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ORIGINAL PAPER

Characteristics of cocoa butter and chocolates obtained from cocoa varieties grown in Bahia, Brazil

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Abstract Due to the attack of cacao (*Theobroma cacao* L.) plants by the fungus *Moniliophthora perniciosa* which caused witches' broom disease in the state of Bahia, Brazil, resistant clonal material with elevated productivity and superior physical characteristics has been selected for use in the genetic improvement program and by the cocoa producers themselves. The present study aimed to characterize the cocoa butter and chocolates produced from nine cacao varieties and also from the *Amelonado* cacao produced in the same region (denominated as Common), with a view to quality improvement. The cocoa butter was characterized for its solid fat content and triacylglycerol composition, and the chocolates for their moisture and protein contents, maximum particle diameter, pH value, acidity, rheological properties and instrumental texture, as well as a sensory test for acceptability with 50 consumers. A relationship was found between the triacylglycerol composition and solid fat curves of the cocoa butters and the instrumental texture of the chocolates, the highest values for the latter parameter being observed for the varieties with greater amounts of symmetrical triacylglycerols and greater solid fat contents at the temperatures evaluated. From a sensory point of view, it was shown that overall acceptance and buying intention were mainly determined by the attributes of "astringency," "bitterness," "acidity" and "chocolate flavor." It was also shown that chocolates produced from varieties descendent from the *Trinitario* group were differentiated from those descendent from the *Forastero* group, showing the preference of consumers for these materials and the possibility of a gain in quality in the genetic improvement of cacao by including varieties descended from the *Trinitario* and *Criollo* groups in the crossing programs, associating disease resistance with product quality.

Keywords Cacao · Chocolate · Varieties · Quality · Evaluation

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Introduction

In the twentieth century, Brazil stood out as one of the main world cocoa (*Theobroma cacao* L.) producers, with a production of 400,000 tons of cocoa beans in the 1980s and productivity of 750 kg/ha, the most productive in the world context at that time. In the 1990s, with the spread of the witches' broom disease (caused by the fungus *Moniliophthora perniciosa*) among the cocoa plantations in the southern region of the state of Bahia, plus economic and environmental factors, there was an accentuated fall in Brazilian production and productivity. Between 1986 and 2009, a reduction of approximately 53 % was observed in the production of cocoa beans in Brazil [11]. By way of a genetic improvement program carried out in Brazil plus the efforts of cocoa producers in the region, new materials not only disease resistant but also with elevated productivity and superior physical characteristics have been used. Thus, it has become relevant to evaluate the technological and sensory performance of these materials in the production of

chocolate, especially searching for an improvement in quality and consequent increase in value of Brazilian cacao.

Few studies have evaluated the influence of cocoa genotypes on the sensory attributes. Luna et al. [20], searching to relate the flavor to the chemical and genetic composition of the materials in Ecuador, demonstrated a correlation between the genetic characteristics and chemical composition (organic acids, sugars, polyphenols and methylxanthines) and the sensory characteristics (astringency, bitterness, acidity, cocoa flavor intensity and the floral and fruity notes). Clapperton et al. [7, 8] also showed the relationship existing between the polyphenol and methylxanthine contents and the astringency and cocoa aroma and flavor intensities perceived sensorially. Using an optimized sensory evaluation protocol, Sukha et al. [29] identified genotypes with flavor potential from materials produced in Ghana (cocoa considered as common) and from Trinidad (cocoa considered as fine), covering three periods of agricultural production.

Cocoa butter has great importance in the sensory profile of chocolate. It is responsible for the melting characteristics and hardness, which influence the stability at room temperature. The average daily temperature during the last few months of pod development affects the fatty acid and triacylglycerol compositions, and consequently the hardness of the cocoa butter, with lower temperatures resulting in softer butters or with lower melting points [12, 18]. Pires et al. [25], Tucci et al. [31] and Khan et al. [17] demonstrated the influence of the genetic characteristics on the cocoa butter content, with important information for breeding programs.

Considering the immense fall in national productivity since the appearance of witches' broom disease, the main ways to maintain a viable cocoa culture in Brazil are by increasing productivity, using pest- and disease-resistant varieties and value aggregation, which could be obtained by using varieties that allow one to obtain higher-quality products. Within this context, the objective of the present study was to process chocolates from cacao varieties used commercially in the state of Bahia, Brazil, and make a physicochemical and sensory characterization of the chocolates and physicochemical characterization of the cocoa butter obtained from the cultivars, given the importance of the cocoa butter in determining the physical and sensory characteristics of the chocolate.

Materials and methods

Material

Healthy, ripe fruits were used, obtained by open pollination from the following varieties: *CEPEC42* (hybrid of TSA644 × SIC19), *EET397* (originated from SCA), *TSA654* (hybrid of SCA6 × IMC67), *TSA656* (hybrid of SCA6 × IMC67), *TSAN792* (hybrid of TSA641 with unknown genotype), *TSH516* (hybrid of SCA6 × ICS1), *TSH565* (hybrid of SCA6 × ICS1), *TSH774* (unknown origin) and *TSH1188* (hybrid selection from a Pound 18 × (TSA641 × Unknown)), selected in Bahia based on the productivity and resistance to witches' broom disease of the original genotypes. The fruits were obtained from CEPLAC (*Comissão Executiva para o Plano da Lavoura Cacaueira*) clones in multiplication fields in Itabuna BA. The cacao known as *Common*, of the *Amelonado* type and characteristic of the region, was also evaluated, since it is widely used despite its susceptibility to witches' broom disease.

