Multivariate analysis of chemical parameters of cocoa: a contribution to origin designation

Análise multivariada de parâmetros químicos do cacau: uma contribuição para a denominação de origem

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ABSTRACT
Brazil has been trying to become a key player in the specialized market through the production of fine chocolate. However, there is still no rules about the chemical quality of cocoa beans that allows it to be grouped by micro-regions. In this context, the objective of this work was to determine some chemical parameters of cocoa samples, aiming to contribute to the creation of the designation of origin for the cocoa produced in the south of Bahia. For this propose, proteins, lipids, total minerals, and fatty acid profile analysis were performed. The results obtained were correlated using multivariate statistical tests. The chemical composition of the cocoa beans, allowed to differentiate samples of cocoa, revealing the formation of three groups of samples. The two main components (lipids and proteins) were analysed together, characterizing the producing region. Regarding the analysis of the fatty acid profile, they showed that the cocoa harvested in the main season presents a higher influence of saturated fatty acids, while in the early season the higher influence is of unsaturated fatty acids. Multivariate techniques were able to group the different types of cocoa according to their chemical profile, helping to create an origin denomination for those produced in Southern Bahia.

Keywords: fine Cocoa, fatty acids, chemical composition, designation of origin.

RESUMO
O Brasil vem tentando se destacar no mercado especializado de chocolates através da produção de chocolates finos. No entanto, ainda não há regulamentação sobre a qualidade química de amêndoas de cacau que permita seu agrupamento por microrregiões. Nesse contexto, o objetivo deste trabalho foi determinar alguns parâmetros químicos de amostras de cacau, visando contribuir para a criação da denominação de origem do cacau produzido no sul da Bahia. Para tanto, foram realizadas análises de proteínas, lipídios, minerais totais e perfil de ácidos graxos. Os resultados obtidos foram correlacionados por meio de testes estatísticos multivariados. A composição química dos grãos do cacau, permitiu diferenciar as amostras, revelando a formação de três grupos. Os dois componentes principais (lipídios e proteínas) foram analisados em conjunto, permitindo caracterizar a região produtora. Quanto à análise do perfil de ácidos graxos, foi observado que o cacau colhido na safra principal apresenta maior influência dos ácidos graxos saturados, enquanto na safra temporã a maior influência são dos ácidos graxos insaturados. Técnicas multivariadas conseguiram agrupar os diferentes tipos de cacau de acordo com seu perfil químico, ajudando a criar uma denominação de origem para as amêndoas produzidas no Sul da Bahia.

Palavras-chave: Cacau fino, ácidos graxos, composição química, denominação de origem.

1 INTRODUCTION

In Brazil, cocoa production is carried out in eight regions and two of them (Bahia and Pará) are responsible for practically all the national production of 176 thousend tons in 2020 (ICCO, 2020). Currently, the cocoa producers in Bahia comprises approximately

110 municipalities, ten of which are responsible for the largest production of the fruit in the State (IBGE, 2017).

Faced by the worst crisis in cocoa production, beginning in 1989, with the spread of witches' broom disease, regional producers had to seek for new alternatives. In this context, it was necessary to diversify the crops with the production of monovarietal cocoa, more resistant to disease, and the exploration of new market niches such as the production of fine cocoa. However, the production of this fine cocoa is still punctual and is specializing, seeking higher gains than those of the common grain market (ICCO, 2020).

In Brazil, the official standard for the classification of cocoa beans refers only to their physical quality parameters, following the maximum defect tolerance limits, which are established by the Normative Instruction No. 38 of June 23, 2008 of the Ministry of Agriculture (Brasil, 2008). It is noteworthy to mention that the high quality cocoa still does not have a standard that characterizes and recognizes this type of product, according to the physical, chemical, and sensory parameters.

In this context, the Association of Cocoa Producers in the South of Bahia was able to certify the Indication of Origin (IO) of cocoa beans, which establishes a new way to control and track the cocoa beans grown in the State, following the origin and quality of the final product (INPI, 2018).

Furthermore, it is emphasized that the “IO Sul da Bahia” is the first quality standard in Brazil that aims to establish the parameters and conditions for the production, obtaining, and use of the Origin and Quality Seal of Southern Bahia for cocoa beans produced in production units from 83 municipalities in Bahia.

Regarding the official international standards for cocoa, the ISO 2451:2014 specifies the requirements for classification, sampling, testing methods, packaging, and identifying cocoa beans, with recommendations related to storage and disinfestation. In addition, it addresses parameters of size, color, moisture content, preparation, and classification of beans (ISO, 2017).

