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Volatile compounds and protein profiles analyses of fermented cocoa beans and chocolates from different hybrids cultivated in Brazil

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ABSTRACT

Cocoa beans from different geographical and genetic origins show distinct fermentation dynamics which result in different chocolate qualities. In order to understand the effects of genetic improvement of cocoa plants, in this work volatile compounds and proteins profiles of beginning and end of the fermentation from different cocoa hybrids (CEPEC2004, PH15, PS1319, SJ02) were searched. Moreover, sensorial characterization of the produced chocolate from these hybrids was performed. According to the results obtained, different volatile compounds were identified in fermented beans and in the chocolate produced. Chocolate from CEPEC2004 was the most accepted by judges and correlated with sweet and bitter taste which can be explained by the presence of desired flavor compounds, such as 2,3-butanediol and 2-methyl-1-butanol. A higher presence of acids (undesirable compounds) was observed in chocolates samples from PS1319 hybrid, that have resulted in the low acceptance by judges. In addition, MALDI-TOF MS analysis showed that during fermentation the protein profile was different among the hybrids, which indicates this kind of compounds also contributes to the cocoa-specific flavor.

1. Introduction

Cocoa is an important commodity for some countries located in tropical regions. Brazil was among the five largest cocoa producer countries until mid-1980s, when a disease called witches' broom emerged mainly in the southern region of Bahia State (Freire, Schwan, & Mororó, 1999). Witches' broom is a disease caused by the fungus *Moniliophthora perniciosa*, which attacks cocoa trees and causes an excessive budding in the final parts of plants and destroys the plantations (Lopes et al., 2011).

In order to recover the cocoa production in Brazil, a fast and efficient strategy for production of disease resistant hybrids was developed (Lopes et al., 2011). However, hybrids cocoa plants lead to a very variable cocoa fruit with different size, color, quantity and weight of the seeds, pulp content, chemical composition and flavor (Lopes & Pires, 2014). Many previous works confirmed the influence of genetic variability on cocoa and chocolate quality (Clapperton, Lockwood, Yow, & Lim, 1994; Efraim et al., 2013; Moreira, Miguel, Duarte, Dias, & Schwan, 2013; Ramos, Dias, Miguel, & Schwan, 2014; Moreira et al., 2016; Moreira et al., 2017).

Fermentation process of cocoa is essential for formation of precursors of chocolate specific flavor (Moreira et al., 2017; Schwan & Wheals, 2004). Voigt and Lieberei (2014) stated in a previous work that during the process of cocoa fermentation proteolytic reactions inside the beans start after 3 days of fermentation. According to these authors, small peptides and free amino acids are released, which influence chocolate flavor in the later process stages, such as beans roasting. As recently reported by Hue et al. (2016), the proteins indeed get degraded with a concomitant increasing in amino acids content during the fermentation. Furthermore, the cocoa samples from different geographical origins presented a kinetic variable of protein degradation which resulted in the formation of a small fraction of peptides (> 3 kDa) and a great quantity of amino acids.

According to Crafack et al. (2014), the presence of peptides and reducing sugars in chocolate production promote the Maillard reaction and intermediate compounds produced from this reaction such as furans, aldehydes, ketones, pyrroles and others volatile compounds. These greatly influence the aroma profile of cocoa and chocolate.

The aim of the present study was to evaluate the volatile compounds and proteins profiles of beginning and end of fermentation from

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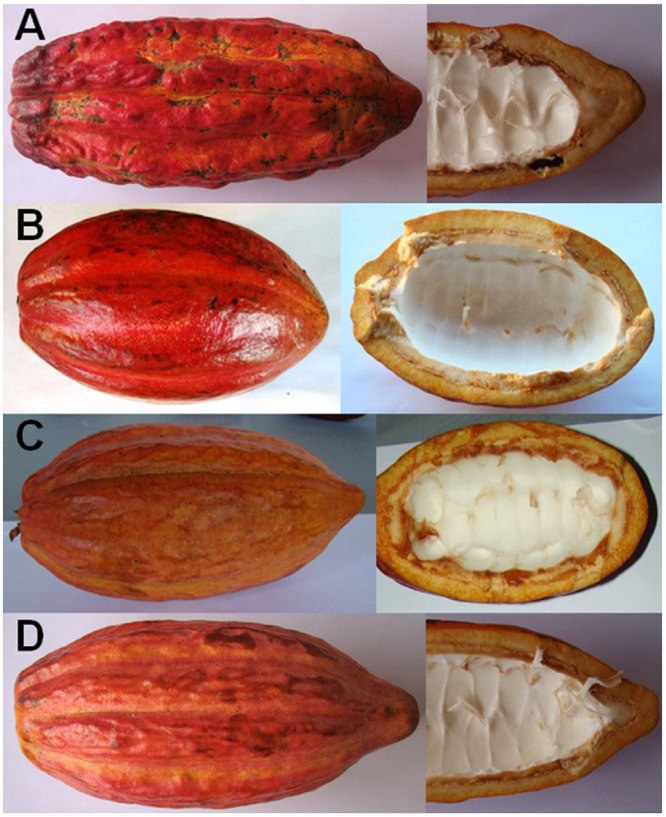

Fig. 1. Pod of four different cocoa hybrids. CEPEC2004 (A), PH15 (B), PS1319 (C) and SJ02 (D).

different cocoa hybrids. Moreover, in order to characterize the produced chocolates, sensory analyzes were also assessed.

