

Original article

Relationship between bioactive compounds and sensory properties of dark chocolate produced from Brazilian hybrid cocoa

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Summary This work evaluated the influence of bioactive compounds on the sensory profile and acceptability of dark chocolate produced from Brazilian hybrid cocoa. The results showed that higher contents of catechin, epicatechin, caffeine, total phenolic compounds and flavonoids contributed to higher intensity of bitterness, cocoa flavour, acid taste, and astringency, and negatively affected the acceptance of chocolate samples from the TSH1188, PH16, and CEPEC2002 varieties. Other varieties SR162 and BN34 showed lower contents of caffeine and phenolic compounds, and higher concentration of theobromine, revealed notes of sweet/caramel and fruity flavour, presented a higher acceptance. This research showed the great potential of the ‘Catongo’ (SR162) and BN34 hybrids from southern Bahia (Brazil) for the production of fine cocoa. The chemical and sensory evaluations may be a strategic tool to help cocoa producers in deciding which genetic varieties should be marketed as fine cocoa, adding value to the product and highlighting promising cocoa varieties.

Keywords Acceptability, hybrid cocoa, methylxanthines, phenolic compounds, sensory profile.

Introduction

After the arrival of ‘Witch’s Broom’ disease, caused by the fungus *Moniliophora perniciosa*, in the Bahia State, Brazil lost its position as the second largest cocoa producer in the world. Strategically, cacao farmers from the southern Bahia have adopted the production of new varieties resistant to this fungus, in a partnership with the Cocoa Research Center (CEPLAC-Comissão Executiva para o Plano da Lavoura Cacaueira) that maintains one of largest cocoa germplasm banks in the world (Lopes *et al.*, 2011). Cocoa production has great economic importance for the Bahia State, which is the main producer in Brazil, currently with a predicted annual cocoa bean production in 190 000 tons for 2019/2020 (International Cocoa Organization, 2020).

There are three main genetic varieties of cocoa (*Theobroma cacao* L.) used in chocolate making:

Forastero (violet colour of the cotyledons), Criollo (identified by the white colour of the cotyledon cells) and Trinitario (presenting cotyledons ranging from white to pale-violet), which are distinguished by their aroma, an attribute that determines their quality. Compared to *Criollo* (highly aromatic) and *Trinitario* groups, which produce a chocolate with excellent quality due to its the special aroma and flavour, *Forastero* has a more bitter and astringent taste (Castro-Alayo *et al.*, 2019). Fine cocoa is characterised mainly by the presence of fruity and/or floral flavour notes, in contrast to bulk cocoa, which has a strong basic cocoa taste [Afoakwa *et al.*, 2008; Beckett *et al.*, 2017; Rottiers *et al.*, 2019]. The cocoa grown in Bahia has suffered a great change in its genetic composition, with approximately 30% of the *Trinitario* group being suitable for fine cocoa production (Santos *et al.*, 2016). The Bahia State has sought to develop as a fine cocoa and chocolate producer through innovative farming techniques, new cocoa

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varieties (hybrid seeds), and research on the physico-chemical and sensory characteristics of these products in order to become the major producer of fine cocoa in Brazil.

Various chemical components from raw cocoa beans participate in the formation of specific cocoa flavours by changes occurring during processing. These components are methylxanthines, polyphenols, proteins, and carbohydrates. The content of polyphenols and their derivatives in the beans may vary due to the variety and origin of the cacao plants. Consequently, the flavour characteristics in the chocolate depend on the origin of the cocoa; the growing conditions, such as the climate, the amount and length of sunshine and rainfall; soil conditions; ripening; harvest time; the time between harvesting and bean fermentation; and processing factors. All of them contribute to variations in the flavour (Afoakwa *et al.*, 2008; Aprotosoai *et al.*, 2016; Castro-Alayo *et al.*, 2019; Muñoz *et al.*, 2020).

Some previous works reported the influence of genetic variability on the characteristics of cocoa, such as size, colour, composition of pulp, volatile components and flavour of the liquor, cocoa butter, and chocolate (Efraim *et al.*, 2013; Moreira *et al.*, 2016; Moreira *et al.*, 2018; Rottiers *et al.*, 2019). However, there is a lack of studies about the effect of genotype on the nonvolatile components that contribute to cocoa flavour and sensory quality of dark chocolates.

This study aimed to investigate the relationship between bioactive compounds and the descriptive sensory attributes of dark chocolate samples produced from hybrid cocoa grown in southern Bahia (Brazil), and to assess their correlation with consumer acceptance of the dark chocolate samples.

Materials and methods

Cocoa samples

The cocoa beans came from a commercial farm (Ibirataia, Bahia) located in the Northeast Brazil (14°41'96"S and 39°12'109"W), in a tropical climate. The temperatures in the crop area ranged from 14 to 28 °C, relative humidity from 90% to 95%, rainfall between 200 and 300 mm. Healthy, ripe and selected fruits were used based on the productivity and resistance to diseases. The selected varieties were BN34 (hybrid of unknown origin), TSH1188 (hybrid from Trinidad and Tobago), PH16 (hybrid from the crossing between the *Amazonian Forastero* and *Trinitario*), SR162 ('Catongo', originated from a genetic mutation of the *Lower Amazon Forastero*, with white seeds), and CEPEC2002 (hybrid of the *Forastero*).

Preprocessing of cocoa beans and chocolate processing

The cocoa beans were pre-selected and transported at room temperature, from the producing region to the fermentation house. The fermentation was carried out in 80 kg lots of seeds with pulp for 5 days in 50 × 50 × 50 cm wooden boxes, with around twenty holes (1.2 cm diameter) at the bottom and the lower sides of the boxes, in order to allow the liquefied pulp to flow during the first days of fermentation. The process was performed at the fermentation house (with room temperature ranging from 23 to 28 °C, humidity > 60%). Turnings for oxygenation and mixing of the mass were performed, every 48 h from the beginning of each fermentation, with a temperature control of approximately 45 °C (with a maximum of 48 °C), until the end of the process. After fermentation, the seeds were dried in the sun-roof surfaces with movable timber for 5–7 days up to 8% of moisture (Cruz *et al.*, 2015).

The fermented and dry beans were roasted in a rotary electric roaster (Jaf Inox-TX, São Paulo, Brazil), at 120 °C, for 60 min (Rocha *et al.*, 2017). The seeds were crushed to remove the peel and germs, in order to obtain cocoa nibs. The nibs were ground in a knife-grinder (Jaf Inox-FX, São Paulo, Brazil), with sugar added in this phase. The dark chocolate samples were obtained using the following proportions: cocoa mass (66%), cocoa butter (4%), sugar (29.6%), and lecithin (0.4%). Then, the cocoa mass was refined in a 3-roller refiner (Jaf Inox-MX, São Paulo, Brazil) to obtain particle sizes varying from 20 to 25 µm. The refined mass and remaining cocoa butter remained for 24 h in a conch (Inco, Avaré, Brazil) at 60 °C, and the lecithin was added during this phase. The chocolates were hand tempered and was held until the mass reached 42 °C, when stable crystals of cocoa butter appeared. The chocolate samples were moulded in rectangular polyethylene moulds (5 g bars), cooled in a cooling tunnel, wrapped in an aluminium foil, and stored at 18 °C until the sensory and chemical evaluation.

