylpyrazine, 2,3-butanediol, dodecanoic acid, β-phenylethyl alcohol, 2-acetylpyrrole, and benzeneacetaldehyde.

On the whole, the compounds of the cocoa liquors studied represent 57.89 to 65.79% of OAV >1, and 32.61 to 42.11% represent OAV < 1. Among these compounds, fruity, floral, chocolate, buttery, and undesirable aromas are highlighted (Table 5). Several compounds account for 17 to 40% of fruit flavors with a VAO > 1, including isoamyl acetate (banana), 2-heptanol (citrus), ethyl phenylacetate, isoamyl alcohol (banana), furaneol (strawberry), pentyl 2-acetate (orange), ethyl 3-methylbutanoate, 2-nonanone, 2-heptanol, 2-heptanone (coconut), pentylacetate, and 3-methylbutyl acetate. From 14 to 25% of the floral compounds, 2-phenylethyl alcohol, linalool, 2-phenylethylacetate, acetophenone, 2-propanone, 2-phenylacetaldehyde, and β-myrcene are included. From 20 to 33% provide chocolate notes, including 2- and 3-methylbutanal, 5-ethyl-2,3-dimethylpyrazine, trimethylpyrazine, 2,3,5-trimethylpyrazine, benzaldehyde, 2-ethyl-5 and 6-methylpyrazine, 2,6-dimethylpyrazine, 2,5-dimethylpyrazine, tetramethylpyrazine, methylpyrazine, and 2-methylpropanal. From 3 to 14% provide creamy and buttery tones, such as ethylpyrazine, furfural, 3-hydroxy-2-butanone, 2,3-butanedione, 2,3-pentanedione, and 2-methylpropanoic acid. Finally, the compounds providing caramel and earthy notes in the 3% to 15% range include gamma-butyrolactone and 3-ethyl-2,5-dimethylpyrazine, respectively. Figure 4 details the set of OAV > 1 compounds described above, which is common among the studies of Streker aldehydes, linalool, isoamyl acetate, and 2-heptanol.



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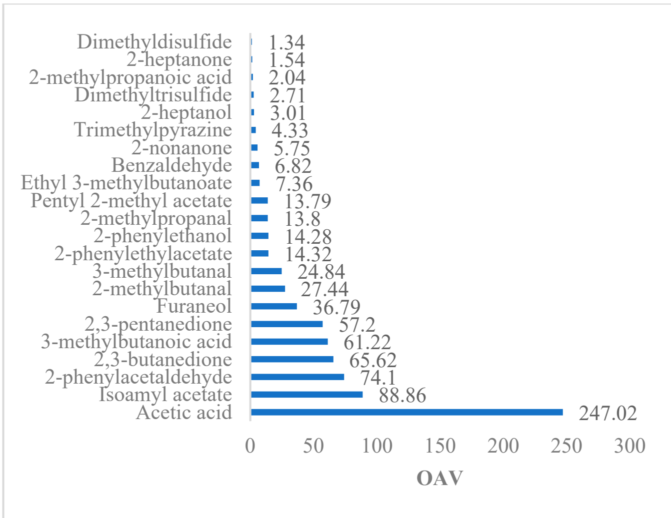

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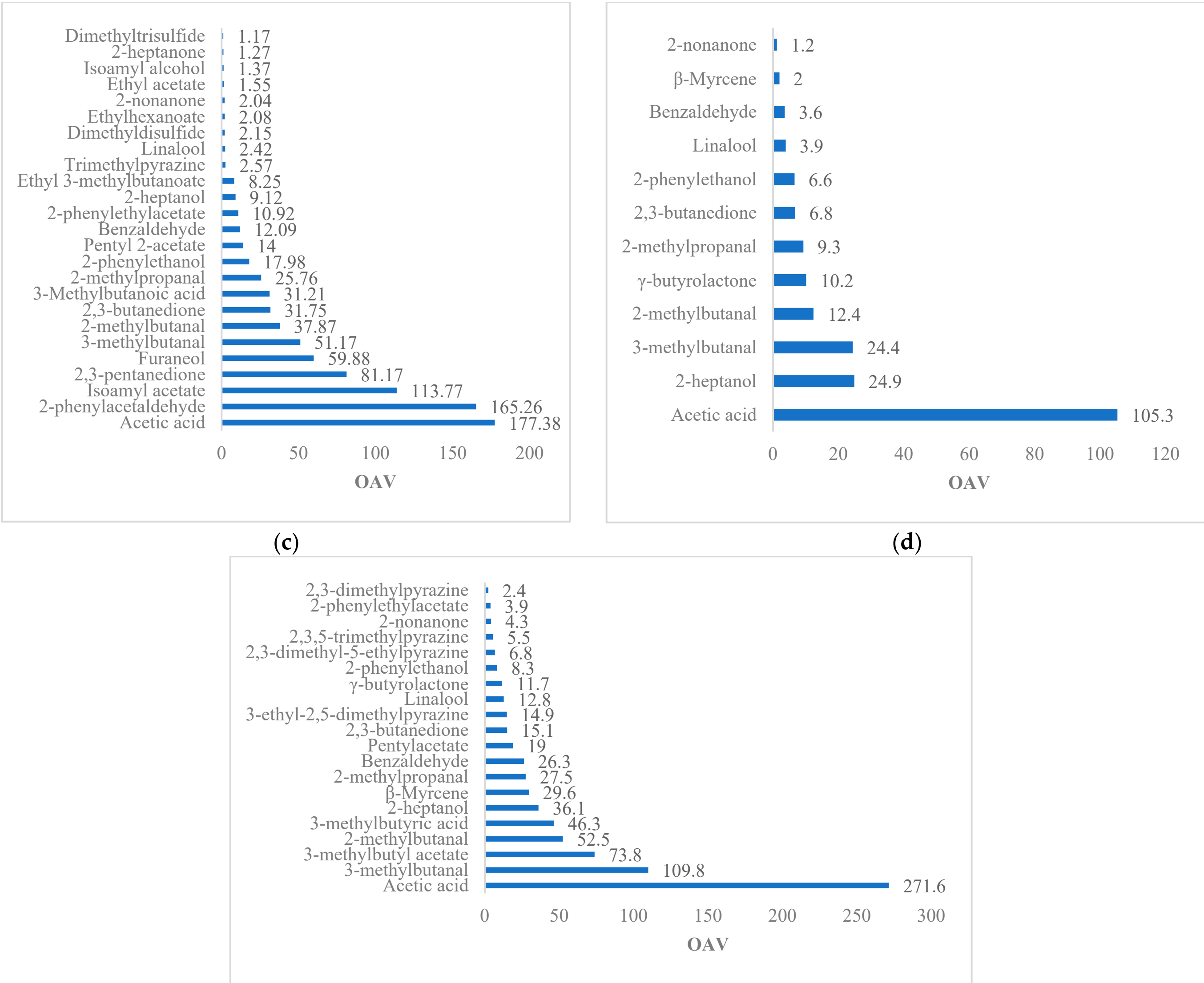

