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Bioactive changes in cocoa nibs and chocolate due to fermentation

Abstract – The objective of this work was to evaluate how cocoa beans subjected to different fermentation indexes influence their physicochemical properties, antioxidant activity (DPPH) and the profile of bioactive compounds in nibs and chocolates, besides carrying out a sensory evaluation of chocolates produced. Chocolates with high cocoa content were produced from beans at 50%, 70%, and 90% fermentation indexes. Nibs and chocolates were characterized using high-performance liquid chromatography (HPLC) and mid-infrared spectroscopy (MIR) techniques. The chocolates were subjected to a sensory evaluation using an optimized descriptive profile. Data were subjected to the principal component analysis (PCA) and the Tukey's test, to establish the difference between means of treatments. Nibs and chocolates produced with cocoa beans at lower fermentation rates showed higher values of epicatechin, catechin, methylxanthines, condensed tannins, and total phenolics. The use of MIR and HPLC in conjunction with PCA makes it possible to distinguish between nibs and chocolates. Chocolates produced with cocoa beans at about 50% fermentation index are a viable alternative, when it comes to producing cocoa derivatives with higher levels of bioactive compounds, without major influences on the characteristics of astringency and bitterness.

Index terms: *Theobroma cacao,* antioxidant capacity, phenolic compounds, principal components.

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Alterações bioativas em *nibs* de cacau e chocolate resultantes da fermentação

Resumo – O objetivo deste trabalho foi avaliar a influência de grãos de cacau, submetidos a diferentes índices de fermentação, sobre as propriedades físico-químicas, a atividade antioxidante (DPPH) e o perfil de compostos bioativos em *nibs* e chocolates, e realizar uma avaliação sensorial dos chocolates produzidos. Chocolates com alto teor de cacau foram produzidos a partir de grãos com índices de fermentação de 50%, 70% e 90%. *Nibs* e chocolates foram caracterizados por técnicas de cromatografia líquida de alta eficiência (HPLC) e espectroscopia no infravermelho médio (MIR). Os chocolates foram submetidos à avaliação sensorial, por meio de um perfil descritivo otimizado. Os dados foram submetidos à análise de componentes principais (ACP) e ao teste de Tukey, para determinar diferenças entre as médias dos tratamentos. *Nibs* e chocolates produzidos com grãos de cacau com menor taxa de fermentação apresentaram os maiores valores de epicatequina, catequina, metilxantinas, taninos condensados e fenólicos totais. A utilização de MIR e HPLC, em conjunto com a ACP possibilita a distinção entre *nibs* e chocolates. Chocolates produzidos com grãos de cacau com aproximadamente 50% de fermentação são uma alternativa viável para a produção de derivados de cacau com maiores teores de compostos bioativos, sem maiores influências sobre as características de adstringência e amargor.

Termos para indexação: *Theobroma cacao*, capacidade antioxidante, compostos fenólicos, componentes principais.

Introduction

Chocolate is widely consumed worldwide, it derives from cocoa (*Theobroma cacao* L.) processed beans that includes ingredients such as sugar, cocoa butter, milk, and lecithin (Kruszewski & Obiedziński, 2018). Due to its natural antioxidants, cocoa beans have been the focus of numerous scientific studies on their cardiovascular health benefits, anti-inflammatory action, antioxidant protection, and cholesterol regulation (Perez et al., 2021). These properties are linked to the presence of phenolic compounds (epicatechin and catechin) and methylxanthines, such as theobromine and caffeine, which also contributes to the bitter and astringent flavors of chocolate (McClure et al., 2022).

Consumers are demanding cocoa products with retained beneficial compounds, driving the food industry to focus on preserving these elements during processing. However, significant transformations occur during the postharvest stages, like fermentation and drying, when the polyphenol content can decrease by 30% in the first 48 hours, and up to 90% by the end of the process (Lončarević et al., 2018). Additionally, during drying, the cotyledons darken due to oxidation and enzymatic reactions involving polyphenol oxidase (Escobar et al., 2021).

However, the use of cacao beans at different fermentation indexes, in the production of nibs and chocolates, remains an underexplored area, despite the significant impact the chemical composition of the beans can have on both the functional and sensory characteristics of the final product (Delgado et al., 2018).

The objective of this work was to evaluate how cacao beans subjected to different fermentation indexes influence their physicochemical properties, antioxidant activity (DPPH), and the profile of bioactive compounds in nibs and chocolates, in addition to carrying out a sensory evaluation of chocolates produced.

Materials and Methods

Cocoa beans from the cacao variety *parazinho* (a Brazilian *forasteiro amelonado* variety) from Agrícola Condurú Ltda., in the municipality of Ilhéus, in the state of Bahia (BA), Brazil, were fermented in *putumuju* (*Centrolobium tomentosum* Guillem. ex Benth.) wooden troughs (0.70x0.70x70 m) with drainage holes. Beans were sampled at various stages of fermentation. After 48 hours of fermentation, the collected cocoa beans showed about 50% (N50) fermentation index; those collected after 96 hours showed about 70% (N70) fermentation rate; and those left to ferment for 144 hours showed a fermentation index exceeding 90% (N90). After 48 hours of fermentation, cacao beans were turned every 24 hours, for 6 days, to ensure oxygenation and homogenization. Each treatment was replicated three times, totaling 9 kg samples. Then, beans were dried on solar dryers by manual aeration for 13 days.