Quantification of phenolic compounds in the chocolate samples

The shredded chocolate samples were defatted five times with petroleum ether at a ratio of 1:2 (sample: solvent). The quantification of total phenolic compounds (TPC) was performed using the Folin-Ciocalteu method. In this method, the phenolic compounds in the sample reduce a reagent and form a blue complex whose intensity increases linearly to 765 nm. Swain & Hillis (1959) described this method, which is mentioned by Roesler *et al.* (2007). Extraction was

carried out using 80 mL of a methanol:water solution (80:20 v/v), according to Maciel *et al.* (2017).

The sealed tubes were agitated in a shaker type vortex for 5 min and then centrifuged for 20 min.

The total amount of phenols from each extract was quantified using a standard curve prepared with gallic acid ($y = 0.0011x + 0.0105$; $R^2 = 0.99893$). For the colour reaction, an aliquot from the 0.5 mL of aqueous extract (concentration 10 mg mL^{-1}) was added to 2.5 mL of a 10% aqueous solution of Folin-Ciocalteu reagent and to 2.0 mL of 7.5% sodium carbonate. The mixture was incubated for 5 min in a water bath at 50 °C, and the absorbance was measured at 765 nm (using the blank as reference). The quantification of the TPC in the sample extracts was performed in triplicate.

The quantification of the flavonoid group of phenolic compounds was made according to Lee *et al.* (2003). The total concentration of flavonoids was determined using a colorimetric reaction (1 mL of sample was transferred using a 10 mL measuring flask containing 4 mL of water). Then, 0.3 mL of 5% sodium nitrite solution was added. After 5 min, 0.3 mL of 10% aluminium chloride solution was added. One minute later, 2 mL of a 1 M sodium hydroxide was added and the volume completed with distilled water and measuring flask. The amount of flavonoids in each extract was quantified using a standard curve prepared with epicatechin ($y = 0.0044x + 0.0048$; $R^2 = 0.99978$). Subsequently, the absorbance was measured at 510 nm using the blank as reference.

Quantification of monomeric phenols and methylxanthines in the chocolate samples

The determination of monomeric phenolic compounds (gallic acid, catechin, and epicatechin) and methylxanthines (caffeine and theobromine) was performed according to methodology described by Maciel *et al.* (2017). Ten microlitres of each sample solution was analysed by High Performance Liquid Chromatography (HPLC) (Perkin Elmer® Model Flexar) equipped with VI Flow injector, C 18 column ($100 \text{ mm} \times 4.6 \text{ mm O.D.S.-2}$, $3 \mu\text{m}$), and using the solvents (A) 2% acetic acid in water and (B) a mixture of acetonitrile, water, and acetic acid (400:90:10 v/v/v), in isocratic elution. The compounds were monitored by UV detection at 280 nm wavelength. The total run time was 20 min at the temperature of 26 °C. All standards used for quantitative determinations were from Sigma-Aldrich (St. Louis, MO, USA). The results were expressed as mg g^{-1} of sample.

Sensory analysis

The descriptive attributes of the dark chocolate samples were quantified using the methodology of

Quantitative Descriptive Analysis (QDA®), described by Stone *et al.* (2012). All sensory analyses were carried out in individual sensory booths at 22 °C with fluorescent lamps. Approval for the study was obtained from the Research Ethics Committee (Process n.1.231.812), and a written consent form was signed by all the volunteers.

In the recruitment stage, fifty volunteers were evaluated regarding their interests, no allergic reactions to cocoa/chocolate, schedule availability, ability to verbalise descriptions of sensory perceptions, and use of scales. In the pre-selection stage, a triangle test was used to check for differences (Stone *et al.*, 2012), in which volunteers evaluated the perception of basic tastes (sweet, salty, sour, bitter), and astringency (tactile sensation) and the recognition of odours of some products (Liu *et al.*, 2015). Five replications were made, in which the candidates had to demonstrate at least 80% accuracy. Thirty candidates (ages between 21 and 52) were selected as potential assessors. The Kelly's repertory grid method (Moskowitz, 1983) was used in the developing stage of the descriptive terminology. All samples were presented in pairs of samples, and the participants described the similarities and differences for each pair. After discussion, the redundant terms were excluded by consensus of all participants, and a total of seventeen descriptive terms were defined, with their respective references (Table 1).

Training for the formation of sensory memory was carried out by direct contact of the assessors with the maximum and minimum references for each attribute. This stage consisted of sixteen sessions lasting 2 h each, and data were obtained through an evaluation sheet containing an unstructured 9-cm line scale for each attribute, with the extremes being 'weak/strong' or 'none/much' (McMahon *et al.*, 2017). The performance of each participant was monitored at the end of the training sessions. After the training, the participants were asked to evaluate five chocolate samples in three replicates, using the definitive sheet for QDA. The results were evaluated by a two-way ANOVA and the participants who exhibited discriminatory ability ($p_{F_{\text{sample}}} \leq 0.50$) and good repeatability of the results ($p_{F_{\text{replicate}}} > 0.05$) for all the attributes were selected for the final stage, and the individual consensus was also considered (Cadena *et al.*, 2013). This process resulted in the formation of a trained team with four men and eight women. The final evaluation was performed with twelve selected participants in three replicates, according to a randomised complete block design (Lawless & Heymann, 2010) to avoid artefacts due to order of sample presentation. The samples (5 g) were identified by random three-digit numbers and presented in a sequential monadic way. Water for cleansing the palate was provided at all stages.

Table 1 Descriptive terms and reference materials used by each panellist during the training stage and quantitative descriptive analysis sessions