With respect to the genetic ancestry of the materials studied, they could be grouped as follows: (1) materials descendent from the *Forastero* group—TSA654, TSA656 and CEPEC42; (2) materials descendent from the *Forastero* and *Trinitario* groups—TSH516, TSH565, TSH774, TSH1188 and TSAN792; and (3) *Forastero Amelonado* cacao (Common). The variety EET397 due to its unknown paternity and green color of the fruit could not be classified: it could just as well have a purely *Forastero* origen as be the result of pollen with *Trinitario* ancestry. The clones TSAN792 and TSH774, despite being of undefined origin, could be classified as group 2, since the unripe fruits were red, a characteristic of the *Trinitario* group [10].

Processing

Cocoa bean processing

The fruits of the cultivars studied were all harvested in November 2004 in the same harvest period with a maximum of 3 days of difference, to avoid climatic variations suffered during their development influencing the characteristics evaluated. The maximum and minimum temperatures in the crop area during the period of fruit maturation were 14° and 28 °C, respectively, the relative humidity between 90 and 95 % and the rainfall between 200 and 300 mm. The fermentations were carried out simultaneously in duplicate, in 50 kg batches of seeds with pulp for 6 days in wooden 50 × 50 × 50 cm (125 L) boxes. The bottom and lower sides of the boxes had 1 cm diameter holes drilled every 5 cm for removal of the liquefied pulp during the first days of fermentation. The pulp was then sun-dried to 8.0 % moisture content on a 5 × 10 m platform constructed with a wooden surface covered with a movable zinc roof (7–8 days of drying). The dried cocoa beans were broken in a knife mill (Icma, Campinas, BR), and the nibs separated by granulometry in a vibratory apparatus with sieves, and in an air column by difference in

density. The nibs were roasted in 300 g batches in a rotary electric roaster (Probat Werke Pre17, Emmerich am Rhein) at 150 °C (heating jacket) for the following times, predetermined in a sensory ranking test for preference carried out with consumers (data not presented):

- CEPEC42, COMMON, EET397, TSA656, TSH516, TSH774: 36 min;
- TSA654, TSAN792, TSH565: 38 min;
- TSH1188: 40 min.

The nibs were ground in a knife grinder and in a three horizontal cylinder refiner (Draiswerke, Mannheim and Waldhof) up to a maximum particle diameter of 25 μm. After refining, the liquors were heat treated in a longitudinal conche (Hans Ströter Bege BV, Dusseldorf, KG) for 3 h at 75 °C to eliminate moisture and acetic acid and aid in flavor development. To obtain the cocoa butter, 200 g of liquor from each material was heated to 60 °C, placed in canvas sacks and pressed in a manual hydraulic press (Carver-C, Wabash, IN) with a maximum pressure of 9 t (5 min at 3 t, 15 min at 6 t and 10 min at 9 t).

Chocolate processing

In order to produce the chocolates, the cocoa butter content of the liquors of each variety (determined by method 963.15—[13]) was corrected to 57.2 % by the direct addition of butter or powder extracted from each material.

Bitter-type chocolates were produced (56 % liquor, 42.6 % icing sugar [União], 1 % cocoa butter and 0.4 % soybean lecithin [Solec CH-Solae] in 700 g batches. Part of the molten liquor (35 %) and icing sugar (42.6 %) were homogenized in a planetarium mixer (Kitchenaid K5SS). Refining and conching were carried out in the same three-cylinder refiner and conche (60 °C/24 h) aforementioned. The rest of the liquor (21 %) was incorporated into the refined mass at the start of conching and the mixture maintained in agitation/shearing for 20 h, at which point the emulsifier and cocoa butter were added and conching continued for a further 4 h. Tempering was carried out in a laboratory temper machine (ACMC/D45134) [19, 30]. The tempering degree was maintained between 4.5 and 5.0, controlled by a Sollich E3 tempermeter. The chocolates were molded in rectangular molds and cooled in a single stage in an 8-m cooling tunnel (Siaht, São Paulo, BR) with belt velocity of 1.0 m/min and air temperatures of approx. 10 °C at the center of the tunnel and 15 °C at the extremities. After removal from the molds, the chocolates were wrapped and stored in a chamber at 23 °C for 15 days for the tensile strength (Sect. 2.3.2) and sensory (Sect. 2.4) tests.

Analytical methods

Characterization of the cocoa butter

Solid fat content It was determined using a nuclear magnetic resonance spectrophotometer Minispec pc120 (Burker Spectrospin, Germany) according to AOCS Cd 16b-93 [1] with the following tempering for the fat samples: heating for 15 min/100 °C; 5 min/60 °C; 90 min/0 °C; 40 h/26 °C; and 90 min/0 °C. The values were measured at 10, 20, 25, 30 and 35 °C.

Triacylglycerol composition This was determined according to AOCS Ce 5–86 [1] using an Agilent 6850 capillary gas chromatograph (Agilent Technologies, Wilmington, USA), with a DB-17 HT—Agilent 122–1811 capillary column; dimensions 15 m; Ø int 0.25 mm; film 0.15 μm; operational conditions: column flow rate 1.00 mL/min; linear velocity 40 cm/s; detector temperature 375 °C; injector temperature 360 °C; oven temperature 250–350 °C (5 °C/min), 350 °C/20 min; stripping gas helium; volume injected 1.0 μL, split 1:50; sample concentration 10 mg/mL tetrahydrofuran.

The cocoa butter content present in the liquors before pressing was also determined by method 963.15 [13].