2. Materials and methods

2.1. Fermentation experiments and sampling

The fermentation experiments were conducted at the Vale do Juliana cocoa farm in Igrapiúna, Bahia, Brazil. The ripe cocoa pods from five different hybrids (CEPEC2004; PH15; PS1319; SJ02) (Fig. 1) were harvested during the main crop of 2013 (September–December).

The cocoa pods were manually opened with a machete, and the beans were immediately transferred to the fermentation house. Fermentation started approximately 3 h after breaking pods and was performed in 0.06 m^3 wooden boxes (Moreira et al., 2017). Fermentation was evaluated during 6 days (144 h), and the amount used for each one of the hybrids was 60 kg. Temperatures were evaluated during fermentations by an average of five different points into the fermentation boxes, without humidity control. The samples were taken (every 24 h) approximately 40 cm from the surface of the center of the fermenting cocoa mass and placed in sterile plastic pots. The samples were stored at −20 °C.

2.2. Characterization of volatile compounds by headspace-solid phase microextraction gas chromatography-mass spectrometry

The volatile compounds from cocoa samples were extracted using the Headspace-Solid Phase Microextraction (HS–SPME) Gas Chromatography-Mass Spectrometry (GC–MS) technique, as previously described on the literature (Rodriguez-Campos, Escalona-Buendía, ORozco-Avila, Lugo-Cervantes, & Jaramillo-Flores, 2011), with modifications. For headspace analysis, cocoa samples (2.0 g) from the beginning (0 h) and end of fermentation (144 h) and chocolate samples (2.0 g) were macerated under liquid nitrogen in a mortar and pestle.

In order to extract volatile constituents from the cocoa and chocolate headspace, a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 mm SPME fiber (Supelco Co., Bellefonte, PA, USA) was used. The fiber was equilibrated for 15 min at 60 °C and then exposed to the samples (beans and chocolates) for 30 min at the same temperature.

The compounds were analyzed using a Shimadzu GC model QP2010 equipped with a mass spectrometry and a capillary column of silica OV Carbonwax 20M (0.25 µm × 0.25 µm × 30 m). The temperature program began with 5 min at 40 °C, followed by a gradient of 40 °C to 200 °C at 10 °C/min and then maintained at 200 °C for 20 min. The injector and detector temperatures were maintained at 230 °C. The carrier gas (He) was used at a flow rate of 1.4 mL/min.

Injections were performed by fiber exposition for 2 min. Volatile compounds were identified by comparing the mass spectra of the compounds in the samples with the database of the National Institute of Standards and Technology (NIST library, Gaithersburg, MD, USA) and the retention time with literature data using the n-Alkane index. All samples were analyzed in duplicate.

2.3. Extraction and analysis of protein profile by Matrix Assisted Laser Desorption Ionization Time of flight (MALDI-TOF)

Before protein extraction, from 0 h and 144 h of fermentation of each hybrid, all samples were subjected to extraction of lipids and polyphenols. For extraction of lipids, 6 g of cocoa beans were macerated using liquid nitrogen and then placed in papers cartridges. A Soxhlet apparatus was used as proposed by Voigt and Biehl (1993). After 6 h, the solvent [n-hexane (bp 68 °C)] was evaporated and recovered later in rotary evaporator.

For extraction of polyphenols, acetone was used to prepare a dried defatted cocoa beans powder and prevent influence of polyphenols in analysis of protein profile (Hue et al., 2016). Three g samples were extracted successively by 70% acetone, 80% acetone and 100% acetone using each time a volume of 60 mL. Acetone solutions were supplemented by 5 mM ascorbic acid. Mixtures were vortexed for 20 min and supernatant was discarded by centrifugation (12.000 rpm for 20 min at 4 °C).

After the extraction of lipids and polyphenols, 0.5 g of each sample was over again macerated with liquid nitrogen, until obtain very small particles. The particles were transferred into tubes containing glass beads to help protein extraction and 2 mL of organic solution (water/acetonitrile/trifluoro-acetic acid, 50:47.5:2.5). The tubes were immediate and vigorously vortexed for 10 min. Afterwards, the tubes were centrifuged at 9.000 rpm for 2 min at 4 °C and proteins were quantified by Bradford's method.

The protein suspension (1 µL) was transferred into the 96-well MALDI flex target plate (Bruker Daltonics, Bremen, Germany). When the liquid phase was almost evaporated 1 µL matrix solution [saturated solution of a-cyano-4-hydroxy-cinnamic acid (CHCA, Fluka, Buchs, Switzerland) saturated in a solution with 33% ethanol, 33% acetonitrile, 31% H_2O and 3% TFA], was added and gently mixed (Oliveira et al., 2015; Santos, Ventura, Costa, Fernandes, & Lima, 2015; Santos, Ventura, & Lima, 2016).