Descriptors	Definition	Reference materials
Brown colour	Characteristic colour of chocolate/ similar colour to mahogany brown	Weak: mixture of 10 g cocoa powder with 100 g starch cream (Unilever Ltda, Brazil) Strong: mixture of 80 g cocoa powder with 100 g starch cream
Brightness	Light reflectivity on the product surface. Light emits shine or greatly reflects shine	Weak: dark chocolate bar, 100% cocoa (AMMA Ltda, Brazil) Strong: Danette chocolate dairy dessert (Nestlé S.A.)
Cocoa aroma	Odour related to chocolate/cocoa	Weak: cocoa powder (100% cocoa) diluted in water 1:20, w/v (AMMA Ltda, Brazil) Strong: cocoa powder diluted in water 1:4, w/v
Sweet/caramel aroma	Odour obtained from caramel/sugar	Weak: cocoa powder diluted in water 1:20, w/v with addition of 0.1% sucrose (AMMA Ltda, Brazil) Strong: mixture of 20 g milk chocolate diluted in 50 mL water with melted caramel (Hershey's Company)
Fruity aroma	Characteristic odour of citric fruits (tangerine, lemon, orange, mandarin)	None: cocoa powder diluted in water 1:20, w/v. Much: mixture of 20 g chocolate bar (60% cocoa) diluted in 50 mL water with addition of citric essential oil (Arcolor Ltda, Brazil)
Burnt aroma	Odour related to very toasted cocoa beans	None: dry cocoa beans without toasting. Much: cocoa beans toasted at 160 °C for 60 min
Vanilla aroma	Characteristic sweet odour of <i>Vanilla</i> Pod	None: cocoa powder diluted in water 1:20, w/v. Much: mixture of 20 g milk chocolate bar diluted in 50 mL water with <i>Vanilla</i> essence (Food Degree Company)
Buttery aroma	Odour related to butter/fat	None: cocoa powder diluted in water 1:20, w/v. Much: mixture of 20 g chocolate bar (60% cocoa) diluted in 50 mL water with 3 g butter (Tirolez Ltda, Brazil)
Sweet taste	Characteristic taste of sucrose or caramel	Weak: cocoa powder diluted in water (1:8, w/v) with addition of 0.15% sucrose (União Ltda, Brazil) Strong: cocoa powder diluted in water (1:8, w/v) with addition of 1.5% sucrose
Bitter taste	Characteristic taste of caffeine. Unpleasant taste of caffeine	Weak: cocoa powder diluted in water (1:8, w/v) with addition of 0.01% caffeine (Food Degree, Brazil) Strong: cocoa powder diluted in water (1:8, w/v) with addition of 0.08% caffeine
Acid taste	Characteristic taste of citric acid	None: water. Much: cocoa powder diluted in water (1:8, w/v) with addition of 0.07% citric acid (Food Degree, Brazil)
Cocoa flavour	Flavour related to chocolate/cocoa	Weak: milk chocolate bar (Nestlé S.A., Brazil) Strong: dark chocolate bar, 80% cocoa (AMMA Ltda)
Fruity flavour	Flavour related to citric fruits (tangerine, orange, lemon, mandarin)	None: water. Much: mixture of dark chocolate bar (70% cocoa) with lemon and orange essential oil (Arcolor Ltda, Brazil)
Astringency	Drying sensation from tasting green fruit. Contraction of mouth mucus	None: water Much: dried unripe banana (Grand Naine, Brazil)
Firmness	Force required biting the chocolate sample	Weak: milk chocolate bar (Nestlé S.A., Brazil) Strong: dark chocolate bar (70% cocoa) stored at 10 °C for at least 1 h (AMMA Ltda, Brazil)
Fracturability	Easily broken or rupture	Little: 'rapadura' (Rapadurinha de Minas Ltda, Brazil) Much: 'suspiro' (Benno Alimentos Ltda, Brazil)
Melting	Length of time for chocolate to melt in the mouth/Liquefied	Weak: dark chocolate bar (70% cocoa) (AMMA, Brazil) Strong: milk chocolate bar (Nestlé S.A.)

The acceptance test was carried out with 100 consumers (seventy-two women and twenty-eight men, aged 21–52 years) randomly recruited from two local universities. Consumers were invited to participate according to their interest, available time, and absence of allergy to cocoa or chocolate. Ethical clearance approval was granted for the sensory evaluation of this study by the same Research Ethics Committee. Prior to testing, each participant signed a consent form. The test was performed in individual sensory booths,

according to the same conditions described above to the descriptive test, using a 9-point structured hedonic scale (9 = liked extremely to 1 = disliked extremely) to assess the attributes: appearance, aroma, flavour, texture, and overall liking (Meilgaard *et al.*, 2006).

Statistical analysis

The acceptance test results ($n = 100$) were analysed by a two-way ANOVA, both for consumers and samples

and for the results ($n = 3$) of chemical evaluation, with parameters versus samples, followed by the Tukey's test for averages ($P < 0.05$) in order to assess differences among the samples (Granato & Ares, 2014). The experimental data ($n = 12$) of the QDA were submitted to a two-way ANOVA (sources of variation: chocolate samples, judges, chocolate * judge) and their interaction followed by the Tukey's test for multiple mean comparisons ($P < 0.05$). Data from the QDA (descriptive information) were correlated with the consumer's acceptance data using a partial least-square (PLS) regression, considering the overall liking as the dependent variable (Y-matrix), and the descriptive information as the independent variable (X-matrix). Some studies have used PLS to report hedonic judgments and product characteristics (Bayarri *et al.*, 2012; Cadena *et al.*, 2013; Morais *et al.*, 2015; Alencar *et al.*, 2016; Ferreira *et al.*, 2017). Consumer preference mapping is the technique most widely used in the food and beverage industries to evaluate the preferences of customers and their clustering on homogeneous groups. Additionally, this methodology provides information on the food attributes that explain the preference of the consumers. External preference mapping (PRE-FMAP) was conducted by first using principal component analysis (PCA) on the descriptive sensory analysis and chemical data, then relating each of the consumers to this PCA space by regression analysis (Kaaki *et al.*, 2012; Cadena *et al.*, 2012; Ferreira *et al.*, 2017). Consumers are usually represented as points on the sample map as in the internal analysis. The vector model was used, which implies a direct relationship between one or more characteristics that are increasing or decreasing across the stimulus space. The statistical analyses were conducted using the Statistical Analysis System-SAS (version 9.4; SAS Institute Inc., Cary, NC, USA, 2013) and the XLSTAT for Windows 2013 software (Addinsoft, New York, NY, USA), both at a 5% significance level.

Results and discussion

Bioactive compounds in the dark chocolate samples

The mean values of bioactive compounds are showed in Table 2, where it is possible to note that the dark chocolate samples differed ($P \leq 0.05$) from each other in all the evaluated parameters. In general, the TSH1188, PH16, and CEPEC2002 varieties presented higher amounts of total phenolic compounds, gallic acid, catechin, and epicatechin, demonstrating a potential trend for these hybrids from the *Forastero* and *Trinitario* groups. The SR162 variety presented lower values. A similar tendency was found by Cruz *et al.* (2015) regarding the phenolic compounds, gallic acid, catechin, and epicatechin contents for the PH16 (higher content) and SR162 (lower content) cocoa varieties. The study of Efraim *et al.* (2006) about Brazilian varieties (grown in southern Bahia) reported higher values of catechin and epicatechin for the TSH1188 and CEPEC2002 varieties, and lower values for the SR162 variety. The authors found similar amounts of total phenolic compounds (86.75 – 149.49 mg g⁻¹) as the ones observed in this study. Maciel *et al.* (2017) observed a similar trend in cocoa beans for catechin (0.55 – 1.20 mg g⁻¹) and epicatechin (0.87 – 2.50 mg g⁻¹) in samples from the TSH1188, BN34, PH16, CEPEC2002, and SR162 varieties. Leite *et al.* (2013a) reported values ranging from 0.76 – 0.93 mg g⁻¹ (catechin) to 0.99 – 1.15 mg g⁻¹ (epicatechin) in Brazilian cocoa (PH16 and SR162 varieties) grown in southern Bahia.

