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Influence of under-fermented cocoa mass in chocolate production: Sensory acceptance and volatile profile characterization during the processing

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ABSTRACT

Under-fermented cocoa mass (UCM) presents, as well as the fresh cocoa seed, a high content of phenolics compounds. For this reason, a chocolate with UCM added to the fermented cocoa mass (FCM) was developed. The sensory quality of chocolate is broadly determined by the composition of volatile compounds resulted from microbial metabolism during fermentation and Maillard reactions, that occur during drying, roasting, and conching. The aim of this work was to investigate the effect of adding UCM (20%–80%) to the FCM on the sensory characteristics of the chocolates produced and their volatile profiles during the process chain. The UCM and FCM were obtained through fermentation (48 h and 144 h, respectively), drying, roasting, and grinding processes. In general, the chocolate samples with a higher content of UCM presented lower scores for flavor acceptance, due to their higher bitterness and astringency. The great acceptance was observed on samples with 80% and 65% of FCM. A total of 55 different volatile compounds were identified by HS-SPME-GC-MS. The PCA analyses showed that the profile of the volatile compounds in the chocolate samples was influenced by the fermentation process, as well as the chocolate quality (flowery, honey, fruit, roasted, and chocolate flavors).

1. Introduction

Chocolate is a worldwide food appreciated by consumers, obtained through the processing of cocoa beans (*Theobroma cacao* L.) with addition of other ingredients such as sugar, cocoa butter, lecithin, and milk (depending on the type of chocolate) (Kruszewski & Obiedziński, 2018; Liu et al., 2015). Cocoa beans are the target of several scientific researches, due to their potential cardiovascular health benefits, antioxidant protections, and help in the cholesterol balance (Gültekin-Özgüven, Berktaş& Özçelik, 2016; Ooi, Ting, & Siow, 2020). These pro-health properties are due to the presence of menthylxantines, phenolic compounds such epicatechin, polyphenols, and anthocyanins, that are often responsible for the bitterness and astringency flavors of the fresh cocoa seed in the chocolate (Batista, de Andrade, Ramos, Dias, & Schwan, 2016; Urbańska & Kowalska, 2019).

The processing of cocoa beans comprises a series of transformations for chocolate production via pre-processing steps (harvesting, fermentation, and drying) and technological processing such as roasting, refining, conching, tempering, and crystallizing (Crafack et al., 2014; Tan & Kerr, 2019). The fermentation and drying processes are critical steps that determine the quality of the cocoa and the development of the precursors of the chocolate aroma and flavors (Beg, Ahmad, Jan, & Bashir, 2017; Pereira, Stellari, Vilela, Schwan, & Sant'Ana, 2020). However, the formation of the desirable aroma and flavor with chocolate notes is related to the decrease of total phenol content (395.15 up to 154.96 mgECE g^{-1}) during these stages (Melo et al., 2020). The fermentation process promotes a 30% reduction of polyphenol content in the first 48 h, reaching up to 90% at the end of the process (Albertini et al., 2015; Efraim et al., 2010), while the drying process is responsible for the browning of the cotyledons, as a result of the oxidation and catalysis reactions, carried out by the polyphenol oxidase (Kongor et al., 2016).

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Roasting, on other hand, is the first industrial process used for attaining the brown color and the chocolate aroma and flavor that are due to the presence of aldehydes, ketones, pyrazines, pyrroles, pyranones, and furans, formed by amino acids and sugars through the Maillard reaction and Strecker degradation (Afoakwa, Paterson, Fowlez & Ryan, 2008; Di Carro, Ardini, & Magi, 2015; Rocha, Santana, Soares, & Bispo, 2017). Through decades of investigation, the impact of the chocolate manufacturing chain on the final aroma and flavor, including the geographical origin of the cocoa, post-harvest storage, fermentation, drying (natural or artificial), roasting (temperature and time), and conching (time) process have been established (Acierno, Yener, Alewijn, Biasioli, & Van Ruth, 2016; Aculey et al., 2010; Afoakwa, 2014; Hinneh et al., 2019; Miguel et al., 2017; Tan & Kerr, 2019). However, the addition of under-fermented cocoa beans as an ingredient for chocolate production has not been studied yet.

The under-fermented cocoa beans, obtained at 48 h of fermentation, already present some volatile compounds (1-pentanol, 2,3-butanediol, 2-heptanol, phenethyl alcohol, 2-pentanone, 2-heptanone, benzaldehyde, phenylethyl acetate, 2-acetylpyrrole, and linalool) (Moreira, Vilela, Santos, Lima, & Schwan, 2018), with a fermentation index lower than 0.7, and a higher total phenol content (260.06 mgECE g^{-1}), because they have not gone through the entire fermentation process (Melo et al., 2020). Phenolic compounds have beneficial effects on human health, which have raised an interest in maintaining them during the processing of cocoa products, like chocolate (Efraim et al., 2010; Lončarević et al., 2018). Moreover, they can impart sensory properties such as color and taste, especially regarding bitter and astringent flavors. For this reason, the use of under-fermented cocoa beans can increase the concentration of antioxidant compounds, severely affecting the sensory characteristics of chocolates. Thefore, the aim of this study was to evaluate the effect on the sensory characteristics of chocolates of adding under-fermented cocoa mass using acceptance tests and to identify the presence of volatile compounds formed during the fermentation and roasting processes used in the process of chocolate making.

2. Materials and methods

2.1. Raw material

The conventional cocoa (mixture of several cocoa species), used as raw material in this study, was obtained from farms located at the south of Bahia, Brazil, at 14°41′96″ S and 39°12′109″ W and at 14°30′3″ S and 39°19′5″ W. The fruits were harvested in the second crop (August 2018) at the maturation stage, determined by the color of the fruit epidermis. The fruits were manually opened and the fresh cocoa seeds with their surrounding mucilage pulp were submitted immediately to the fermentation process.

2.2. Fermentation and drying processes

The fermentation and drying processes were performed at the farm following the standards of the producer. The fermentation occurred in duplicate, using batches of 40 kg of fresh cocoa seeds. The raw material was placed in 50 cm × 40 cm x 70 cm wooden boxes, with holes with a diameter of 1 cm in the bottom, spaced 5 cm from each other, and covered with plantain leaves for natural fermentation. The fermentation was carried out under two conditions: under-fermentation stage (until 48 h) and complete fermentation (144 h), both conditions carried out at room temperature (25 °C). According to Melo et al. (2020), the highest content of phenolic compounds can be obtained at 48 h fermentation. After this period, there is a great reduction of these substances due to changes in the cocoa beans. Therefore, the condition of 48 h was chosen.

During the fermentation process, the parameters of temperature, total acidity, and pH were monitored (AOAC, 1995). Based on the Barel (2016) methodology, the cocoa beans were daily transferred from one box to another one manually, in order to ensure their aeration and to homogenize the fermentation of the cocoa mass. Then, the under-fermented and the fermented cocoa beans of each batch were sun dried in barges for 5–7 days, until the final moisture of 8% (Afoakwa, Paterson, Fowler, & Ryan, 2008). The beans were manually moved once a day in order to obtain a uniform drying. After the drying process, the cocoa beans were bagged and transported to the Pharmacy School at UFBA, where they were stored at room temperature until the analysis was performed.