Physicochemical characterization of the chocolates

The following parameters were evaluated according to the AOAC reference methods (Association of Official Analytical Chemists) [13]: moisture content in a Titroline Alpha Shott TZ128 equipment (method 931.04); pH (Tecnal TE-2 pH meter, method 970.21); total titratable acidity (method 942.15); and the total nitrogen content (method 970.22). Also determined were the water activity using a Decagon-Aqualab CX-2 hygrometer coupled to a water bath at 25 ± 0.3 °C; Cassons plastic viscosity and yield value in a rheometer (Brookfield RVDV III+, Stoughton, USA) coupled to a water bath at 40 ± 0.5 °C and using the S15 cylindrical spindle [15, 32]; maximum particle diameter after conching using a digital micrometer (Mitutoyo S15, scale from 0 to 25 mm) and the tensile strength of the chocolate bars using a TA.XT2i Texture Analyser (Stable Micro System Ltda., Surrey, UK), with the SMS P/W base and HDP/3PB probe [16, 19] at 25 °C. The determinations were carried out in triplicate with the exception of the water activity (nine replicates) and the maximum particle diameter and tensile strength (10 replicates).

Sensory evaluation

The sensory evaluation of the chocolates consisted of an acceptance test and was carried out according to a random

complete block design using 50 consumers with no age, sex or social class limits [21, 22]. The samples were identified by random three-digit numbers and presented in a sequential monadic way. The evaluations were carried out in individual booths equipped with fluorescent lamps and the Compusense Five 4.6 data collection system. The "Just Right" and hedonic scales were used. The samples were evaluated for their chocolate aroma and flavor, bitterness, acidity, astringency and the strength required to break them using a seven-point scale (7 = "much stronger than I like" to 1 = "much weaker than I like"). Melting was also evaluated using a seven-point scale (7 = "much more difficult than I like" to 1 = "much easier than I like"). Overall acceptance was evaluated using a nine-point scale (9 = liked extremely to 1 = disliked extremely) and buying intention on a five-point scale (1 = certainly buy to 5 = certainly not buy). The consumers also answered questions related to their chocolate-eating habits.

Statistical analysis and data analysis

The data were submitted to an analysis of variance (ANOVA) and the means compared using Tukey's test at a 5 % level of significance. A multivariate analysis (MANOVA) was also carried out, Wilks' lambda value being used to determine the significance of the test. The analyses were carried out using the SAS® statistical package [26].

Results and discussion

Characterization of the cocoa butter

Table 1 shows the total lipid contents and triacylglycerol profiles of the cocoa butter samples, Fig. 1 the solid profiles of the cocoa butter in the solid state at different temperatures, and Table 2 the physicochemical characterization of the chocolates.

A wide variation in the cocoa butter content of the liquors from the varieties studied was observed (Table 1). Due to this variation, the lipid content was standardized for the production of the chocolates as described at the start of Sect. 2.2.2 (Chocolate processing).

In the evaluation of the solid fat content at the different temperatures, the Common material showed the lowest contents and the cultivar TSH774 the sharpest fall, presenting the lowest content at 25 °C as compared to the other materials studied. On the other hand, the cultivars TSAN792 and TSH1188 showed the highest solid fat contents. These results were corroborated by those for the triacylglycerol contents (Table 1). The materials TSH1188, TSAN792, TSH565 and EET397 were those presenting the highest symmetrical triacylglycerol contents (>57 %), characterized by a high structural order, and therefore responsible for forming a more compact crystalline network in the chocolates. The materials CEPEC42 and Common showed the lowest symmetrical triacylglycerol contents (<55 %). With respect to the sum of the unsaturated triacylglycerols (UUU), the highest values were found in the cultivars TSA656, TSH516 and TSH774 (>18 %) and the lowest values in TSAN792 and TSH1188 ($\approx$15 %).

The solid fat content at room temperature is related to the hardness of the cocoa butter, which, for its part, is related to chain length, degree of unsaturation and position of the fatty acids on the glycerol molecule [9]. Considering that all the materials studied came from fruits harvested in November 2004 and that the development and ripening of the cacao fruits occurred between the months of June and November of that year at temperatures lower than those observed in the cocoa producing countries of Africa and Asia, an increase in the proportion of the unsaturated acids oleic and linoleic was expected, making the cocoa butter less hard (Berbert and Alvim [3]; Berbert [2], as cited by [24]). According to Slabas and Fawcett [28] and Ohlrogge et al. [23], as cited by Pires [24] and demonstrated by Lehrian et al. [18] and by Tucci et al. [31], various plants are capable of adjusting the degree of unsaturation of the fatty acids in their cells in response to lower temperatures, aiming to maintain fluidity.

Physicochemical characterization of the chocolates

The moisture content of the chocolates remained between 0.95 and 1.07 % and the maximum particle diameter between 20 and 21 μm, the ranges recommended for chocolates [34]. Both these parameters together with the total lipid and emulsifier contents influence the rheological properties and flow rate in industrial processing lines and sensory aspects such as texture (melting) and flavor release [5, 33, 34]. The pH values of the samples were in the range from 5.14 to 5.52 and the total acidity from 3.62 to 4.81 mEq NaOH/100 g, the differences possibly being caused by the characteristics of the actual varieties, such as the content and composition of the pulp surrounding the seeds, which influences the evolution of the fermentation step [4, 5, 9, 14]. There was a significant difference in the total nitrogen content of the chocolates from TSH1188 and those from TSH516, TSA654, EET397 and Common. Ripe cocoa seeds contain between 10 and 16 % (dry wt.) of proteins, and during fermentation, these proteins are degraded by enzymatic hydrolysis, giving rise to 1–2 % of free amino acids (dry wt.) [35].