Cocoa beans were roasted at 100°C, for 1 hour, in a rotary oven with an exhaust fan (Proservice, Itabuna, BA, Brazil), then they were ground and shelled, using Transfornibs equipment (Proservice, Itabuna, BA, Brazil), to produce cocoa nibs with fermentation index of 50% (N50), 70% (N70) and 90% (N90). From these nibs, 1.5 kg of 70% cocoa chocolate was produced, classified as C50, C70, and C90, using a Melanger multifunctional machine (Spectra 11, Coimbatore, TN, India).

The chocolate formulations consisted of 61% cocoa nibs, 9% deodorized cocoa butter (Barry Callebaut, Ilhéus, BA, Brazil), 29.6% sugar (União, Araquari, SC, Brazil), and 0.4% soy lecithin (Adicel Ind. e Com. ltda., Belo Horizonte, MG, Brazil). After mixing in a chocolate tempering machine (Mini Chocomachine, Finamac, São Paulo, SP, Brazil), the chocolate mass was molded, cooled at 5°C, for 4 hours, wrapped in laminated paper (Cromus Embalagens Ind. e Com. Ltda., Mauá, São Paulo, SP, Brazil), and stored at 5°C for further analysis.

The physicochemical characterization and chemical composition of nibs and chocolates was carried out in triplicate, using methods of analysis of AOAC (2016), for the following parameters: pH (AOAC 970.21), using a digital pH meter (model Q400AS, 32 Quimis, Diadema, SP, Brazil); titratable acidity, using the potentiometric method (AOAC 981.12); moisture (AOAC 931.04); fat (AOAC 963.15); protein (AOAC 970.22); and fixed mineral residue (AOAC 972.15). The DNS (3,5-dinitrosalicylic acid) method (Sigma-Aldrich, St. Louis, MO, USA) was used to assess total sugars and reducing sugars, using a spectrophotometer (Shimadzu UV - 1800, Duisburg, Germany) at 540 nm (Cecchi, 2003).

The total phenolic compound content of nibs and chocolates was determined using spectrophotometric analysis according to the Folin-Ciocalteu method. The extraction of phenolic compounds was carried out in aqueous solvent, adapted from Lee et al. (2003), and the absorbance was measured on a spectrophotometer (Shimadzu UV – 1800), at 773 nm for chocolate, and 740 nm for nibs.

Condensed tannins in nibs and chocolates were determined using the vanillin method (Tiitto-Julkunem, 1985) in a spectrophotometer (Quimis, model Q898UV2, Diadema, São Paulo, Brazil).

The antioxidant capacity of nibs and chocolates by radical scavenging assay was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), by the Molyneux method, and the absorbance was measured at 517 nm in a spectrophotometer (Shimadzu UV – 1800).

Contents of theobromine, caffeine, epicatechin, and catechin in the nibs and chocolates were quantified using the HPLC technique. The filtered sample extracts were separated on an RP-LC column (Zorbax SB-C18, 4.6 mm ID x 250 mm, 5 µm, and Zorbax SB-C 18 guard column, 4.6 mm ID x 12.5 mm, 5 µm), using an HP Agilent 1260 Infinity II system. The mobile phase consisted of 2.5% acetic acid – considered the solvent (A) –, and acetonitrile – considered the solvent (B) –, at 1mL per min flow rate. The elution gradient was as follows: 0–13 min, 3% solvent B; 13–18 min, 9% B; 18–25 min; 11% B; 25–45 min, 18% B; 45–50 min, 30% B; and in 50 min, 3% B.

The nibs and chocolate were analyzed by mid-infrared spectroscopy (MIR), using a Fourier transform infrared-attenuated reflectance (FTIR-ATR) equipment with a total attenuated reflectance cell (Cary 630 FTIR, Agilent Technologies Inc., Santa Clara, CA, USA). Before each collection, the background spectrum was read. Approximately 0.5 g of the crushed samples were placed individually on the diamond crystal of the ATR accessory, where incident rays on the spectral region was from 4000 cm^{-1} to 600 cm^{-1} wave number, in absorbance mode of 4 cm^{-1} resolution, and 64 scans of the sample.

Microbiological analyses were carried out for the presence of total coliforms, thermotolerant coliforms, and *Salmonella* sp., complying with the requirements of the Resolution RDC n.º 331 (Anvisa, 2019). The study was submitted to the Research Ethics Committee of the Universidade Estadual do Sudoeste da Bahia (UESB), BA, Brazil, and it was approved under the opinion number 5.481.027.