Twelve defined ribosomal proteins of *Escherichia coli* strain DH5α cells (4365.4, 5096.8, 5381.4, 6241.4, 6255.4, 6316.2, 6411.6, 6856.1, 7158.8, 7274.5, 7872.1, 9742 and 12227.3 Da) were used as external standard of MALDI-TOF MS equipment (Lima-Neto et al., 2014; Oliveira et al., 2015; Passarini, Santos, Lima, Berlinck, & Sette, 2013). Each analysis was developed in triplicate to evaluate reproducibility. A MALDI-TOF Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) was used for the spectra acquisition.

Raw spectra data (mzXML format) were preprocessed and analyzed using the software Mass-Up following the protocol presented by López-Fernández et al. (2015). Mass-Up is an open-source mass spectrometry

software for proteomics designed to support the preprocessing and analysis of MALDI-TOF MS data. Data treatment involved the following parameters: none for intensity transformation, smoothing by Savitzky-Golay method, baseline correction by Snip method, standardization by TIC (Total Ion Current) method and detection of peaks by Maldiquant method using signal-to-noise ratio (SNR) of 3.

After preprocessing and peak matching operations, analysis of "Peak List Quality Control" and "Biomarker Discovery" were performed to generate a list with *m/z* values of each sample and thus evaluate presence and absence of each peak in the spectra.

2.4. Chocolate production and sensorial analysis

After fermentation, the beans from different hybrids were sun dried in drying greenhouses during five days, every 24 h the beans were mixed for complete drying. Dried beans were sent for chocolate production at the enterprise Sartori e Pedroso Alimentos Ltda. (São Roque, SP, Brazil). Bitter-type chocolates were produced (62% liquor, 30% icing sugar, 8% cocoa butter). Fermented and dried beans were roasted in a roaster cylinder at 120 °C for 90 min. After roasting, cocoa liquor was obtained and transferred to conching step at 80 °C for 29 h. The chocolates molded were wrapped and stored at 4 °C for 4 weeks before sensory analysis.

Sensory analyses of chocolates were performed using a consumer acceptance test followed by a check-all-that-apply (CATA) question. The tests were conducted on 71 adults over 18 years old, 41% male and 59% female. All of them were consumers of dark chocolate.

For the acceptance test, the consumers evaluated how much they liked each sample using a 9-point hedonic scale (1 = dislike extremely and 9 = like extremely) (Stone & Sidel, 1993). For the CATA question, the consumers were asked to evaluate seven sensory attributes (sour, fruity, bitter, astringent, coffee, nut and sweetness) and select those they considered appropriate to describe the chocolate. The tests were performed as previously described elsewhere (Batista, Ramos, Ribeiro, Pinheiro, & Schwan, 2015).

2.5. Statistical analysis

Data obtained from acceptance test and proteins quantification were subjected to statistical analysis based on ANOVA. The means were compared using Tukey's test at a 5% level of significance. The analyses were carried out using the SISVAR 5.1 software (Federal University of Lavras, Department of Statistic, Lavras, MG, Brazil).

Agglomerative hierarchical clustering (AHC) was carried out using XLSTAT version 7.5.2. AHC graph was assembled with results of Biomarker Discovery analysis (presence and absence of peaks). Pearson correlation coefficient was used for showing similarities among samples.

3. Results

3.1. General characteristics of cocoa fruit

The cocoa pods of different hybrids are shown in Fig. 1. Characteristics of PH15 and PS1319 hybrids were previously described elsewhere (Moreira et al., 2013; Ramos et al., 2014). The CEPEC2004 hybrid (Fig. 1A) is the largest fruit with 22 ± 2 cm in length and 11 ± 0.3 cm in diameter, rind weigh 590 ± 121 g and contains the highest amount of seeds per fruit (53 ± 4). The PH15 fruit (Fig. 1B) is the smallest among the hybrids (14.5 ± 1 cm of length, 9 ± 0.1 cm of diameter and 433 ± 60 g of rind weight) and showed 45 ± 2 seeds per fruit, the least number.

Although rind of PS1319 (Fig. 1C) is the heaviest (782 ± 123 g), size of the fruit is between the CEPEC2004 and PH15 hybrids, 19 ± 1 cm of length and 10 ± 0.5 cm of diameter, and 49 ± 3 seeds per fruit. The SJ02 hybrid (Fig. 1D) produces fruits measuring 20 ± 1 cm and 9 ± 0.5 cm in length and diameter, respectively, has a rind weight of 506 ± 79 g and 47 ± 5 seeds per fruit.

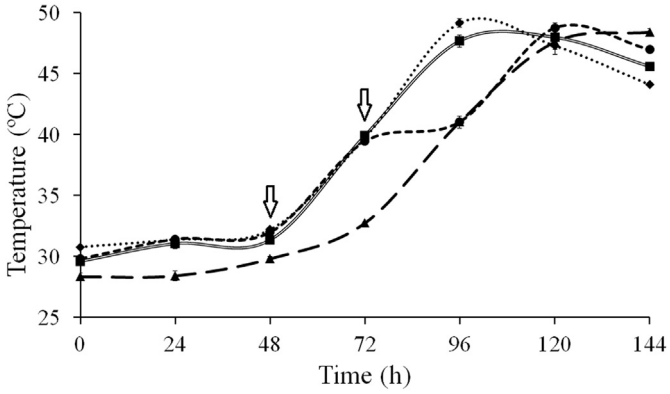

Fig. 2. Measurements of the temperature in cocoa mass during the 144 h of fermentation for the different hybrids. Temperature values are averages collected at different points of cocoa mass. CEPEC2004 (—●—), PH15 (••◆••), PS1319 (—■—) and SJ02 (—▲—). Mixing (⇩) of the cocoa mass was performed at 48 and 72 h of fermentation process.