2.3. Cocoa and chocolate processing

The cocoa beans were submitted to the cleaning and roasting steps (Drying oven, DeLeo, model A35EAF8, 0612), at 120 °C for 25 min, following the methodology established by the chocolate industries. Then, the roasted cocoa beans were crushed by a manual press with a wooden roll to remove the peel and germ and obtain the cocoa nibs.

The cocoa mass content was determined according to the simplex planning, through the independent variables X_1 and X_2 (Table 1), which represent the percentage of under-fermented and fermented cocoa nibs, respectively. The levels ranged from 20% to 80%, according to preliminary experiments. The control treatment was identified as FCN (100% fermented cocoa nibs).

The base formulation for the production of chocolates was 66.3% of cocoa content: cocoa mass (610 g), commercial refined sugar (333.5 g), cocoa butter (53 g) (Barry Callebaut, Ilheus), and soy lecithin (3.5 g) (Our Creator, Pantec Food Technology, São Paulo). The cocoa nibs were ground (NutriNinja, model BL480BR30, HAI XIN Technology (Shezhen)) in order to obtain the liquor, which was transferred with the other ingredients to the conching step in the Melanger equipment (Model Spectra 11, USA). The conching and refining process lasted 24 h, following the methodology of Leite, Bispo, and Santana (2013). The chocolates were produced with the same proportions of cocoa mass mentioned in Table 1. The control chocolate was identified as CC. Then, the chocolates were molded in a polyethylene mold, producing 5 g bars. The chocolates were cooled, wrapped in aluminum packaging, and stored at 8 °C for later analyses.

2.4. Sensory evaluation of chocolates

The judges received 5 g squares of each chocolate sample, served in plastic plates labelled with 3-digit random numbers, according to a randomized block design. Water and cracker biscuit without salt were used to cleanse the palate. The judges were untrained volunteers without allergy to cocoa or chocolate, and regardless of age, sex, or social class, were recruited from the Federal University of Bahia, Brazil, and invited to participate in the sensory tests according to their available time. Ethical approval was granted by the Research Ethics Committee of the Universidade Estadual da Bahia - UNEB (Process n.1.231.812) and CAAE (Presentation Certificate for Ethical Appreciation), under the

Table 1
List of identifiers of cocoa nibs and chocolate samples with the independent variables obtained from the simplex planning.

Samples		Independent variables	
Nibs	Chocolates	X_1 (%)	X_2 (%)
N1	C1	80	20
N2	C2	20	80
N3	C3	65	35
N4	C4	35	65
N5	C5	50	50
UCN	–	100	0
FCN	CC	0	100

X_1: under-fermented cocoa nibs (UCN) and under-fermented cocoa mass (UCM) for chocolate samples; X_2: fermented cocoa nibs (FCN) and fermented cocoa mass (FCM) for chocolate samples.

number 47478315.0.0000.0057. Prior to testing, each participant signed an informed consent form.

A sensory evaluation using the Just-about-right Test was performed for the evaluation of the bitterness and astringency attributes in the chocolate samples using a five point scale ranging from 5 = much more bitter/astringent than the ideal to 1 = much less bitter/astringent than the ideal. The test was carried out in individual sensory booths at 22 °C with white light using 75 volunteers. The acceptance test was carried out with 120 volunteers that evaluated the appearance, aroma, flavor, texture, and overall acceptability of the chocolates using a 9-points hedonic scale (9 = liked extremely to 1 = disliked extremely); and in the same session, the judges also evaluated the purchase intention using a purchase intention test (Meilgaard, Carr, & Civille, 2006), using a 5-points scale, ranging from 5 = certainly would buy to 1 = certainly would not buy.

2.5. Analysis of volatile compounds

2.5.1. Extraction of volatile compounds

The volatile compounds from samples (2.0 g) shown in Table 1 were extracted, separated and identified using the Headspace-Solid Phase Micro Extraction (HS-SPME) Gas Chromatography-Mass Spectrometry (GC–MS) technique, as previously described (Rodriguez-Campos, Escalona-Buendía, Orozco-Avila, Lugo-Cervantes, & Jaramillo-Flores, 2011). A 50/30 μm divinylbenzene/carboxen/polydimentylsiloxane (DVB/CAR/PDMS) SPME fiber provided by Supelco (Bellefonte, PA, USA) was used to extract the volatile compounds from the seeds, cocoa beans, cocoa nibs, and chocolates. The fiber was equilibrated for 5 min at 250 °C, while the sample was equilibrated for 20 min at 60 °C and then exposed to the fiber for 40 min at the same temperature. The chocolate samples analyzed by the HS-SPME-GC-MS were chosen (C1, C2, and C4), as well as their respective cocoa nibs. The samples unfermented cocoa beans (UCS), under-fermented and dried cocoa beans (UCB), fermented and dried cocoa beans (FCB), and control chocolate (CC), were also analyzed in duplicate.

2.5.2. Separation and identification of volatile compounds

The volatile compounds were analyzed by the Gas Chromatography–Mass Spectrometry (GC–MS) (Perkin Elmer Clarus 500, Model CT 06484, Shelton, EUA), equipped with an Omegawax 250 capillary column (30 m × 0.25 mm x 0.25 μm film thickness). The oven temperature was set at 50 °C for 1 min, which was then increased up to 240 °C at a rate of 3 °C min^{-1}, and finally maintained at 240 °C for 5 min. The carrier gas was high-purity helium at 1.0 mL min^{-1}. The injector, at splitless mode was set at 250 °C for (5 min). The selective mass detector was a quadrupole (Perkin Elmer Clarus 500, Model CT 06484, Shelton, EUA), with an electronic impact ionization system at 70 eV, 230 °C of interface temperature, 150 °C of quadrupole temperature, and 230 °C of source temperature. The injections were performed by fiber exposition for 5 min at 250 °C. Volatile compounds were identified by comparing the mass spectra of the sample compounds with the database of the National Institute of Standards and Technology (NIST Mass Spectral library, version 2.0, 2005), with a spectral matching expressed in at least 80 area%. (Utrilla-vázquez, Rodríguez-Campos, Avendaño-Arazate; Gschaedler & Lugo-Cervantes, 2020). The maximal acceptable coefficient of variation was 30 area% for a given compound (Cevallos-Cevallos, Gysel, Maridueña-Zavala, & Molina-Miranda, 2018). The aroma descriptors were obtained from the literature. The amount of the individual volatile compounds were calculated through the integration of their peak areas (Ascrizzi, Flamini, Tessieri, & Pistelli, 2017; Pereira et al., 2020).

2.6. Statistical analysis

The data were presented as mean ± standard deviation. The scores obtained from the Just-about-right Test was evaluated by the Friedman test at a 5% significance level ($p < 0.05$). The data of the acceptance and purchase intention tests were subjected to ANOVA using Tukey test at 5% significance ($p < 0.05$). The peak area of each volatile compound was evaluated by using the Principal Component Analysis (PCA). The Tukey test at 5% significance level ($p < 0.05$) was also applied to analyze the differences among the total volatile content. All statistical analyses were performed using XLSTAT® (Addinsoft INC., Anglesey, United Kingdom, 2020).