In this perspective, the increasing advances in instrumental techniques of chemical analysis provide a greater data volume, requiring the use of more complex mathematical data processing techniques.

In this perspective, the objective of this work was to establish some chemical parameters of cocoa samples classified as Type 1, aiming to propose analytical methodologies and data correlations that may contribute to the creation of a cocoa

chemical classification for beans from southern Bahia. This will add value to the product and conquer new national and international markets by creating the appellation of origin.

2 MATERIAL AND METHODS

Cocoa samples

Twenty cocoa samples (fermented and dried beans) grown by producers belonging to 11 municipalities from the Southern region of Bahia (Ibicaraí, Porto Seguro, Jaguaquara, Ilhéus, Itacaré, Belmonte, Aurelino Leal, Ubaitaba, Uruçuca, Ibirapitanga, and Apuarema) and provided by the Cocoa Innovation Center – CIC were used in this study. Among the samples, 11 were collected in the early harvest (May to September of 2017 and designated with the letter "T") and 9 were collected in the main harvest (October of 2016 to April of 2017 and designated with the letter "S"). Only beans classified as Type 1 superior were considered in this study (Brasil, 2008).

Sample preparation

The fermented and dried beans were placed in thin layers on hollow trays and submitted to the roasting process using an oven with air circulation (NOVA ÉTICA), at 120°C ± 0.1°C for twenty minutes (Rocha et al., 2017). Then, the beans were fractionated in order to produce the nibs that were ground using an analytical mill. The mass obtained was stored in vacuum packaging, under refrigeration, for further analysis.

Total minerals

The mineral content was determined by the thermogravimetric method with the destruction of the organic matter present in 5g of sample, initially submitted to total carbonization in a heating blanket, followed by incineration in a LAVOISIER oven, at 550ºC, for 12h according to the methodology 900.02 from the AOAC (1996).

Proteins

The protein content was determined by the microKjeldahl method, from the quantification of the total nitrogen contained in 0.5g of the sample. The conversion factor of total nitrogen to protein was 6.25, according to the methodology from the AOAC (1996).

Total lipids

Total lipids were extracted in a Soxhlet apparatus, using hexane as a solvent, according to the methodology described by the AOAC (1996).

Fatty acid profile

Individual fatty acids were determined by gas chromatography according to the direct methodology proposed by Palmquist & Jenkins (2003). About 0.5 g of sample was subjected to the esterification reaction with 10% methanolic HCl. The fatty acid methyl esters (EmAG) were extracted with hexane and transferred to an amber flask and stored in a freezer at -60 ° C.

The separation of fatty acid methyl esters was performed in a gas chromatograph (Perkin Elmer Clarus 680), equipped with flame ionization detector and capillary column of fused silica DB-FFAP (30m × 0.32mm × 0.25µm). The analysis parameters were: injector temperature at 250°C; detector temperature at 280°C; column temperature programmed at 150°C for 16 min., 2°C per minute up to 180°C; remaining at that temperature for 25 minutes and increasing from 5°C to 210°C; and remaining at that temperature for 25 minutes. Helium was used as a carrier gas at 1.0mL/min. The injections were performed in duplicate and the injection volume was 1µL.

The identification of the fatty acid methyl esters was carried out by comparing the peak retention times of the samples with the retention time of the fatty acids mixed standard (189-19, Sigma, USA). Peak areas were determined using the Clarus Chromatography workstation software to normalize the percentage of the total fatty acid areas.

The quantification of fatty acids was performed by adding the internal standard (C19: 0 - nonadecanoic acid, Sigma Aldrich, USA). The calculations were performed according to Equation 1 and expressed in mg.g-1 of sample.

$$\text{Concentration } (mg.g^{-1}) = \frac{AT\text{-}APi}{APi} \text{ X } \frac{MPi}{MA} \qquad \text{(Equation 1)}$$

Where:

AT= peak area of the fatty acid methyl ester in the sample chromatogram;

APi= internal standard area in the sample chromatogram;

MPi= mass of the internal standard added to the sample in mg;

MA= sample mass in grams.

Data analysis

The chemical composition and fatty acid profile data were analyzed using the Statistica Version 7 software and subjected to the Analysis of Variance test (ANOVA) with the Tukey test ($p < 0.05$) to compare the means. The PCA and HCA analyses were applied to evaluate the similarity of the samples according to the chemical composition

and concentration of fatty acids. These multivariate analyses was performed using the XLSTAT Version 2019.1 statistical software.