Before the fermentation step, the highest protein content found in the seeds used in this study (data not shown) was observed in the material TSA654, with no significant

Table 1 Cocoa butter content of the liquors and the triacylglycerol compositions (g/100 g) and solid fat contents of the cocoa butters from the varieties studies

		CEPEC42	COOMMON	EET397	TSA654	TSA656
	Lipid content (%)	56.56 (0.57)^{bc}	62.36 (0.49)^{a}	54.02 (0.32)^{de}	57.07 (0.32)^{b}	57.34 (0.64)^{b}
Trisaturated triacylglycerols (%)	PPS	0.28 (0.05)^{a}	0.22 (0.01)^{a}	0.28 (0.03)^{a}	0.31 (0.05)^{a}	0.25 (0.05)^{a}
	PSS	0.26 (0.00)^{a}	0.23 (0.02)^{a}	0.33 (0.01)^{a}	0.29 (0.01)^{a}	0.33 (0.06)^{a}
	SSS	20.87 (0.07)^{c}	22.61 (0.07)^{a}	19.14 (0.05)^{f}	19.78 (0.06)^{e}	19.75 (0.06)^{e}
Total		21.42	23.06	19.75	20.38	20.33
Disaturated triacylglycerols (%)	POP	17.32 (0.11)^{f}	16.39 (0.02)^{g}	19.37 (0.09)^{c}	18.18 (0.08)^{de}	17.95 (0.04)^{e}
	POS	36.14 (0.01)^{ef}	36.79 (0.10)^{c}	36.56 (0.04)^{cd}	36.35 (0.03)^{de}	36.09 (0.12)^{fg}
	SOS	0.21 (0.01)^{a}	0.21 (0.01)^{a}	0.24 (0.01)^{a}	0.30 (0.10)^{a}	0.23 (0.01)^{a}
	SOA	1.07 (0.04)^{a}	1.15 (0.00)^{a}	1.02 (0.09)^{a}	1.05 (0.01)^{a}	1.02 (0.07)^{a}
Total		54.74	54.54	57.19	55.87	55.28
	PLP	2.39 (0.00)^{c}	2.13 (0.02)^{e}	2.51 (0.04)^{b}	2.51 (0.02)^{b}	2.33 (0.01)^{c}
	PLS	3.51 (0.21)^{ab}	3.32 (0.29)^{ab}	3.34 (0.03)^{ab}	3.42 (0.01)^{ab}	3.19 (0.08)^{b}
Total		5.90	5.45	5.85	5.93	5.52
Tri-unsaturated triacylglycerols (%)	POO	6.08 (0.14)^{b}	5.35 (0.40)^{c}	6.21 (0.00)^{ab}	6.39 (0.12)^{ab}	6.71 (0.00)^{a}
	PLO	0.87 (0.03)^{bc}	0.71 (0.02)^{d}	0.87 (0.02)^{bc}	0.87 (0.03)^{bc}	0.86 (0.00)^{bc}
	SOO	7.61 (0.39)^{ab}	7.28 (0.00)^{abc}	6.90 (0.12)^{bcd}	7.10 (0.03)^{bc}	8.15 (0.43)^{a}
	OOO + SLS	2.17 (0.20)^{a}	2.45 (0.01)^{a}	2.16 (0.07)^{a}	2.38 (0.03)^{a}	2.01 (0.42)^{a}
	SLO	0.87 (0.01)^{b}	0.82 (0.04)^{bcd}	0.75 (0.03)^{de}	0.76 (0.01)^{cde}	0.82 (0.01)^{bcd}
Total		17.61	16.61	16.90	17.50	18.55

		TSAN792	TSH516	TSH565	TSH774	TSH1188	MSD*
	Lipid content (%)	55.16 (0.76)^{cd}	54.30 (0.38)^{de}	53.49 (0.69)^{ef}	52.28 (0.69)^{f}	55.08 (0.32)^{cd}	1.57
Trisaturated triacylglycerols (%)	PPS	0.33 (0.04)^{a}	0.27 (0.03)^{a}	0.21 (0.02)^{a}	0.25 (0.05)^{a}	0.27 (0.02)^{a}	0.15
	PSS	0.32 (0.03)^{a}	0.27 (0.04)^{a}	0.23 (0.00)^{a}	0.24 (0.05)^{a}	0.24 (0.04)^{a}	0.13
	SSS	21.20 (0.02)^{b}	18.00 (0.04)^{h}	18.65 (0.03)^{g}	20.05 (0.02)^{d}	19.25 (0.07)^{f}	0.21
Total		21.85	18.54	19.09	20.55	19.76	
Disaturated triacylglycerols (%)	POP	18.47 (0.11)^{d}	19.52 (0.15)^{bc}	19.70 (0.01)^{ab}	18.29 (0.04)^{d}	20.01 (0.03)^{a}	0.33
	POS	37.47 (0.03)^{a}	35.42 (0.01)^{h}	36.14 (0.04)^{ef}	35.89 (0.06)^{g}	37.04 (0.02)^{b}	0.24
	SOS	0.28 (0.07)^{a}	0.29 (0.08)^{a}	0.22 (0.01)^{a}	0.24 (0.02)^{a}	0.30 (0.05)^{a}	0.20
	SOA	1.06 (0.08)^{a}	0.97 (0.03)^{a}	1.00 (0.06)^{a}	1.05 (0.10)^{a}	1.00 (0.03)^{a}	0.24
Total		57.28	56.19	57.06	55.47	58.35	
	PLP	2.20 (0.01)^{de}	2.78 (0.02)^{a}	2.57 (0.03)^{b}	2.25 (0.03)^{d}	2.75 (0.01)^{a}	0.08
	PLS	3.26 (0.08)^{ab}	3.56 (0.10)^{ab}	3.13 (0.13)^{b}	3.30 (0.00)^{ab}	3.78 (0.22)^{a}	0.58
Total		5.46	6.34	5.70	5.55	6.53	
Tri-unsaturated triacylglycerols (%)	POO	5.18 (0.02)^{c}	6.82 (0.16)^{a}	6.60 (0.14)^{ab}	6.33 (0.01)^{ab}	5.37 (0.11)^{c}	0.63
	PLO	0.71 (0.00)^{d}	1.02 (0.03)^{a}	0.92 (0.03)^{ab}	0.85 (0.04)^{bc}	0.77 (0.00)^{cd}	0.10
	SOO	6.46 (0.05)^{cd}	7.40 (0.05)^{ab}	7.36 (0.08)^{abc}	7.56 (0.42)^{ab}	6.03 (0.01)^{d}	0.91
	OOO + SLS	2.06 (0.00)^{a}	2.41 (0.08)^{a}	2.09 (0.06)^{a}	2.54 (0.47)^{a}	2.18 (0.01)^{a}	0.85
	SLO	0.71 (0.05)^{e}	0.96 (0.01)^{a}	0.85 (0.01)^{bc}	0.80 (0.01)^{bcde}	0.76 (0.00)^{cde}	0.1
Total		15.12	18.60		18.09	15.10	