3.2. Physical-chemical changes

Temperature was measured during 144 h of fermentation and is presented in Fig. 2. Temperatures changed according to the time for all fermentations, which ranged from around 29 °C at 0 h to 48 °C at 144 h.

The maximum temperatures for each fermentation were: 48.8 ± 0.4 °C (at 120 h) for CEPEC2004; 49.18 ± 0.33 °C (at 96 h) for PH15, 48.00 ± 0.1 °C (at 120 h) PS1319 and 48.42 ± 0.3 °C (at 144 h) for SJ02. Lower temperatures (28.32 ± 0.3 °C, 28.40 ± 0.4 °C and 29.80 ± 0.2 °C) during fermentation were observed for SJ02 hybrid; however, at the end of process (144 h) higher temperature (48.42 ± 0.3 °C) was observed for the fermentation of this hybrid, as presented above. The rate of temperature increasing was higher in the fermentations of the hybrids PH15 and PS139, approximately 0.35 °C per hour between fermentation times 48 and 96 h.

3.3. Volatile compounds

All the compounds identified by HS–SPME GC–MS during fermentation and chocolate samples of four hybrids (CEPEC2004, PH15, PS1319 and SJ02) are shown in Table 1. Third-six volatile compounds were detected by HS–SPME GC–MS at the beginning (0 h) of CEPEC2004 fermentation; while 41 volatile compounds were found at 144 h of its fermentation. For fermentation of PH15 hybrid, 35 compounds were identified at 0 h and 42 at the 144 h. The highest amounts of volatile compounds were found in the fermentation of PS1319 hybrid, 38 and 46 at 0 h and 144 h, respectively. Concerning the fermentation of SJ02 hybrid, 31 volatile compounds were detected at 0 h, while 42 volatile compounds were detected at the end of process.

Between hybrids, regarding to chocolates samples, many common compounds were detected. A total of 32 common volatile compounds were detected on chocolates samples of both CEPEC2004 and PS1319 hybrids, while 33 volatile compounds were detected on PH15 and SJ02 chocolates. In all fermentations and chocolate samples, the following groups of compounds were found: organic acids, alcohols, aldehydes and ketones, esters and others (pyrazines, pyrroles and terpenoids).

Considering the sequence, first 0 h of fermentation (Fig. 3A), second 144 h of fermentation (Fig. 3B) and finally chocolate samples (Fig. 3C), percentages of volatile compounds belonging to acids and esters groups increased, while amount of alcohols and aldehyde and ketones decreased for all samples. Alcohols, aldehydes and ketones were most

Table 1
Volatile compounds identified by HS–SPME GC–MS at the beginning and end of the fermentation of the hybrids and in chocolate samples.

