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Phenolic compounds, methylxanthines, and preference drivers of dark chocolate made with hybrid cocoa beans

Compostos fenólicos, metilxantinas e direcionadores de preferência de chocolates com alto teor de cacau elaborados com cacau híbrido

Compuestos fenólicos, metilxantinas y conductores de preferencia del chocolates con alto contenido de cacao elaborados con cacao hibrido

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Abstract
The distinct properties of cocoa beans may be due to their different geographical and genetic origins which lead to chocolates with different characteristics. This study aimed to evaluate dark chocolate samples made with hybrid cocoa cultivated in the Bahia State, Brazil, regarding the composition of bioactive compounds (spectrophotometry, High Performance Liquid Chromatography) and the sensory characteristics (Quantitative Descriptive Analysis-QDA®), and acceptance test). External preference mapping was conducted by first using principal components analysis (PCA) on the QDA data, and then relating consumer preference (overall liking) to this PCA space by regression analysis. Significant differences among the cocoa varieties were found. Chocolate samples that showed lower acceptability also presented higher content of theobromine, caffeine, anthocyanins, and gallic acid which conferred greater intensities of bitterness, astringency, and intense cocoa flavor. Lower levels of methylxanthines, monomeric phenolic compounds, and anthocyanins allowed the highlight of fruity, sweet and caramelized notes, which contributed to greater acceptance of the chocolate samples. Therefore, these characteristics can be recognized as consumer preference drivers (negative and positive, respectively) for the high cocoa chocolates. It seems that higher values of ephicatechin and catechin had no negative contribution to the flavor of the chocolate samples. The findings of this study can be used in agriculture and in the cocoa/chocolate industry, suggesting the potential and applicability of promising hybrid cocoa, with important characteristics, representing a genetic material of great quality, that may be classified as fine flavor cocoa, associating greater value to the product, and contributing to meet consumer needs.
Keywords: *Theobroma cacao*; Fine cocoa; Bioactive compounds; Acceptability; Sensory profile.

Resumo
As propriedades distintas dos grãos de cacau podem ser devidas às suas diferentes origens geográficas e genéticas que levam a chocolates com características diferentes. Este trabalho teve como objetivo avaliar o chocolate com alto teor de cacau feito com amêndoas de cacau híbrido cultivadas no Estado da Bahia, Brasil, quanto à composição de compostos bioativos (espectrofotometria, Cromatografia Líquida de Alta Eficiência) e as características sensoriais (Análise Descritiva Quantitativa-QDA®, e teste de aceitação). O mapa de preferência externo foi realizado usando primeiro a análise de componentes principais (PCA) nos dados QDA e, em seguida, relacionando a preferência do consumidor (impressão global) a este espaço PCA por análise de regressão. Foram encontradas diferenças significativas entre as variedades de cacau. As amostras de chocolate que apresentaram menor aceitabilidade, também, apresentaram maiores teores de teobromina, cafeína, antocianinas e ácido gálico que conferiram maiores intensidades de amargor, adstringência e sabor intenso de cacau. Menores teores de metilxantinas, compostos fenólicos monoméricos e antocianinas permitiram destacar notas frutadas, doces e caramelizadas, o que contribuiu para maior aceitação dos chocolates. Portanto, essas características podem ser reconhecidas como direcionadores da preferência do consumidor (negativa e positiva, respectivamente) dos chocolates com alto teor de cacau. Parece que valores mais altos de epicatequina e catequina não tiveram contribuição negativa para o sabor das amostras de chocolate. Os achados deste estudo podem ser utilizados na agricultura e na indústria cacaueira/chocolate, sugerindo o potencial e aplicabilidade de cacau híbrido promissor, com características importantes, representando um material genético de ótima qualidade, que pode ser classificado como cacau de sabor fino, associando maior valor ao produto e, contribuindo para atender às necessidades do consumidor.
Palavras-chave: *Theobroma cacao*; Cacau fino; Compostos bioativos; Aceitabilidade; Perfil sensorial.

Resumen
Las distintas propiedades de los granos de cacao pueden deberse a sus diferentes orígenes geográficos y genéticos que conducen a chocolates con diferentes características. Este estudio tuvo como objetivo evaluar el chocolate con alto contenido de cacao elaborado a partir de granos de cacao híbridos cultivados en el Estado de Bahía, Brasil, en cuanto a la composición de compuestos bioactivos (espectrofotometría, Cromatografía Líquida de Alta Resolución) y características sensoriales (Análisis Descriptivo Cuantitativo-QDA®, y prueba sensorial de aceptación). El mapa de preferencias externas se realizó utilizando primero el análisis de componentes principales (PCA) en los datos de QDA y luego relacionando la preferencia del consumidor (impresión global) con este espacio de PCA mediante análisis de regresión. Se encontraron diferencias significativas entre las variedades de cacao. Las muestras de chocolate que mostraron menor aceptabilidad también tenían niveles más altos de teobromina, cafeína, antocianinas y ácido gálico, lo que les dio mayores intensidades de amargor, astringencia y sabor intenso a cacao. Los niveles más bajos de metilxantinas, compuestos fenólicos monoméricos y antocianinas destacaron las notas afrutadas, dulces y caramelizadas, lo que contribuyó a una mayor aceptación de los chocolates. Por lo tanto, estas características pueden reconocerse como impulsores de la preferencia del consumidor (negativa y positiva, respectivamente) por los chocolates con alto contenido de cacao. Parece que los valores más altos de epicatequina y catequina no contribuyeron negativamente al sabor de las muestras de chocolate. Los hallazgos de este estudio pueden ser utilizados en la agricultura y en la industria del cacao/chocolate, sugiriendo el potencial y la aplicabilidad de un cacao híbrido promisorio, con importantes características, que representa un material genético de excelente calidad, que puede clasificarse como cacao con un fino sabor, asociando mayor valor al producto y contribuyendo a satisfacer las necesidades del consumidor.
Palabras clave: *Theobroma cacao*; Cacao fino; Compuestos bioactivos; Aceptabilidad; Perfil sensorial.

1. Introduction

Brazil is a country of great agricultural extension and the cocoa (*Theobroma cacao* L.) is an agricultural commodity of relevant economic value, being the seventh largest producer of cocoa in the world. The fungus *Monilioptora perniciosa* caused severe crisis in the cacao plantation and the solution was the use of disease-resistant and highly productive varieties with relevant agricultural characteristics, which were developed by genetic breeding programs as a way to prevent the occurrence of this disease. Through innovative agricultural and preprocessing techniques, and hybrid cocoa seeds (new varieties) the State of Bahia is establishing itself as a fine cocoa and chocolate producer. The concept of fine flavor cocoa indicates that the product presents special aromatic notes, as fruity, floral, woody, nutty, or caramel flavor and aroma, and has a high market value. The “bulk” cocoa is defined as a class of common cocoa or standard, it does not have special aromatic attributes (Aprotosoaie et al., 2016; Muñoz et al., 2020). The trade classifies cocoa as "bulk" or fine cocoa.