Sixty chocolate consumers were recruited and pre-selected, and the triangular difference test was applied to assess their ability to discriminate samples. Chocolates with 60% and 70% cocoa were used, and judges who got at least 75% of the tests correct were selected (Meilgaard et al., 2006). Subsequently, a descriptive terminology was developed using the network method, followed by familiarization sessions to standardize terms. The optimized descriptive profile (ODP) proposed by Silva et al. (2013) was applied to sensorially characterize the chocolates. In each session, one attribute was assessed separately, using an unstructured 9 cm scale. The analysis was carried out in individual cabins with controlled temperature and light, and the results were subjected to the principal component analysis (PCA) to generate the sensory map. Thirty-three sessions were carried out, with three replicates per judge, following a randomized block design, to allow all judges to evaluate all formulations.

We used specific descriptor terms, as follows: "brown color" that evaluates the intensity of chocolate's characteristic brown hue, while "shine" measures its light reflection. "Chocolate aroma" refers to the typical aroma of chocolate, and "toughness" gauges the force needed to bite through it. "Sweet taste" reflects the sweetness from sucrose, and "sour taste" indicates the sensation from acetic acid. "Astringency" describes the dry, rough feeling in the mouth, similar to that caused by green bananas or wine. "Bitter taste" highlights the lingering bitterness of cocoa nibs, and "chocolate flavor" refers to the typical taste of chocolate.

A completely randomized experimental design was employed with three replicates and three treatments (50%, 70%, and 90% fermentation index). Data were analyzed using the analysis of variation; and the Tukey's test was applied to determine significant differences between treatment means at 5% probability.

The bioactive compounds in nibs and chocolates were analyzed by PCA, while Pearson's correlation assessed data correlations. The MIR spectra were analyzed using PCA to correlate functional groups with the literature data. Additionally, the optimized descriptive profile results were mapped with PCA for sensory analysis.

Results and Discussion

Fat was the main component in both nibs and chocolates, followed by protein in nibs, and sugar in chocolates, with significant differences between treatments (Table 1). No significant differences ($p>0.05$) were found for ash, pH, and acidity.

The protein content decreased in the N90 nibs, likely due to proteolytic cleavage triggered by the fermentation process. This reduction is caused by protein degradation, stimulated by microbial fermentation by-products like ethanol, lactic acid, and acetic acid, which activate proteolytic enzymes. In the C50 chocolates, the protein content was 10.18%, which is significantly higher than in the other treatments.

Regarding fat content, there was a significant increase in nibs originating from more fermented cocoa beans. Fermentation causes changes such as increased temperature, formation of alcohol, acidification of cocoa beans, and changes in enzymatic activities, which can result in enzymatic breakdown, hydrolysis or oxidation, with changes in the fat content during the process (Servent et al., 2018).

Total acidity was similar among nibs, but total sugars varied notably between N50 and N90. Reducing sugars (fructose and glucose) showed significant differences for nibs among all treatments. Sucrose levels decreased during fermentation, due to the endogenous invertase activity, leading to higher glucose and fructose concentrations (Balcázar-Zumaeta et al., 2023). For chocolates, there was no significant difference for the parameters of humidity, ash, pH, total acidity, and fat, between the groups evaluated (Table 1).

The N50 nibs showed a greater antioxidant capacity than the N70 and N90 ones, indicating that the lower was the fermentation rate, the greater was the capacity to eliminate the DPPH radical. The antioxidant activity is related to the content of polyphenols, in which monomeric phenols, mainly epicatechin and catechin – the main compounds that contribute to this activity – are more preserved in less fermented cocoa beans and, consequently, in their derivatives (Chagas Junior et al., 2021).

The results indicate that the degree of fermentation influences the total phenolic levels, of which N50 showed a higher content, highlighting the importance of fermentation in the chemical composition and sensory quality of the final products (Nguyen et al., 2022). DPPH antioxidant activity did not vary significantly among chocolates, indicating a stable antioxidant capacity, despite differences in cocoa bean fermentation and phenolic levels. This underscores that cocoa bean quality, origin, genetic variety, and processing methods are crucial for determining the content of polyphenols in high-cocoa chocolate (Penido et al., 2021).

The score graph (Figure 1) shows the dispersion of nibs and chocolates. Data were subjected to PCA, showing distinct results, in which the two principal components explained 98.66% of the total variation (PC1 97.37% and PC2, 1.29%).

Table 1. Mean values and standard deviation of physicochemical properties and antioxidant activities of nibs (N50, N70, and N90) and chocolates (C50, C70, and C90) produced from *Theobroma cacao* beans at different fermentation indexes[(1)].