(**c**) (**d**)

(**e**)

Figure 4. Studies of the volatile compounds with OAV > 1 in the liquor of the cocoa determined by (**a**) [9] in forastero cocoa beans, (**b**) [4] in CCN51 cocoa beans, (**c**) [4] in nacional cocoa beans, (**d**) [12] Ecuadorian liquor and (**e**) [12] African liquor.

The compounds with an OAV < 1 include isoamyl alcohol, ethyl acetate, ethylhexanoate, limonene, acetophenone, linalool, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-5-methylpyrazine, tetramethylpyrazine, propionic acid, 2-butanone, ethanol, 2-butanol, 2-methyl-1-propanol, 2-pentylfuran, 3-hydroxy-2-butanone and ethanol, with fruity, floral, chocolate, buttery, and mainly undesirable aromas. The compounds exclusive to the study include rose oxide and β-myrcene that reveal floral notes in cocoa of the forastero and national variety, respectively.

Smoky, acidic, hammy, or musty aromas are the common compounds in cocoa liquors, and are present throughout the postharvest process [79]. From 8 to 24% of the total active odor aromas (OAV > 1) correspond to undesirable compounds in cocoa liquors and dried beans, such as acetic acid, 2,3-pentanedione, 2- and 3-methylbutanoic acid, and 2-methoxyphenol. In addition, 2,3-butanedione, dimethyl di- and trisulfide, methylpropanoic acid, and butanoic acid are in roasted beans and persist in cocoa liquor. During chocolate processing with these liquors, the OAV partially decreases or is undetected. For instance, in acetic acid and 2-nonanone, the OAV decreases by 5 to 14 times, and the concentration of 2,3-butanedione is undetected, so their OAV cannot be calculated [12]. The same occurs with butyric acid and 3-methylbutyric acid, which are only detected in cocoa liquors and disappear in processed chocolate. It is worth mentioning that although acids present a lower number of compounds in cocoa liquors, they have the highest VAOs, affecting the liquor's pH. Cocoa liquor with a low pH (4.75 to 5.19) is more likely to have off-flavors.

Table 5. Odor activity values of key aroma compounds in cocoa liquor.

Variety	OAV *	Odor Contribution %								Reference
		Fruity	Floral	Chocolate	Buttery	Spices	Caramel	Undesira-bles	Earthy	
CCN51	>1	31.82	13.64	22.73	13.64	-	-	18.18	-	[4]
National		4-	16.00	2-	8.00	-	-	16.00	-	
Forastero		17.24	13.79	27.59	3.45	3.45	6.90	24.14	3.45	[9]
National		16.67	25.00	33.33	8.33	-	8.33	8.33	-	[12]
**		2-	2-	3-	5.00	-	15.00	1-	-	
CCN51	<1	25.00	12.50	25.00	-	-	-	37.50	-	[4])
National		7.69	7.69	30.77	-	-	-	53.85	-	
Foastero		35.29	17.65	11.76	11.76	-	5.88	17.65	-	[9]
National		25.00	12.50	37.50	-	-	12.50	12.50	-	[12]

* Odor activity values. ** African cocoa.

3.2.4. Chocolate

The flavor of chocolate depends on the way the series of processes described above are carried out. Conching is the last of these processes, whereby the manufacturer can obtain the flavor and aroma required for a particular product. However, this process cannot correct previous mistakes, like an unpleasant smoky or moldy flavor due to poor drying, nor can it turn an inferior cocoa taste into a perfect one [80]. Specifically, the function of conching is to evaporate volatile acids, achieve adequate viscosity, remove excess moisture, and develop a desirable color [81,82], as well as remove off-flavors and aromas while retaining desirable ones [80]. Chocolate producers often use different conching temperatures and times depending on cocoa bean varieties and the origin of the chocolate products with the desired aromatic properties [83]. In particular, levels of the most important odors decrease significantly by rising conching duration (from 6 to 10 h at 80 °C). Prolonged times reduce most pyrazines (including 2,5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, and 2,3,5-trimethylpyrazine) and the levels of alcohol, acid, aldehydes, and small esters [25]. Similarly, [11] reported that prolonged conching reduces volatile acid concentration, alcohol, 3-methylbutanal, benzaldehyde, and several lesser volatile pyrazines, like trimethylpyrazine, tetramethylpyrazine, and acetylpyrrole. They also noted that this treatment increases the furfural content and does not affect the isobutanal, 2-methylbutanal, and phenylacetaldehyde levels because of the additional reaction compensation to form Strecker aldehydes during conching. Other components with significant contributions to chocolate (Figure 5) and that are highly odorous based on their low odor thresholds are 2-methylbutanal (2.2 ng/g), 3,5-diethyl-2-methylpyraniza, furaneol (27 ng/g), 2,3-diethyl-5-methylpyrazine (7.2 ng/g), ethyl 2- and 3-methylbutanoate (0.37 and 0.98 ng/g), 2-methylpropanal (3.4 ng/g), 3-isobutyl-methoxypyrazine (0.04 ng/g), 3-isopropyl-2-methoxypyrazine (0.01 ng/g), and linalool (37 ng/g) [10–12,44,84].