The triacylglycerols are formed from the following fatty acids: *P* palmitic acid (saturated), *O* oleic acid (monounsaturated), *S* stearic acid (saturated), *L* linoleic acid (bi-unsaturated), *A* arachidic acid (saturated)

Values expressed as mean (SD)

* *MSD* minimum significant difference at the 5 % error level by the Tukey's test; samples with the same letters in the same row are not significantly different at the 5 % level

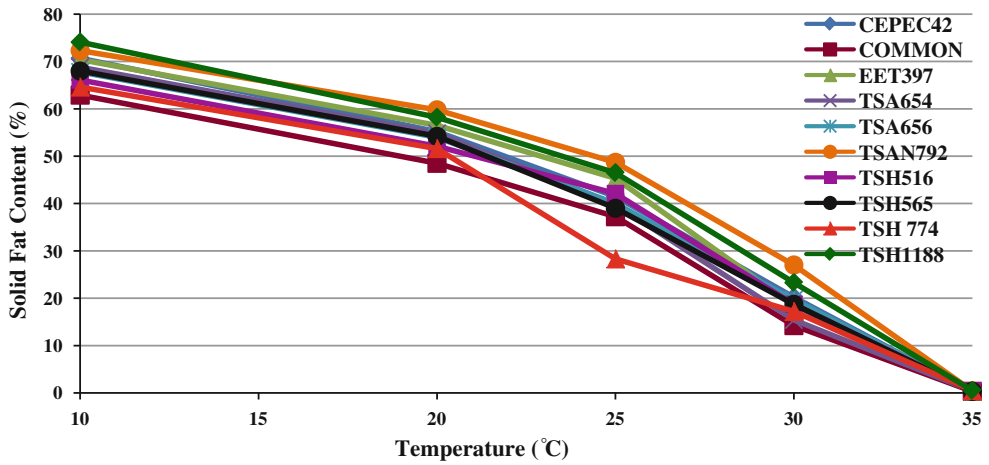

Fig. 1 Solid fat content profiles (%) of the cocoa butter samples from the varieties evaluated

Table 2 Physical and physicochemical characterizations of the chocolates produced from the ten cacao cultivars

Chocolates	Moisture content (%)*	Max. particle diameter (μm)*	pH*	Titratable acidity (mEqNaOH/100 g)*	Total nitrogen content (%)*,•	Plastic viscosity (Pa.s)*	Flow limit (Pa)*	Tensile strength (Kgf/cm^2) *
CEPEC42	1.07 (0.11)^{abcd}	21 (2.1)^{a}	5.29 (0.12)^{bc}	4.10 (0.09)^{bc}	7.63 (0.12)^{ab}	4.01 (0.16)^{b}	33.15 (0.74)^{b}	2.361 (0.127)^{abc}
COMMON	1.04 (0.04)^{cde}	21 (2.3)^{a}	5.14 (0.03)^{c}	4.81 (0.13)^{a}	6.39 (0.06)^{b}	5.85 (0.65)^{a}	51.86 (7.28)^{a}	2.042 (0.120)^{de}
EET397	1.03 (0.07)^{bcd}	21 (1.8)^{a}	5.30 (0.05)^{bc}	3.96 (0.22)^{bc}	6.40 (0.13)^{b}	4.31 (0.61)^{b}	24.74 (2.66)^{bcd}	2.392 (0.169)^{abc}
TSA654	1.07 (0.10)^{abc}	20 (2.3)^{a}	5.52 (0.08)^{a}	3.62 (0.35)^{c}	6.43 (1.01)^{b}	5.76 (0.18)^{a}	21.03 (0.97)^{cd}	2.371 (0.153)^{abc}
TSA656	1.05 (0.03)^{ab}	21 (2.4)^{a}	5.38 (0.05)^{ab}	3.89 (0.20)^{bc}	7.87 (0.09)^{ab}	4.72 (0.38)^{ab}	26.90 (0.97)^{bc}	2.278 (0.232)^{bcd}
TSAN792	0.95 (0.03)^{e}	21 (2.4)^{a}	5.22 (0.07)^{bc}	4.33(0.15)^{ab}	7.80 (0.31)^{ab}	5.88 (0.21)^{a}	24.45 (1.62)^{bcd}	2.504 (0.116)^{ab}
TSH516	1.06 (0.05)^{cde}	20 (1.7)^{a}	5.41 (0.04)^{ab}	3.83 (0.11)^{bc}	6.89 (0.42)^{b}	5.68 (0.68)^{a}	25.48 (4.54)^{bcd}	2.337 (0.148)^{abc}
TSH565	1.04 (0.11)^{de}	21 (2.5)^{a}	5.41 (0.03)^{ab}	3.98 (0.14)^{bc}	6.95 (0.51)^{ab}	5.85 (0.35)^{a}	17.31 (2.50)^{d}	2.533 (0.159)^{a}
TSH774	1.04 (0.12)^{cd}	21 (2.6)^{a}	5.21 (0.08)^{bc}	4.09 (0.22)^{bc}	7.86 (0.13)^{ab}	3.66 (0.21)^{b}	32.65 (1.26)^{b}	2.031 (0.114)^{e}
TSH1188	1.01 (0.05)^{a}	21 (1.9)^{a}	5.54 (0.10)^{a}	3.70 (0.24)^{c}	8.59 (1.29)^{a}	3.68 (0.21)^{b}	32.55 (2.44)^{b}	2.502 (0.180)^{ab}
MSD**	0.15	3.2	0.20	0.58	1.66	1.20	9.21	0.242