Some factors directly impact the characteristic flavor of chocolate such as cocoa beans origin, soil conditions, climate, rainfall, amount of exposure to sunlight, ripening, harvest time, post-harvest processing, and manufacturing conditions, that contribute to variations, leading to a unique flavor. The various chemical components that take part in the formation of the distinctive cocoa flavor are methylxanthines, polyphenols, proteins, carbohydrates, organic acids, among others (Aprotosoaie et al., 2016).

The correlation between the genetic characteristics, chemical parameters, and sensory properties was assessed in some studies. Researchers in Ecuador have demonstrated a strong relationship between the genetic material, chemical compounds, and the sensory characteristics of cocoa (Luna et al., 2002). In another study, the impact of the ancestry of the cocoa genotypes on its flavor attributes was verified, thus the material obtained from Ghana was regarded as common, and from Trinidad was regarded as fine (Sukha et al., 2008). A Brazilian study concluded that chocolates manufactured with varieties descendant from the Trinitario group were more preferred than those samples elaborated with varieties from the Forastero group (Efraim et al., 2013). Research carried out in Ecuador compared the CCN51 variety (most common in the country) with fine Nacional hybrids in terms of the flavor profile, they identified correlations between sensory attributes and volatile compounds using partial least squares analysis (Rottiers et al., 2019). Recently, researchers of Bahia showed the relationship of bioactive compounds and sensory characteristics of 5 varieties grown in Brazil (Das Virgens et al., 2020).

This study aimed to provide a better knowledge about the relations between the content of bioactive compounds that influence the cocoa flavor and the sensory properties of the dark chocolate and, associated with multivariate analysis to correlate with the acceptability of these chocolate samples made with hybrid cocoa cultivated in southern Bahia, Brazil.

2. Methodology

2.1 Cocoa samples and preprocessing of cocoa beans

The cocoa beans were obtained from a commercial production in the municipality of Ibirataia (149 m above sea level, 14º04’01” S and 39º38’26” W), Bahia State, Brazil. The fruit maturation occurred in a cocoa plantation area, where the climate conditions were: relative humidity corresponded to 90 - 95 %, the minimum and maximum temperatures were 14° - 28° C, and the rainfall corresponded to 200 - 300 mm. The selected cocoa varieties showed good productivity and were free of witches’ broom disease, they were recommended by Cocoa Research Center (CEPEC/CEPLAC): ‘Pará’ (hybrid originated of crossing between Amelonado and Lagarto cultivars, from Pará State, Brazil), ‘CCN51’ (variety obtained of crossing between ICS95 and IMC67 cultivars, from Ecuador), ‘PS1319’ (hybrid obtained of genetic mutation of the cocoa beans from Upper Amazon Forastero), and ‘Ipiranga’ (hybrid obtained by open pollination, from producers in the Southern Bahia, Brazil).

The fruits were harvested ripe, healthy, preselected, and transported to fermentation house at ambient temperature. The fermentation of the seeds with pulp (60 kg) lasted 5 days and was carried out in wooden boxes with approximate dimensions of width (50 cm) x length (50) x height (50). The fermentation process conditions: humidity was greater than 60 %, the minimum and maximum room temperatures were 23° - 28° C, respectively. The cocoa beans were stirred every 48 h to oxygenate and mix the material, the temperature of the mass was controlled between 45° - 48° C throughout the process. A sun-roof surface was used to dry the cocoa seeds to 8% moisture for 5 - 7 days. (Das Virgens et al., 2020; Cruz et al., 2015).

2.2 Processing of the chocolate samples

After the fermentation and drying process, the cocoa beans were toasted in a rotary roaster machine (Jaf Inox-TX, São Paulo, Brazil), under the following conditions: temperature of 120° C for 60 min. In order to obtain the cocoa nibs, the seeds were crushed to remove the peel and germs. In the next stage, sugar was added to the nibs to grind in a knife grinding equipment (Jaf Inox-FX). To prepare the chocolates with high cocoa content, the following formulation was used: cocoa mass in the proportion of 66.0 %, cocoa butter of 4.0 %, sugar of 29.6 %, and lecithin of 0.4 %. The next step was refining of the cocoa mass in a 3-roller machine (Jaf Inox-MX, São Paulo, Brazil) to obtain uniform particles with a size between 18 and 20 µm. The remaining cocoa butter was added to the refined mass in a conching equipment (Inco, Avaré, Brazil); at this stage, lecithin was added and the final mass remained heated at 70º C for 24 h. Using rectangular polyethylene molds the chocolate samples were molded (5 g bars), then they were cooled in a chiller tunnel. The samples were packed in aluminum foil, stored at 18 ºC, then chemical and sensory analyzes were carried out.

2.3 Quantification of methylxanthines and monomeric phenolic compounds

First, the chocolate samples were crushed, were degreased with petroleum ether (ratio at 1 sample**:** 2 solvent), under shacking during 30 min, five times. From defatted samples (5 g) were obtained methanolic extracts with 80 mL of a solution of methanol**:**water (80**:**20 v/v), and in a bench top homogenizer were stirred for 1 h, for the extraction of phenolic compounds (Oliveira et al., 2011). These methanolic extracts were filtrated, then were placed in amber flasks under nitrogen atmosphere, and stored at -18 ºC until the analyzes were carried out. For the determination of methylxantines (caffeine and theobromine) and monomeric phenols (catechin, epicatechin and gallic acid) was followed the method recommended by Elwers et al. (2009). The samples (10 µL) were analyzed in a High Performance Liquid Chromatography -HPLC system (Shimadzu, Tokyo, Japan, PE-M Flexar) equipped with a VI Flow injector, DAD detector and a C 18 column (4.6 x 100 mm O.D.S.-2, 3 µ; Discovery, USA), and were used the solvents: (A) acetic acid (2%) in water and a mixture (B) of acetic acid, water and acetonitrile (10:90:400 v/v/v), in the following proportions: 90% solution (A) and 10% solution (B), a 3 µL injection volume, and a flow rate of 0.5 mL min^{-1}. The compounds were monitored by UV detection at 280 nm, the total run time was 20 min, and the temperature was 26° C. The chocolate samples were evaluated in triplicate, and the results were presented in mg g^{-1} of sample. Standard reagents were used (Sigma-Aldrich, St. Louis, MO).