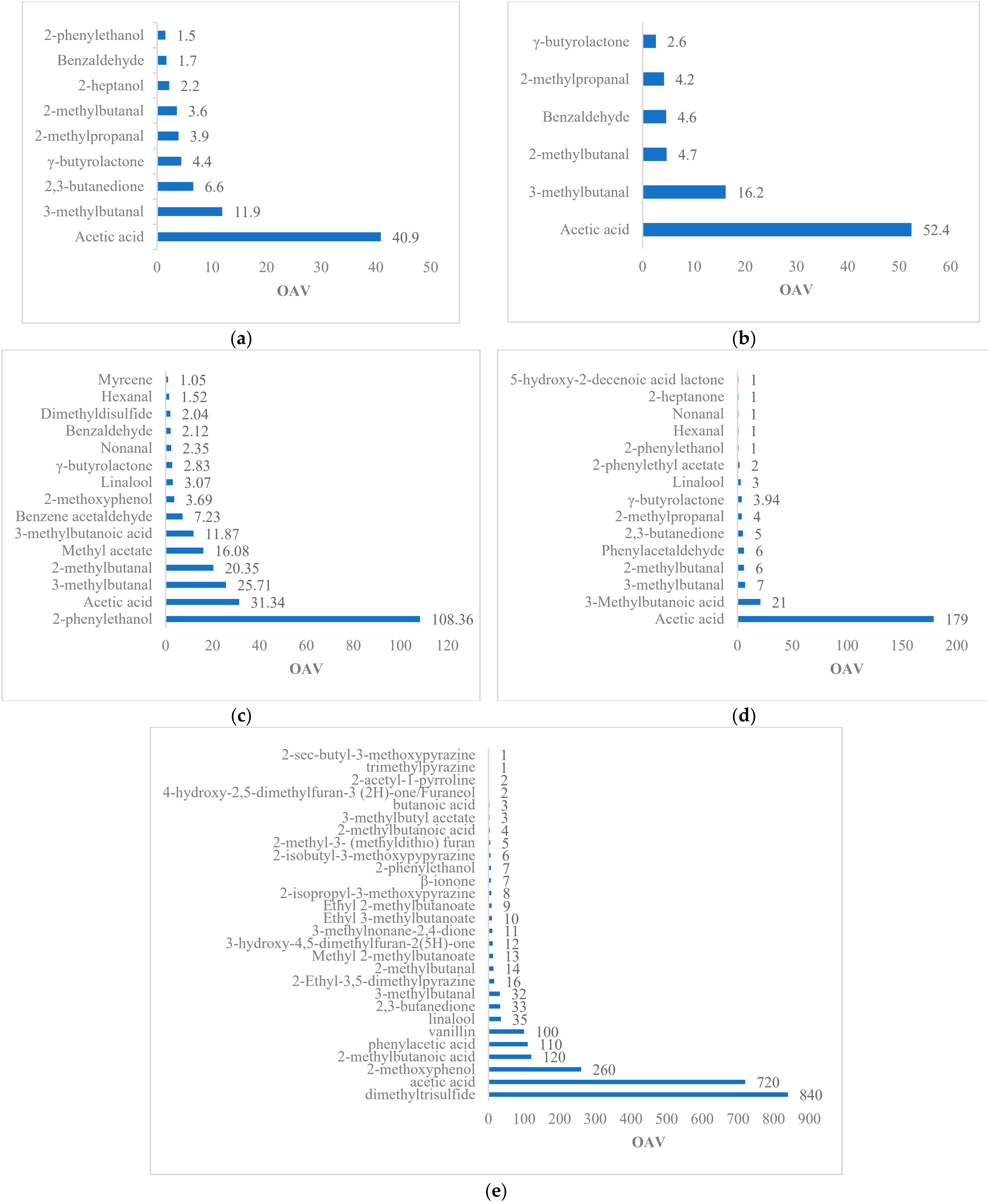

Figure 5. Studies of volatile compounds with OAV > 1 in dark chocolate determined by (**a**) [12] in chocolate from national cocoa beans, (**b**) [12]in chocolate from African cocoa beans, (**c**) [44] in chocolate from forastero cocoa beans, (**d**) [11] in Vietnamese cocoa beans and (**e**) [10] in commercial chocolate.

Other major aroma compounds remaining in chocolates include 2- and 3-methylbutanal (chocolate, malt), benzaldehyde (roasted almonds, malt), gamma-butyrolactone (sweet, caramel), 2-methylpropanal (unroasted cocoa, malt), linalool (flowery, fruity, tea-like), acetic acid (bitter, vinegar), and 2-phenylethanol (honey, rose) (Figure 5). [84] reported similar compounds, such as 3-methylbutanal, 2-methylpropanal, phenylacetaldehyde, tetramethylpyrazine, 2-acetyl-1-pyrroline, trimethylpyrazine, 3-methylbutanoic acid, acetic acid, and vanillin. The uncommon compound is vanillin, which is a highly odorous compound with OAV = 100 and was found only in chocolate with 90% cocoa (Figure 5e). In fact, the most uncommon aromatic compounds among the studies are found in this chocolate. Likewise, 2-acetyl-1-pyrroline (popcorn-like aroma) is found in roasted beans (OAV = 39) [6], cocoa liquor (OAV = 207.55) and chocolate (OAV= 396.23) [85]. Seyfried and Granvogl (2019) [10] reported OAV = 2 in 90% cocoa chocolate, and this was unidentified in 99% cocoa chocolate. This compound is highly volatile and mainly generated during roasting.

The greater the availability of the compounds, the more complex the chocolate aroma is due to a wide volatile matrix. The studies in this paper show that acetic acid is the most abundant compound, and the rest of the compounds represent a mixture from different families (acids, alcohols, pyrans, aldehydes, esters, furans, and pyrazines). Table 6 shows that the chocolates made with 40 and 90% cocoa have an almost complete distribution of aromas ranging from fruity to undesirable in a range of 13 to 25%.

Table 6. Odor activity values of key aroma compounds in dark chocolate.

Description	OAV	Odor Contribution %								Reference
		Fruit	Floral	Chocolate	Buttery	Spice	Caramel	Undesirable	Earthy	
Chocolate 90% cocoa	>1	25.00	14.29	21.43	3.57	7.14	3.57	25.00	-	[10]
Chocolate 40% cocoa liquor		13.33	26.67	2.00	13.33	6.67	6.67	13.33	-	[11]
* Chocolate 51.6% cocoa		11.11	11.11	44.44	11.11	-	11.11	11.11	-	[12]
** Chocolate 51.6% cocoa		-	-	66.67	-	-	16.67	16.67	-	
Chocolate 70% cocoa liquor		6.67	26.67	2.00	-	6.67	6.67	33.33	-	[44]
Chocolate 90% cocoa	<1	23.81	4.76	9.52	23.81	9.52	14.29	14.29	-	[10]
Chocolate 40% cocoa liquor		11.11	-	66.67	11.11	-	-	11.11	-	[11]
* Chocolate 51.6%cocoa		36.36	27.27	18.18	-	-	18.18	-	-	[12]
** Chocolate 51.6% cocoa		35.71	28.57	14.29	7.14	-	14.29	-	-	
Chocolate 70% cocoa liquor		40.91	13.64	18.18	-	-	4.55	18.18	4,55	[44]

* Made with Ecuadorian national cocoa liquor. ** Made from African cocoa liquor.