* Values expressed as mean (SD)

** *MSD* minimum significant difference at the 5 % error level according to the Tukey's test; samples with the same letters in the same row are not significantly different at the 5 % level

• Values expressed on a dry weight basis

difference between CEPEC42 and TSA656. The lowest content was observed in the TSH565 seeds, with no significant difference between TSH1188, TSH774, EET397 and the Common material. After the drying stage, TSA654 continued to show the highest levels, with no significant difference between TSH1188 and CEPEC42, and the lowest value was found for TSH774, with no difference between TSAN792 and EET397. Thus, a higher consumption of nitrogen compounds was observed in the processing steps of the liquor and chocolate especially for TSA654, which, together with EET397, showed the lowest levels of nitrogen; and a lower consumption for TSH1188, which showed the highest content in the chocolate as compared to the other samples. It is important to mention that the method used for the determination of the protein content (micro Kjeldahl) evaluates the nitrogen compound content and not that of the protein itself. Further analyses of the protein profiles of each material studied would be important to evaluate their influence on the formation of the desirable flavor precursors formed in the cocoa processing steps.

Despite the variation in the values for plastic viscosity and flow limit, they are still characteristic of bitter chocolates containing 0.4 % soybean lecithin [6, 27]. Since the differences observed could affect the industrial process for the manufacture of chocolates, corrections in the dosage and/or types of emulsifier could be carried out industrially [27].

In relation to the force required to break the chocolates, the relationships observed with the solid fat contents at 25 °C and also between those at 35 °C and those at 25 °C (Fig. 1) stand out, as also the sum of the symmetrical triacylglycerols POP, POS and SOS (Table 1). The values for tensile strength of the chocolates TSH565, TSAN792 and TSH1188 were greater and differed significantly from those of the chocolates TSH774 and Common (Table 2), which also presented the lowest solid fat contents at 25 °C and between 35 and 25 °C and the smallest values for the symmetrical triacylglycerols (Table 1).

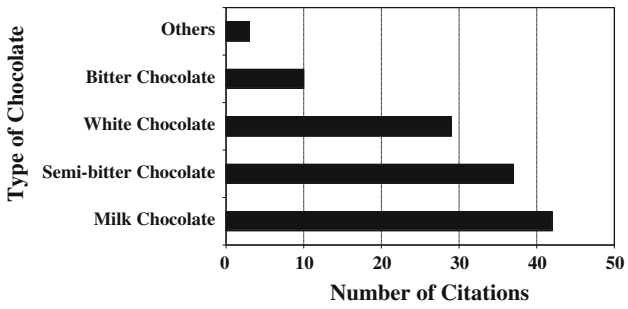

Fig. 3 Types of chocolate preferred by the consumer group recruited to evaluate the samples of bitter chocolate from the ten cacao cultivars

Sensory evaluation

The consumer group that evaluated the chocolates was composed of 18 % men and 82 % women, and the age range was predominantly between 21 and 30 (Fig. 2). The majority of the consumer group preferred milk- or semi-bitter-type chocolates (Fig. 3).

With respect to chocolate flavor, TSH1188 was the one that stood out, with 74 % of the responses considering its flavor to be "ideal," and EET397 and COMMON, with 60 % of the responses considering their flavor to be "more intense than ideal." The same was found for bitterness and acidity, with 72 and 78 % of the responses considering the bitterness and acidity of TSH1188 to be ideal, and, respectively, 70–76 % and 58 % of the consumers considering the chocolates EET397 and COMMON to be bitterer and more acid than the ideal. More than 91 % of the responses considered the astringency of the chocolates to be between ideal and more than ideal and 76 % of the consumers considering EET397 to be more astringent than the ideal in comparison with only 20 % for TSH1188.

For the attributes of chocolate aroma and flavor, astringency, acidity, bitterness, force required to break the chocolate and melting characteristics, scores above 4.0 were computed as "More than ideal," scores equal to 4.0 as "Ideal" and scores below 4.0 as "Below the ideal."