2.4 Quantification of total anthocyanins

The quantification of the total anthocyanins followed the methodology presented by Maciel et al. (2017). Priorly, a solution of ethanol**:**HCl 1.5 N in a proportion of 85**:**15 (v/v) was prepared and 50 mL of this solution were added to 2 g of sample, then were stirred in a bench homogenizer during 30 min, after the sample was refrigerated for 12 h. The homogenized mixture was doubly filtered using no.4 Whatman filter paper and a glass microfibre (Whatman filter), the filtrate was placed in a volumetric flask (100 mL) which was filled with a solution of ethanol**:**HCl 1.5 N in a proportion of 85**:**15 v/v, and placed at rest for 60 min to stabilize. Absorbance of the samples (triplicate) was measured at 528 nm using a UV-M51 spectrophotometer (Bel Photonics, San Diego, CA, USA), also was performed a absorbance measure (used as blank) with a solution of ethanol**:**HCl 1.5 N (85**:**15, v/v). The result of the total anthocyanins (AT) was expressed in mg of cyanidin-3-glucoside (major anthocyanin) in 100 g of sample, whose quantification used the Equation 1.

$$AT = \left(\frac{Abs_{528} \text{ x } PM_{cyanidin-3-glucoside} \text{ x fd}}{\varepsilon}\right) X\ 100 \qquad \text{(Equation 1)}$$

The parameters were Abs_{528}: absorbance (at 528 nm) of the filtered substrate, PM: molecular weight (445.2) of the cyanidin-3-glucoside, fd: dilution factor according to volume and mass, ε: coefficient of molar extinction of the cyanidin-3-glucoside in acidified ethanol solution at 528 nm, and 100 to express the results per 100 g sample.

2.5 Sensory evaluation

All sensory analyses were carried out in individual booths, at 23 °C, with fluorescent lamps. Approval for the study was obtained from the Research Ethics Committee, CAEE N.1.231.812 (UNEB), and all participants signed a consent term.

Quantitative Descriptive Analysis (QDA®) was performed according to the principles described by Stone & Sidel (2004) in order to quantify the descriptive attributes of the dark chocolate samples. The acceptability of the chocolate samples was also evaluated by consumers.

2.5.1 Quantitative Descriptive Analysis

In the recruitment stage, 48 volunteers were evaluated regarding their interests, no allergic reactions to cocoa/chocolate, schedule availability, ability to verbalize descriptions of sensory perceptions, and use of scales. In the pre-selection stage, a triangle test was used to check for differences of basic tastes (sweet, salty, sour, bitter), and astringency, and for the recognition of the odors of some products, the concentration of the materials needed to prepare the samples followed Brazilian technical standards (ISO, 2012). Five replications were carried out, in which the candidates had to demonstrate at least 80% accuracy. Twenty candidates (age between 21 and 52 yr) were selected for the next stage. The development of descriptive terminology was performed using Kelly's Repertory Grid method (Moskowitz 1983). All samples were presented by pairs, and the participants described the similarities and differences of the attributes (aroma, appearance, flavor, texture) for each pair of samples. Guided by a leader, after a good discussion, the redundant terms were excluded by consensus of all participants. Table 1 shows the 18 descriptive terms that were defined to characterize the chocolate samples, as well as the reference materials and the intensity terms (major and minor) for each extreme point of the scale.

The next stage aimed at training the participants' sensory memory regarding the descriptor terms and their respective maximum and minimum intensities, which was carried out by direct contacting the assessors with the reference materials for each attribute. The samples were evaluated using an evaluation form composed of 9-cm unstructured line with anchors "little/much" or "none/much" at the extremes, on the left/right, respectively (Das Virgens et al., 2020; McMahon et al., 2017). The training stage consisted of 16 sessions lasting 1 h each, and the performance of each assessor was monitored. After training, and when the participants had no more doubts in the evaluation of the samples, they were invited to evaluate the chocolate samples (3 replicates), using the definitive sheet for QDA. Those who exhibited good sample discrimination (probabilities $p\ F_{sample} \leq 0.50$) and replicate capacity ($p\ F_{replicate} > 0.05$), and consensus with the group (interaction effect of taster*sample) for all attributes were selected (Damasio & Costell, 1991; Cadena et al., 2013). The final evaluation was performed with twelve selected panelists (4 men and 8 women), which evaluated four chocolate samples. The samples (5 g) were served in disposable plastic plates which were coded with random 3-digit numbers, according to a random complete block design (3 replicates), using individual sensory cabins (white light and temperature at 23 °C). Filtered water was provided at all stages.

Table 1 - Descriptors terms and references used by panelists during training and QDA sessions.

Attributes	Definition	References
Brown color	Characteristic color of chocolate, similar color to mahogany brown	Little intense: alkaline cocoa powder- 10 % added in 100 g starch cream (Unilever) Much intense: alkaline cocoa powder- 80 % added in 100 g starch cream.
Brightness	Light reflectivity on the product surface. Light emits shine or greatly reflects shine.	Little: dark chocolate 80 % cocoa (AMA). Much: dark chocolate 80 % cocoa with butter on surface.
Acid aroma	Characteristic odor of a blend of acid (acetic, citric, succinic).	None: distilled water. Much: acetic acid solution- 0.5 % (Castelo)
Cocoa aroma	Odor related to chocolate/ cocoa.	Little: powder cocoa- solution 0.5 % (Mãe Terra). Much: powder cocoa- solution 5.0 %.
Sweet aroma	Odor obtained from caramel/sugar.	None: distilled water. Much: sucrose warmed at 120 ºC for 4 min (brown color)
Fruity aroma	Characteristic odor of citric fruits (tangerine, lemon, orange).	None: distilled water. Much: citric essential oil- solution 0.5 % (Arcolor).
Toasted aroma	Odor related to very toasted cocoa beans.	None: dry cocoa beans without toasting. Much: cocoa beans toasted at 160 ºC for 60 min.
Buttery aroma	Odor related to butter/fat.	None: distilled water. Much: powder cocoa with butter (1:1) added in 100 g starch cream (Mãe Terra)
Woody aroma	Characteristic of woody, oak moss, cinnamon and earth tones, dry odor	None: distilled water. Much: Woody essence- solution 2.0 % (Food Degree)
Sweet taste	Sweet taste obtained from sugar. Caramel flavor	Little: sucrose solution at 1.0 %. Much: sucrose solution at 10.0 %.
Bitter taste	Characteristic taste of caffeine. Unpleasant taste of caffeine.	None: distilled water. Much: caffeine solution at 0.5 % (Food Degree)
Acid taste	Characteristic citric acid taste.	None: distilled water. Much: citric acid solution- 1.0 % (Food Degree)
Cocoa flavor	Flavor related to chocolate/cocoa	Little: powder cocoa solution- 0.5 %. (Mãe Terra). Much: powder cocoa solution- 5.0 % (Mãe Terra)
Fruity flavor	Flavor related to citric fruits (tangerine, orange, lemon)	None: distilled water. Much: citric essential oil- solution 0.5 % (Arcolor)
Buttery flavor	Flavor related to butter.	None: distilled water. Much: powder cocoa with butter (1:1) added in 100 g starch cream (Mãe Terra)
Astringency	Drying sensation from tasting green fruit. Contraction of mouth mucus.	None: milk chocolate (Nestlé). Much: pieces of unripe banana
Firmness	Force required biting the chocolate sample.	Little Firm: milk chocolate (Nestlé). Much Firm: Dark chocolate 85 % cocoa (AMA)
Melting	Length of time for chocolate to melt in the mouth /Liquefied.	Little: chocolate (75 % cocoa) warmed to 80 ºC for 20 sec (AMA). Much: chocolate (75 % cocoa) warmed to 80 ºC for 40 sec.