Parameter	Nibs			Chocolate		
	N50	N70	N90	C50	C70	C90
Humidity (%)	3.81±0.30a	4.01±0.18a	3.38±0.21a	2.0±0.15A	1.91±0.06A	1.89±0.12A
Ash (%)	2.27±0.07a	2.33±0.08a	2.30±0.03a	1.44±0.02A	1.47±0.04A	1.51±0.02A
Fat (%)	36.38±0.48a	38.69±0.31b	41.91±0.83c	47.61±0.01A	49.45±0.01A	49.81±0.01A
Protein (%)	16.20±0.08a	16.01±0.13a	15.71±0.20b	10.18±0.20A	9.95±0.16B	9.80±0.11B
pH	4.85±0.06a	4.93±0.11a	4.89±0.14a	5.27±0.06A	5.24±0.13A	5.18±0.04A
Acidity	7.60±05a	8.0±0.01a	8.5±0.01a	7.0±0.01A	6.0±0.01A	6.2±0.01A
Total sugars (%)	14.97±0.21a	13.25±0.34ab	11.82±1.12b	32.24±0.45A	30.25±0.72B	30.21±1.12B
Reducing sugars (%)	0.39±0.06a	0.71±0.11b	0.82±0.05c	4.75±0.15A	5.28±0.64A	6.0±0.34B
DPPH (%)	53.52±3.9a	48.95±2.25ab	45.02±2.62b	48.58±1.17A	47.77±0.96A	45.89±4.68A

[(1)]Means followed by equal lowercase letters, in the rows, do not differ for nibs, and means followed by equal uppercase letters, in the rows, do not differ for chocolates, by the Tukey's test, at 5% probability. N50, N70, and N90: nibs produced from cocoa beans at 50%, 70%, and 90% fermentation indexes, respectively.C50, C70, and C90: chocolates produced from N50, N70, and N90 nibs, respectively. Acidity expressed in meq NaOH 100g-1. DPPH: 2,2-diphenyl-1-picrylhydrazyl.

The PCA identified a clear separation between the nibs and chocolate, based on their chemical compositions. Furthermore, the samples differed within each group, suggesting variations for the concentrations of bioactive compounds between nibs and chocolates.

The grouping of nibs (N50, N70, and N90) along the positive PC1 axis showed significant variations for the concentration of bioactive compounds, in which N50 had the highest score, followed by N70 and N90 with the lowest scores.

These differences in composition are influenced by fermentation conditions, such as temperature, pH, oxidation and polymerization reactions (condensation), and polyphenol oxidase activity. These factors result in a considerable content decrease of these constituents, with increasing fermentation time, besides contributing to the reduction of astringency and bitterness of cocoa beans and, consequently, of nibs and chocolates (Balcázar-Zumaeta et al., 2023).

All bioactive compounds exhibited positive scores for PC1, indicating that nibs were characterized by a higher concentration of epicatechin, catechin, methylxanthines, condensed tannins and total phenolics in relation to chocolates.

Chocolate (C50, C70 and C90) clustered on the negative axis of PC1 showed lower scores than nibs did. This difference is probably attributed to losses during the processing steps, which include exposure to high temperatures (Ramos-Escudero et al., 2021). Among the chocolate, C50 showed the highest score, while C90 recorded the lowest one (Figure 1).

In chocolate production, nibs undergo grinding, refining, and conching. The conching stage is crucial, involving mixing and aerating the chocolate mass at approximately 60°C for 48 hours. This process reduces humidity, removes some volatile acids, and significantly affects the content of bioactive compounds, notably through the oxidation of flavan-3-ols (Toker et al., 2023).

The MIR spectra of nibs and chocolate showed similar patterns, but with notable differences in band intensities (Figure 2 A). The N50 and C50 had higher absorption intensities in bands related to the phenol group, specifically in the 3558-3330 cm^{-1} (O-H vibrations) and 1247-1044 cm^{-1} (C-H vibrations)