Many of the compounds that remain in chocolate have an increased concentration along the processing chain or remained unchanged, as has been reported; the influence of the manufacturing process is greater than that of the difference in the cocoa production area, providing a diversity of aroma profiles [86]. Particularly, criollo cocoa roasting increases acetophenone, tetramethylpyrazine, 2,3,5-trimethylpyrazine, and 2,5-

dimethylpyrazine concentration, whereas 2-heptanol, phenylethyl alcohol, 2,3-butanedione, 2-phenylphenylpyrazine, 2,3-butanedione, 2,3-butanedione and 2,3-butanedione, 2,3-butanedione, 2-phenyl-2-butenal, 5-methyl-2-phenyl-2-hexanal, ethyl octanoate, ethyl phenylacetate, ethyl decanoate, and trans-linalool oxide remain stable during roasting [2]. In addition, some of the samples' volatile compounds are affected by each brand formulation, masking or enhancing a specific volatile. [26] considered three of the predominant masses of chocolates, namely, mass 33, 43, and 61, which were identified as methanol, a fragment of diverse origin, and acetic acid, respectively, and because of their high concentration, he suggested these three masses can have a huge impact on the fingerprint analysis that differentiates them by regions and brands.

4. Conclusions

Throughout the cocoa processing chain, aromatic precursor compounds give rise to characteristic aromas in each cocoa matrix. The way aroma compounds contribute to a specific matrix is estimated by the compound odor activity value. Initially, the dry fermented beans had acidic notes for which the OAV was high. The diversity of acids depends on the fermentation method used and also on bean preconditioning to reduce the fermentable sugar and, therefore, the final acidity of the bean, whereas fruity and floral aromas were characteristic of dry fermented beans. However, their concentration and abundance of compounds depends on the variety used. Compounds that exude chocolate aromas are scarce in dry fermented beans as they arise from Maillard reactions during roasting. The roasting parameters, like temperature, roasting time, and the roasting method, influence the appearance of new compounds and the preservation of those already found in the dry beans. Temperatures above 160 °C for a period of 35 min favor the appearance of pyrazines but reduce the compound concentration responsible for fruity and floral aromas, such as esters and ketones. In cocoa liquor, on average, 61.84% of the compounds represent fruity and floral aromas. The abundance of compounds in chocolate is directly related to the conching process, whereby the remaining fraction of moisture and undesirable aroma is eliminated, and the desirable aromas are concentrated.

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References

1. Kongor, J.E.; Hinneh, M.; de Walle, D.V.; Afoakwa, E.O.; Boeckx, P.; Dewettinck, K. Factors influencing quality variation in cocoa (Theobroma cacao) bean flavour profile—A review. *Food Res. Int.* **2016**, *82*, 44–52. https://doi.org/10.1016/j.foodres.2016.01.012.
2. Ascrizzi, R.; Flamini, G.; Tessieri, C.; Pistelli, L. From the raw seed to chocolate: Volatile profile of Blanco de Criollo in dif-ferent phases of the processing chain. *Microchem. J.* **2017**, *133*, 474–479. https://doi.org/10.1016/j.microc.2017.04.024.
3. Castro-Alayo, E.M.; Idrogo-Vásquez, G.; Siche, R.; Cardenas-Toro, F.P. Formation of aromatic compounds precursors during fermentation of Criollo and Forastero cocoa. *Heliyon* **2019**, *5*, e01157 . https://doi.org/10.1016/j.heliyon.2019.e01157.
4. Rottiers, H.; Sosa, D.A.T.; Lemarcq, V.; De Winne, A.; De Wever, J.; Everaert, H.; Jaime, J.A.B.; Dewettinck, K.; Messens, K. A multipronged flavor comparison of Ecuadorian CCN51 and Nacional cocoa cultivars. *Eur. Food Res. Technol.* **2019**, *245*, 2459–2478. https://doi.org/10.1007/s00217-019-03364-3.
5. Rottiers, H.; Sosa, D.A.T.; De Winne, A.; Ruales, J.; De Clippeleer, J.; De Leersnyder, I.; De Wever, J.; Everaert, H.; Messens, K.; Dewettinck, K. Dynamics of volatile compounds and flavor precursors during spontaneous fermentation of fine flavor Tri-

nitario cocoa beans. *Eur. Food Res. Technol*. **2019**, *245*, 1917–1937. https://doi.org/10.1007/s00217-019-03307-y.