Others studies found higher values, like Meng *et al.* (2009) who reported approximately 1.85 and 2.74 mg g⁻¹ for catechin and epicatechin respectively, in the dark chocolate from Malaysia. Bordiga *et al.* (2015) found low values for catechin and epicatechin contents in dark chocolate samples from the following different geographic areas, Cameroon (0.25 and

Table 2 Mean values of bioactive compounds for the dark chocolate samples¹

Bioactive compounds	BN34	TSH1188	PH16	SR162	CEPEC2002	SMD ²
Total phenolics	109.16 ± 1.93 ^a	134.29 ± 1.15 ^a	111.28 ± 1.27 ^b	83.87 ± 1.37 ^d	86.96 ± 1.20 ^c	2.12
Gallic acid	2.76 ± 0.06 ^c	3.69 ± 0.05 ^a	3.08 ± 0.01 ^b	0.01 ± 0.00 ^d	2.96 ± 0.05 ^b	0.17
Catechin	1.02 ± 0.00 ^b	1.59 ± 0.01 ^a	0.95 ± 0.00 ^b	0.01 ± 0.00 ^c	1.52 ± 0.04 ^a	0.13
Epicatechin	1.44 ± 0.01 ^c	1.92 ± 0.01 ^a	1.41 ± 0.01 ^c	0.01 ± 0.00 ^d	1.68 ± 0.03 ^b	0.23
Flavonoids	21.85 ± 1.07 ^c	30.03 ± 1.08 ^a	21.09 ± 1.12 ^d	19.54 ± 1.03 ^e	22.32 ± 1.02 ^b	0.47
Caffeine	3.59 ± 0.02 ^d	11.96 ± 0.03 ^a	10.16 ± 0.02 ^b	0.01 ± 0.00 ^e	5.05 ± 0.04 ^c	1.12
Theobromine	11.91 ± 0.06 ^a	11.19 ± 0.07 ^b	8.69 ± 0.01 ^c	8.67 ± 0.03 ^c	7.17 ± 0.02 ^d	0.70

Total Phenolics (mg GAEs g²)-GAE(Gallic acid equivalent).

¹(Means and standard deviation) Significant differences between samples ($P < 0.05$) are marked with a–e.

²Significant Minimum Difference.

0.41 mg g⁻¹, respectively), Nigeria (0.27 and 0.44 mg g⁻¹), Ecuador (0.29 and 0.58 mg g⁻¹), Ivory Coast (0.25 and 0.38 mg g⁻¹), and Ghana (0.28 and 0.47 mg g⁻¹). Concerning phenolic compounds the authors reported a negative trend from the beans to the chocolate bar. Technological processes have a significant impact on cocoa quality, with the largest observed losses of phenolic compounds being observed during the roasting stage. Fernández-Romero *et al.* (2020) determined the degradation kinetics parameters of total phenolic compounds (TPC), epicatechin, and catechin during the roasting process of Criollo cocoa. The results showed a lower degradation of TPC (11%) and epicatechin (8%) at 130 °C for 10 min of roasting, while a total degradation of epicatechin and a 92% degradation of TPC occurred at 200 °C for 50 min.

Literature reviews indicated that the content of phenolic compounds, even within one variety is very diverse, and the level of (–)-epicatechin and (+)-catechin may vary independently from one sample to another, depending on many factors. Among them are the geographical region of cultivation, the varietal characteristics of cocoa, beans maturity, climatic conditions, post-harvest processing, and the manufacturing process (Aprotosoiaie *et al.*, 2016; Urbanska & Kowalska, 2019).

Theobromine and caffeine are often referred to as alkaloids. Theobromine (3,7-dimethylxanthine) is the major alkaloid present in cocoa, and caffeine (1,3,7-trimethylxanthine) is found in small amounts (Aprotosoiaie *et al.*, 2016). Significant differences ($P < 0.05$) were found in the content of theobromine and caffeine of the dark chocolate samples. The same trend was observed to the TSH1188, PH16, and CEPEC2002 varieties that presented higher caffeine content than the SR162 and BN34 varieties. Also, Cruz *et al.* (2015) and Leite *et al.* (2013a) reported higher caffeine content for cocoa beans and chocolate produced from the PH16 variety and lower amount for the SR162 variety, both grown in southern Bahia (Brazil). Others studies found lower values, Maciel *et al.* (2017) recorded caffeine values varying from 0.083–0.24 mg g⁻¹ for cocoa beans from the TSH1188, CEPEC2002, BN34, and PH16 varieties. Deus *et al.* (2018) registered values from 1.60–2.33 mg g⁻¹ for the Brazilian cocoa beans submitted to different drying processes. Regarding theobromine, the BN34 variety exhibited higher values, followed by TSH1188, SR162, PH16, and CEPEC2002 that presented lower amount. Similar results were found by Deus *et al.* (2018) for theobromine that varied from 11.14–14.96 mg g⁻¹, and lower values were obtained by Maciel *et al.* (2017) ranging from 1.8–1.4 mg g⁻¹. Indeed, the caffeine content was lower than the theobromine content in the chocolate samples, confirming the general trend in *Theobroma cacao* beans (Aprotosoiaie *et al.*, 2016).

Further, Bordiga *et al.* (2015) found high values for theobromine and low caffeine content in dark chocolate samples from the following different geographic origins: Cameroon (8.26 and 0.16 mg g⁻¹, respectively), Nigeria (7.67 and 0.25 mg g⁻¹), Ecuador (6.14 and 0.35 mg g⁻¹), Ivory Coast (6.82 and 0.25 mg g⁻¹) and Ghana (6.15 and 0.23 mg g⁻¹). The authors reported that besides the technological impact of the processing method, the development and fermentation steps are critical for the accumulation of purine alkaloids in cocoa. In addition, small differences were observed from beans to cocoa mass as well as a significant reduction in chocolate and tablet.

Methylxanthines and polyphenols contribute to the flavour of cocoa and chocolate. They are responsible for the bitterness of cocoa products (De Taeye *et al.*, 2016; Muñoz *et al.*, 2020). The cocoa genotype determines the chemical composition of the bean, specifically the contents of proteins, polysaccharides, and polyphenols. This determines the quantities and type of precursors formed during fermentation and drying processes leading to flavour formation, hence, influencing both flavour type and intensity (Muñoz *et al.*, 2020).

Quantitative descriptive analysis and acceptance test of the dark chocolate samples

The mean values of the sensory descriptors obtained from the trained panellists using QDA and the mean scores of the acceptance test for the dark chocolate samples are showed in the Table 3. The results of the QDA showed that there was a significant difference ($P \leq 0.05$) in the sensory characteristics of the five chocolate samples in the experimental conditions. No significant chocolate sample*panellist interaction ($P > 0.05$) was detected, revealing that the training for the descriptive panel was adequate.