Compounds	Odor description[a]	Hydrids detection		Chocolate
		0 h	144 h	
Acids				
Acetic acid	Sour, astringent	CEPEC2004, PH15, PS1319	–	All hybrids
Caprinic acid		–	–	All hybrids
Caproic acid	Sweat, pungent	CEPEC2004, PH15, PS1319	All hybrids	All hybrids
Caprylic acid	Sweaty, fatty	–	All hybrids	All hybrids
Isobutyric acid	Rancid, butter, cheese	PH15, PS1319	All hybrids	All hybrids
Isovaleric acid	Sweat, rancid	PH15, PS1319	All hybrids	All hybrids
Phenylacetic acid		–	–	All hybrids
Propanoic acid		PS1319	All hybrids	All hybrids
Valeric acid	Sweat, rancid	–	PS1319	PS1319
Alcohols				
1-Butanol		PH15	–	–
1-Hexadecanol	Fruity, Green	–	–	All hybrids
1-Pentanol		CEPEC2004, PH15, PS1319	CEPEC2004, PS1319, SJ02	–
1-Phenylethanol		All hybrids	All hybrids	–
2,3-Butanediol	Cocoa butter	CEPEC2004	CEPEC2004	CEPEC2004
2-Ethyl-1-hexanol		All hybrids	All hybrids	–
2-Heptanol	Sweet, citrusy	All hybrids	All hybrids	–
2-Hexanol	Fruity, Green	All hybrids	All hybrids	–
2-Methyl-1-butanol	Malty, chocolate	All hybrids	All hybrids	CEPEC2004
2-Methyl-3-buten-2-ol		All hybrids	All hybrids	–
2-Nonanol		PS1319	PS1319	–
2-Octanol		PS1319	PS1319	–
2-Pentanol		All hybrids	All hybrids	–
2-Phenylethanol		All hybrids	All hybrids	–
3-Methyl-1-butanol		All hybrids	All hybrids	PH15, SJ02, CEPEC2004
4-Methyl-1-pentanol		CEPEC2004, PS1319	CEPEC2004, PS1319	–
Benzyl alcohol	Sweet, flower	All hybrids	All hybrids	–
Ethyl alcohol		All hybrids	All hybrids	All hybrids
Furfuryl alcohol		–	–	All hybrids
Guaiacol	Smoke, sweet	–	PH15	–
Isobutyl alcohol		All hybrids	All hybrids	–
Phenethyl alcohol	Honey, rose, caramel	–	–	All hybrids
α-Terpineol		–	PS1319	–
Aldehydes and Ketones				
(E)-2-butenal		CEPEC2004, PS1319	–	–
1-Phenylethanone	Honey	All hybrids	All hybrids	–
2,3-Butanedione	Butter	PS1319	All hybrids	–
2-Furaldehyde		–	–	All hybrids
2-Heptadecanone		–	–	All hybrids
2-Heptanone		All hybrids	PH15, PS1319, SJ02	–
2-Hexanone		SJ02	–	–
2-Octanone		PS1319	PS1319	–
2-Pentanone	Fruit	All hybrids	All hybrids	–
2-Propanone		All hybrids	All hybrids	–
2-Pyrrolidone		–	–	All hybrids
2-Vinylfuran		All hybrids	All hybrids	–
3-Methyl-2(5H)-furanone		SJ02	–	–
3-Methylbutanal	Chocolate	All hybrids	All hybrids	–
5-Methyl-2-hexanone		All hybrids	PH15, PS1319, SJ02	–
5-Methyl-2-phenyl-2-hexenal		–	CEPEC2004, SJ02	–
6,10,14-Trimethyl-2-pentadecanone		PS1319	–	–
Acetaldehyde	Sherry, nutty, apple	All hybrids	All hybrids	–
Acetoin	Butter, cream	CEPEC2004, PH15, SJ02	All hybrids	All hybrids
Benzaldehyde	Bitter	All hybrids	All hybrids	All hybrids
Benzeneacetaldehyde		All hybrids	All hybrids	–
Butyrolactone		–	–	All hybrids
Hexanal	Green, grass	CEPEC2004, PS1319	–	–
Pentanal		PH15, PS1319	All hybrids	–
Esters				
2-Pentyl acetate	Fruity	–	PH15, SJ02	–
2-Phenylethyl isobutyrate		CEPEC2004, PH15, SJ02	All hybrids	–
Allyl acetate	Fruity, banana	–	All hybrids	–
Amyl phthalate		–	–	All hybrids
Dibutyl phthalate		–	–	All hybrids
Diisobutyl phthalate		–	–	All hybrids
Ehtyl acetate		PS1319	–	–
Ethyl benzoate		SJ02	PH15, SJ02	–
Ethyl caprate	Pear, grape	–	–	All hybrids
Ethyl caprylate	Fruity, flowery	–	All hybrids	–
Ethyl laurate	Fruity, floral	–	–	All hybrids

(*continued on next page*)

Table 1 (*continued*)

Compounds	Odor description[a]	Hydrids detection		Chocolate
		0 h	144 h	
Ethyl myristate	Waxy, soapy	FA13, PH15	All hybrids	All hybrids
Ethyl palmitate	Waxy, green	–	–	All hybrids
Ethyl phenylacetate		CEPEC2004, PH15	All hybrids	–
Ethyl pyruvate		CEPEC2004, PH15, SJ02	All hybrids	All hybrids
Isoamyl acetate		–	All hybrids	–
Methyl palmitate		–	–	All hybrids
Phenylethyl acetate	fruity, sweet	–	–	All hybrids
Others[b]				
2,3,5,6-Tetramethylpyrazine	Chocolate, coffee	–	–	All hybrids
2,3,5-Trimethylpyrazine	Cocoa, rusted nuts	–	–	All hybrids
2-acetyl Pyrrole	Chocolate, hazelnut	–	–	All hybrids
Linalool	Flower, lavender	CEPEC2004, PH15, SJ02	All hybrids	–

[a] Obtained from literature.
[b] Include: Pyrazines, pyrroles and terpenes.

found at the beginning (Fig. 3A) of fermentation process, while acids and esters were most predominant in the chocolate samples (Fig. 3C).

3.4. Protein analysis by MALDI-TOF MS and its kinetic of changing over the fermentation process

After extraction, proteins were quantified by Bradford's method and results are presented in Table 2. The amount of proteins decreased during the fermentation time for all hybrids studied. The highest difference was observed for PS1319 hybrid, i.e. 0.49 mg of protein per mL of extract, while the lowest was 0.21 mg/mL of extract for the SJ02 hybrid.

Analyze of Peak List Quality Control, performed in Mass-Up software, allowed to identify a minimum molecular mass (m/z) and a maximum molecular mass (m/z) for each sample. Moreover, it shows the total of peaks after treatment of the spectra obtained by MALDI-TOF