6. Frauendorfer, F.; Schieberle, P. Key aroma compounds in fermented Forastero cocoa beans and changes induced by roasting. *Eur. Food Res. Technol*. **2019**, *245*, 1907–1915. https://doi.org/10.1007/s00217-019-03292-2.
7. Qin, X.-W.; Lai, J.-X.; Tan, L.-H.; Hao, C.-Y.; Li, F.-P.; He, S.-Z.; Song, Y.-H. Characterization of volatile compounds in Criollo, Forastero, and Trinitario cocoa seeds (Theobroma cacao L.) in China. *Int. J. Food Prop*. **2017**, *20*, 2261–2275. https://doi.org/10.1080/10942912.2016.1236270.
8. Utrilla-Vázquez, M.; Rodríguez-Campos, J.; Avendaño-Arazate, C.H.; Gschaedler, A.; Lugo-Cervantes, E. Analysis of volatile compounds of five varieties of Maya cocoa during fermentation and drying processes by Venn diagram and PCA. *Food Res. Int*. **2020**, *129*, 108834. https://doi.org/10.1016/j.foodres.2019.108834.
9. Hinneh, M.; Van de Walle, D.; Tzompa-Sosa, D.A.; De Winne, A.; Termote, S.; Messens, K.; Van Durme, J.; Afoakwa, E.O.; De Cooman, L.; Dewettinck, K. Tuning the aroma profiles of FORASTERO cocoa liquors by varying pod storage and bean roasting temperature. *Food Res. Int*. **2019**, *125*, 108550. https://doi.org/10.1016/j.foodres.2019.108550.
10. Seyfried, C.; Granvogl, M. Characterization of the Key Aroma Compounds in Two Commercial Dark Chocolates with High Cocoa Contents by Means of the Sensomics Approach. *J. Agric. Food Chem*. **2019**, *67*, 5827–5837. https://doi.org/10.1021/acs.jafc.8b06183.
11. Tran, P.D.; Van Durme, J.; Van De Walle, D.; De Winne, A.; Delbaere, C.; De Clercq, N.; Phan, T.T.Q.; Nguyen, C.P.; Dewettinck, K. Quality Attributes of Dark Chocolate Produced from Vietnamese Cocoa Liquors. *J. Food Qual*. **2016**, *39*, 311–322. https://doi.org/10.1111/jfq.12200.
12. Tuenter, E.; Delbaere, C.; De Winne, A.; Bijttebier, S.; Custers, D.; Foubert, K.; Van Durme, J.; Messens, K.; Dewettinck, K.; Pieters, L. Non-volatile and volatile composition of West African bulk and Ecuadorian fine-flavor cocoa liquor and chocolate. *Food Res. Int.* **2019**, *130*, 108943. https://doi.org/10.1016/j.foodres.2019.108943.
13. Füllemann, D.; Steinhaus, M. Characterization of Odorants Causing Smoky Off-Flavors in Cocoa. *J. Agric. Food Chem*. **2020**, *68*, 10833–10841. https://doi.org/10.1021/acs.jafc.0c04633.
14. Rodriguez-Campos, J.; Escalona-Buendía, H.; Orozco-Avila, I.; Lugo-Cervantes, E.; Jaramillo-Flores, M. Dynamics of volatile and non-volatile compounds in cocoa (Theobroma cacao L.) during fermentation and drying processes using principal components analysis. *Food Res. Int.* **2011**, *44*, 250–258. https://doi.org/10.1016/j.foodres.2010.10.028.
15. Koua, B.K.; Koffi, P.M.E.; Gbaha, P. Evolution of shrinkage, real density, porosity, heat and mass transfer coefficients during indirect solar drying of cocoa beans. *J. Saudi Soc. Agric. Sci*. **2019**, *18*, 72–82. https://doi.org/10.1016/j.jssas.2017.01.002.
16. Afoakwa, E.O.; Kongor, J.E.; Takrama, J.; Budu, A.S. Changes in nib acidification and biochemical composition during fermentation of pulp pre-conditioned cocoa (Theobroma cacao) beans. *Int. Food Res. J.* **2013**, *20*, 1843–1853.
17. Souza, C.D.S.; Block, J.M. Impact of the addition of cocoa butter equivalent on the volatile compounds profile of dark chocolate. *J. Food Sci. Technol*. **2018**, *55*, 767–775. https://doi.org/10.1007/s13197-017-2989-6.
18. Marty-Terrade, S.; Marangoni, A.G. Impact of Cocoa Butter Origin on Crystal Behavior. *Cocoa Butter Relat. Compd*. **2012**, 245–274. https://doi.org/10.1016/b978-0-9830791-2-5.50014-1.
19. Afoakwa, E.O.; Paterson, A.; Fowler, M.; Ryan, A. Matrix effects on flavour volatiles release in dark chocolates varying in particle size distribution and fat content using GC–mass spectrometry and GC–olfactometry. *Food Chem*. **2009**, *113*, 208–215. https://doi.org/10.1016/j.foodchem.2008.07.088.
20. Valle-Epquín, M.G.; Balcázar-Zumaeta, C.R.; Auquiñivín-Silva, E.A.; Fernández-Jeri, A.B.; Idrogo-Vásquez, G.; Castro-Alayo, E.M. The roasting process and place of cultivation influence the volatile fingerprint of Criollo cocoa from Amazonas, Peru. *Sci. Agropecu*. **2020**, *11*, 599–610. https://doi.org/10.17268/sci.agropecu.2020.04.16.
21. Hinneh, M.; Semanhyia, E.; Van de Walle, D.; De Winne, A.; Tzompa-Sosa, D.A.; Scalone, G.L.L.; De Meulenaer, B.; Messens, K.; Van Durme, J.; Afoakwa, E.O.; et al. Assessing the influence of pod storage on sugar and free amino acid profiles and the implications on some Maillard reaction related flavor volatiles in Forastero cocoa beans. *Food Res. Int*. **2018**, *111*, 607–620. https://doi.org/10.1016/j.foodres.2018.05.064.
22. Marseglia, A.; Musci, M.; Rinaldi, M.; Palla, G.; Caligiani, A. Volatile fingerprint of unroasted and roasted cocoa beans (Theobroma cacao L.) from different geographical origins. *Food Res. Int*. **2020**, *132*, 109101. https://doi.org/10.1016/j.foodres.2020.109101.
23. Hinneh, M.; Van de Walle, D.; Tzompa-Sosa, D.A.; Haeck, J.; Abotsi, E.E.; De Winne, A.; Messens, K.; Van Durme, J.; Afoakwa, E.O.; De Cooman, L.; et al. Comparing flavor profiles of dark chocolates refined with melanger and conched with Stephan mixer in various alternative chocolate production techniques. *Eur. Food Res. Technol*. **2019**, *245*, 837–852. https://doi.org/10.1007/s00217-018-3221-2.
24. Engeseth, N.J.; Pangan, M.F.A. Current context on chocolate flavor development—A review. *Curr. Opin. Food Sci*. **2018**, *21*, 84–91. https://doi.org/10.1016/j.cofs.2018.07.002.
25. Owusu, M.; Petersen, M.A.; Heimdal, H. Effect of Fermentation Method, Roasting and Conching Conditions on the Aroma Volatiles of Dark Chocolate. *J. Food Process. Preserv*. **2012**, *36*, 446–456. https://doi.org/10.1111/j.1745-4549.2011.00602.x.
26. Acierno, V.; Yener, S.; Alewijn, M.; Biasioli, F.; Van Ruth, S. Factors contributing to the variation in the volatile composition of chocolate: Botanical and geographical origins of the cocoa beans, and brand-related formulation and processing. *Food Res. Int.* **2016**, *84*, 86–95. https://doi.org/10.1016/j.foodres.2016.03.022.
27. Caligiani, A.; Marseglia, A.; Palla, G. Cocoa: Production, Chemistry, and Use. *Encycl. Food Health* **2015**, 185–190 . https://doi.org/10.1016/b978-0-12-384947-2.00177-x.
28. Cevallos-Cevallos, J.M.; Gysel, L.; Maridueña-Zavala, M.G.; Molina-Miranda, M.J. Time-Related Changes in Volatile Compounds during Fermentation of Bulk and Fine-Flavor Cocoa (Theobroma cacao) Beans. *J. Food Qual*. **2018**, *2018*, 1–14 . https://doi.org/10.1155/2018/1758381.