The brown colour and brightness attributes presented higher intensities in the samples from the TSH1188, PH16, and CEPEC2002 varieties, which differed significantly ($P \leq 0.05$) from the others two varieties. Barbin *et al.* (2018) also studied the PH16, BN34, SR162, and CEPEC2002 cocoa varieties by traditional methods in relation to their chemical composition and colour features. The authors reported that the results of the colorimetric measurements showed significant differences among the varieties. Variety SR162 presented the highest values for parameters L^* , a^* , and b^* , which was characterised as a lighter sample due to the highest colour reflectance (L^*). BN34 sample showed the lowest values for L^* and b^* , which characterise their cocoa beans as darker. Regarding the attributes buttery and burnt aroma were found lower intensities for all chocolate samples, but were able to differentiate the varieties.

The samples from the BN34 and SR162 varieties showed higher intensities of the attributes sweet/caramel and vanilla aroma, and sweet taste. The SR162 also presented higher intensities of fruity aroma and flavour, which are very important characteristics of cocoa products. The international market classifies cocoa as 'bulk' or fine cocoa, and the latter is defined as the one with special aroma (fruity, floral, woody, nutty, and caramel notes), being produced from the *Criollo* or *Tritario* cocoa group. In contrast, 'bulk' cocoa (common cocoa) is a type of standard cocoa produced from the *Forastero* group, without specific aromatic characteristics (Rottiers *et al.*, 2019).

On the other hand, the samples from the TSH1188, PH16, and CEPEC2002 varieties showed higher intensities of bitter and acid taste, cocoa aroma and flavour, and astringency, differing significantly ($P \leq 0.05$) from the others two varieties. This low aromatic complexity may suggest the need for an adjustment in the postharvest protocol or a special management of this material in order to obtain a high quality chocolate. It is important to highlight that the aforementioned samples presented higher intensities of cocoa aroma and flavour, which are very important characteristics to cocoa trading. These varieties can be used by the

cocoa/chocolate industry to provide the products with a higher intensity of cocoa/chocolate aroma and flavour.

The presence of alkaloids (caffeine, found higher amounts in samples of the TSH1188, PH16, and CEPEC2002 varieties), among others as diketopiperazines, polyphenols, free L-amino acids, or peptides are the main source of bitterness and astringency. Astringency is often confused with bitterness because many consumers do not clearly understand its nature, and many polyphenols exhibit both bitterness and astringency (Aprotosoiaie *et al.*, 2016). Vázquez-Ovando *et al.* (2015) found that the scores were higher for the sour taste than for the bitter taste for cocoa beans from the Sonosco cultivar (Chiapas, Mexico). The authors reported that cocoa from different regions of the world present different contents of organic acids, monosaccharides, disaccharides, oligosaccharides, and some L-amino acids, depending on the latitude and climate, which have a determining impact on attributes as sourness and sweetness.

Regarding the texture properties, samples from the BN34 and SR162 varieties showed lower scores for firmness and higher scores for fracturability and melting, which are important quality factors for the texture

Table 3 Mean values of sensory descriptors that characterised the dark chocolate samples generated by the trained panellists using the QDA and mean scores of the acceptance test¹

Attributes	BN34	TSH1188	PH16	SR162	CEPEC2002	SMD ²
Descriptors- QDA ($n = 12$)						
Brown colour	6.1 ± 0.9 ^b	7.0 ± 0.3 ^a	7.3 ± 0.4 ^a	5.8 ± 1.1 ^c	7.1 ± 0.3 ^a	0.31
Brightness	6.0 ± 1.0 ^b	6.5 ± 0.5 ^a	6.7 ± 0.5 ^a	5.6 ± 1.0 ^c	6.8 ± 0.5 ^a	0.26
Cocoa aroma	4.9 ± 0.6 ^b	6.0 ± 1.2 ^a	6.3 ± 0.5 ^a	5.0 ± 1.3 ^b	5.8 ± 1.1 ^a	0.51
Sweet aroma	4.8 ± 0.7 ^a	1.7 ± 0.8 ^b	1.8 ± 0.6 ^b	5.1 ± 0.9 ^a	1.8 ± 1.1 ^b	0.36
Fruity aroma	4.5 ± 0.4 ^b	0.8 ± 0.3 ^d	0.9 ± 0.5 ^{cd}	4.9 ± 0.9 ^a	1.1 ± 0.6 ^c	0.24
Burnt aroma	0.9 ± 0.5 ^{cd}	1.4 ± 0.6 ^{ab}	1.6 ± 0.9 ^a	0.8 ± 0.4 ^d	1.2 ± 0.6 ^{bc}	0.31
Vanilla aroma	3.0 ± 0.9 ^a	0.8 ± 0.4 ^b	0.7 ± 0.5 ^b	3.1 ± 0.5 ^a	0.9 ± 0.5 ^b	0.38
Buttery aroma	1.1 ± 0.4 ^b	1.4 ± 1.0 ^{ab}	1.6 ± 0.6 ^a	1.1 ± 0.5 ^b	1.6 ± 0.7 ^a	0.38
Sweet taste	4.4 ± 0.7 ^a	1.7 ± 0.6 ^c	1.6 ± 0.4 ^c	4.5 ± 0.9 ^a	2.5 ± 0.7 ^b	0.34
Bitter taste	3.6 ± 0.9 ^b	6.1 ± 0.6 ^a	6.1 ± 0.7 ^a	3.3 ± 0.7 ^b	6.0 ± 1.0 ^a	0.45
Acid taste	1.8 ± 0.4 ^b	5.8 ± 1.3 ^a	5.8 ± 0.9 ^a	1.6 ± 0.5 ^b	5.6 ± 1.4 ^a	0.27
Cocoa flavour	4.8 ± 0.8 ^b	5.8 ± 1.0 ^a	6.0 ± 0.7 ^a	4.6 ± 0.7 ^b	5.8 ± 0.5 ^a	0.41
Fruity flavour	3.7 ± 1.1 ^b	0.8 ± 0.6 ^c	0.8 ± 0.5 ^c	4.2 ± 0.9 ^a	1.0 ± 0.5 ^c	0.40
Astringency	1.5 ± 0.7 ^b	3.2 ± 0.6 ^a	3.4 ± 0.7 ^a	1.3 ± 0.4 ^b	3.1 ± 0.8 ^a	0.42
Firmness	3.9 ± 0.8 ^b	5.8 ± 0.4 ^a	5.9 ± 0.3 ^a	3.7 ± 0.2 ^b	5.6 ± 0.9 ^a	0.41
Fracturability	3.1 ± 0.9 ^a	2.7 ± 0.9 ^b	2.7 ± 0.7 ^b	3.2 ± 1.2 ^a	2.7 ± 1.1 ^b	0.35
Melting	4.9 ± 0.6 ^a	3.0 ± 0.7 ^b	3.1 ± 0.4 ^b	5.1 ± 0.6 ^a	3.1 ± 0.9 ^b	0.54
Attributes- acceptance test ($n = 100$)						
Appearance	7.9 ± 0.8 ^a	8.0 ± 0.9 ^a	7.9 ± 1.0 ^a	7.9 ± 1.0 ^a	7.9 ± 1.1 ^a	0.22
Aroma	7.0 ± 1.6 ^{ab}	6.7 ± 1.5 ^{bc}	6.8 ± 1.1 ^{abc}	7.2 ± 1.3 ^a	6.5 ± 1.5 ^c	0.47
Flavour	6.4 ± 1.2 ^a	5.8 ± 1.2 ^b	5.4 ± 1.5 ^b	6.5 ± 1.2 ^a	5.9 ± 1.1 ^{ab}	0.48
Texture	7.2 ± 1.5 ^{ab}	7.0 ± 1.5 ^b	7.0 ± 1.6 ^b	7.4 ± 1.2 ^a	7.1 ± 1.4 ^{ab}	0.37
Overall liking	6.8 ± 1.6 ^a	6.3 ± 1.2 ^b	6.2 ± 1.4 ^b	6.9 ± 1.3 ^a	6.4 ± 1.6 ^b	0.34