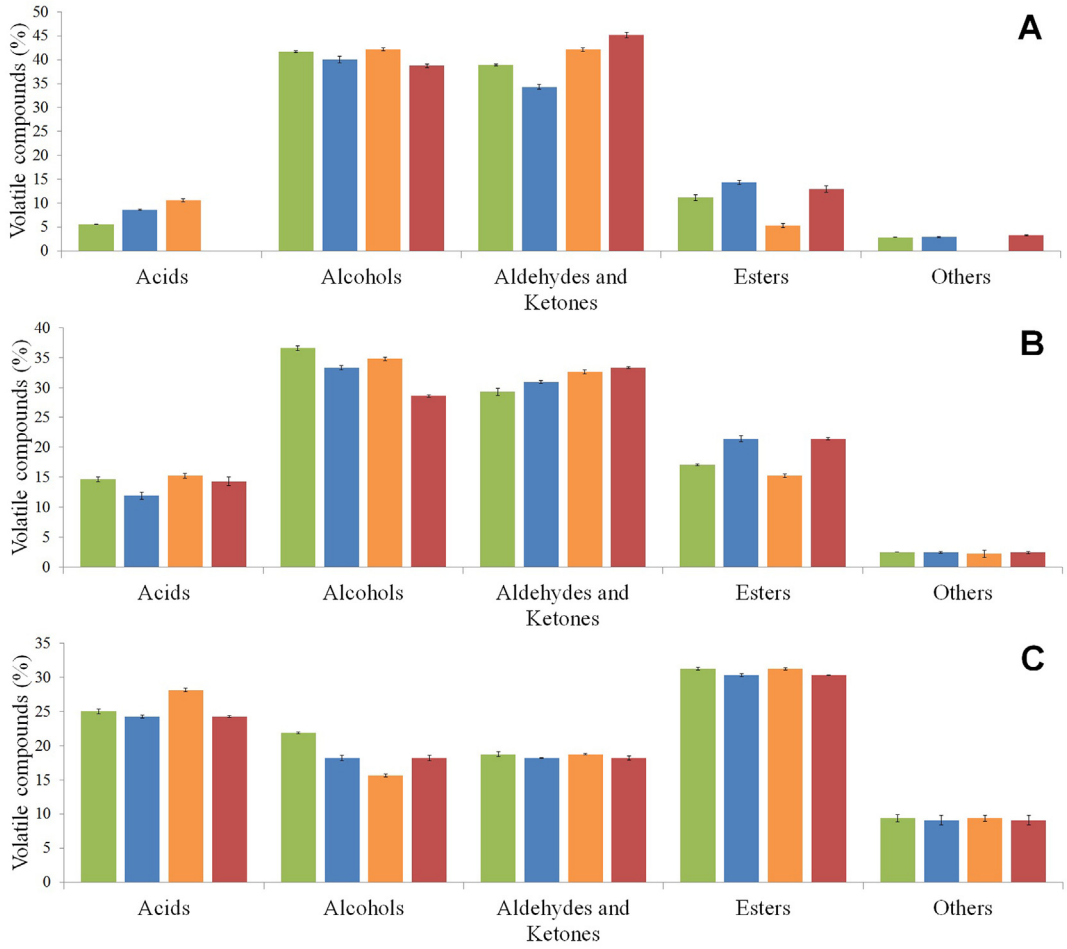

Fig. 3. Profile of volatile compounds identified by HS-SPME GC–MS during fermentation of CEPEC2004 (), PH15 (), PS1319 (), and SJ02 (), and in the chocolate samples. Fermentation times: 0 h (A) and 144 h (B). Chocolate samples (C). Total of compounds: CEPEC2004 0 h (36), CEPEC2004 144 h (41), CEPEC2004 Ch (32), PH15 0 h (35), PH15 144 h (42), PH15 Ch (33), PS1319 0 h (38), PS1319 144 h (46), PS1319 Ch (32), SJ02 0 h (31), SJ02 144 h (42), SJ02 Ch (33).

Table 2
Protein concentration of each hybrid at beginning and end of fermentation.

Samples	Protein (mg/mL)	
	0 h	144 h
CEPEC2004	0.80 ± 0.02	0.41 ± 0.08
PH15	0.70 ± 0.05	0.37 ± 0.05
PS1319	0.61 ± 0.01	0.12 ± 0.01
SJ02	0.58 ± 0.07	0.37 ± 0.02

MS analysis. Results are presented in the Table 3. A total of 144 distinct molecular mass peaks (m/z) were observed in the different MALDI-TOF mass spectra. Four common peaks were found in all spectra of fermentations samples, m/z 2515.385, 2570.638, 4194.994, 9537.834.

Sample from SJ02 at the beginning of fermentation showed the highest number of peaks (63), while the sample CEPEC2004 at 0 h showed the lowest number of peaks (41) (Table 3). Peaks with molecular mass values of m/z 4635.140 and 5821.460 were found only at the beginning of all fermentations (CEPEC2004, PH15, PS1319 and SJ02), while mass values m/z 2818.639 and 9698.426 appeared only at the end of fermentation process for all studied hybrids.

The presence and absence of peaks indicate variability between the two times of fermentation process and among the different hybrids. Fig. 4 shows the clustering by similarity of the samples in case.

3.5. Sensorial analysis

The results of sensory analyzes of the chocolates produced from each hybrid are shown in Table 4 and Fig. 5. There were significant differences ($p < 0.5$) in acceptance between the four samples of chocolate. Chocolate from CEPEC2004 hybrid was the most appreciated (score 7.29), while chocolate from PS1319 hybrid was the less appreciated (score 6.55) by consumers.