29. Samaniego, I.; Espín, S.; Quiroz, J.; Ortiz, B.; Carrillo, W.; García-Viguera, C.; Mena, P. Effect of the growing area on the methylxanthines and flavan-3-ols content in cocoa beans from Ecuador. *J. Food Compos. Anal*. **2020**, *88*, 103448. https://doi.org/10.1016/j.jfca.2020.103448.
30. Anecacao, 2019. Cacao Nacional | Anecacao Ecuador. Cacao Nac. Available online: http://www.anecacao.com/index.php/es/quienes-somos/cacao-nacional.html (accessed on 8 December 2020).
31. Dang, Y.K.T.; Nguyen, H.V.H. Effects of Maturity at Harvest and Fermentation Conditions on Bioactive Compounds of Cocoa Beans. *Plant Foods Hum. Nutr*. **2019**, *74*, 54–60. https://doi.org/10.1007/s11130-018-0700-3.
32. Pedan, V.; Weber, C.; Do, T.; Fischer, N.; Reich, E.; Rohn, S. HPTLC fingerprint profile analysis of cocoa proanthocyanidins depending on origin and genotype. *Food Chem*. **2018**, *267*, 277–287. https://doi.org/10.1016/j.foodchem.2017.08.109.
33. Scollo, E.; Neville, D.C.; Oruna-Concha, M.J.; Trotin, M.; Cramer, R. UHPLC–MS/MS analysis of cocoa bean proteomes from four different genotypes. *Food Chem*. **2020**, *303*, 125244. https://doi.org/10.1016/j.foodchem.2019.125244.
34. Afoakwa, E.O. Cocoa Cultivation and Practices. In *Chocolate Science and Technology*; Wiley Blackwell: Singapore, 2010; pp. 16–18.
35. Rottiers, H.; Sosa, D.A.T.; Van de Vyver, L.; Hinneh, M.; Everaert, H.; De Wever, J.; Messens, K.; Dewettinck, K. Discrimination of Cocoa Liquors Based on Their Odor Fingerprint: A Fast GC Electronic Nose Suitability Study. *Food Anal. Methods* **2019**, *12*, 475–488. https://doi.org/10.1007/s12161-018-1379-7.
36. Scalone, G.L.L.; Textoris-Taube, K.; De Meulenaer, B.; De Kimpe, N.; Wöstemeyer, J.; Voigt, J. Cocoa-specific flavor components and their peptide precursors. *Food Res. Int*. **2019**, *123*, 503–515. https://doi.org/10.1016/j.foodres.2019.05.019.
37. Calva-Estrada, S.; Utrilla-Vázquez, M.; Vallejo-Cardona, A.; Roblero-Pérez, D.; Lugo-Cervantes, E. Thermal properties and volatile compounds profile of commercial dark-chocolates from different genotypes of cocoa beans (Theobroma cacao L.) from Latin America. *Food Res. Int*. **2020**, *136*, 109594. https://doi.org/10.1016/j.foodres.2020.109594.
38. Hamdouche, Y.; Meile, J.C.; Lebrun, M.; Guehi, T.; Boulanger, R.; Teyssier, C.; Montet, D. Impact of turning, pod storage and fermentation time on microbial ecology and volatile composition of cocoa beans. *Food Res. Int*. **2019**, *119*, 477–491. https://doi.org/10.1016/j.foodres.2019.01.001.
39. Rodriguez-Campos, J.; Escalona-Buendía, H.; Contreras-Ramos, S.; Orozco-Avila, I.; Jaramillo-Flores, E.; Lugo-Cervantes, E. Effect of fermentation time and drying temperature on volatile compounds in cocoa. *Food Chem*. **2012**, *132*, 277–288. https://doi.org/10.1016/j.foodchem.2011.10.078.
40. Torres-Moreno, M.; Tarrega, A.; Blanch, C. Effect of cocoa roasting time on volatile composition of dark chocolates from different origins determined by HS-SPME/GC-MS. *CyTA—J. Food* **2021**, *19*, 81–95. https://doi.org/10.1080/19476337.2020.1860137.
41. Lemarcq, V.; Van de Walle, D.; Monterde, V.; Sioriki, E.; Dewettinck, K. Assessing the flavor of cocoa liquor and chocolate through instrumental and sensory analysis: A critical review. *Crit. Rev. Food Sci. Nutr*. **2021**, *62*, 5523–5539. https://doi.org/10.1080/10408398.2021.1887076.
42. Schlüter, A.; Hühn, T.; Kneubühl, M.; Chatelain, K.; Rohn, S.; Chetschik, I. Novel Time- and Location-Independent Postharvest Treatment of Cocoa Beans: Investigations on the Aroma Formation during "Moist Incubation" of Unfermented and Dried Cocoa Nibs and Comparison to Traditional Fermentation. *J. Agric. Food Chem*. **2020**, *68*, 10336–10344. https://doi.org/10.1021/acs.jafc.9b06119.
43. van Ruth, S.M. Methods for gas chromatography-olfactometry: A review. *Biomol. Eng.* **2001**, *17*, 121–128. https://doi.org/10.1016/s1389-0344(01)00070-3.
44. Hinneh, M.; Abotsi, E.E.; Van de Walle, D.; Tzompa-Sosa, D.A.; De Winne, A.; Simonis, J.; Messens, K.; Van Durme, J.; Afoakwa, E.O.; De Cooman, L.; et al. Pod storage with roasting: A tool to diversifying the flavor profiles of dark chocolates produced from 'bulk' cocoa beans? (Part I: Aroma profiling of chocolates). *Food Res. Int*. **2019**, *119*, 84–98. https://doi.org/10.1016/j.foodres.2019.01.057.
45. Parker, J. Introduction to aroma compounds in foods. In *Flavour Development, Analysis and Perception* In *Food and Beverages*; Elsevier Ltd.: Amsterdam, The Netherlands , 2015. https://doi.org/10.1016/b978-1-78242-103-0.00001-1.
46. Servent, A.; Boulanger, R.; Davrieux, F.; Pinot, M.-N.; Tardan, E.; Forestier-Chiron, N.; Hue, C. Assessment of cocoa (Theobroma cacao L.) butter content and composition throughout fermentations. *Food Res. Int*. **2018**, *107*, 675–682. https://doi.org/10.1016/j.foodres.2018.02.070.
47. Afoakwa, E.O.; Quao, J.; Takrama, J.; Budu, A.S., Saalia, F.K. Chemical composition and physical quality characteristics of Ghanaian cocoa beans as affected by pulp pre-conditioning and fermentation. *J. Food Sci. Technol*. **2013**, *50*, 1097–1105. https://doi.org/10.1007/s13197-011-0446-5.
48. Afoakwa, E.O.; Quao, J.; Budu, A.S.; Takrama, J.; Saalia, F.K. Effect of pulp preconditioning on acidification, proteolysis, sugars and free fatty acids concentration during fermentation of cocoa (Theobroma cacao) beans. *Int. J. Food Sci. Nutr*. **2011**, *62*, 755–764. https://doi.org/10.3109/09637486.2011.581224.
49. Afoakwa, E.O.; Quao, J.; Takrama, F.S.; Budu, A.S.; Saalia, F.K. Changes in total polyphenols, o-diphenols and anthocyanin concentrations during fermentation of pulp pre-conditioned cocoa (Theobroma cacao) beans. *Int. Food Res. J*. **2012**, *19*, 1071–1077.
50. D'Souza, R.N.; Grimbs, A.; Grimbs, S.; Behrends, B.; Corno, M.; Ullrich, M.S.; Kuhnert, N. Degradation of cocoa proteins into oligopeptides during spontaneous fermentation of cocoa beans. *Food Res. Int.* **2018**, *109*, 506–516. https://doi.org/10.1016/j.foodres.2018.04.068.
51. Kumari, N.; Kofi, K.J.; Grimbs, S.; D'Souza, R.N.; Kuhnert, N.; Vrancken, G.; Ullrich, M.S. Biochemical fate of vicilin storage protein during fermentation and drying of cocoa beans. *Food Res. Int.* **2016**, *90*, 53–65. https://doi.org/10.1016/j.foodres.2016.10.033.
52. Voigt, J.; Textoris-Taube, K.; Wöstemeyer, J. pH-Dependency of the proteolytic formation of cocoa- and nutty-specific aroma