3. Results and discussion

3.1. Sensory evaluation of chocolates

The scores for the appearance and texture attributes corresponded to the hedonic term "liked moderately" (Table 2). The samples C1–C5 were not significantly different ($p > 0.05$) for these attributes. The scores for the aroma attribute varied between "liked slightly" and "liked moderately", and the flavor attribute varied between "disliked slightly" and "liked slightly". The C1 sample presented a low score for flavor and overall acceptability. The scores of overall acceptability varied between "neither liked/neither disliked" and "moderately liked". The relationship between the presence of under-fermented cocoa mass (UCM) and sensory acceptability seems to be highly significant ($p < 0.05$). The chocolate samples with higher content of UCM showed low scores for consumer acceptance, possibly due to the presence of bitter and astringent flavors caused by higher levels of phenolic compounds. High acceptance scores were observed on chocolate samples with fermented cocoa mass (FCM) proportions of 20% (C2) and 35% (C4).

Regarding the Just-about-right Test for the intensity of bitterness and astringency attributes, samples C1 and C3 presented high scores of bitteress and astringency. This result was already expected, since both samples were developed with the highest percentage of UCM, which contains a high content of phenolic compounds not oxidized during the uncomplete fermentation and roasting processes. Samples C4 and C5 indicated intermediate intensities for the bitter and astringent tastes, respectively. Sample C2 obtained the lowest intensity for both attributes, caused by the lower amount of UCM used in the formulation.

A study conducted by Cruz, Andrade, Marques, Bispo, and Maciel (2019) proved the high content of phenolic compounds in chocolate samples produced by mixtures of under-fermented and fermented cocoa

Table 2
Averages of Acceptance Test, Just-about-Right test of bitter and astringency parameters, and Purchase Intention of chocolates produced.

Samples/Average scores					
Attributes	C1	C2	C3	C4	C5
Acceptance Test					
Appearance	7.64 ± 1.41^{a}	7.87 ± 1.29^{a}	7.68 ± 1.35^{a}	7.86 ± 1.09^{a}	7.69 ± 1.33^{a}
Aroma	6.75 ± 1.64^{b}	7.34 ± 1.46^{a}	6.73 ± 1.64^{b}	$7.24 \pm 1.38^{a,b}$	$7.00 \pm 1.47^{a,b}$
Flavor	4.88 ± 2.36^{c}	$6.36 \pm 2.19^{a,b}$	$5.60 \pm 2.17^{b,c}$	6.56 ± 2.02^{a}	$5.84 \pm 2.13^{a,b}$
Texture	7.02 ± 1.85^{a}	7.50 ± 1.53^{a}	7.09 ± 1.89^{a}	7.58 ± 1.40^{a}	7.23 ± 1.59^{a}
Overall Acceptability	5.35 ± 2.07^{c}	6.81 ± 1.81^{a}	$6.03 \pm 2.03^{b,c}$	6.90 ± 1.79^{a}	$6.36 \pm 1.97^{a,b}$
Just-about-right Test*					
Bitter (%)	3.75 ± 1.40^{c}	2.09 ± 1.36^{a}	$3.11 \pm 1.43^{b,c}$	2.72 ± 1.40^{a}	2.91 ± 1.31^{b}
Astringency (%)	3.75 ± 1.45^{b}	2.01 ± 1.26^{a}	3.17 ± 1.46^{b}	2.56 ± 1.35^{a}	3.13 ± 1.29^{b}
Purchase Intention	2.38 ± 1.26^{a}	3.56 ± 1.44^{c}	2.89 ± 1.36^{b}	3.56 ± 1.32^{c}	2.93 ± 1.33^{b}

Mean ± Standard Deviation. Means with the same letter are not significantly different by ANOVA (Tukey test; $P > 0.05$; *Friedman test; $P > 0.05$). C1 (80% under-fermented cocoa mass (UCM): 20% fermented cocoa mass (FCM)); C2 (20%: 80%); C3 (65%: 35%); C4 (35%: 65%), and C5 (50%: 50%).

masses, with proportions of 20–80%. All samples showed high phenolic content: C1 (87.82 mg/g), C2 (65.46 mg/g), C3 (85.04 mg/g), C4 (70.74 mg/g), and C5 (72.13 mg/g). In addition, the authors also evaluated the total flavonoid content: C1 (19.45 mg/g), C2 (12.90 mg/g), C3 (17.40 mg/g), C4 (14.73 mg/g), and C5 (15.07 mg/g). The highest contents of phenolic compounds and flavonoids were observed in samples C1 and C3, produced with 80% and 65% of UCM, respectively. Considering these results, it is possible to observe the influence of the phenolic compounds content on the sensory acceptance of the chocolates. The samples produced with higher amounts of UCM had a higher content of phenolic compounds and flavonoids and, consequently, higher bitterness and astringency, which resulted in a lower acceptance by the judges.

Jinap, Jamilah, and Nazamid (2005) studied changes in the polyphenol ability to produce astringency during the roasting of cocoa beans. Their results indicated that astringency reduction in cocoa products is determined by the loss of polyphenols through their oxidation and exudation from the cocoa beans, and by interactions with proteins during the process. The polyphenol–protein interaction would reduce the ability of polyphenol to precipitate salivary proline-rich proteins, thus decreasing the astringent sensation.

Regarding the purchase intention, samples C2 and C4 showed the same positive score, corresponding to the hedonic term “certainly would buy”, followed by sample C5, with a high intention of “maybe buying/maybe not buying”, and sample C1 exhibited the lowest purchase intention represented by the category “certainly would not buy”. There was a significant difference ($p < 0.05$) between the evaluated attributes from the just-about-right test and purchase intention test.

Fig. 1 shows the frequency distribution of “acceptance” (scores 6.0 to 9.0), “indifference” (scores equal to 5.0), and “rejection” (scores 1.0 to 4.0), associated with chocolate samples for attributes such as aroma, flavor, texture, and overall acceptability through the hedonic scale used. Also, the frequencies of “ideal” (score 3.0), “more intense than the ideal” (scores 4.0 to 5.0), and “less intense than the ideal” (scores 1.0 to 2.0) are found for the bitterness and astringency attributes. Finally, the figure shows the frequencies of “positive” (scores 4.0 to 5.0), “indifferent” (score 3.0), and “negative” (scores 1.0 to 2.0) responses for purchase intention.

Regarding the optimal bitterness and astringency, the C5 sample stood out with 37% and 30%, respectively. The purchase intention was considered adequate for all samples, since those produced with a higher content of UCM were already expected to have the lowest purchase intention. Consequently, samples C2 and C4 showed 58.5% positive purchase intention. In contrast, sample C2 showed 22.88% of negative intention while sample C4 presented only 19.49%, being the preferred chocolate sample for all the judges. In overall assessment, the best acceptance for the attributes appearance, aroma, flavor, texture, overall acceptability, bitterness, astringency, and purchase intention was presented by the C4 sample, followed by the C2 and C5 samples.

Fig. 2 shows the internal preference map of 120 judges for the overall acceptability attribute, explaining 62.43% of the total variation, shown in two components; F1 (39.96%) and F2 (22.48%). The preference of most judges for the C4, C2, and C5 samples (lowest % of UCM). Fig. 2A exhibits the samples preferred by the judges, plotted on the positive and negative axes, whilst in Fig. 2B the dots represent the 120 judges, located close the samples of their preference. The preference for these chocolate samples probably was due to their lower bitterness and astringency. Conversely, the less accepted chocolate samples (C1 and C3) presented 80% and 65% of UCM, respectively.