3 RESULTS AND DISCUSSION

Chemical characterization of samples

The results of chemical characterization of the evaluated cocoa samples are shown in Table 1.

Among the samples, the mineral values ranged from 1.96% to 2.98%, showing significant differences ($p < 0.05$) for most of the samples under study. However, there was a greater homogeneity among the early harvest cocoa samples, because of the 11 samples studied, seven (T1, T3, T7, T8, T9, T10, and T11) were not statistically different. For the samples of the main harvest, among the nine studied, only three (S4, S5, and S8) were not statistically different. Another important point to be considered is that, regarding the mineral content, there was no pattern to differentiate the beans harvested in the main and early season.

Table 1. Means and standard deviations of the chemical composition of cocoa samples (lipids are shown in dry basis)

Samples	Lipids (%)	Proteins (%)	Minerals (%)
T1	52.35 ^{cde*}	10.67 ^{i}	2.39 ^{hi}
T2	57.00 ^{ab}	15.45 ^{ab}	2.47 ^{efg}
T3	47.68 ^{fgh}	12.29 ^{fg}	2.43 ^{ghi}
T4	52.88 ^{cd}	13.05 ^{def}	2.45 ^{fgh}
T5	43.79 ^{i}	12.38 ^{efg}	2.82 ^{b}
T6	52.27 ^{cde}	12.35 ^{fg}	2.54 ^{de}
T7	50.70 ^{def}	13.09 ^{def}	2.39 ^{hi}
T8	48.73^{efgh}	13.55 ^{d}	2.44 ^{ghi}
T9	58.50 ^{a}	13.22 ^{def}	2.41 ^{ghi}
T10	54.94 ^{abc}	11.89 ^{gh}	2.36 ^{i}
T11	55.58 ^{abc}	10.85 ^{hi}	2.38 ^{i}
S1	46.58 ^{ghi}	16.26 ^{a}	2.98 ^{a}
S2	45.92 ^{hi}	11.86 ^{gh}	1.96 ^{j}
S3	57.93 ^{a}	12.24 ^{fg}	2.89 ^{b}
S4	46.73 ^{ghi}	12.36 ^{efg}	2.42 ^{ghi}
S5	49.77 ^{defg}	13.48 ^{de}	2.38 ^{hi}
S6	56.78 ^{ab}	13.20 ^{def}	2.72 ^{c}
S7	49.70 ^{defg}	13.70 ^{cd}	2.51 ^{def}
S8	53.49 ^{bcd}	12.18 ^{fg}	2.40 ^{hi}
S9	50.74 ^{def}	14.70 ^{bc}	2.58 ^{d}

*- Different letters in the same column differ significantly by the Tukey test ($p < 0,05$)
T: early harvest; S: main harvest

Significant differences ($p < 0.05$) were also observed in the protein content of all the samples, which showed a variation of 10.67% for sample T1 and 16.26% for sample

S1. As evidence of this great variation, only one sample was not statistically different from the others, the T11 sample, which was similar to sample T1 and sample T2, which was similar to sample S1. The others presented values that were statistically different from these.

In addition, no protein pattern that could differentiate the samples collected in the main and early seasons was identified, just like with minerals.

Variation in protein content can occur for a number of factors. During fermentation, for example, biochemical reactions occur that lead to protein reduction due to the action of enzymes or complexation with phenolic compounds (Silveira et al., 2017; D'Souza et al., 2018). However, there are studies that attribute variations in protein content to the total nitrogen content of the soil where the cocoa was grown, as well as to the genetic variety of cocoa (Adewole et al., 2011).