precursors. *Food Chem*. **2018**, *255*, 209–215. https://doi.org/10.1016/j.foodchem.2018.02.045.
53. Sunoj, S.; Igathinathane, C.; Visvanathan, R. Nondestructive determination of cocoa bean quality using FT-NIR spectroscopy. Comput. *Electron. Agric*. **2016**, *124*, 234–242. https://doi.org/10.1016/j.compag.2016.04.012.
54. Afoakwa, E.; Jennifer, Q.; Agnes, S.B.; Jemmy, S.T.; Firibu, K.S. Influence of pulp-preconditioning and fermentation on fermentative quality and appearance of ghanaian cocoa (Theobroma cacao) beans. *Int. Food Res. J.* **2012**, *19*, 127–133.
55. Ilangantileke, S.G., Wahyudi, T., Bailon, M.G. Assessment methodology to predict quality of cocoa beans for export. *J. Food Qual.* **1991**, *14*, 481–496. https://doi.org/10.1111/j.1745-4557.1991.tb00088.x
56. Brunetto, M.D.R.; Gallignani, M.; Orozco, W.; Clavijo, S.; Delgado, Y.; Ayala, C.; Zambrano, A. The effect of fermentation and roasting on free amino acids profile in Criollo cocoa (*Theobroma cacao* L.) grown in Venezuela. *Braz. J. Food Technol.* **2020**, *23*, 1–12. https://doi.org/10.1590/1981-6723.15019.
57. Crafack, M.; Keul, H.; Eskildsen, C.E.; Petersen, M.A.; Saerens, S.; Blennow, A.; Skovmand-Larsen, M.; Swiegers, J.H.; Petersen, G.B.; Heimdal, H.; et al. Impact of starter cultures and fermentation techniques on the volatile aroma and sensory profile of chocolate. *Food Res. Int.* **2014**, *63*, 306–316. https://doi.org/10.1016/j.foodres.2014.04.032.
58. Reineccius, G.A.; Andersen, D.A., Kavanagh, T.E., Keeney, P.G. Identification and Quantification of the Free Sugars in Cocoa Beans. *J. Agric. Food Chem*. **1972**, *20*, 199–202. https://doi.org/10.1021/jf60180a033.
59. Domínguez-Pérez, L.A.; Beltrán-Barrientos, L.M.; González-Córdova, A.F.; Hernández-Mendoza, A.; Vallejo-Cordoba, B. Artisanal cocoa bean fermentation: From cocoa bean proteins to bioactive peptides with potential health benefits. *J. Funct. Foods* **2020**, *73*, 104134. https://doi.org/10.1016/j.jff.2020.104134.
60. Kumari, N.; Grimbs, A.; D'Souza, R.N.; Verma, S.K.; Corno, M.; Kuhnert, N.; Ullrich, M.S. Origin and varietal based proteomic and peptidomic fingerprinting of Theobroma cacao in non-fermented and fermented cocoa beans. *Food Res. Int.* **2018**, *111*, 137–147. https://doi.org/10.1016/j.foodres.2018.05.010.
61. Tchouatcheu, G.A.N.; Noah, A.M.; Lieberei, R.; Niemenak, N. Effect of cacao bean quality grade on cacao quality evaluation by cut test and correlations with free amino acids and polyphenols profiles. *J. Food Sci. Technol.* **2019**, *56*, 2621–2627. https://doi.org/10.1007/s13197-019-03749-y.
62. Bonvehí, J.S.; Coll, F.V. Factors Affecting the Formation of Alkylpyrazines during Roasting Treatment in Natural and Alkalinized Cocoa Powder. *J. Agric. Food Chem.* **2002**, *50*, 3743–3750. https://doi.org/10.1021/jf011597k.
63. Caligiani, A.; Marseglia, A.; Prandi, B.; Palla, G.; Sforza, S. Influence of fermentation level and geographical origin on cocoa bean oligopeptide pattern. *Food Chem.* **2016**, *211*, 431–439. https://doi.org/10.1016/j.foodchem.2016.05.072.
64. Marseglia, A.; Palla, G.; Caligiani, A. Presence and variation of γ-aminobutyric acid and other free amino acids in cocoa beans from different geographical origins. *Food Res. Int.* **2014**, *63*, 360–366. https://doi.org/10.1016/j.foodres.2014.05.026.
65. ICCO. (n.d.). Cultivo de cacao - Organización Internacional del Cacao. Available online: https://www.icco.org/growing-cocoa/ (accessed on 27 May 2021)
66. Niether, W.; Smit, I.; Armengot, L.; Schneider, M.; Gerold, G.; Pawelzik, E. Environmental Growing Conditions in Five Production Systems Induce Stress Response and Affect Chemical Composition of Cocoa (*Theobroma cacao* L.) Beans. *J. Agric. Food Chem.* **2017**, *65*, 10165–10173. https://doi.org/10.1021/acs.jafc.7b04490.
67. Selamat, J.; Harun, S.M.; Ghazali, N.M. Formation of Methyl Pyrazine during Cocoa Bean Fermentation. *Pertanika* **1994**, *17*, 27–32.
68. Counet, C.; Ouwerx, C.; Rosoux, D.; Collin, S. Relationship between procyanidin and flavor contents of cocoa liquors from different origins. *J. Agric. Food Chem.* **2004**, *52*, 6243–6249. https://doi.org/10.1021/jf040105b.
69. Rentería, O.; Pliego-Arreaga, R.; Regalado, C.; Amaro-Reyes, A.; García-Almendárez, B.E. Enhancing isoamyl acetate biosynthesis by Pichia fermentans. *Rev. Mex. Ing. Química* **2021**, *12*, 505–511. https://doi.org/10.24275/rmiq/bio2125.
70. Schwab, W.; Davidovich-Rikanati, R.; Lewinsohn, E. Biosynthesis of plant-derived flavor compounds. *Plant J.* **2008**, *54*, 712–732. https://doi.org/10.1111/j.1365-313x.2008.03446.x.
71. Ziegleder, G. Linalool contents as characteristic of some flavor grade cocoas. *Z. Lebensm. Unters. Forsch.* **1990**, *191*, 306–309. https://doi.org/10.1007/bf01202432.
72. Maga, J.A. Pyrazine update. *Food Rev. Int*. **1992**, *8*, 479–558. https://doi.org/10.1080/87559129209540951.
73. Ziegleder, G. Composition of flavor extracts of raw and roasted cocoas. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 521–525. https://doi.org/10.1007/bf01202506.
74. Perotti, P.; Cordero, C.; Bortolini, C.; Rubiolo, P.; Bicchi, C.; Liberto, E. Cocoa smoky off-flavor: Chemical characterization and objective evaluation for quality control. *Food Chem.* **2020**, *309*, 125561. https://doi.org/10.1016/j.foodchem.2019.125561.
75. Lemarcq, V.; Tuenter, E.; Bondarenko, A.; Van de Walle, D.; De Vuyst, L.; Pieters, L.; Sioriki, E.; Dewettinck, K. Roasting-induced changes in cocoa beans with respect to the mood pyramid. *Food Chem.* **2020**, *332*, 127467. https://doi.org/10.1016/j.foodchem.2020.127467.
76. Spizzirri, U.G.; Ieri, F.; Campo, M.; Paolino, D.; Restuccia, D.; Romani, A. Biogenic Amines, Phenolic, and Aroma-Related Compounds of Unroasted and Roasted Cocoa Beans with Different Origin. *Foods* **2019**, *8*, 306. https://doi.org/10.3390/foods8080306.
77. Liu, M.; Liu, J.; He, C.; Song, H.; Liu, Y.; Zhang, Y.; Wang, Y.; Guo, J.; Yang, H.; Su, X. Characterization and comparison of key aroma-active compounds of cocoa liquors from five different areas. *Int. J. Food Prop.* **2017**, *20*, 2396–2408. https://doi.org/10.1080/10942912.2016.1238929.
78. Misnawi, J.; Ariza, B.T.S. Use of gas Chromatography-Olfactometry in combination with solid phase micro extraction for cocoa liquor aroma analysis. *Int. Food Res. J.* **2011**, *18*, 829–835.
79. Dand, R. Cocoa bean processing and the manufacture of chocolate. In *The International Cocoa Trade*, 3rd ed.; Woodhead Publishing: Sawston, UK, 2011; pp. 268–289. https://doi.org/10.1016/b978-0-85709-125-3.50009-4.
80. Beckett, S.T.; Paggios, K.; Roberts, I. Conching. In *Beckett's Industrial Chocolate Manufacture and Use*; John Wiley & Sons: Hoboken,