3.2. *Total volatile compounds content*

Fifty-five volatile compounds were identified by the HS-SPME-GC-MS during the fermentation and drying processes in the cocoa nibs and in the chocolate samples. Their functional chemical groups are shown in Table 3. Some of these compounds have been reported as responsible for the desirable flavors and off-flavors in cocoa beans during the fermentation, drying, and roasting processes (Rodriguez-Campos et al., 2011). Twenty-five volatile compounds were detected at the beginning (0 h) of the fermentation process; while 29 and

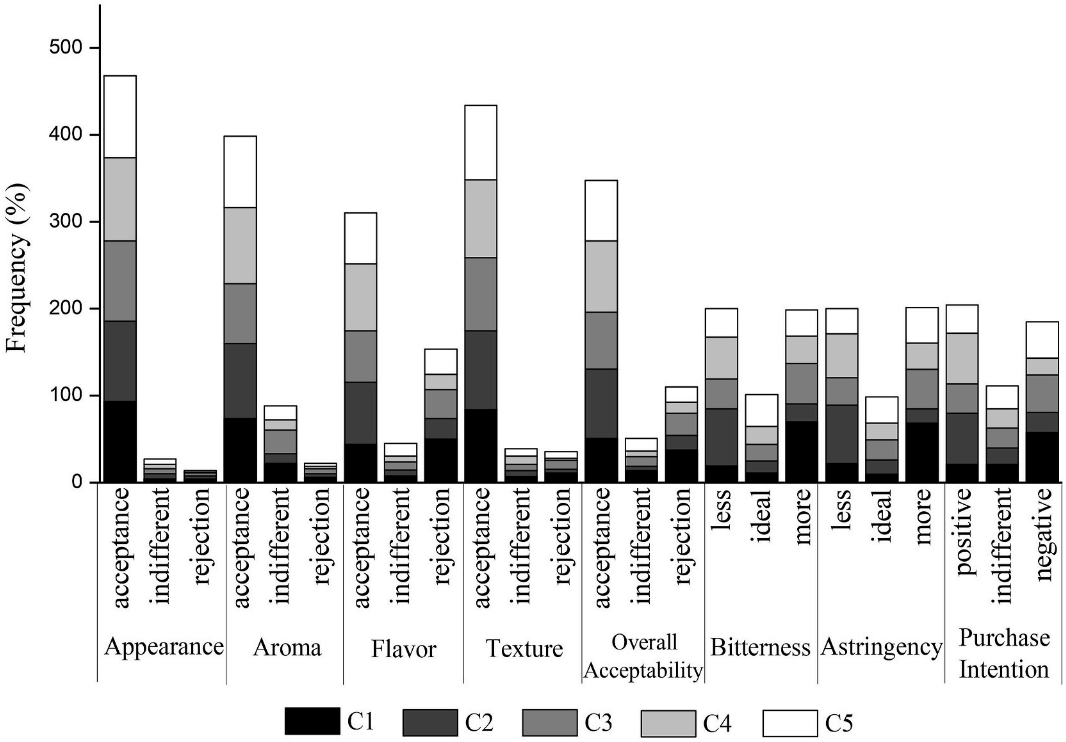

Fig. 1. Frequency (%) of scores assigned to the chocolate samples in relation to acceptance, just-about-right test and purchase intention.
C1 (80% under-fermented cocoa mass (UCM): 20% fermented cocoa mass (FCM)), C2 (20%: 80%), C3 (65%: 35%), C4 (35%: 65%) and C5 (50%: 50%).

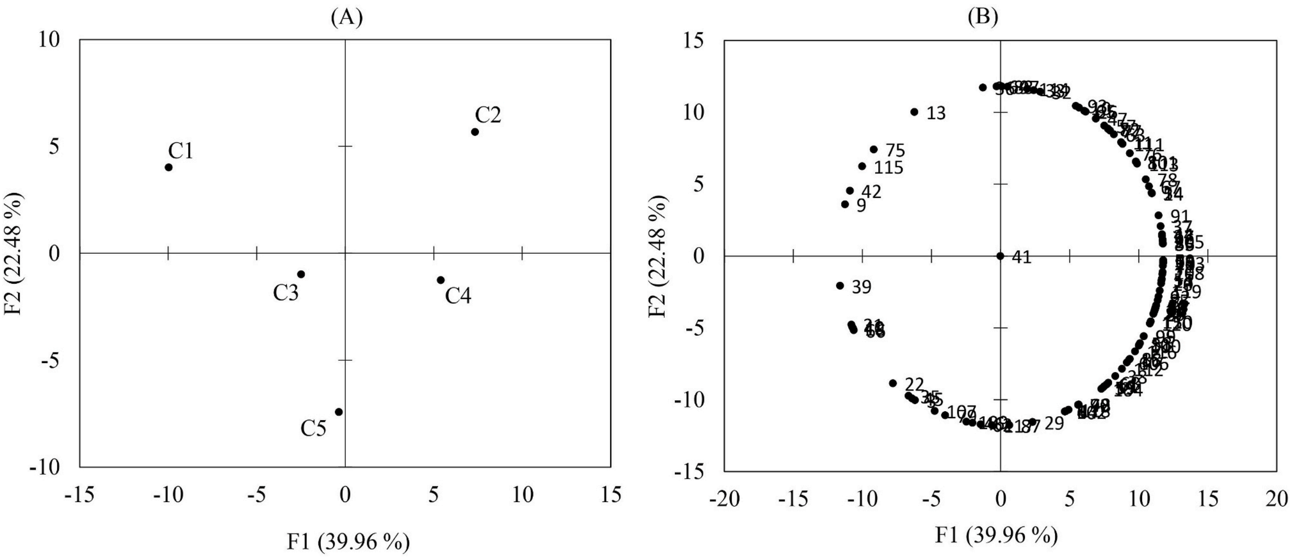

Fig. 2. Internal Preference Mapping obtained with the results of the overall acceptability of the chocolates. Samples preferred by the judges (A) and representation of judges (B).
C1 (80% under-fermented cocoa mass (UCM): 20% fermented cocoa mass (FCM)); C2 (20%: 80%); C3 (65%: 35%); C4 (35%: 65%) and C5 (50%: 50%).

35 volatile compounds were found at 48 h and 144 h of fermentention, respectively. Forty-nine volatile compounds were detected in the cocoa nib samples and 49 volatile compounds were detected in the chocolate samples.

In general, the total content of alcohols, acids, pyrazines, and esters was higher than the content of aldehydes and ketones, pyrroles, furans, furones, pyrans, pyranones, terpene alcohols, and other substances (Fig. 3). The ester (13) group showed a higher number of individual compounds than alcohols (7), pyrazines (7), ketones (7), furans, furones, pyrans, pyranones (6), acids (4), aldehydes (3), pyrroles (3), terpene alcohols (3), and other compounds (2). A higher number of compounds was found by Moreira et al. (2018) at the beginning and end of the fermentation of hybrid cocoa (CEPEC2004, PH15, PS1319 and SJ02) and chocolate samples. They found 23 alcohols, 18 esters, 13 ketones, 11 aldehydes, 9 acids, 2 pyrazines, 1 pyrrole, and 1 compound of another group.

3.2.1. *Acids*

Four volatile acids were detected in all samples and the total content of acids increased significantly ($p < 0.05$) during the fermentation and drying processes (Fig. 3). This high acid content is the result of the metabolization of the sugars contained in the cocoa pulp (Bonvehí, 2005). The highest total acid content was found in the FCB samples (17.04 area%) due to the presence of the nonanoic acid. The chocolate samples were characterized by the presence of the following identified acids: nonanoic acid (5.52–12.23 area%), octanoic acid (1.11–4.62 area%), 2-ethylhexanoic acid (0.23–1.46 area%), and decanoic acid (0.29–0.74 area%).