In the evaluation of the difference between the chocolates for the group of variables considered in the sensory evaluation (Wilks' lambda value/PROC–GLM/MANOVA—SAS®) (Table 3), it was observed that the chocolate EET397 differed from the others at the significance levels of 1 or 5 %, with the exception of chocolate TSA656, which only differed from chocolate TSH1188 at the 5 % level of significance. The chocolates CEPEC42 and TSA654 differed from EET397 and TSH1188 and the chocolates TSH565 and TSH774 only differed from EET397. It can also be seen that the chocolate COMMON differed from EET397 and from all the materials descendent from *Trinitario* (TSAN792, TSH516, TSH565, TSH774 and TSH1188) at 1 % of significance. On the other hand, TSH1188 differed from all the materials originating purely from the *Forastero* group (CEPEC42, COMMON, TSA654 and TSA656), and also from TSH516 and EET397.

In the evaluation of the contrast between the groups (Table 4), it can be seen that Groups 1 and 3 (materials originating purely from the *Forastero* group) did not present a significant difference at a level below 5 % (the result was 8.61 %), such that, sensorially, the consumers did not clearly differentiate the samples of these two groups. On the other hand, the contrast between Group 2

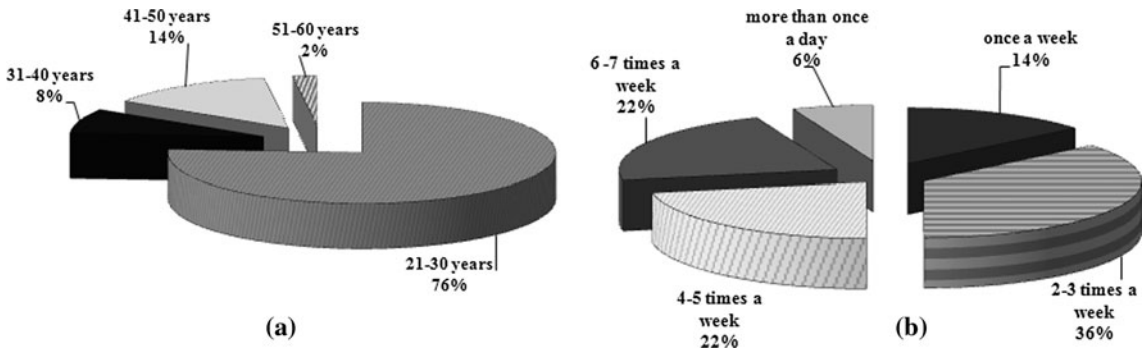

Fig. 2 Age range (**a**) and chocolate consumption frequency (**b**) of the consumer group recruited to evaluate the samples of bitter chocolate from the ten cacao cultivars

Table 3 Probability of error for rejection of the null hypothesis (of equality) between the samples of chocolate from the varieties studied regarding the sensory attributes evaluated

	COMMON	EET397	TSA654	TSA656	TSAN792	TSH1188	TSH516	TSH565	TSH774
CEPEC42	ns	*	ns	ns	ns	**	ns	ns	ns
COMMON		*	ns	ns	*	**	*	**	ns
EET397			*	ns	**	**	**	**	**
TSA654				ns	ns	**	ns	ns	ns
TSA656					ns	**	ns	ns	ns
TSAN792						ns	*	ns	ns
TSH1188							*	ns	ns
TSH516								ns	ns
TSH565									ns

"ns" difference between the samples not significant at the levels evaluated; "*" statistical difference between the samples at 5 % of significance and "**" statistical difference between the samples at 1 %—Wilks' lambda test

Table 4 Probability of error for rejection of the null hypothesis (of equality) between the samples of chocolate evaluated

	Group 3	Group 1
Group 1	ns	
Group 2	**	**
Groups 1 and 2	**	

"ns" difference between the samples not significant at the levels evaluated; "**" statistical difference between the samples at 1 %—Wilks' lambda test. Group 1: materials descendent from the *Forastero* group*: TSA654, TSA656 and CEPEC42;* Group 2: materials descendent from the *Forastero* and *Trinitario* groups: TSH516, TSH565, TSH774, TSH1188 and TSAN792; and Group 3: *Forastero Amelonado* (Common)

and the Groups 1 and 3 resulted, in the two cases, in a significant difference at the 1 % level.

According to the mean scores given by the consumers in the acceptability test for the chocolates (Table 5), there was no difference at the 5 % significance level in the values given by the consumers for the attributes of "chocolate aroma" and "melting." However, with respect to the attributes of "chocolate flavor" and "acidity," a difference was found at the 5 % significance level for the samples TSH565 and TSH1188 in relation to EET397 and Common, which presented means above the ideal, whereas the former were closer to the ideal. With respect to bitterness, the samples in Groups 1 and 3 differed significantly from the samples in Group 2. In relation to astringency, TSH1188 presented the lowest value and differed from all the samples in Groups 1 and 3, with the exception of EET397, and its mean value was also the closest to the ideal. Overall, in the evaluation of the chocolates, EET397 differed from the others and was more appreciated. The highest mean for this attribute was that of TSH1188, which did not differ significantly from the other samples in Group 2. It can be seen that both the buying intention and overall evaluation of the chocolates were influenced and possibly determined by the attributes "bitterness," "astringency" and "acidity" and also by the attribute "chocolate flavor."

The chocolates in Groups 1 and 3 presented greater frequencies of scores "above the ideal" for the attributes of bitterness, acidity and astringency, whereas for the same attributes, with the exception of "bitterness" and including "chocolate flavor," the chocolates of Group 2 presented a greater frequency of scores considered to be of "ideal" intensity.