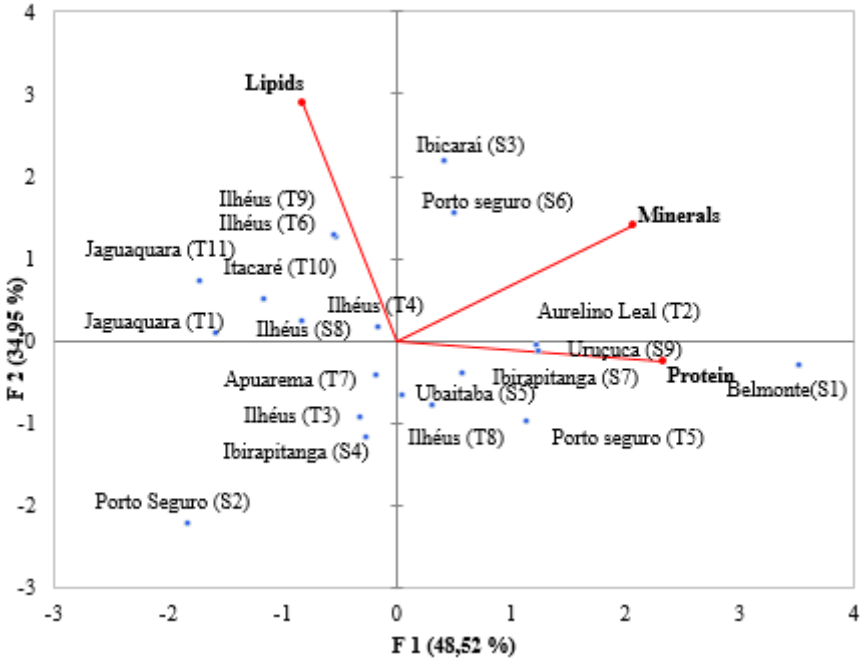
Regarding the lipid content, Table 1 shows variations from 43.79%, for sample T5, to 58.50%, for sample T9. Thus, as observed for minerals and proteins, there was great variability in the results, since only 3 more samples were not statistically different from the T5 sample (S1, S2, and S4) and 4 samples were not different from the T9 (T10, T11, S3, and S6). Once again, there was no difference in lipid content regarding the harvest season (main or early). The variation in the lipid content of the samples is related to factors such as the fruit ripening process, the genetic origin, and the climatic conditions for cultivation (Amorim et al., 2019).

Despite of the few studies on the lipid content in roasted cocoa beans and the influence of the roasting process (Kongor et al., 2016), there are reports in the literature that correlate the reduction in fat content with the roasting method used. According to Krysiak (2011), the roasting process by convection of whole beans causes a migration of cocoa butter from the seed to the shell, which is eliminated in the production process of the nibs. This fact is disadvantageous, mainly for economic reasons, since shells are not used in the production of cocoa derivatives.

Considering that there was no difference in the chemical composition with respect to the harvesting period, the PCA was performed (Figure 1) correlating the composition parameters with the production areas of the samples. In this case, the first main component (PC1) described 48.52% and the second described 34.95% of the variation contained in each main component, expressed by the eigenvalues of the standardized matrix.

Therefore, the two main components together explained 83.47% of the total variance observed between the samples. Those with similar characteristics occupy close regions in the graph, while those with different characteristics are more distant from each other.

Figure 1. Principal Components Analysis of the twenty cocoa samples according to their chemical composition and production areas



The vector closest to the sample describes its characteristics with greater intensity, making it possible to observe the formation of three groups due to the similarity between the analyzed data.

Group I was formed by samples that were grouped according to the higher lipid content: T1, T4, T6, T9, T10, T11, and S8 and Group II consisted of T2, T5, T8, S5, S7, S9, and S1 samples, which are grouped according to the protein content. A third small group assembled the samples according to the mineral content, being formed by samples S3 and S6. There are also some samples that were little correlated with the analyzed parameters which were not associated with any of the vectors, such as samples T7, T3, S4, and S2.

Of the three variables under study, the protein and lipid content were the ones that were able to describe the percentage of 83.47% of the variation of cocoa samples.

Thus, the samples grouped according to the lipid content were those in which their average levels were between 52.35 g.100g– 1 and 58.50 g.100g– 1. In addition, it is

observed that four of these samples were produced in the same municipality (Ilhéus), and two in another one (Jaguaquara), a fact that implies some correlation due to specific characteristics of the region where the plants were grown, the genetic origin or type, or the post harvest processing of cocoa ("terroir").

It is well known that for a good development of the cocoa crop it is necessary to apply techniques that target the excellent development of the plants. Some examples are the clearing, conducted from December to March, in which the plants that compete for water are removed, and the organic fertilization, which is carried out at the beginning of the early harvest time and at the end of the main harvest, in order to improve physico-chemistry quality of the soil (Santos et al, 2015).

Employing this management, the plants from which the fruits were collected in the early harvest, suffered less water stress, since the competition for water with the other plants is reduced. According to Kirnak et al. (2019), and Alizadeh Yeloojeh et al. (2020), when plants are subjected to water stress there is a reduction in the oil content of oilseeds.