3.2.2. *Alcohols*

Alcohols were the most prevalent group (>30%) of volatile compounds detected in most samples (Fig. 3). This chemical group is generally desirable in chocolates with flowery and candy notes (Aculey et al., 2010). They are produced during the fermentation process as a result of microbial activity and heat degradation of amino acids (Rodriguez-Campos et al., 2011). However, the alcohol content decreases through the chemical degradation or volatilization during the drying and roasting processes. This fact was confirmed by the higher total alcohol content ($p < 0.05$) found in the UCB sample (69.95 area%), collected during the intense period of microbiological activity in the fermentation process and, consequently, in the UCN (48.20 area%), N1 (43.24 area%), and C1 (42.75 area%) samples. The elevated content was due to the presence of the phenylethyl alcohol (flowery, spicy, and honey-like aroma), a superior alcohol that increased over the fermentation and drying processes (4.77–49.48 area%), and decreased during roasting (21.90–35.83 area%) and in the chocolate samples (25.69–36.87 area%). The 2,3-butanediol, another alcohol desirable for high quality cocoa products, was observed in samples with a high content of fermented dry cocoa beans: FCB (5.81 area%), FCN (5.54 area%), N2 (8.62 area%); decreasing after the conching process in C2 (5.13 area%) and CC (4.40 area%). The influence of the under-fermented dry cocoa beans was also observed in 1-pentanol, which presented a high content in the UCB (17.30 area%), UCN (5.47 area%), N1 (5.61 area%), and C1 (1.04 area%) samples.

3.2.3. *Aldehydes and ketones*

Aldehydes and ketones are formed by the Strecker degradation of the free amino acids during the roasting process (Afoakwa et al., 2008; Aprotosoaie, Luca, & Miron, 2016; Ziegleder, 2009). A high content of aldehydes and ketones is favorable for the cocoa quality, due to the desirable cocoa and malty flavors. The mainline aldehyde found in all samples was benzaldehyde (2.23–20.37 area%), which is responsible for an intense bitter taste (Bonvehí, 2005). The 2-methylbutanal (malty and chocolate-like flavors) was also detected in the processing steps, albeit with a low content that varied between 0.21 area% - 3.84 area%. The highest total aldehyde content ($p < 0.05$) was found in the N4 sample (21.50 area%).

The influence of the under-fermented cocoa mass (UCM) was observed in the ketone group. The 2-nonanone and acetophenone were found in all samples. The concentration of 2-nonanone was higher in the samples with a high amount of fermented dry cocoa beans: FCB (16.00 area%), FCN (3.55 area%), N2 (3.43 area%), C2 (2.68 area%), and CC (3.66 area%); while the acetophenone showed a higher content in samples with a high amount of under-fermented dry cocoa beans: UCB (7.87 area%), UCN (4.37 area%), N1 (3.91 area%), and C1 (3.00 area%). These compounds produced cocoa with flowery and sweet flavor notes (Utrilla-Vázquez, Rodríguez-Campos, Avendaño-Arazate, Gschaedler, & Lugo-Cervantes, 2020). However, the highest total content of ketones ($p < 0.05$) was identified in UCS (52.78 area%), decreasing during the processing steps (Fig. 3).

Table 3
Volatile compounds identified by the Headspace-Solid Phase Microextraction-Gas Chromatography Mass Spectrometry (HS-SPME-GC-MS) during different fermentation periods, in roasted cocoa beans (nibs) and chocolate samples.

Group	Retention time (min)	Compound	Odor description	Samples found		
				Cocoa beans	Cocoa nibs	Chocolates
Acids						
1	37.49	2-ethyl hexanoic acid		all		all
2	41.39	octanoic acid	Sweet, cheese, oily, fatty[b,e]	all	FCN, N1, N2, N4	all
3	44.61	nonanoic acid	Green, fatty[b]	all	FCN, N1, N2, N4	all
4	47.91	decanoic acid	Rancid, fatty, metal[b]	FCB		all
Alcohols						
5	5.00	prenol		UCS		
6	6.74	3-methyl-2-butanol			UCN, N1	C1
7	9.37	1-pentanol	Flowery, sweet[d], nail polish, pungen[e]	all	all	all
8	13.45	2-heptanol	Citrus, fresh, lemon grass-like[a], sweet, citrusy[b,c,e,f]	all	all	all
9	15.60	3-ethoxy-1-propanol		UCB	UCN	
10	22.25	2,3-butanediol	Cocoa butter[c,f], sweet, flowery[d], creamy, buttery[e]	UCB, FCB	all	all
11	35.67	phenylethyl alcohol	Flowery[d], spicy, honey-like, rose[a,b], caramel[e]	all	all	all
Aldehydes						
12	3.25	2-methylbutanal	Malty, chocolate-like[a,b,c,f], cocoa[d,e]	UCS, FCB	FCN, N1, N2, N4	all
13	21.36	benzaldehyde	Bitter[a,c], grass[d], candy, almond, burnt sugar[e]	all	all	all
14	40.98	5-methyl-2-phenyl-2-hexenal	Cocoa[a,e], sweet, roasted cocoa[d]		FCN, N1, N2, N4	C2, C4, CC
Esters						
15	2.93	ethyl acetate	Pineapple[e,f], jasmine[b], nail polish, fruity[d]	FCB	FCN, N1, N2, N4	
16	4.44	isobutyl acetate	Fruit[,f], apple, banana[b,d,e]	FCB	N1, N2, N4	
27	5.65	2-pentanol acetate = amyl acetate		FCB	FCN, N1, N2, N4	
18	6.88	isoamyl acetate	Banana[b], pear[d], fruit[f]	FCB	all	C1, C2, CC
19	17.98	ethyl octanoate	Fruity, floral[,f], pineapple[a,e], flowery[e]	UCB, FCB	all	all
20	25.90	ethyl decanoate	Pear, grape[,f], brandy[a,b,d,e]	FCB	FCN, N2, N4	all
21	26.64	1,3-propanediol diacetate			FCN	C2, C4, CC
22	27.40	diethyl succinate	Pleasant aroma[f]		FCN, N1, N2, N4	C2, C4, CC
23	29.25	benzyl acetate	Floral, jasmine[b,f], rose[d], fresh[e]	UCB, FCB	FCN, N2, N4	C2, C4, CC
24	31.30	ethylphenyl acetate	Fruit, sweet[,f], honey-like[a,b], flowery, rose[d,e]	UCB, FCB	all	all
25	32.33	2-phenylethyl acetate	Fruity, sweet[c], honey[a], floralf, flowery[d]	all	all	all
26	33.13	isopenthyl acetate		all	all	all
27	33.37	ethyl laurate	Leaf, fruit, floral[b,c,f]	UCB, FCB	all	all
Furans, Furones, Pyrans & Pyranones						
28	19.18	Furfural	Bread, almond, sweet[e]		UCN, N1	C1
29	23.40	5-methyl furfural	Caramellic, bready, coffee-like[e]		all	
30	26.84	furfuryl alcohol			UCN, N1	C1
31	37.43	3-hydroxy-2-methyl-4-pyrone (maltol)	Nutty[f]		FCN, N1, N4	
32	40.66	5-acetyldihydro-2(3H)-furanone			all	all
33	47.18	3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one			FCN, N4	CC
Ketones						
34	3.97	2-pentanone	Fruit[f], sweet, cheesy[b,d,e]	all	UCN	
35	8.60	2-heptanone	Fruity[f], cheese-like[a], flowery[b], green[d], pear, grape, brandy[e]	all	all	
36	16.13	2-nonanone	Flowery, fatt[d],fruit, musty[e]	all	all	all
37	23.54	3,6-heptanedione		UCS		
38	25.39	Butyrolactone	Must, flowery, almond, sweet, aromatic creamy[b,e]	UCB, FCB	all	all
39	26.21	Acetophenone	Sweet, almond, flowery[f], must-like[a,b], almond, pungent[e]	all	all	all
40	38.51	3-methoxy-3-methyl-2-butanone		UCS		
Pyrazines						
41	11.37	Methylpyrazine	Cocoa, green, hazelnut[d], nutty, chocolate, roasted[e]	UCB, FCB	all	all
42	13.87	Ethylpyrazine	Peanut-butter, musty, nutty[d]		UCN, N1, N2, N4	
43	14.34	2,3-dimethylpyrazine	Caramel, cocoa[a,b,e], sweet[f], baked[d]	all	all	all
44	15.86	2-ethyl-6-methylpyrazine	Roasted, green, coca-like[a]		UCN, N2, N4	
45	16.57	Trimethylpyrazine	Cocoa, roasted nuts, peanut[e]	all	all	all
46	19.36	Tetramethylpyrazine	Roasted, green, coffee, cocoa[a], milk-coffee, chocolate[b,e]	all	all	all
47	20.88	2,3,5-trimethyl-6-ethylpyrazine	Candy, sweet[b,d,f]		FCN, N1, N2, N4	all
Pyrroles						