Source: Authors (2023).

2.5.2 Acceptance test

The acceptance test was carried out with 100 randomly recruited consumers (68 women and 32 men, aged 19 to 61 yr) from two local universities, the participants were recruited spontaneously according to their availability and consumption habits of intense chocolate (Ethical clearance approval and participant's signature in a consent form were obtained). For the tests, 5g of each sample was served to consumers, at room temperature and in disposable trays, coded with three-digit numbers, following an incomplete block design. Consumers were instructed to taste the samples from left to right and mineral water was available to clean the palate. The evaluations took place in sensory cabins (fluorescent lamps and temperature at 23 °C). The attributes appearance, aroma, flavor, texture, and overall liking were evaluated using a nine-point structured hedonic scale, being 9 = liked extremely to 1 = disliked extremely (Meilgaard et al., 2006; Morais et al., 2014).

2.6 Statistical analysis

The data from the chemical evaluation and acceptance test were evaluated by Analysis of Variance, 2-way ANOVA (repetition/consumer and samples), followed by the Tukey's test to verify significant differences ($p < 0.05$) between samples, according to each sensory attribute and chemical parameter (Granato and Ares, 2014). QDA results were also analyzed by ANOVA, using two factors (panelist and sample) and their interaction, followed by a Tukey's means test (P < 0.05).

Descriptive information obtained from the trained panel was organized into a matrix of i rows (4 samples) and j columns (18 sensory descriptors), and External preference mapping (PREFMAP) was conducted by first using principal components analysis (PCA) on the QDA data and then relating consumer preference (overall liking) to this PCA space by regression analysis (Cadena et al., 2013). The vector model, which implies a direct relationship between one or more characteristics that are increasing or decreasing across the stimulus space, was used. The consumer preference is represented by "overall liking", and the closer a particular attribute is to overall liking indicates that this descriptive term is important for acceptance of the dark chocolate samples. The chemical datasets were also organized into a matrix of rows (samples) and columns (bioactive compounds), and PCA was carried out. External preference mapping was also performed by means of a regression where hedonic rating (overall liking) are the response (dependent) variable and chemical parameters are the independent variable (Ferreira et al., 2017). All statistical analyses were carried out using XLSTAT® software for Windows, version 2021 (Addinsoft, Paris, France), at a 5% significance level.

3. Results and Discussion

3.1 Chemical properties of dark chocolate samples

The mean values of the chemical parameters of the dark chocolate samples produced from hybrid cocoa are presented in the Table 2. Significant differences (p<0.05) were observed in the theobromine and caffeine content of the samples. These substances are often referred to as alkaloids, and in cocoa beans the theobromine (3,7-dimethylxanthine) is found in predominance when compared to caffeine (1,3,7-trimethylxanthine), which appears in minor content (Boza et al., 2014; Aprotosoaie et al., 2016). In this study, the samples from the 'CCN51' variety presented lower amounts of theobromine and caffeine, consequently higher value to the theobromine/caffeine ratio, which was significantly different ($p < 0.05$) from the others varieties 'Pará', 'Ipiranga', and 'PS1319' (no significant difference among each other).

Table 2 - Chemical parameters of the dark chocolate samples produced from hybrid cocoa.

	Samples				
Parameters	**Pará**	**Ipiranga**	**CCN51**	**PS1319**	**SMD**[*]
Theobromine (mg/g)	13.66 ± 0.37[**a]	10.32 ± 0.66[b]	7.67 ± 0.34[c]	9.46 ± 0.68[b]	1.79
Caffeine (mg/g)	6.86 ± 1.40[a]	6.25 ± 1.27[a]	1.08 ± 0.20[b]	5.25 ± 0.73[a]	2.17
Theobromine/Caffeine	1.99[b]	1.65[b]	7.10[a]	1.04[b]	2.39
Gallic Acid (mg/g)	27.84 ± 1.60[a]	18.65 ± 1.10[a]	8.91 ± 0.43[b]	12.04 ± 0.22[b]	3.39
Catechin (mg/g)	0.85 ± 0.06[c]	1.29 ± 0.20[ab]	0.91 ± 0.07[bc]	1.36 ± 0.02[a]	0.44
Epicatechin (mg/g)	0.24 ± 0.01[c]	0.73 ± 0.07[a]	0.25 ± 0.02[c]	0.58 ± 0.03[b]	0.13
Anthocyanins (mg/g)	0.51 ± 0.06[a]	0.44 ± 0.05[b]	0.41 ± 0.03[c]	0.44 ± 0.02[b]	0.02

[*]SMD= Significant Minimum Difference. **Mean ± standard deviation (n=3). Means in the row followed by different letters represents significant differences, for each parameter (Tukey test, p< 0.05). Source: Authors (2023).

Maciel et al. (2017) found lower values of these substances for the 'CCN51' and 'Pará' varieties (theobromine: 1.74 and 1.27 mg g^{-1}, caffeine: 0.19 and 0.13 mg g^{-1}, respectively). Calva-Estrada et al. (2020) revealed predominance in the theobromine concentration in chocolate samples of different cultivars from Latin American (theobromine: 9.25–12.19 mg g^{-1}, caffeine: 1.49–2.34 mg g^{-1}).

Some studies have shown a relevant correlation between the content of methylxanthines and the cocoa's genotype.