Regarding the nutrition of the plant and soil fertility, studies have shown that the use of green manure associated with mineral fertilization affects positively the oil content in plants (Adewole et al., 2011; Khademian et al., 2019). Therefore, this could be one of the factors responsible for a higher content of lipids in the fruits collected in the early harvest. Nevertheless, further studies are needed about the genetic origin, planting, and processing conditions for a better understanding of the similarity between the samples.

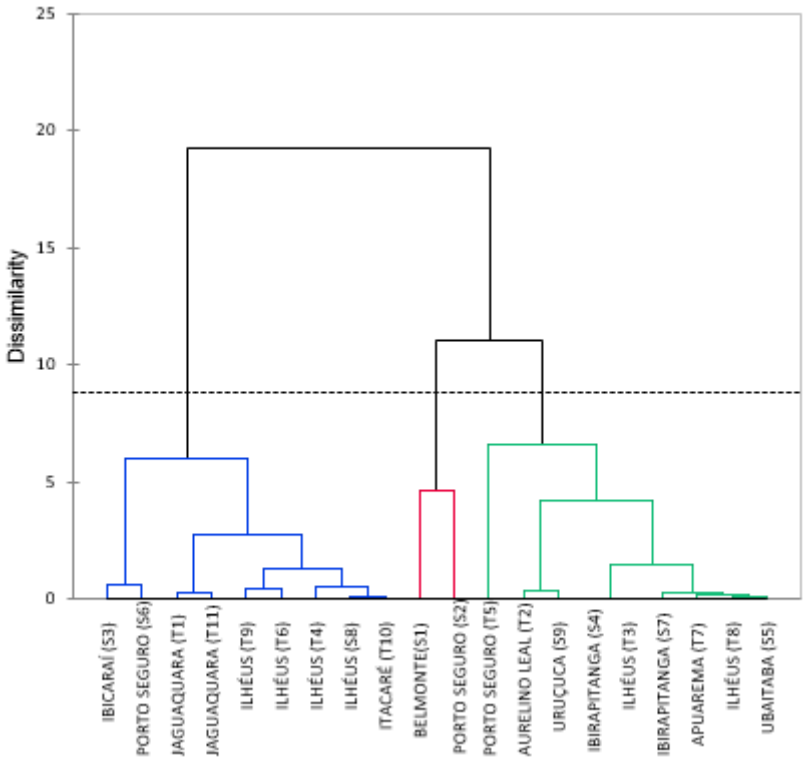
The trends observed in the PCA were confirmed by the dendrogram obtained in the HCA (Figure 2), in which it is possible to observe the formation of three main groups. However, the groups that showed the best data correlation were groups I and II. In the former, the samples that stood out were those from the municipalities of Ilhéus and Jaguaquara (T1, T4, T6, T9, T11, and S8) with the HCA including also the T10, S3, and S6 samples in this group, which clustered according to their lipid content. The lipid content of these samples ranged from 52.35% to 58.50%.

Thus, it is possible to infer that in the municipality of Ilhéus, cocoa can be produced with slightly higher content of lipids, probably due to the edaphoclimatic and management conditions of the region.

Further, Figure 2 presents a second group, formed by samples T2, T3, T5, T7, T8, S4, S5, S7, and S9, which was formed based on their protein content, without presenting any local specificicity, since several municipalities were grouped according to this

parameter. In addition, a group was formed by samples S1 and S2, which were not associated with any parameter since they are dispersed in PCA. Thus, these results

Figure 2. Dendogram (HCA) representing the dissimilarity between different cocoa samples depending on the chemical composition and production location



corroborate those obtained in the PCA analysis, in which the samples were grouped according to the levels of lipids and proteins. However, the protein content was not homogeneous for each production site, as occurred with lipids.

Identification and quantification of fatty acids

The results regarding the fatty acids profile of the samples are shown in Table 2. The samples presented the same profile, although there was a significant difference ($p < 0.05$) for all samples related to the fatty acids concentrations.

The major fatty acids were palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (18:1n9). It should be noted that these results are similar to those found by Servent et al. (2018), who analyzed the fatty acid profile of cocoa from different countries, showing higher concentrations of the same acids, Although the samples analyzed in this study showed slightly higher levels of unsaturated fatty acids and slightly lower of saturated.

Oleic acid was the major fatty acid, and the sample with the highest content of this acid was S4 (232.22mg.g-1), with no other sample being statistically similar to that. This can be explained, among other factors, by the specific climatic conditions of the cultivating region, since the chemical and physical characteristics of cocoa butter are influenced by several factors as the country of origin, climatic conditions, and season (Kongor et al., 2016; Firmanto & Muhammadiyah, 2018).