(*continued on next page*)

Table 3 (*continued*)

Group	Retention time (min)	Compound	Odor description	Samples found		
				Cocoa beans	Cocoa nibs	Chocolates
48	37.68	2-acetylpyrrole	Chocolate, hazelnut[b,c,d,e,f]	all	all	all
49	39.45	1H-pyrrole-2-carboxaldehyde	Nutty[f]		all	all
50	42.01	2-formyl-1-methylpyrrole			all	CC
Terpene alcohols						
51	18.16	*cis*-linalool oxide (furanoid)	Sweet, nutty[a,f], flowery[d]	UCS, UCB		
52	22.49	linalool	Flowery[a], lavender[c], rose[d]	all	all	all
53	30.54	*trans*-linalool oxide (pyranoid)	Floral[a]	all	All	all
Others						
54	33.96	2-methoxy phenol (guaiacol)	Smoke, sweet[c], phenol, spicy[e]	FCB	FCN, N4	CC
55	63.87	xanthone		all	All	all

UCS: unfermented cocoa seed; UCB: under-fermented dry cocoa beans; FCB: fermented dry cocoa beans; UCN: under-fermented cocoa nibs; FCN: fermented cocoa nibs; N1 (80% of UCN: 20% of FCN); N2 (20%: 80%); N4 (35%: 65%); C1 (80% under-fermented cocoa mass (UCM): 20% fermented cocoa mass (FCM)); C2 (20%: 80%); C4 (35%: 65%), and CC (100% of FCM).

[a] Ascrizzi et al. (2017).

[b] Rodriguez-Campos et al. (2011) and Rodriguez-Campos et al. (2012).

[c] Moreira et al. (2018).

[d] Crafack et al. (2014).

[e] Utrilla-Vázquez et al. (2020).

[f] Aprotosoaie et al. (2016).

3.2.4. *Esters*

Esters are the second most important class of volatile compounds after pyrazines, which result in a fruity flavor for cocoa and its derivatives (Rodriguez-Campos et al., 2012). They originate from the reaction of an organic acid with an alcohol during the anaerobic phase of fermentation (Aprotosoaie et al., 2016; Qin et al., 2017). Thirteen esters were identified in this study (Table 3). The highest total content of esters ($p < 0.05$) was observed in the FCB (21.41 area%) sample due to 2-phenylethyl acetate. This substance was found in high concentration in most of the samples with a high amount of fermented dry cocoa beans: FCB (7.50 area%), FCN (9.87 area%), N2 (9.03 area%), C2 (9.54 area%), and CC (11.13 area%). This volatile compound is also responsible for one of the key aromas. Ethyl phenylacetate (fruit, sweet, and honey-like flavors) was another desirable ester found in samples with a high content of fermented cocoa beans (0.11–2.62 area%).

3.2.5. *Pyrazines*

The group of pyrazines is one of the most important volatile compounds in roasted cocoa beans, representing 40% of the aroma in roasted cocoa (Bonvehí, 2005). Most pyrazines are obtained from the Strecker degradation and Maillard reactions, mainly present in foods processed under high temperature and low-moisture conditions (Yu & Zhang, 2010). However, tetramethylpyrazine (cocoa and chocolate flavors), trimethylpyrazine (cocoa and roasted nuts flavors), and methylpyrazine (nutty, roasted, and chocolate flavors) were reported to result partially from microbiological activities during fermentation (Hinneh et al., 2019). In this study, the total pyrazine group content ($p < 0.05$) ranged from 4.52 area% to 29.13 area% (Fig. 3), and its highest total content was found in the CC (29.13 area%), C2 (26.11 area%), and C4 (24.03 area%) samples, respectively, which might explain the positive acceptance of these chocolates. The highest concentrations observed

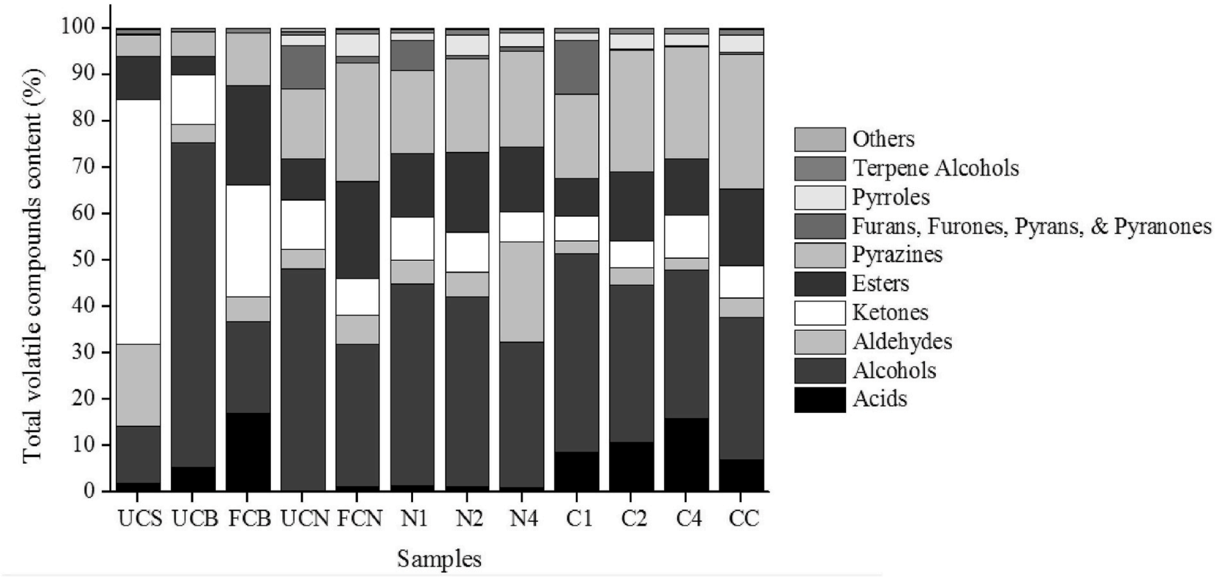

Fig. 3. Total relative concentration of the major classes of volatile compounds identified by the HS-SPME-GC–MS from different process stages.
UCS: unfermented cocoa seed; UCB: under-fermented dry cocoa beans; FCB: fermented dry cocoa beans; UCN: under-fermented cocoa nibs; FCN: fermented cocoa nibs; N1 (80% of UCN: 20% of FCN); N2 (20%: 80%); N4 (35%: 65%); C1 (80% under-fermented cocoa mass (UCM): 20% fermented cocoa mass (FCM)); C2 (20%: 80%); C4 (35%: 65%), and CC (100% of FCM).

were those of tetramethylpyrazine (3.49–24.89 area%), followed by trimethylpyrazine (0.33–13.22 area%), methylpyrazine (0.42–3.99 area %), and 2,3-dimethylpyrazine (0.15–1.11 area%).