NJ, USA, 2017.
81. Barišić, V.; Kopjar, M.; Jozinović, A.; Flanjak, I.; Ačkar, Đ.; Miličević, B.; Šubarić, D.; Jokić, S.; Babić, J. The Chemistry behind Chocolate Production. *Molecules* **2019**, *24*, 3163. https://doi.org/10.3390/molecules24173163.
82. Toker, O.S.; Palabiyik, I.; Konar, N. Chocolate quality and conching. *Trends Food Sci. Technol.* **2019**, *91*, 446–453. https://doi.org/10.1016/j.tifs.2019.07.047.
83. Owusu, M.; Petersen, M.A.; Heimdal, H. Relationship of sensory and instrumental aroma measurements of dark chocolate as influenced by fermentation method, roasting and conching conditions. *J. Food Sci. Technol.* **2013**, *50*, 909–917. https://doi.org/10.1007/s13197-011-0420-2.
84. Toker, O.S.; Palabiyik, I.; Pirouzian, H.R.; Aktar, T.; Konar, N. Chocolate aroma: Factors, importance and analysis. *Trends Food Sci. Technol.* **2020**, *99*, 580–592. https://doi.org/10.1016/j.tifs.2020.03.035.
85. Liu, J.; Liu, M.; He, C.; Song, H.; Guo, J.; Wang, Y.; Yang, H.; Su, X. A comparative study of aroma-active compounds between dark and milk chocolate: Relationship to sensory perception. *J. Sci. Food Agric.* **2015**, *95*, 1362–1372. https://doi.org/10.1002/jsfa.6831.
86. Kitani, Y.; Putri, S.P., Fukusaki, E. Investigation of the effect of processing on the component changes of single-origin chocolate during the bean-to-bar process. *J. Biosci. Bioeng.* **2022**, *134*, 138–143. https://doi.org/https://doi.org/10.1016/j.jbiosc.2022.05.007.