Tuenter et al. (2020) found significant diferences between chocolate samples produced from cocoa beans of different origin. The caffeine content in the samples from Ecuador (Nacional variety fine flavor cocoa) was twice as high as in the West African samples (Forastero "bulk" cocoa). The theobromine/caffeine ratio was 4.7:1 for the samples from National variety and 9:1 for African varieties, typically the ratio was higher in the chocolate samples from "Bulk" cocoa. A similar trend was reported by Carrillo et al. (2014) when evaluating cocoa beans from different areas in Colombia, the authors found higher theobromine and lower caffeine content (higher theobromine/caffeine ratios) for Forastero group, when compared to Trinitario and Criollo groups. Also, Brunetto et al. (2007) studied the theobromine/caffeine ratio of genotype of cocoa grown in Venezuela under different seasons and subject to several stages of fermentation. The findings permitted classify in cocoa ''Forastero'' (high ratio), ''Trinitario'' (intermediate ratio) and ''Criollo'' (low ratio).

According to Aprotosoaie et al. (2016), difference in the levels of methylxanthines may significantly affect the flavor of cocoa products, being that the higher the theobromine/caffeine ratio the more the sensory profile of cocoa approaches the "bulk" flavor. Todorovic et al. (2015) found values ranging from 10.70 mg g^{-1} to 14.60 mg g^{-1} for theobromine and from 0.56 mg g^{-1} to 1.36 mg g^{-1} for caffeine in dark chocolates from Serbia. The authors reported that the bitterness of these products might be due to methylxanthines concentrations.

Significant differences ($p < 0.05$) and a relevant variation in the content of phenols compounds (catechin, epicatechin, gallic acid, anthocyanins) were observed in the chocolate samples. The products from the 'PS1319' and 'Ipiranga' cocoa varieties showed higher concentration of catechin and epicatechin, while the 'Para' and 'CCN51' varieties presented lower content. Maciel et al. (2017) in their study with Brazilian varieties found content of catechin and epicatechin that were 0.26 mg g^{-1} and 0.46 mg g^{-1}, respectively, for the 'CCN51' variety, and values of 0.064 mg g^{-1} and 0.092 mg g^{-1} for the 'Pará' variety.

In general, higher values were found in others studies. Meng et al. (2009) reported to content of catechin and epicatechin, respectively, 1.85 mg g^{-1} and 2.74 mg g^{-1}, in the intense chocolate from Malaysia. Leite et al. (2013) found concentrations in the range of 0.95 mg g^{-1} to 1.73 mg g^{-1} for catechin and 1.55 mg g^{-1} to 1.85 mg g^{-1} for epicatechin in Brazilian cocoa grown in the Southern Bahia. Todorovic et al. (2015) reported lower values for both substances in the range of 0.06 - 0.18 mg g^{-1} for catechin, and 0.18 - 0.26 mg g^{-1} for epicatechin in dark chocolates from Serbia. Conversely, Calva-Estrada et al. (2020) observed greater proportions for epicatechin of 0.45 - 1.03 mg g^{-1} compared to catechin 0.06 - 0.25 mg g^{-1}. Intrinsic factors of the cocoa genotype, mainly varietal characteristics and maturity of the beans can affect the value of polyphenolic compounds, as the level of -epicatechin and -catechin (Cambrai et al., 2017). Several authors also mentioned that others parameters such as geographical region of plantation, climatic conditions, and even the post-harvest processing, and different ways of the chocolate manufacturing, roasting, and conching may be related to variations in the levels of phenolic compounds (Urbanska & Kowalska, 2019; Oracz et al., 2015; Oracz et al., 2013).

The anthocyanins provide the specific color to the cocoa beans from the Forastero group, but not to the Criollo group. Anthocyanins are phenolic substances contained on cells, and divided into sessions/groups in the cotyledons of the cocoa beans (De Taeye et al., 2016). In this study, the 'Pará' variety presented the highest values and the 'CCN51' variety presented the lowest amounts of anthocyanins. According to Boza et al. (2014), the 'CCN51' variety presents specific ancestries in the genetic groups: Iquitos (45 %), Criollo (23 %), and Amelonado (22 %), based on population structure analyze. The lower amounts of anthocyanins found in the 'CCN51' variety when compared to others varieties that have predominant ancestries in Forastero group might be explained by the lack of anthocyanins in the Criollo group. Calva-Estrada et al. (2020) found values in the range of 0.04–0.08 mg CyE g^{-1} for the dark chocolate samples made with different cocoa genotypes from regions of Latin America, and Bordiga et al. (2015) reported content in the range of 0.002–0.005 mg of cyanidin-3-galactoside equivalentes g^{-1} for dark chocolates prepared with cocoa from different origins (Ecuador, Cameroon, Ivory Coast, Ghana and Nigeria).

3.2 Quantitative descriptive analysis and acceptability of the dark chocolate samples

The mean values of the sensory descriptors (QDA data) and the mean scores of the attributes evaluated in the acceptance test for each dark chocolate sample are showed in the Table 3. Appearance of chocolates is an important sensory characteristic, which influences the acceptance of these products. In relation to the color attribute, the 'Pará' variety samples presented brown color significantly (p < 0.05) more intense, these findings may be explained by its higher value of anthocyanins in relation to the other cocoa varieties. Some authors indicated significant differences in the anthocyanin content of cacao clones from different geographic origins (Niemenak et al, 2006; Elwers et al, 2009). In a study with Brazilian cocoa beans, higher content of anthocyanins was found for cocoa from the Forastero group when compared to cocoa from the Trinitario and Criollo groups (Maciel et al, 2017). The data for brightness attribute showed that there was no significant difference (p > 0.05) among the studied cocoa varieties, this attribute was not able to contribute to the differentiation of the products.

Table 3 - Mean values of sensory descriptors of the dark chocolate samples produced from hybrid cocoa obtained by QDA, and mean scores of the acceptance test.