3.2.6. Others

Other compounds were also identified in this study, such as 5-methylfurfual (caramellic and coffee-like flavors), that was found in cocoa nibs with a concentration that ranged from 0.19 area% to 0.50 area%. Furfuryl alcohol and furfural (bread, almond, and sweet flavors) were found only in samples with higher amounts of under-fermented cocoa nibs: UCN (1.08 area% and 7.66 area%), N1 (0.99 area% and 4.93 area %), and C1 (1.25 area% and 10.37 area%), respectively. The 5-acethyl-dihydro-2(3H)-furanone was detected in all cocoa nibs (0.12–0.31 area %) and chocolate (0.16–0.27 area%) samples. According to Ziegleder

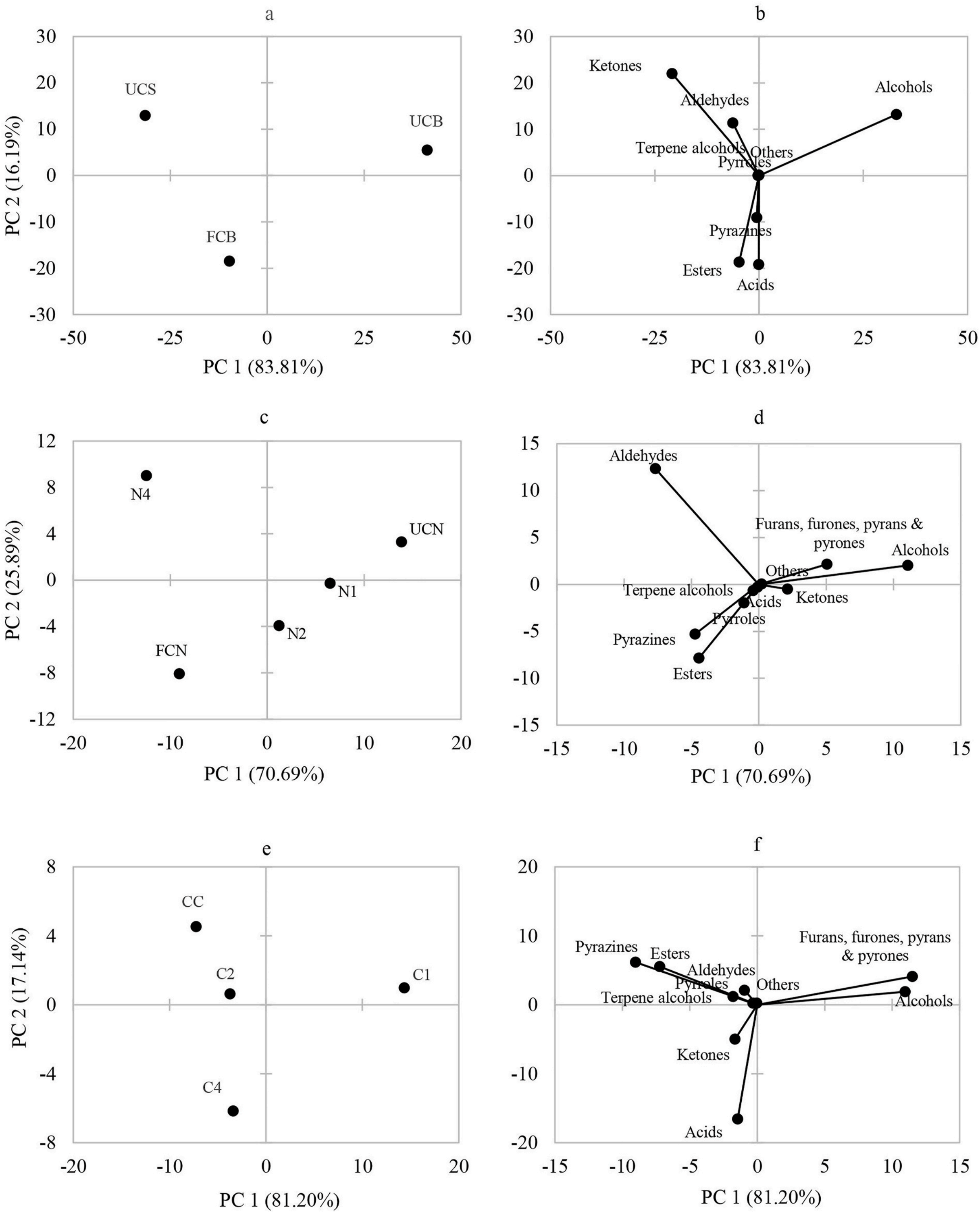

Fig. 4. Principal Component Analysis. Score plot (a) and loadings plot (b) of PC1 and PC2, from volatile compound after the fermentation and drying processes. Score plot (c) and loadings plot (d) of PC1 and PC2, from volatile compounds in the samples of cocoa nibs. Score plot (e) and loadings plot (f) of PC1 and PC2, from volatile compounds in chocolate samples.

(2009), furanones and pyranones are generally formed during the roasting process via degradation of monosacharides, with an optimal condition at 130 °C for 20 min. The total content of furans, furones, pyrans, and pyranones ranged from 0.22 area% to 11.77 area% ($p < 0.05$), while the total concentration of the pyrroles group ranged from 0.02 area% to 4.75 area% ($p < 0.05$). The 2-acetylpyrrole (chocolate and hazelnut flavors) was found in all samples, with an increase after roasting (0.02 area% - 3.87 area%) and a decrease after the conching process (1.12 area% - 3.19 area%).

Other volatiles identified included linalool, pyranoid, and furanoid in the terpene alcohol group ($p < 0.05$). Linalool is generally considered a product of biosynthesis, being found in its glycosidically bound form in the fruit pulp and in the cocoa bean cotyledons (Crafack et al., 2014; Ziegleder, 1990). Linalool and pyranoid were found in all samples ranging from 0.54 area% to 1.01 area% and from 0.06 area% to 0.39 area%, respectively. Furanoid was found only in the UCS (0.08 area%) and UCB (0.03 area%) samples. These compounds give flowery, floral, and nutty flavors, respectively. Another compound identified was guaiacol, ranging from 0.07 area% to 0.21 area%.