¹Means and standard deviations. Significant differences between samples ($P < 0.05$) are marked with a–d.

²Significant Minimum Difference.

of chocolates. In contrast, samples from the TSH1188, PH16, and CEPEC2002 varieties showed higher firmness and lower fracturability and melting intensity.

Similar results were found by Leite *et al.* (2013) on their study about the sensory quality of dark chocolates using the QDA methodology. The chocolate samples from the PH16 variety were characterised by more intense brown colour, cocoa aroma and flavour, bitterness and firmness, while the samples of the SR162 variety was characterised by greater fruity aroma and flavour, and melting. Moreira *et al.* (2016) also found differences in the descriptive attributes and acceptability of the chocolates produced from the three cocoa hybrids grown in Bahia, Brazil (PH9, PH15, and PH16 varieties). The study concluded that the chocolate samples from the PH16 variety were bitter, acidic and astringent. The authors recommended that the manufacturing practices should be controlled to obtain high-quality final products.

Flavour is one of the most important quality attributes, and is decisive to the acceptability of cocoa products, such as chocolate. The complex composition involved in the flavour of cocoa beans depends on the genotype; postharvest treatments such as pulp pre-conditioning, fermentation, and drying; industrial processes such as roasting; as well as the type of soil, and age of the cocoa trees (Oracz *et al.*, 2015; Kongor *et al.*, 2016).

According to the mean scores assigned by the consumers in the acceptance test (Table 3), there was no significant difference ($P > 0.05$) among the chocolate samples regarding the attribute appearance. All the samples presented great acceptability, and the scores corresponded to the hedonic term 'liked much'. In relation to the aroma and flavour attributes, the samples from the BN34 and SR162 varieties scored highest (no difference between both), corresponding to 'liked moderately', with a significant difference compared to the others samples ($P < 0.05$). The consumers mentioned in their sheets that these varieties had a pleasant sweet taste and fruity flavour, respectively. The samples from the TSH1188, PH16, and CEPEC2002 varieties received lower scores in aroma and flavour acceptability (no significant difference among them), with the scores corresponding to the hedonic term 'liked slightly' and 'neither liked/neither disliked', respectively. The remarks made by the consumers were about their unpleasant bitter and acid taste. Regarding texture, all the samples obtained great acceptability, and the scores corresponded to the hedonic term 'liked moderately'. For the overall liking, there was a significant difference among the samples, with the chocolate from the BN34 and SR162 varieties being the most accepted by consumers ('liked moderately'), and the chocolate from the TS1188, PH16, and CEPEC2002 varieties

receiving slightly lower scores, corresponding to the hedonic term 'liked slightly'.

Torres-Moreno *et al.* (2012) on their study about sensory acceptability of dark chocolate samples from different origins (Ghana and Ecuador) and processing conditions also concluded that the differences in acceptability of the samples were mainly related to differences in flavour attribute. Efraim *et al.* (2013) reported that the dark chocolate samples produced from the *Forastero* group were less accepted and differed significantly from the *Trinitario* group samples, showing the preference of the consumers for these materials and the possibility to obtain quality through the genetic improvement of cocoa plants.

Drivers of liking of the dark chocolate samples

The sensory descriptors (QDA data) were correlated with the hedonic data (overall liking) using partial least-square (PLS) regression (Fig. 1). PLS is a modelling approach that should be used when predictive variables are inter-correlated (Ferreira *et al.*, 2017). The Fig. 1 shows the attributes that contributed positively or negatively to the acceptance of the samples. The columns with descriptive terms located on the positive portion of the *Y* axis were positively correlated with the acceptance, while those located on the negative portion were negatively correlated with the acceptance. The magnitude of the columns also represents the effect of the attributes on the sample acceptance. However, when the vertical line crosses the *X* axis, no effect of the attribute was observed on the driver of consumer preferences (Ferreira *et al.*, 2017). Thus, the attributes sweet aroma, fruity aroma, vanilla aroma, sweet taste, fruity flavour, and melting contributed positively to the acceptance of dark chocolate samples. Inversely, the attributes bitter taste, acid taste, cocoa flavour, astringency, and firmness correlated negatively with the acceptance of the samples. The attributes brown colour, brightness, cocoa aroma, burnt aroma, buttery aroma, and fracturability were sensory descriptors with no significant contribution to the acceptance of the consumers.

The results of the external preference mapping (PREFMAP) are shown in Fig. 2. In this technique, preferences are expressed as a function of two or more principal components, not as simple analytical variables. This technique allows the identification of the hedonic ratings of each consumer in a set of product characteristics (Cadena *et al.*, 2012; Bonany *et al.*, 2014), which in this study were descriptive sensory attributes and chemical parameters. Data were represented by two principal components. The first factor explained approximately 53% of the variation while the second factor accounts for 19% of it. Most of the overall liking scores are located in the upper and lower