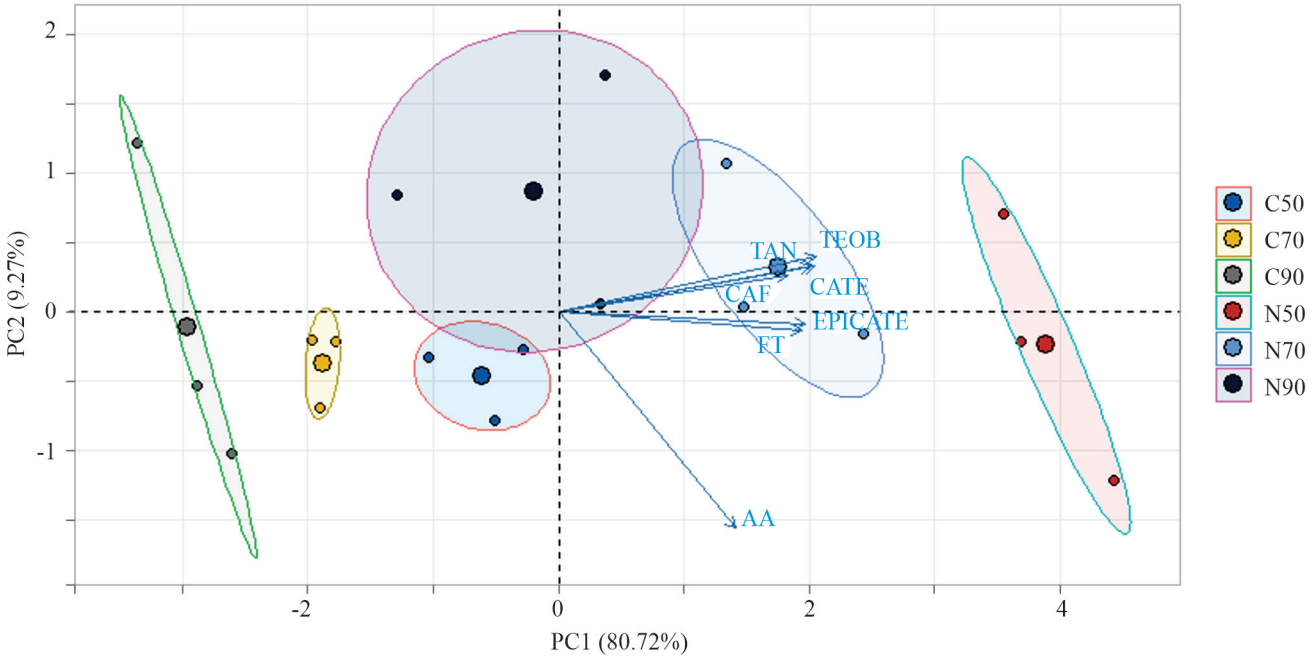

Figure 1. Scoring graph of bioactive compounds in nibs and chocolates produced from cocoa (*Theobroma cacao*) beans at different fermentation indexes, in relation to the main components PC1 and PC2. CAF, caffeine; THEOB, theobromine; CATE, catechin; EPICAT, epicatechin; TP, total phenolics; TAN, condensed tannins.

regions. They also exhibited stronger signals in bands associated with the aromatic ring, particularly in the 2915-2849 cm^{-1} (C-H stretching) and 988-680 cm^{-1} (C-H angular deformation) regions (Deus et al., 2021).

The expanded spectra (Figure 2 B, C) highlight the regions associated with the phenolic compound groups. The absorption bands identified at 1650 cm^{-1} and 1520 cm^{-1} (Figure 2 C), together with the band at 1171 cm^{-1} (Figure 2 B), were associated with the C-C bond in the aromatic ring and the related CH vibrations of the phenolic compounds and the phenol group, respectively (Onelli et al., 2024). It is possible to observe that nibs and chocolates produced with cocoa beans at a lower fermentation index showed higher intensities, possibly due to higher concentrations of bioactive compounds.

Based on the PCA results (Figure 3), the first two components explained 98.62% of the total variance, with PC1 accounting for 96.58%, and PC2, for 2.04%. The PC1 was particularly effective in distinguishing between nibs and chocolates. Nibs clustered strongly on the negative side of PC1, and showed similar spectra characterized by peaks, as follows: at 2915 and 2849 cm^{-1} (C-H stretching of the aromatic ring); at 1733 cm^{-1} (CO); 1650 cm^{-1} (C=O); at 1467 and 1247 cm^{-1} (C-H), related to phenolic compounds;