Answers to the CATA questions highlighted that the main parameters used to describe chocolates were bitter, sweetness, coffee and nutty (Fig. 5). Chocolates from CEPEC2004 and SJ02 hybrids were described with a bitter taste, although chocolate of CEPEC2004 was sweeter than SJ02 chocolate. Fermentation of PH15 hybrid resulted in a chocolate with higher values of sweet and nut tastes. Chocolates produced by fermentation of PS1319 hybrid were related to coffee and fruit taste, however, a sour taste was reported by the consumers (Fig. 5).

4. Discussion

As a way to recover the Brazilian cocoa productivity, disease-resistant hybrids has been developed by farmers and research centers over the last years (Moreira et al., 2016). In order to overcome the lower quality of chocolate produced with fruits of cocoa plants hybrids, a better knowledge about fermentation process and chocolates production from these new hybrids is essential.

In the present study fruits of four different cocoa hybrids (CEPEC 2004, PH15, PS1319 and SJ02) were selected and evaluated according to the volatile compounds present in both (a) during the cocoa fermentation and (b) on final chocolates samples. Moreover, qualitative

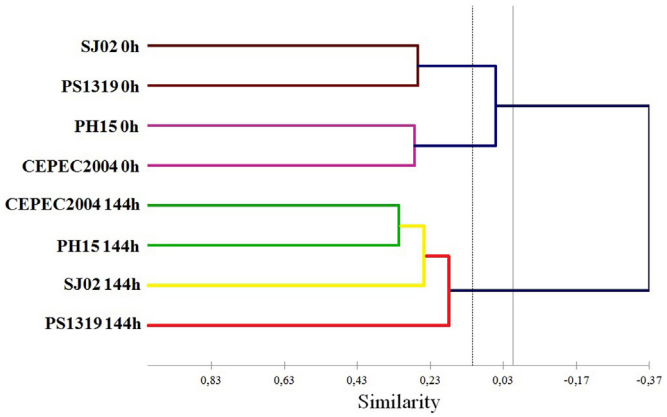

Fig. 4. Agglomerative hierarchical clustering by presence and absence of peaks with different m/z values.

Table 4
Acceptance test for the chocolate samples of each cocoa hybrid.

Chocolates samples	Acceptance test
CEPEC2004	7.29 ± 1.18[a]
PH15	6.90 ± 1.57[b]
PS1319	6.55 ± 1.82[c]
SJ02	7.06 ± 1.48[b]

Values followed by the same letter in the same row are not different at the 5% level of significance by Tukey's test.

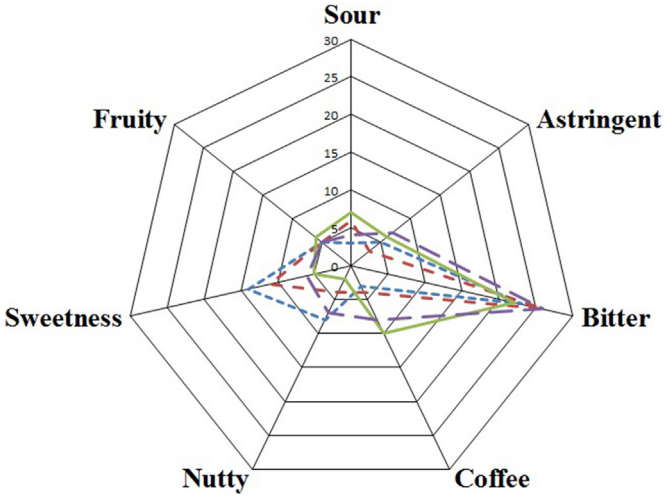

Fig. 5. Flavor profiles of the chocolates produced from four different hybrids [CEPEC 2004 (—), PH15 (– – –), PS1319 (—), and SJ02 (—)]. The center of the diagram corresponds to the lowest flavor intensity and the perimeter to the highest flavor intensity.

Table 3
Minimum and maximum molecular masses, and number of mass peaks found in each hybrid at beginning and end of fermentation.

Samples	Min. Mol. Mass (m/z)		Max. Mol. Mass (m/z)		No. of mass peaks	
	0 h	144 h	0 h	144 h	0 h	144 h
CEPEC2004	1968.749	1980.906	9697.598	12078.339	41	50
PH15	1970.005	1980.906	9864.243	11864.830	52	54
PS1319	1968.330	1980.067	13242.174	11598.089	62	59
SJ02	1968.749	1980.067	11517.646	12076.257	63	59

protein profile during cocoa fermentation and sensorial characterization of chocolate produced from the fermented beans were assessed.

According to the CATA results, chocolate from CEPEC2004 hybrid was correlated with sweet and bitter taste. Furthermore, it was the most accepted by judges (score 7,29) (Fig. 5 and Table 4). These characteristics coincide with results for volatile compounds analysis presented in Table 1 and Fig. 3.

Samples of CEPEC2004 fermentation showed a greater number of alcohols compounds which confers desirable flavor, such as 2,3-butanediol (cocoa butter flavor) and 2-methyl-1-butanol (malty and chocolate flavor) found only in this hybrid (Table 1).

Esters are correlated to fruity flavors, while acids are considered undesirable compounds which confer unpleasant odors (e.g. sweat, pungent, fatty and rancid flavors) (Frauendorfer & Schieberle, 2008; Luna, Crouzillat, Cirou, & Bucheli, 2002). Fig. 3 shows that in the fermentation process the amount of esters was greater than organic acids. In fact, yeasts are good producers of esters and these are the most important microorganisms found in cocoa fermentation. Furthermore, some acids are consumed by yeasts during the fermentative process, as citric acid (Schwan & Wheals, 2004; Crafack et al., 2014).