Descriptors	**Samples**				
	Pará	**Ipiranga**	**CCN51**	**PS1319**	**SMD[*]**
Brown color	6,94 ± 0,30[**a]	5,31 ± 0,20[b]	6,06 ± 0,30[b]	5,36 ± 0,20[b]	0,76
Brightness	5,76 ± 0,40[a]	5,71 ± 0,40[a]	5,72 ± 0,30[a]	5,82 ± 0,50[a]	0,15
Acid aroma	5,28 ± 0,50[a]	2,95 ± 0,40[c]	1,59 ± 0,40[d]	3,28 ± 0,60[b]	0,21
Cocoa aroma	6,46 ± 0,40[a]	4,67 ± 0,50[d]	5,24 ± 0,50[c]	5,74 ± 0,50[b]	0,19
Sweet aroma	1,16 ± 0,30[c]	1,25 ± 0,30[c]	3,06 ± 0,90[a]	2,05 ± 0,40[b]	0,17
Fruity aroma	1,15 ± 0,50[c]	4,37 ± 0,90[a]	1,16 ± 0,40[c]	3,43 ± 0,90[b]	0,18
Toasted aroma	1,41 ± 0,40[a]	0,72 ± 0,30[b]	0,73 ± 0,30[b]	1,37 ± 0,30[a]	0,14
Buttery aroma	0,81 ± 0,50[d]	1,05 ± 0,60[c]	3,11 ± 0,80[a]	1,79 ± 0,60[b]	0,16
Woody aroma	1,08 ± 0,70[b]	1,07 ± 0,40[b]	0,89 ± 0,50[c]	2,82 ± 0,70[a]	0,17
Sweet taste	1,82 ± 0,50[d]	4,08 ± 0,50[b]	5,08 ± 0,90[a]	3,72 ± 0,90[c]	0,27
Bitter taste	6,37 ± 0,70[a]	4,75 ± 0,50[b]	3,83 ± 0,90[c]	4,72 ± 1,10[b]	0,31
Acid taste	4,35 ± 0,70[a]	3,93 ± 0,40[b]	2,41 ± 0,90[c]	4,60 ± 0,60[a]	0,31
Cocoa flavor	6,04 ± 0,60[a]	5,18 ± 0,60[c]	4,81 ± 0,50[d]	5,48 ± 0,80[b]	0,22
Fruity flavor	1,14 ± 0,50[d]	4,85 ± 0,60[a]	1,41 ± 0,60[c]	2,99 ± 0,30[b]	0,22
Buttery flavor	1,39 ± 0,90[c]	1,16 ± 0,80[d]	2,85 ± 0,70[a]	2,10 ± 0,80[b]	0,17
Astringency	4,49 ± 0,70[a]	1,59 ± 0,20[c]	1,41 ± 0,40[c]	1,99 ± 0,30[b]	0,25
Firmness	5,84 ± 0,60[c]	6,29 ± 0,40[b]	7,06 ± 0,30[a]	5,50 ± 0,40[d]	0,14
Melting	3,52 ± 0,30[b]	5,43 ± 0,50[a]	3,10 ± 0,50[c]	5,30 ± 0,40[a]	0,19
Acceptance[***]					
Appearance	8,26 ± 0,88[a]	8,21 ± 0,89[a]	8,23 ± 0,84[a]	7,99 ± 1,04[a]	0,28
Aroma	7,51 ± 1,34[a]	7,41 ± 1,30[a]	7,16 ± 1,56[a]	7,13 ± 1,53[a]	0,45
Flavor	5,76 ± 2,08[c]	6,41 ± 2,19[b]	7,16 ± 1,58[a]	5,50 ± 2,19[c]	0,54
Texture	7,47 ± 1,30[ab]	7,59 ± 1,11[a]	7,52 ± 1,42[ab]	7,16 ± 1,62[b]	0,39
Overall liking	6,45 ± 1,91[b]	7,09 ± 1,74[a]	7,53 ± 1,36[a]	6,21 ± 2,03[b]	0,49

[*]SMD=Significant Minimum Difference. **Mean ± standard deviation. [***]n=100. Means in the row followed by different letters represents significant differences, for each attribute (Tukey test, p< 0.05). Source: Authors (2023).

In relation to the aroma attribute, the 'Pará' variety reached higher intensities of acid, roasted and cocoa aroma compared to the others varieties, although similar intensity was observed for the roasted aroma in the 'PS1319' variety. On the other hand, the samples from the 'CCN51' variety showed expressive intensities of sweet and buttery aroma, and there was significant difference ($p < 0.05$) in relation to the other samples. In a study on volatile profile and sensory characteristics of Ecuadorian varieties was reported that the 'CCN51' variety presented higher levels of volatile compounds which confer buttery/creamy aroma, the authors identified in the liquor of this variety distinct odor-active ketones, as 2,3-butanedione (diacetyl) and 2,3-pentanedione, illustrating the genotype effect (Rottiers et al., 2019). The samples from the 'Ipiranga' variety stood out by high intensity of fruity aroma and the 'PS1319' variety samples were associated to woody aroma, these attributes are very important to indicate good quality for chocolate and cocoa products. For the international market, fine flavor cocoa is defined as one that presents special aromas, such as with fruity, flowery, woody, green note, buttery, creamy, honey, sweet, caramel, nutty, and roasted almond notes (Muñoz et al., 2020; Aprotosoaie et al., 2016). The "bulk" cocoa (common cocoa) represents more than 90% of the world cocoa production, is a type of cocoa without specific aromatic characteristics (Oractz et al., 2013; Santos et al., 2016; Beckett et al., 2017).

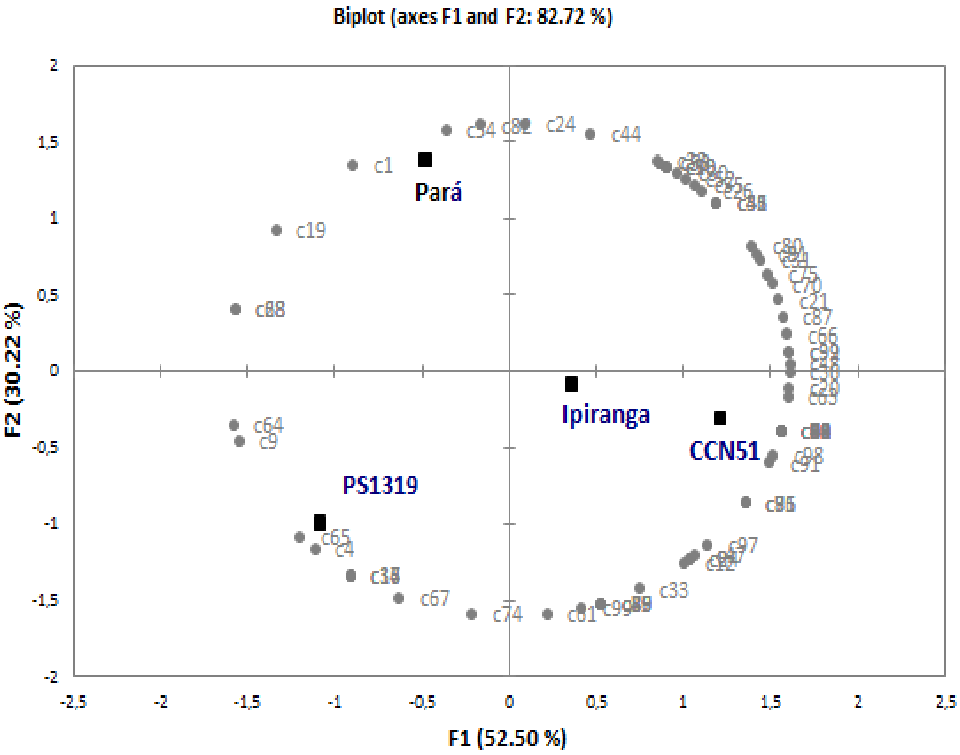
In this study, significant differences were observed in the flavor intensity of the chocolate samples, as acidity, bitterness, sweetness, fruity, buttery, cocoa flavor, and astringency. Samples from the 'Pará' variety stood out due to high intensity of cocoa flavor, acid and bitter taste, and astringency. Higher contents of methylxanthines and anthocyanins were found for this variety, possibly explaining these sensory findings. The presence of methylxanthines among other substances such as diketopiperazines, peptides, and certain amino acids, may contribute to the perception of the bitter taste of cocoa beans (Muñoz et al., 2020). Regarding astringency, many consumers do not understand this attribute very well and confuse it with bitterness, because many polyphenols can induce both astringency and bitterness (Tuenter et al., 2020; Counet-Kersch et al., 2004). The last authors reported that cocoa beans were characterized by a pronounced astringency due to the high content of phenolic compounds, mainly anthocyanins. However, the samples from the 'Ipiranga' variety showed high intensity of fruity flavor and the samples from the 'CCN51' variety presented high intensity of sweet taste and buttery flavor. Brazilian researchers (Reges et al., 2021; Alexandre et al, 2015) reported that the 'CCN51' variety presented higher values to reducing sugar content and soluble solid (º Brix). Another study carried out with Ecuadorian cocoa varieties found significant difference in the acidity, the 'CCN51' variety was considered the least acid compared to the other varieties studied (Rottiers et al., 2019).