In differentiating cocoa quality, ordinary materials (known as "Bulk") generally originate from fruits of the *Forastero* group, and, due to their greater availability, are generally used by the manufacturers for the production of liquor and cocoa butter on a large scale for use in conventional chocolates. On the other hand, beans from "fine" grades of cocoa, generally obtained from the groups *Criollo* and *Trinitario*, have greater market value, reaching prices two to three times higher than those of the "Bulk" type, since they give rise to chocolates with differentiated flavors, valued for the production of premium type chocolates with higher cocoa contents in their formulations [29].

Conclusions

By characterizing the cocoa butters of the varieties studied, it could be seen that the triacylglycerol composition and solid fat curves of some materials such as TSH1188, TSAN792 and EET397 presented properties of greater interest for the manufacture of chocolates from the technological point of view, especially as compared to the Common material. These could provide greater product stability at room temperature, better melting properties and a more compact crystalline network. It could be seen from the sensory analysis that the non-trained consumer panel was able to conclusively differentiate the varieties studied,

Table 5 Means of the scores given by the consumers in the sensory evaluation (acceptability test) for the chocolates produced with the different varieties

Varieties	Sensory attributes								
	Chocolate aroma	Snap (breaking force)	Melting	Chocolate flavor	Bitterness	Acidity	Astringence	Overall mode	Buying intention
CEPEC42	4.0 (1.0)^{a}	4.5 (0.8)^{abc}	3.5 (0.6)^{a}	4.3 (1.1)^{ab}	4.7 (1.1)^{abc}	4.6 (0.9)^{ab}	4.7 (0.9)^{bc}	6.3 (1.8)^{ab}	2.9 (1.2)^{b}
COMMON	4.0 (1.1)^{a}	4.6 (0.9)^{ab}	3.6 (0.5)^{a}	4.8 (1.0)^{a}	5.0 (1.0)^{ab}	4.9 (1.0)^{a}	4.8 (0.9)^{ab}	6.2 (1.7)^{b}	3.0 (1.2)^{ab}
EET397	3.9 (1.0)^{a}	4.4 (0.7)^{abc}	3.6 (0.8)^{a}	4.8 (1.4)^{a}	5.2 (1.2)^{a}	4.9 (1.2)^{a}	5.3 (1.1)^{a}	4.9 (2.3)^{c}	3.7 (1.3)^{a}
TSA654	3.9 (0.8)^{a}	4.4 (0.7)^{abc}	3.4 (0.8)^{a}	4.5 (0.8)^{ab}	4.7 (0.7)^{abc}	4.4 (0.7)^{ab}	4.7 (0.7)^{ab}	6.6 (1.4)^{ab}	2.6 (1.1)^{bc}
TSA656	4.0 (1.0)^{a}	4.4 (0.7)^{abc}	3.6 (0.8)^{a}	4.5 (1.0)^{ab}	4.8 (1.0)^{abc}	4.7 (0.8)^{ab}	4.9 (0.9)^{ab}	6.2 (1.8)^{b}	2.9 (1.3)^{b}
TSAN792	4.0 (0.7)^{a}	4.7 (0.8)^{a}	3.6 (0.7)^{a}	4.2 (0.8)^{b}	4.4 (0.9)^{bcd}	4.4 (0.6)^{ab}	4.5 (0.6)^{bc}	6.7 (1.6)^{ab}	2.6 (1.0)^{bc}
TSH516	4.0 (1.1)^{a}	4.2 (0.6)^{c}	3.6 (0.6)^{a}	4.4 (1.0)^{ab}	4.5 (1.1)^{bcd}	4.6 (0.8)^{ab}	4.6 (0.9)^{bc}	6.4 (1.7)^{ab}	2.9 (1.2)^{b}
TSH565	3.9 (0.8)^{a}	4.3 (0.6)^{abc}	3.7 (0.6)^{a}	4.1 (0.8)^{b}	4.3 (0.8)^{cd}	4.3 (0.8)^{b}	4.5 (0.8)^{bc}	6.7 (1.5)^{ab}	2.6 (1.0)^{bc}
TSH774	3.9 (0.6)^{a}	4.2 (0.5)^{c}	3.6 (0.6)^{a}	4.4 (0.9)^{ab}	4.6 (0.8)^{bcd}	4.5 (0.9)^{ab}	4.5 (0.7)^{bc}	6.9 (1.6)^{ab}	2.4 (1.2)^{bc}
TSH188	3.8 (0.8)^{a}	4.3 (0.7)^{abc}	3.8 (0.6)^{a}	3.9 (0.8)^{b}	4.0 (0.8)^{d}	4.2 (0.5)^{b}	4.2 (0.7)^{c}	7.3 (1.3)^{a}	2.0 (0.9)^{c}
MDS*	0.6	0.4	0.4	0.6	0.6	0.5	0.5	1.1	0.7

Values expressed as the mean (SD)

* *MSD* minimum significant difference at the 5 % error level by the Tukey's test; samples with the same letters in the same row are not significantly different at the 5 % level

suggesting the possibility and interest in producing and commercializing mono-varietal chocolates, and in the current Brazilian case, monoclonal ones. The results also confirmed the possibility of a gain in quality by the genetic improvement of cacao, with the inclusion of varieties from the *Trinitario* and *Criollo* groups in the cross-breeding programs, associating disease resistance with other characteristics of interest, such as product quality.

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Conflict of interest None.

Compliance with Ethics Requirements Considering the sensory analysis performed in the article, we declare that all procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. All of the consumers recruited for the sensory analysis were informed and signed a term of consent for being included in the study.

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