On the other hand, 85% of the samples showed values ranging from 172.46 mg.g-1 to 195.08mg.g-1 and only one sample (S5) presented a lower concentration, with 160.3446mg.g-1. According to Santos (2019), Brazilian cocoa butter is considered softer compared to other cocoa-producing countries, such as Malaysia and Ivory Coast, due to a higher oleic acid content. In this way, samples from southern Bahia with high oleic acid content may confer greater softness to the cocoa butter.It is emphasized that the rate of unsaturation level and, consequently, the hardness of cocoa butter is an aspect of particular interest for the genetic improvement of these fruits (Talbot, 2017). Furthermore, low cultivation temperatures increase the proportion of unsaturated fatty acids, as oleic and linoleic acids, making the butter from fruits harvested during the early period (from August to October) softer (Castro-Fettermann et al., 2017; Barisic et al., 2019).

In this context, the high content of unsaturated fatty acids can make cocoa butter softer. However, this fact can result in problems during the production process, since fats with a higher rate of unsaturation are more susceptible to oxidation (Santos, 2019).

On the other hand, regarding the main saturated fatty acids, palmitic acid (C16: 0) presented a high variability in its levels, but of the nine studied samples collected during the main harvest period, seven presented the highest levels of this acid (about 78%), while in the early harvest of the eleven samples studied, six presented the lowest values (55%). Thus, the early harvest actually produces beans with lower levels of palmitic acid. Regarding stearic acid (C18:0), a high variability was also observed, although its behavior does not follow that of the palmitic acid. In this case, both the highest and lowest concentrations were found during the main harvest period while in the palmitic acid the highest and lowest values are spread over the two harvest periods.

In this perspective, the perfomed PCA analysis was able to discriminate the samples according to their fatty acid profile (Figure 3). The first main component (PC1) described 37.83% of the variance and the second main component (PC2) described

30.01%. Therefore, the two main components explained together 67.84% of the total variance observed between the samples.

Table 2. Fatty acid profile in cocoa samples

Samples	Fatty acids (mg.g^{-1})									
	C14:0	C16:0	C16:1n7	C17:0	C18:0	C18:1n9c	C18:1n9t	C18:2n6c	C18:3n3	C20:0
T1	0.34 cdef*	114.32 efg	0.88 g	0.78 h	152.22 cde	191.04 bc	1.66 a	21.00 cde	1.10 cd	4.40 cdef
T2	0.38 abcd	123.86 bcdef	0.96 efg	0.86 gh	146.08 def	172.46 bcd	1.64 a	20.10 cdef	1.10 cd	4.46 abcde
T3	0.36 bcdef	118.18 cdefg	1.10 bcde	1.02 bcde	160.02 abcd	192.88 b	1.74 a	22.14 bcd	1.12 bcd	4.66 abcde
T4	0.34 cdef	114.74 defg	1.00 defg	0.86 gh	151.50 cdef	185.12 bc	1.66 a	21.06 cde	1.04 cd	4.40 def
T5	0.38 abcd	123.72 bcdef	1.20 abc	1.14 ab	155.96 bcd	163.26 cd	1.52 a	15.92 gh	0.96 d	4.72 abcde
T6	0.36 bcde	122.76 bcdef	1.20 abc	0.98 cdefg	160.46 abcd	195.08 b	0.92 a	22.38 bcd	1.12 bc	4.58 bcdef
T7	0.30 ef	124.46 bcde	1.10 bcde	0.90 efgh	149.54 cdef	187.80 b	1.80 a	23.00 bc	0.96 bc	2.27 bcdef
T8	0.34 cdef	111.74 efg	1.00 defg	0.92 defgh	150.86 cdef	184.08 bcd	1.80 a	21.82 cd	1.12 cd	4.58 def
T9	0.34 cdef	111.74 efg	1.06 bcdef	0.96 defg	151.24 cdef	182.50 bcd	1.64 a	20.98 cde	1.12 cd	4.40 def
T10	0.34 cdef	139.88 a	1.32 a	0.84 gh	174.20 ab	177.34 bcd	1.92 a	19.02 defg	1.04 cd	5.06 abc
T11	0.30 f	113.38 fg	0.96 efg	0.82 h	150.36 cdef	183.76 bcd	1.68 a	22.34 bcd	1.06 bcd	4.26 ef