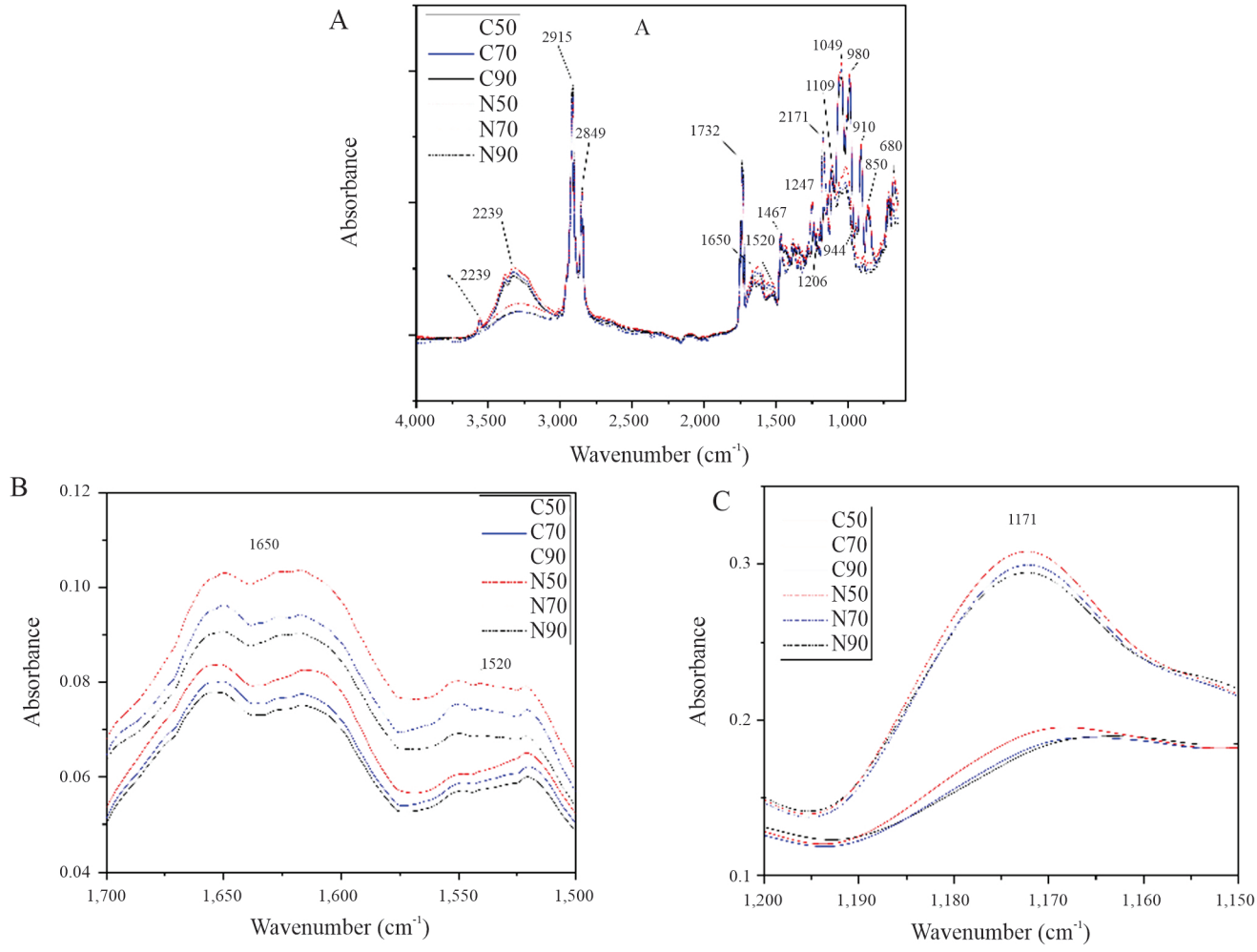

Figure 2. Spectra of nibs (N50, N70, and N90) and chocolates (C50, C70, and C90) from *Theobroma cacao* beans, obtained by mid-infrared spectra: A, complete spectrum; B, expansion of the 1700−1500 cm^{-1} region; C, expansion of the 1200−1140 cm^{-1} region. N50, N70, N90: nibs produced from cocoa beans respectively at 50%, 70%, and 90% fermentation indexes. C50, C70, and C90: chocolates produced respectively from N50, N70, N90 nibs.

and 1171 and 1109 cm^{-1} (C-O), which are associated with phenols (Santos et al., 2021; Onelli et al., 2024).

This separation can be attributed to the concentration variation of bioactive compounds between cocoa nibs and chocolates. In addition, chocolates differed from the cocoa nibs, clustering on the positive PC1 axis in the following spectra: 3558, 3384, 3330, 1044, 988, 860 cm^{-1} (O-H/C-O/C=C/C-O-C vibrations), and between 944 and 901 cm^{-1} (OH and CO). These spectra with positive scores for PC1 are mainly associated with carbohydrate index and lipids, indicating differences in the chemical composition between nibs and chocolates, possibly due to the addition of other ingredients, such as cocoa butter and sugar.

Nibs and chocolates derived from cocoa beans with lower fermentation index showed higher scores on the PC2 axis than those produced from beans with higher fermentation index. This differentiation was evident in relation to PC2, where the 1109 cm^{-1} wavelength (Figure 3) associated with the C-O functional group of phenols recorded the highest score, being the main factor responsible for this distinction (Santos et al., 2021).

These findings suggest that combining MIR with chemometric analysis effectively distinguished nibs and chocolates from cocoa beans with varying fermentation index, particularly regarding the content of bioactive compounds. This approach enables a quick, accurate assessment of compound losses, during processing, and highlights potential technological alternatives for preserving these valuable constituents.

The microbiological analyses of all chocolate formulations were within the current legal standards, which according to Anvisa (2019) are 10NMP g^{-1} for coliforms at 45ºC, absence of *Salmonella* sp. 25 g^{-1}, and for *Staphylococcus aureus* at 1 UFC g^{-1}.

There were no significant differences for the parameter descriptors shine, snap, hardness, toughness, and sweet taste (Table 2). As the chocolates were made using the same formulation and the same technological production process, treatments seemed to not affect the judges' perception of these attributes.

The C90 chocolates exhibited significantly higher intensities of chocolate flavor and chocolate aroma than the other treatments (Table 2). This difference is linked