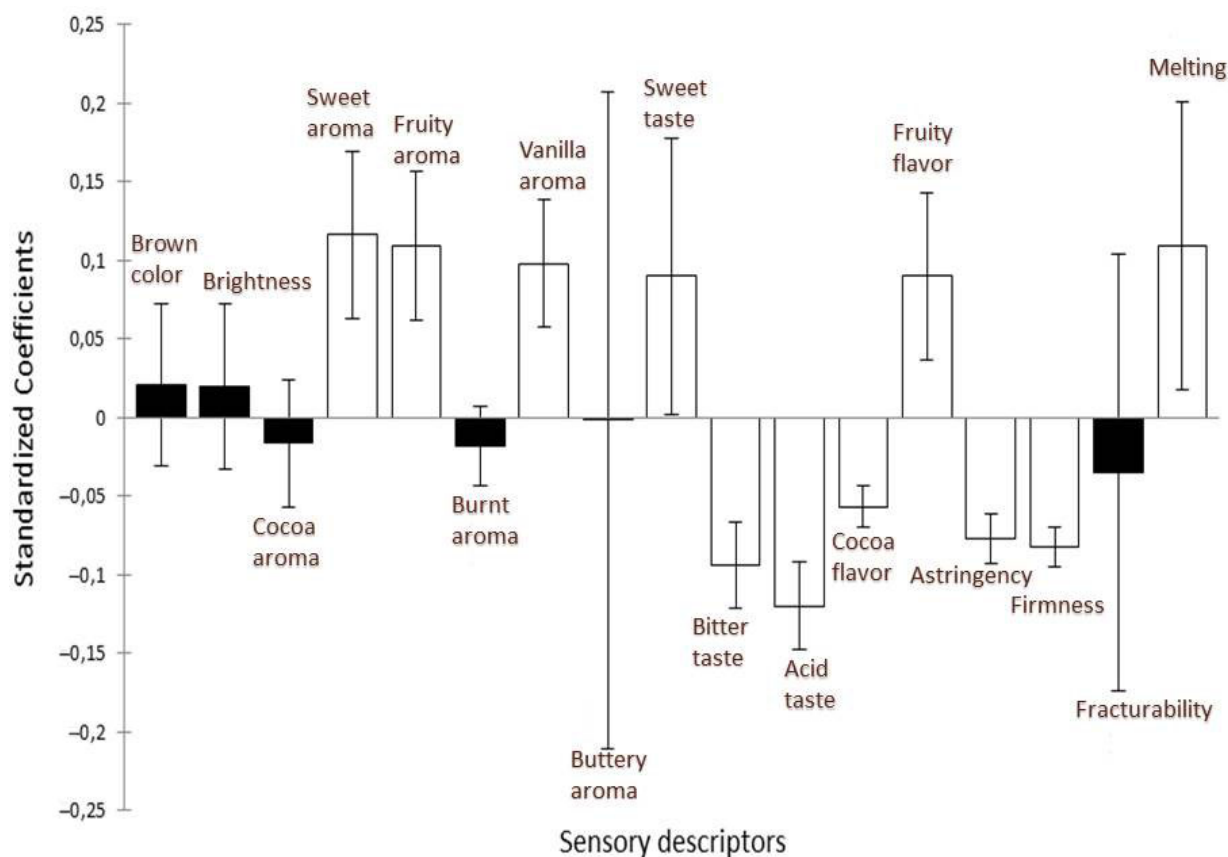


Figure 1 Standardised coefficients from the regression analysis by partial least squares (PLS) between the sensory descriptors and the overall liking means for dark chocolate samples. White bars are sensory descriptors that contribute positively or negatively to the acceptance by consumers at $P \leq 0.05$; black bars are sensory descriptors with no significant contribution to the acceptance by consumers. [Colour figure can be viewed at wileyonlinelibrary.com]

right quadrant. The variables that presented a positive coordinate value on the first factor (positive x -axis) showed a direct correlation with preference, while variables with a negative coordinate value on factor 1 (negative x -axis) were negatively correlated to it. In the dark chocolate samples evaluated in this study, the overall liking was positively correlated to sweet taste, vanilla aroma, sweet aroma, fruity aroma, fruity flavour, fracturability, and melting; and negatively correlated to burnt aroma, cocoa aroma, brown colour, bitter taste, acid taste, astringency, cocoa flavour, and firmness. These attributes may be considered as major drivers of liking and disliking of dark chocolate samples, respectively. Thus, the samples from the TSH1188, PH16, and CEPEC2002 varieties were the least preferred compared to the samples from the BN34 and SR162 varieties. Cocoa producers should pay particular attention to the descriptors that negatively affected the hedonic dimension the chocolate samples, since these are probably the most critical factors for

the acceptability of consumers and, consequently, purchase intentions.

In addition, the results showed that chemical parameters as total phenolic compounds, gallic acid, catechin, epicatechin, caffeine, and total flavonoids were positively correlated with sensory descriptors as bitter taste, acid taste, cocoa flavour, and astringency, which probably contributed to the lower acceptance scores of the chocolate samples from the TSH1188, PH16, and CEPEC2002 varieties. Indeed, the samples from the BN34 and SR162 varieties are located in the opposite region of the aforementioned varieties and presented sweet/caramel and fruity notes that contributed to their higher acceptance scores. Our findings suggest that lower intensities of the phenolic compounds and caffeine (consequently, lower intensity or absence of bitterness, acidity, and astringency) probably allowed the perception of sweet and fruity notes, making the products more pleasant for the consumers that participated in the sensory test.