Table 2. continuation

Samples	Fatty acids (mg.$^{g-1}$)									
	C14:0	C16:0	C16:1n7	C17:0	C18:0	C18:1n9c	C18:1n9t	C18:2n6c	C18:3n3	C20:0
S1	0.40 ^{abcd}	134.66 ^{ab}	0.84 ^{cdefg}	0.86 ^{gh}	132.57 ^{f}	181.36 ^{bcd}	2.12 ^{a}	25.60 ^{ab}	1.48 ^{a}	4.76 ^{abcde}
S2	0.40 ^{abcd}	125.04 ^{abcde}	1.16 ^{abcd}	1.06 ^{abcd}	161.35 ^{abcd}	173.60 ^{bcd}	1.70 ^{a}	17.30 ^{fgh}	1.06 ^{cd}	4.96 ^{abcd}
S3	0.44 ^{a}	132.38 ^{abc}	1.10 ^{bcde}	1.02 ^{bcdef}	156.27 ^{bcd}	181.54 ^{bcd}	1.50 ^{a}	19.66 ^{cdef}	1.08 ^{bcd}	4.74 ^{abcde}
S4	0.30 ^{ef}	129.48 ^{abcd}	1.32 ^{a}	1.02 ^{abcde}	177.12 ^{a}	232.22 ^{a}	1.56 ^{a}	28.70 ^{a}	1.30 ^{b}	5.12 ^{ab}
S5	0.34 ^{def}	105.14 ^{g}	0.92 ^{fg}	0.88 ^{fgh}	136.04 ^{ef}	160.34 ^{d}	1.58 ^{a}	18.16 ^{efgh}	1.16 ^{d}	3.94 ^{f}
S6	0.44 ^{a}	132.38 ^{abc}	1.10 ^{bcde}	1.02 ^{bcde}	156.27 ^{bcd}	181.54 ^{bcd}	1.50 ^{a}	19.66 ^{cdef}	1.16 ^{bcd}	4.74 ^{abcde}
S7	0.40 ^{abc}	131.54 ^{abc}	1.22 ^{ab}	1.12 ^{abc}	166.48 ^{abc}	177.06 ^{bcd}	1.52 ^{a}	14.84 ^{h}	1.08 ^{cd}	5.28 ^{a}
S8	0.38 ^{abcd}	121.58 ^{bcdef}	1.14 ^{bcd}	1.18 ^{a}	165.10 ^{abcd}	176.66 ^{bcd}	1.64 ^{a}	17.44 ^{fgh}	1.10 ^{cd}	4.92 ^{abcd}
S9	0.42 ^{ab}	130.82 ^{abc}	1.06 ^{bcdef}	1.02 ^{bcdef}	160.54 ^{abcd}	193.30 ^{bcd}	1.72 ^{a}	19.20 ^{defg}	1.08 ^{cd}	4.90 ^{abcde}

* Different letters in the same column differ significantly by the Tukey test ($p < 0.05$)

The PCAa analysis showed a trend to group the samples of the main harvest period and early crops. In order to confirm this fact, the HCA analysis was also performed. Nevertheless, according to this analysis, three groups were formed (Figure 4).

Figure 3. Principal Components Analysis (PCA) of the twenty cocoa samples according to their fatty acid composition and harvest time