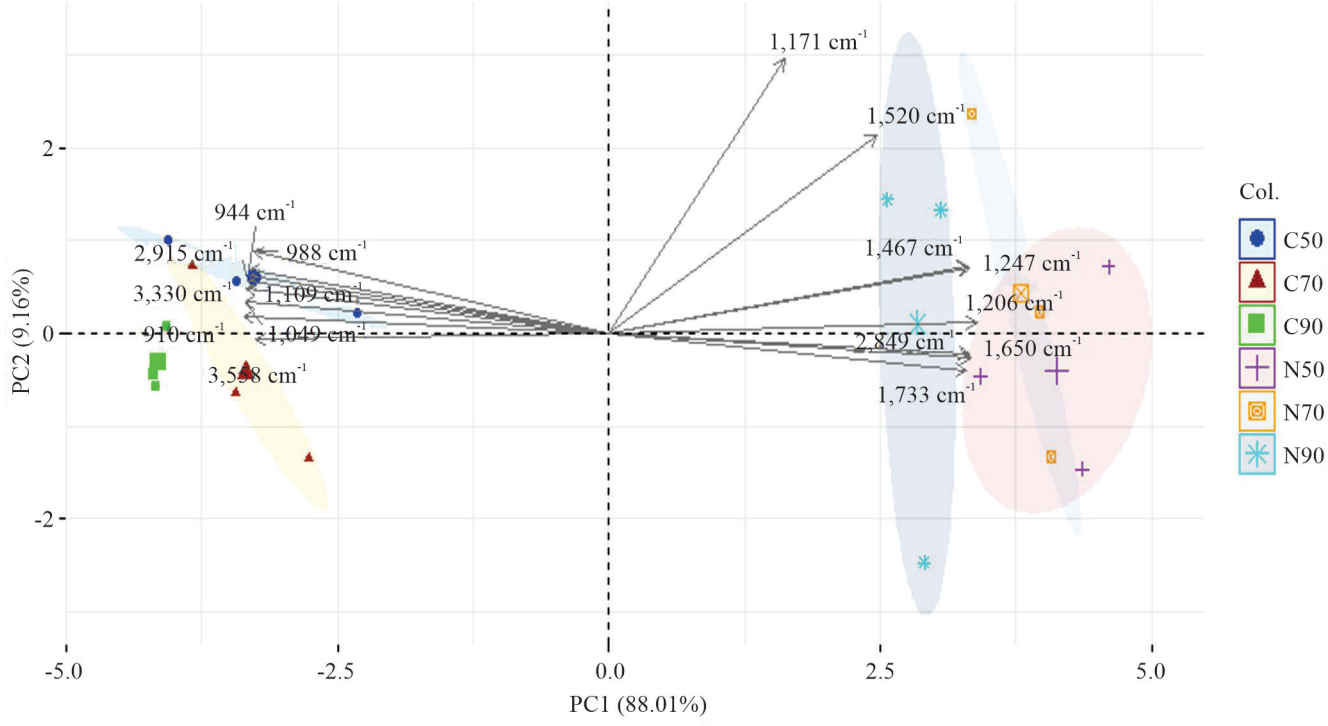

Figure 3. Score graph of nibs and chocolates produced from *Theobroma cacao* beans, at different fermentation indexes, in relation to the main components PC1 and PC2. N50, N70, N90: nibs produced from cocoa beans respectively at 50%, 70%, and 90% fermentation indexes. C50, C70, C90: chocolates produced respectively from N50, N70, N90 nibs.

Table 2. Values for the scores obtained by the optimized descriptive profile for chocolates (C50, C70, and C90) produced with *Theobroma cacao* beans at different fermentation indexes[(1)].

Characteristics	Chocolates (means±standard deviation)		
	C50	C70	C90
Brown color	6.58±0.36a	7.21±0.49b	7.51±0.41b
Shine	1.81±0.60a	1.95±0.60a	2.32±1.64a
Snap	7.19±0.50a	7.63±0.54a	7.20±0.23a
Chocolate croma	5.68±0.35a	6.61±0.45b	7.21±0.24c
Toughness	7.19±0.50a	7.63±0.54a	7.21±0.23a
Sandy	0.08±0.04a	0.06±0.09a	0.11±0.04a
Sweet taste	1.54±0.26a	1.50±0.36a	1.52±0.35a
Acidic taste	7.00±0.49a	6.65±0.49ab	6.12±0.65b
Bitter taste	6.86±0.27a	6.38±0.35b	5.91±0.49c
Astringent	7.34±0.43a	6.82±0.46b	6.01±0.38c
Chocolate flavor	6.81±0.44a	7.62±0.27b	8.01±0.17c

[(1)]Means followed by equal letters in the rows do not differ, by the Tukey's test, at 5% probability. C50, C70, and C90: chocolates produced respectively from N50, N70, and N90 nibs.

to the analysis of constituents (Figure 1 and Figure 2 A), which evidences that a higher fermentation index reduces compounds such as polyphenols and methylxanthines, directly influencing these attributes. The fermentation process is crucial for the development of the chocolate characteristics flavor and aroma, as it metabolizes proteins and carbohydrates into simpler compounds known as flavor precursors (Escobar et al., 2021).

The intensity of acidic, bitter, and astringent tastes increased in chocolates made from cocoa beans with lower fermentation index. Chocolates with higher levels of phenolic compounds and methylxanthines, such as theobromine and caffeine, exhibited greater astringency and bitterness. The C50 chocolate − with higher concentrations of these compounds − resulted in a stronger perception of these attributes.