The texture attribute is very important since it is one of the main sensory properties appreciated by chocolate consumers. In this study, a negative correlation was found between firmness and melting quality, that is, those firmer chocolate samples showed low melting intensity in the consumer's mouth, such as the 'CCN51' and 'Pará' varieties. On the other hand, the samples with higher melting quality also presented lower firmness, such as 'Ipiranga' and 'PS1319' varieties.

According to the mean scores given by the consumers in the acceptance test (Table 3), there was no significant difference ($p > 0.05$) among the chocolate samples regarding the attributes of appearance and aroma. All the samples presented great acceptability, and the scores corresponded to the hedonic terms "liked much" and "liked moderately", to the appearance and aroma attributes, respectively. With regard to the flavor attribute, the samples from the 'CCN51' variety scored highest with significant differences between them ($p < 0.05$). The samples from the 'Ipiranga' variety scored the second highest in flavor acceptability, followed by the 'PS1319' and 'Pará' varieties (no significant difference between them). These results possibly are due to bitter and acid taste, and astringent characteristics referred to the 'Pará' and 'PS1319' varieties (QDA data). However, the scores corresponded to the hedonic term "neither liked/neither disliked". Higher scores of the 'CCN51' and 'Ipiranga' varieties probably are due to sweet taste and buttery flavor, and fruity notes, respectively. Further, the chocolate samples from the 'CCN51' and 'Ipiranga' varieties had higher scores for the overall liking attribute (correspondent to "liked moderately"), being followed by the samples of the 'PS1319' and 'Pará' varieties (correspondent to "liked lightly", and no significant difference between them). Regarding texture, all the samples reached great acceptability, and the scores corresponded to the hedonic term "liked moderately".

Figure 1 shows the internal preference map for the overall liking attribute of the chocolate samples. The first factor explains up to 52.5% of the variation while the second factor accounts for 30.2%, totaling together 82.7%. Most of overall liking scores of consumers are located in the right side of the plot. The greater the density of dots (consumers) near a sample, the more that sample was preferred over others. The map shows that the samples from ‘CCN51’ and ‘Ipiranga’ varieties were most accepted by consumers and the samples from ‘Pará’ and ‘PS1319’ varieties were least accepted, according to the visual density of dots near each sample.

Figure 1 - Internal preference map (dots represent consumers = 100) for the dark chocolate samples produced from hybrid cocoa. The higher the density of dots near a sample, the more that sample was preferred over the others, relating to overall liking.



Source: Authors (2023).

Flavour is one of the most influential factors for the acceptability of the products (consumer’s decision to buy a product). In a study carried out by Torres-Moreno et al (2012) on the influence of different origins of cocoa beans (Ghana and Ecuador) and different conditions of chocolate manufacturing on the sensory attributes as overall impression, color, odor, flavor, texture, the authors reported that the acceptability of the intense chocolate was strongly influenced by the flavor attribute. Paiva et al. (2012) also concluded that flavor was one of the factors that most influenced the acceptance of food products. Brazilian researchers found evidence that intense chocolates prepared with cocoa beans from the Forastero group were less accepted than chocolates from the Trinitario group. The authors concluded that there may be gains in the quality of chocolates with more studies on the genetics of cocoa beans (Efraim et al., 2013).