An exception for this was observed on the fermentation of PS1319 hybrid, which presented more acids than esters at the beginning and equal values of these compounds at the end of fermentation. Furthermore, valeric acid, which is correlated with odors description of sweat and rancid, was only found in PS1319 samples (144 h and chocolate) (Table 1).

Regarding to chocolates produced from fermented beans of the PS1319 hybrid, amounts of organic acids were greater than alcohols (Fig. 3C), this fact explains both the lower score (6.55) in acceptance test (Table 4) and sour taste reported by judges in CATA question analysis (Fig. 5).

The formation of chocolate flavor is complex. More than 500 non-volatile and volatile compounds contribute to chocolate characteristic flavors (Serra-Bonvehí, 2005; Afoakwa, Paterson, Fowler, & Ryan, 2008; Rodriguez-Campos et al., 2011; Ho, Zhao, & Fleet, 2014). It is not surprising that different hybrids have a distinct fermentation dynamic and, consequently, lead to chocolates with different flavor sensations, as previously described elsewhere (Clapperton et al., 1994; Efraim et al., 2013; Moreira et al., 2013; Ramos et al., 2014).

Afoakwa et al. (2008) stated compounds responsible for flavor formation in cocoa beans and final chocolate have various generation sources, such as: intrinsic to the bean (genetic origin, growth location, climatic conditions), post-harvest treatment (mainly fermentation and drying processes) and roasting and coaching processes during chocolate manufacture (Maillard and other reactions) (Misnawi, Jinap, Jamilah, & Nazamid, 2004). Furthermore, according to these authors, pyrazines components are important to flavor and found mainly in chocolates.

In the present study, only two pyrazines compounds were detected for all hybrids, precisely: 2,3,5,6-tetramethylpyrazine and 2,3,5-trimethylpyrazine (Table 1). Recently, Visintin et al. (2017) also observed few pyrazines in chocolates from Brazilian cocoa. Authors stated presence of pyrazines can be influenced by parameters such as weather condition, ripeness of pod, varieties of cocoa, and chocolate processing.

During the fermentation of CEPEC2004, PH15, PS1319 and SJ02 hybrids the total amount of proteins decreased from the beginning to the end. Overall, a drop of 51.25% (CEPEC2004), 47.14% (PH15), 80.32% (PS1319), and 36.20% (SJ02) in the protein concentration in each case was observed.

Hue et al. (2016) evaluated the impact of fermentation on nitrogenous compounds of cocoa beans and observed similar results regarding to protein content. When the seed embryo is killed by acidification of the pulp and temperature increase, as microbial activities consequence, proteolytic processes start (Schwan & Wheals, 2004).

An aspartic endoprotease and a carboxypeptidase degrade the most protein content into the beans which is mainly composed of two proteins: albumin and vicilin (7S)-class globulin (Voigt et al., 1994; Voigt & Biehl, 1993; Voigt & Biehl, 1995; Voigt & Lieberei, 2014). Furthermore, proteolytic processes into the fermented cocoa beans generate smaller peptides and free amino acids that contribute to the formation of cocoa-specific flavor precursors (Hue et al., 2016; Marseglia et al., 2014).

The MALDI-TOF spectra clustering based on the presence and absence of peaks (different values of m/z) (Fig. 4) showed that initial sample fermentation are different from final sample fermentation and also among hybrids. Moreover, regarding to the final fermentation time (144 h) CEPEC2004 and PH15 hybrids were similar between themselves, while PS1319 was different from the other hybrids (Fig. 4). This data is in accordance with results obtained in the sensorial analyzes; chocolates produced from PS1319 hybrid were less accepted, presenting particular sensory characteristics which influenced the judges' decision, such as sweet and nut tastes.

5. Conclusions

Different volatile compounds were identified in the fermented beans of CEPEC2004, PH15, PS1319 and SJ02 hybrids and in the chocolate produced with these beans. The different cocoa hybrid CEPEC2004, PH15, PS1319 and SJ02 showed different chemical compositions regarding to the volatile compounds and protein profile observed during fermentation, which consequently influence the chocolates sensory perception.

Samples of CEPEC2004 fermentation showed a greater number of alcohols compounds that confer desirable flavor to the chocolate, such as 2,3-butanediol (cocoa butter flavor) and 2-methyl-1-butanol (malty and chocolate flavor). These compound were just found for this hybrid. Moreover, chocolate from CEPEC2004 hybrid was correlated with sweet and bitter taste (2,3-butanediol and 2-Methyl-1-butanol) and chocolate produced with CEPEC2004 hybrid was the most accepted by judges (score 7,29). In contrast, a higher concentration of undesirable compounds, such as organic acids, was observed in chocolates samples from PS1319 hybrid. Chocolates samples from PS1319 hybrid generated lower acceptance by judges.

MALDI-TOF MS analysis showed that during fermentation the protein profile was different among the hybrids, which indicates this kind of compound also contributes to the cocoa-specific flavor.

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