3.3. Volatile compounds profile

A Principal Component Analysis (PCA) was performed to compare the profiles of the volatiles in the samples of fermented dry cocoa beans, cocoa nibs, and chocolates. In accordance with Shin, Craft, Pegg, Phillips, and Eitenmiller (2010), PCA can reduce the number of dimensions and define the number of principal components by compressing the data based on their similarities and differences. Explaining 100% of the variation (PC1, 83.81% and PC2, 16.19%), the score plot in Fig. 4a shows a clear separation of the samples obtained from the fermentation and drying processes. Fermented dry cocoa bean samples (FCB) clustered on the negative axis of PC1, whilst the under-fermented dry cocoa beans (UCB) clustered on the positive axis of PC1. Unfermented cocoa seed (UCS), on the other hand, clustered on the positive axis of PC2.

The loadings plot in Fig. 4b shows that the most of the identified chemical groups of volatile compounds have positive loadings on PC1 (clustering on the right in the plot), indicating that the UCB sample was characterized by a higher content of alcohols (phenylethyl alcohol and 1-pentanol). The PC1 negative axis was characterized by esters (isoamyl acetate and 2-phenylethyl acetate), pyrazines (tetramethylpyrazine), and acids (nonanoic acid) identified in the FCB sample. The UCS was located in the PC2 positive axis, being associated with a greater abundance of different ketones (acetophenone, 2-pentatone, 2-heptanone, and 2-nonanone) and aldehydes (benzaldehyde and 2-methylbutanal).

During the roasting process, the typical roasty and chocolate flavors generated by the Maillard reactions, and the specific texture of the beans are developed (Aprotosoaie et al., 2016; Ioannone et al., 2015). The PCA (Fig. 4c and d) explains 96.56% of the total variation of the data for the nib samples in the first two components; PC1 (70.69%) and PC2 (25.89%). According to scores (Fig. 4c) and loading plot (Fig. 4d), UCN and N1 samples were located at the positive axis of PC1 and were associated with a greater abundance of different alcohols (phenylethyl alcohol, 1-pentanol, 2,3-butanediol, and 2-heptanol), furans, furones, pyrans, pyranones (furfural and furfuryl alcohol), and ketones (acetophenone, 2-nonanone, 2-heptanone, and butyrolactone). Many of the compounds identified in these groups (Table 3) have the desirable attributes described as flowery, sweet, fruit, and nutty odor description. The N2 sample was also located at the positive axis of PC1 and exhibited a greater abundance of ketones (acetophenone and 2-nonanone), acids (nonanoic acid), pyrroles (2-acetylpyrrole), and terpene alcohols (linalool). The FCN sample was located at the negative axis of PC1, presenting a high abundance of pyrazines (trimethylpyrazine and tetramethylpyrazine) and esters (2-phenethyl acetate, isoamyl acetate, and ethylphenyl acetate), while the N4 sample, located at the positive axis of PC2, exhibited a greater abundance of aldehydes (benzaldehyde and 2-methylbutanal), responsible for the fruit, honey, and roasted cocoa odor descriptors.

Fig. 4e and f presents the distribution of the scores and the loadings plot obtained from the Principal Component (PC) of the chocolate samples. The PC explains 98.34% of the total variability of the major chemical groups in two components: PC1 (81.20%) and PC2 (17.14%). The CC and C2 samples were located at the positive axis of PC2 and were correlated with the abundance of pyrazines (tetramethylpyrazine and trimethylpyrazine), esters (2-phenylethyl acetate and ethylphenyl acetate), aldehydes (benzaldehyde), pyrroles (acetylpyrrole), and terpene alcohols (linaool and pyranoid). The major presence of these compounds may explain the positive acceptance of the chocolate samples (C4 and C2), due to the volatile profile obtained (buttery, honey-like, sweet, flowery, fruit, roasted, chocolate, and hazelnut flavors). Conversely, C4 was located at the negative axis of PC1, revealing a higher content of ketones (butyrolactone, acetophenone and 2-nonanone) and acids (nonanoic and octanoic acids). The C1 sample was observed at the positive axis of PC1, which was correlated with a greater abundance of alcohols (phenylethyl alcohol, 2,3-butanediol and 1-pentanol), furans, furones, pyrans, and pyranones (furfural and furfuryl alcohol). According to the high content of volatile compounds found in the C1 sample, the volatile profile obtained exhibited green, flowery, bitter, caramel, almond, and roasted flavors, emphasizing the astringent and bitter tastes observed in the Just-about-right Test.

The cause and effect relationship between volatile aroma and sensory attributes cannot be accurately established. However, it was observed that a higher concentration of key-aromas promoted better characteristics in the chocolates, affecting their sensory acceptance. The presence of a high amount of a volatile compound might be correlated with a high intensity of a flavor attribute (Owusu, Petersen, & Heimdal, 2013). Great amounts of volatile compounds such as nonanoic acid, phenylethyl alcohol, acetophenone, butyrolactone, furfural, furfuryl alcohol, and tetramethylpyrazine were found in chocolates produced with 80% and 35% of under-fermented dry cocoa beans. Regarding the volatile compounds found during the processing steps, phenylethyl alcohol, 2-phenylethyl acetate, and tetramethylpyrazine were the volatile compounds with the highest concentrations. The major concentration of phenylethyl alcohol was caused by UCB, while the highest concentrations of 2-phenylethyl acetate and tetramethylpyrazine were from FCB, a characteristic that might be directly linked to the positive and negative acceptability of the chocolate samples.

4. Conclusion

The influence of the under-fermented cocoa mass on the sensory characteristics and volatile profile of the chocolate samples was suggested. Although the under-fermented cocoa mass can provide functional characteristics to chocolates, the use of the highest percentage (80%) had a negative impact on the sensory characteristics, especially on the flavor, bitterness, and astringency attributes. On the other hand, 35% and 20% of under-fermented cocoa mass showed a positive acceptance among the judges for all attributes evaluated, reaching the objective of this study. Forty-eight hours of fermentation were sufficient to develop key aromas in chocolates. The volatile profile obtained was sweet, floral, fruity, toasted, honey-like, buttery, and creamy.

Author contributions

Aurora Britto de Andrade: conceptualization, methodology, project administration, investigation, data curation, writing - original draft, writing – review & editing. **Margarida Lins da Cruz:** data curation. **Fernanda Antonia de Souza Oliveira:** data curation. **Sergio Eduardo Soares:** resources. **Janice Izabel Druzian:** resources. **Ligia Regina Radomille de Santana:** supervision, validation, writing – review & editing. **Carolina Oliveira de Souza:** supervision, validation, resources, writing – review & editing. **Eliete da Silva Bispo:** conceptualization, methodology, supervision, resources, writing – review & editing.

Aurora Britto de Andrade: contributed to the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article and final approval of the version to be submitted.

Margarida Lins da Cruz: contributed to acquisition of data and final approval of the version to be submitted.

Fernanda Antonia de Souza Oliveira: contributed to acquisition of data and final approval of the version to be submitted.

Sergio Eduardo Soares: contributed to resources, revising it critically for important intellectual content and final approval of the version to be submitted.

Janice Izabel Druzian: contributed to resources, revising it critically for important intellectual content and final approval of the version to be submitted.

Ligia Regina Radomille de Santana: contributed to analysis and interpretation of data, revising it critically for important intellectual content and final approval of the version to be submitted.

Carolina Oliveira de Souza: contributed to analysis and interpretation of data, revising it critically for important intellectual content and final approval of the version to be submitted.

Eliete da Silva Bispo: contributed to the conception and design of the study, analysis and interpretation of data and final approval of the version to be submitted.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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