In the PCA analysis, the graphical representation (Figure 4) showed that the first principal component

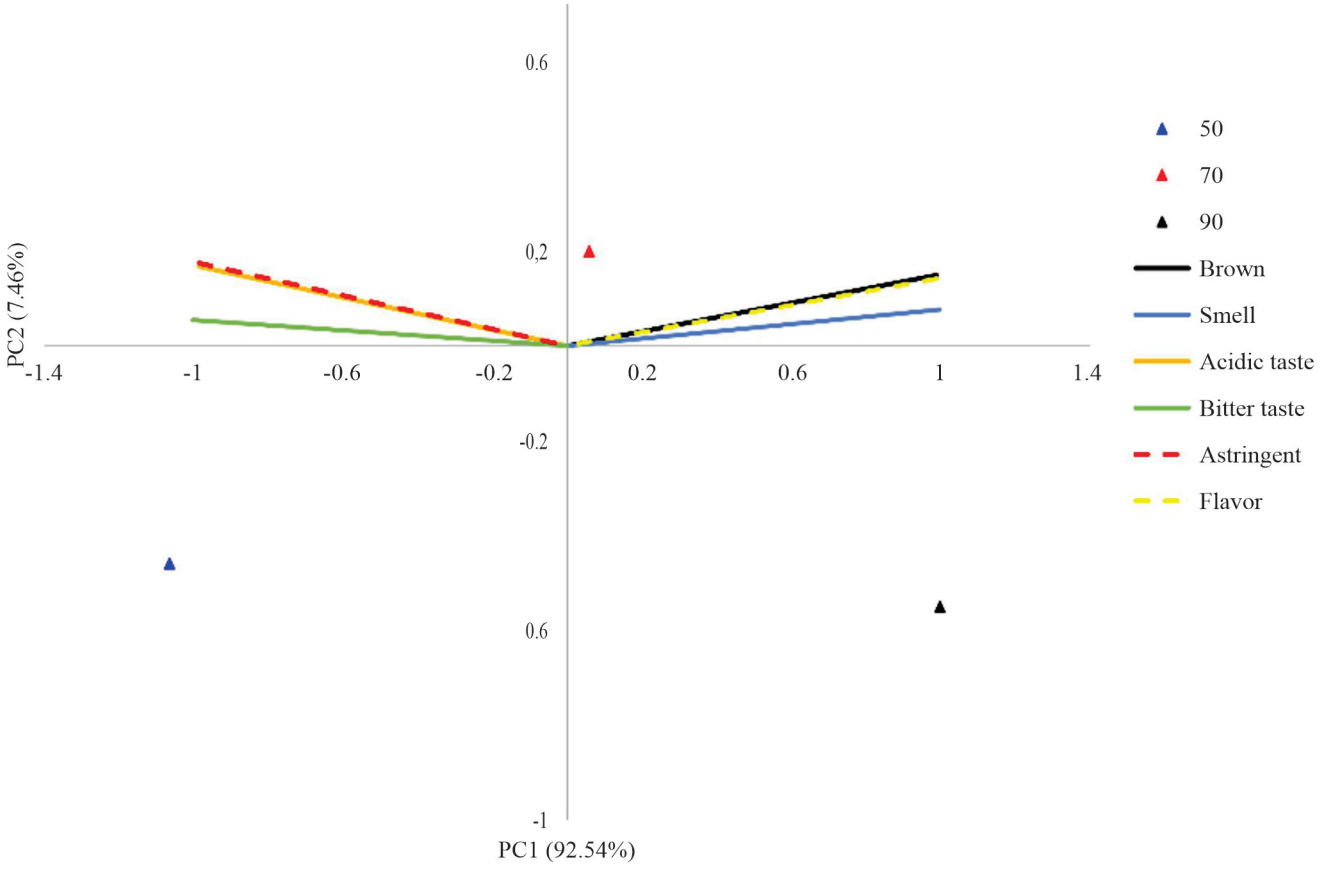

Figure 4. Sensory map of chocolates produced from *Theobroma cacao* beans at different fermentation indexes. C50, C70, C90: chocolates produced respectively from N50, N70, and N90 nibs. Chocolate characteristics: "Brown", brown color; "Smell", chocolate smell; "Flavor", chocolate flavor.

(PC1) accounted for approximately 92.54% of the total variation, while the second component (PC2) explained 7.46%. Only the first dimension was used for interpretation. Attributes such as chocolate smell, flavor, and brown color were positively correlated with PC1 and were more intense in the C90 chocolates, which showed lower levels of bioactive compounds.

Color, aroma, and flavor characteristics of chocolate are developed through postharvest activities such as fermentation, drying, and roasting of cocoa beans that reduce anthocyanins and polyphenols, while promoting the formation of Maillard reaction products (Żyżelewicz et al., 2018). Acidic, bitter, and astringent tastes showed a negative correlation with the first principal component and were more pronounced in the C50 chocolate, in which higher concentrations of polyphenols and methylxanthines compounds, directly linked to these sensory attributes, were observed.

Conclusions

1. Fermentation affects the fat, protein, sugar, and antioxidant activity levels in nibs derived from *parazinho* cocoa (*Theobroma cacao*) beans, and in chocolates produced from these nibs.

2. The content of bioactive compounds − including epicatechin, catechin, methylxanthines, and condensed tannins − is higher in nibs and chocolates produced from cocoa beans with lower fermentation index.

3. Chocolate produced with cocoa beans at about 50% fermentation is an alternative, when it comes to producing cocoa derivatives with higher levels of bioactive compounds, without major influences on the characteristics of astringency and bitterness.

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Declaration of use of AI technologies

No generative artificial intelligence (AI) was used in this study.

Conflict of interest statement

The authors declare no conflicts of interest.

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