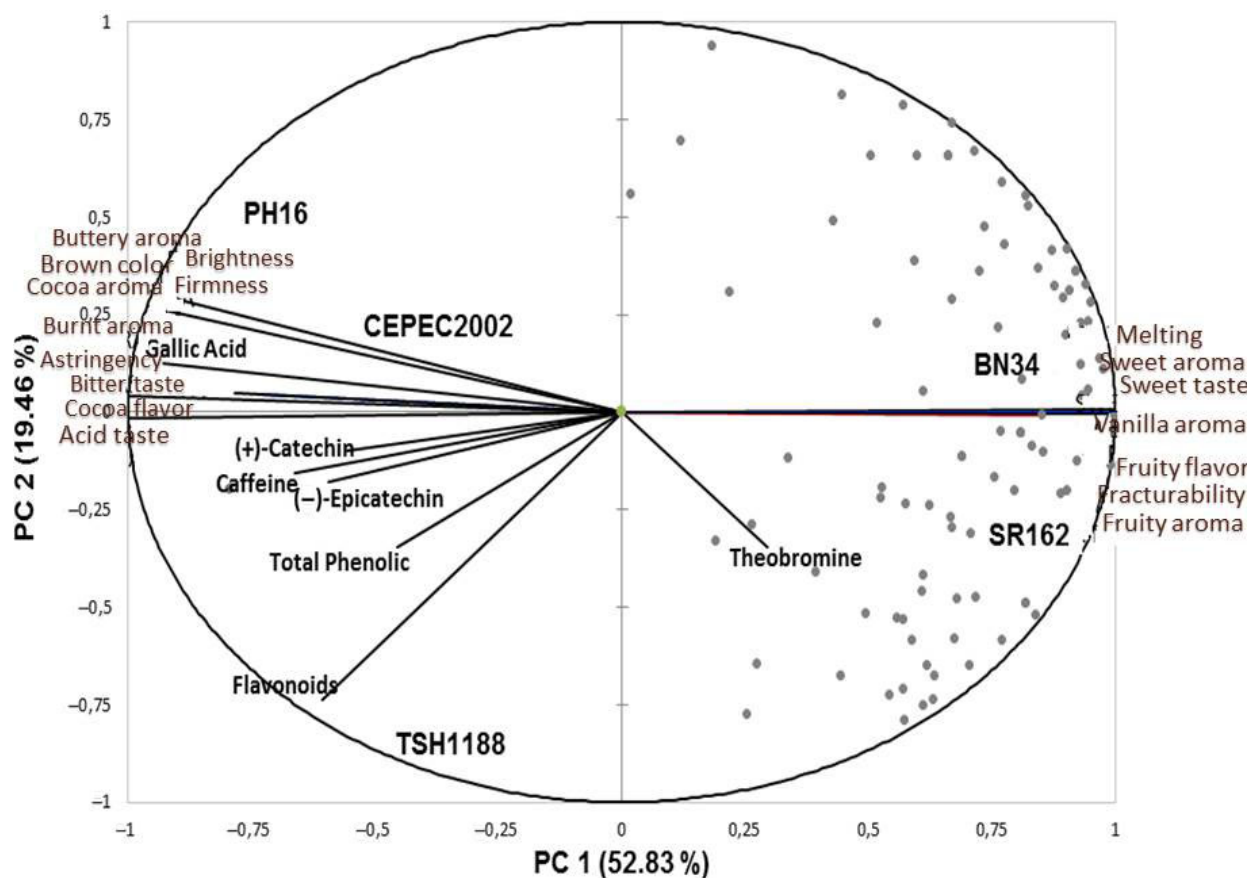


Figure 2 External preference mapping obtained by PLS regression of the bioactive compounds, descriptive attributes, and consumers overall liking of the dark chocolate samples. Circles are consumers; bold words are the bioactive compounds; cocoa varieties: BN34, TSH1188, PH16, SR162, CEPEC2002.

Several research papers mention the pro-health properties of cocoa beans, which result from the presence of polyphenolic compounds (flavonoids, methylxanthines, epicatechin, catechin, and anthocyanins), often responsible for conferring the bitterness and astringency of the raw cocoa seed to chocolate (Aprotosoia *et al.*, 2016; Kongor *et al.*, 2016; Urbanska & Kowalska, 2019). The reduction of phenolic compounds occurs mainly in the steps taken to develop the chocolate flavour, which contribute to the reduction of astringency and bitterness, therefore reducing the antioxidant capacity and bioactive properties of chocolate.

Similar results were found by Luna *et al.* (2002) on a study that determined the relationship between genotypes (Ecuadorian cocoa) and chemical parameters involved in the chocolate aroma and flavour, using a panel of trained tasters. The results indicated that the polyphenols were positively correlated to astringency, bitterness, and negatively correlated to the fruity attribute. Different levels of polyphenols in the products were either due to a genotypic contribution or to the

post-harvest and roasting conditions. Manufacturers have, therefore, developed processing techniques for eliminating the bitterness. Such processes decrease the polyphenol content by up to 10-fold, making the chocolate more palatable (Montagna *et al.*, 2019).

Morais *et al.* (2015) carried out a study that aimed to identify the drivers of liking for chocolate dairy desserts. The authors mentioned that the most desired sensory properties of such products were sweetness; chocolate flavour; sweet aroma; and mouth fill; whereas bitterness and bitter aftertaste were considered undesirable, and were considered drivers of liking and disliking of chocolate dairy desserts.

This study compared cocoa varieties produced in southern Bahia, Brazil, and showed the great potential of the SR162 ('Catongo') and the BN34 varieties for the production of fine cocoa. Farmers have adopted these hybrid varieties due to their good results in productivity, resistance to diseases, and more recently, for their quality. They have stood out and won awards in national and international quality contests. 'Catongo' cocoa

from southern Bahia is increasingly catching the attention of the fine cocoa market, due to its aromatic profile and the attractive shade of its white almonds. The TSH1188, PH16, and CEPEC2002 varieties are also being widely planted by producers, although in this study the results showed less aromatic complexity, which may suggest the need of an adjustment in the postharvest protocol of this material, mainly in the fermentation and drying stages. The chemical and sensory evaluation of chocolate is strategic tool to help cocoa producers in deciding which genetic materials should be traded as fine flavoured cocoa. It also helps producers to adopt the appropriate postharvest methodologies for each genetic material, in order to obtain an increasingly special product with a greater added value.

Conclusions

This work identified a great variability among the Brazilian hybrid cocoa varieties regarding their levels of bioactive compounds. The results showed that a higher content of catechin, epicatechin, caffeine, total phenolic, and flavonoids contributed to a higher intensity of bitter taste, cocoa flavour, acid taste, and astringency, affected negatively the acceptance of chocolate samples by consumers, showing a possible trend of the hybrid varieties (TSH1188, PH16, CEPEC2002) from the *Forastero* and *Trinitario* cocoa groups. Conversely, hybrid varieties (SR162, BN34) that presented a lower content of caffeine and phenolic compounds (consequently, lower intensity or absence of bitterness, acidity, and astringency) probably allowed the perception of sweet and fruity notes, making the products more pleasant for consumers (contributed higher acceptance scores of these chocolate samples). These attributes may be considered as major drivers of liking and disliking of dark chocolates.

This study brings an important contribution to the area of agriculture, with the chemical and sensory evaluations proving to be a strategic tool for cocoa farmers in deciding which genetic material should be traded as fine cocoa, adding value to the product, and highlighting promising cocoa varieties. In this work, the results showed the great potential of the SR162 ('Catongo') and BN34 hybrid clones from southern Bahia (Brazil) for the production of fine cocoa. Both of them presented important sensory characteristics (sweetness/caramel flavour and fruity notes) for chocolate samples.

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Conflict of interest

The authors declare no conflict of interests.

Author contribution

Ivia Araújo das Virgens: Data curation (supporting); Formal analysis (lead); Investigation (supporting); Methodology (supporting); Software (supporting); Writing-original draft (supporting); Writing-review & editing (supporting). **Tássia Cavalcante Pires:** Data curation (supporting); Formal analysis (lead); Methodology (supporting); Software (supporting); Writing-original draft (supporting); Writing-review & editing (supporting). **Ligia Regina Radomille de Santana:** Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Software (lead); Supervision (supporting); Writing-original draft (lead); Writing-review & editing (supporting). **Sérgio Eduardo Soares:** Conceptualization (lead); Data curation (supporting); Formal analysis (supporting); Funding acquisition (lead); Investigation (supporting); Methodology (lead); Project administration (lead); Resources (lead); Supervision (lead); Writing-original draft (supporting); Writing-review & editing (supporting). **Leonardo Fonseca Maciel:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Writing-original draft (supporting); Writing-review & editing (supporting). **Adriana Cristina Reis Ferreira:** Conceptualization (supporting); Data curation (supporting); Investigation (supporting); Resources (supporting). **Aline Camarão Telles Biasoto:** Data curation (supporting); Writing-original draft (supporting); Writing-review & editing (supporting). **Eliete da Silva Bispo:** Conceptualization (supporting); Data curation (supporting); Formal analysis (supporting); Funding acquisition (supporting); Investigation (supporting); Methodology (supporting); Resources (supporting); Supervision (supporting); Writing-original draft (supporting); Writing-review & editing (supporting).

Ethical approval

Ethics approval was not required for this research.

Peer Review

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Data availability statement

No data are available.

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