3.3 Drivers of liking of the dark chocolate samples

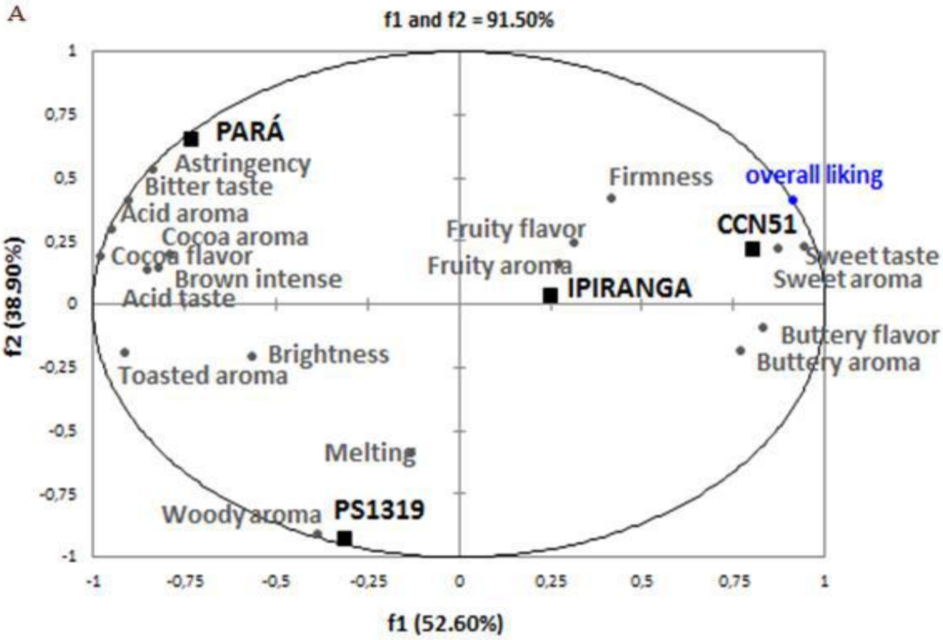
The results of the external preference mapping are shown in Figure 2 (A, B). Data were represented by two principal components, the factor 1 (horizontal axis) divided the chocolate samples on the right side of the axis (‘CCN51’ and ‘Ipiranga’ varieties), and the chocolate samples on the left side ('Pará' and 'PS1319' varieties). Thus, variables that present a positive coordinate on the first factor show a direct correlation with preference, while variables with a negative coordinate on factor 1 are negatively correlated to preference. As can be seen in Figure 2 (A), the first factor f1 (X-horizontal axis) explains up to 52.6 % of the variation, and the second factor f2 (Y-vertical axis) accounts for 38.9 %, totaling together 91,5%. The model shows that for the dark chocolate samples, the overall liking is positively correlated to sweet and buttery aroma, sweet taste, buttery flavor, fruity aroma and flavor, and firmness attributes (which are important preference driving for chocolate), and negatively correlated to bitter and acid taste, cocoa aroma and flavor, intense brown and astringency (drivers of disliking). These are the most important sensory descriptors driving the preference for the dark chocolate, and demonstrates whether consumers based their preference on the same sensory descriptors. In Figure 2 (B) the chemical parameters are plotted, the first factor f1 explains up to 42.6 % of the total variation, and the second factor f2 accounts for 47.0 %, totaling together 89,6%. Primarily, variables that are close to the overall liking make a positive contribution to acceptance of the product, and variables that are located in the opposite part of the graph contribute negatively to the acceptance of the products. The model demonstrates that for the considered dark chocolate samples, the overall liking is negatively correlated to bioactive compounds as theobromine, caffeine, gallic acid and anthocyanins (these compounds imparted bitterness and astringency.

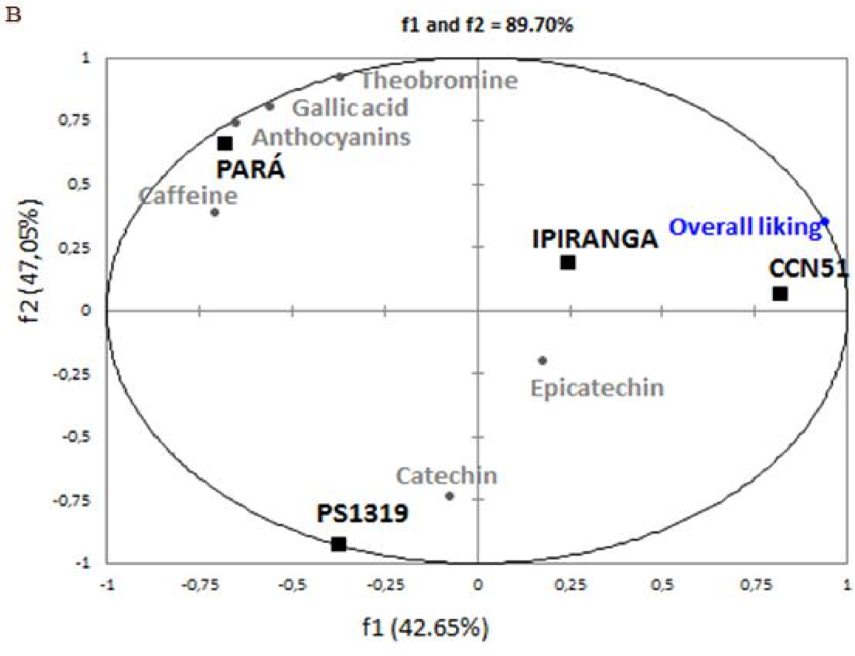
The Figure 2 (A, B) shows that the chemical properties associated with the chocolate samples from 'Pará' variety were theobromine, caffeine, gallic acid and anthocyanins, which negatively correlated with consumers' overall liking, probably contributing to their higher intensity of bitter and acidic taste, and astringency. The chocolate samples from 'CCN51' and 'Ipiranga' varieties are located in the opposite region from the aforementioned compounds and they presented sweet/caramel/buttery and fruity notes that contributed to higher acceptance by the consumers. The chocolate samples from 'PS1319' variety are located in the intermediate region of the graph, and it presented woody notes, melting quality that contributed to intermediate acceptance by consumers.

Although the 'Pará' variety did not show special flavor and aromatic notes, it is important to mention that it demonstrated higher intensities for the cocoa aroma and flavor, these attributes are relevant for the cocoa marketing and for the chocolate industry, this material can be used to intensify the cocoa aroma and flavor in the chocolate products. The results of this study are also important to demonstrate to producers the need to update postharvest and processing protocols for cocoa beans, which can help improve product quality.

In others studies that also aimed to find preference drivers for chocolate and cocoa products, the authors concluded that the favorites sensory characteristics were caramel aroma, sweetness, chocolate flavor, melting quality, and mouth fill; they also reported that the attributes bitterness, roasted flavor, acidity and bitter aftertaste were considered as undesirable characteristics, associated to the drivers of liking and disliking of the cocoa products, respectively (Ferreira et al., 2017; Morais et al., 2014; Mello et al., 2009).

Figure 2 - External preference mapping obtained by regression method of the descriptive sensory profile (A- QDA data) and chemical properties (B- bioactive compounds data) and consumer's responses for overall liking of the dark chocolate samples produced from hybrid cocoa.





Source: Authors (2023).

4. Conclusion

This study identified variability among the dark chocolate samples prepared with hybrid cocoa regarding the content of bioactive compounds. The results suggested that high contents of theobromine, caffeine, anthocyanins, and gallic acid contributed to higher intensities of bitterness, acid taste, cocoa flavor, and astringency, affecting negatively the acceptance of the samples by consumers (possible trend of the 'Pará' variety). Lower values of methylxanthines, monomeric phenolic compounds, and anthocyanins enabled the perception of sweet/caramel/ buttery and fruity notes that contributed to higher acceptance scores (trend of the 'CCN51' and 'Ipiranga' varieties). The 'PS1319' variety was associated to woody notes and reached intermediate acceptance score. These attributes can be considered as major drivers of liking and disliking for dark chocolates.

The findings of this study can be used in agriculture and in the cocoa/chocolate industry, suggesting the possibility and applicability of promising hybrid cocoa with important characteristics and disease resistance, representing a genetic material of great quality that may be classified as fine flavored cocoa, adding value to the product, and meeting the consumer needs. Additional studies must be carried out to detect the existing potential for the production of fine cocoa in the different cocoa producing micro-regions of Brazil.

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