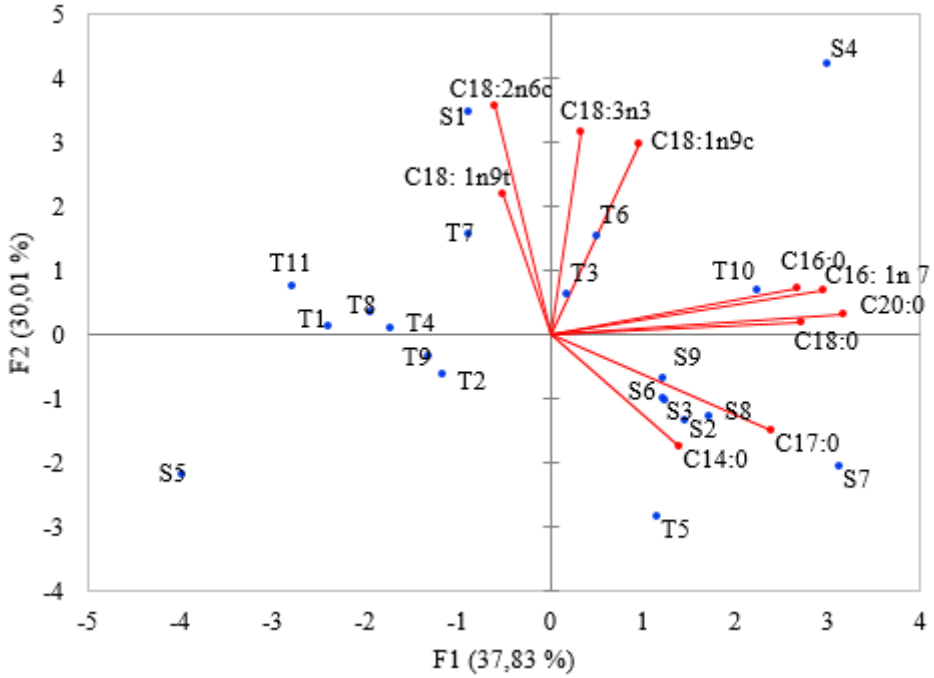
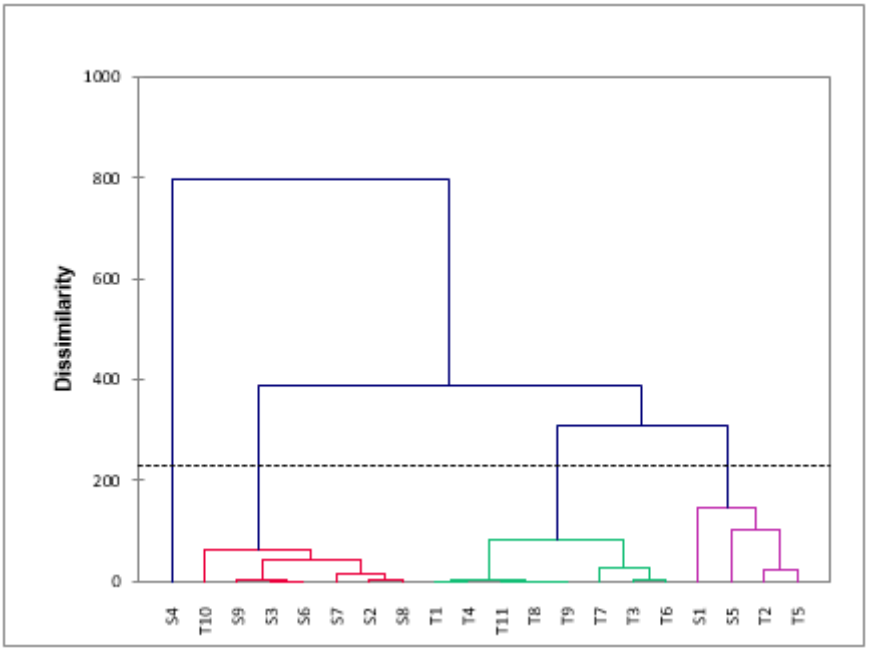


Figure 4. Dendogram (HCA) representing the dissimilarity between different cocoa samples depending on the fatty acid profile and harvest time



Group 1 was formed mainly by samples grown in the main harvest period, consisting of samples S9, S3, S6, S7, S2, S8, and T10. Group 2 was formed exclusively by samples grown in the early period, T1, T4, T11, T8, T9, T7, T3, and T6. Group III was formed by four samples - S1, S5, T2, and T5, which have little or no similarity depending on the variables studied.

Group I samples, grown in the main harvest period, were grouped mainly according to the content of myristic (C: 14: 0) and heptadecanoic saturated fatty acids (C17: 0). Group II is formed by samples grown in the early season, which were similar due to their content of unsaturated fatty acids, such as oleic (C18: 1n9c), elaidic (C18: 1n9t), linoleic (C18: 2n6c), and linolenic acids (C18: 3n3).

On the other hand, the fatty acid profile did not allow the grouping of samples by producers or by municipality, since the same counties presented differences in the main harvest and in the early crop time.

In order to check this statement the sum of saturated, monounsaturated, and polyunsaturated fatty acids and a new PCA made from these results are represented in Figure 5.

According to Figure 5, 97% of the variance is explained when considering the sum of saturated, monounsaturated, and polyunsaturated fatty acids, confirming the formation of two large groups, one for the samples collected in the main harvest time, grouped by the saturated fatty acid profile, and another one collected in the early harvest time, grouped by the polyunsaturated fatty acids.

The dendrogram shown in Figure 6 shows these groups very clearly, with samples from the main crop forming a group and samples from the early crop forming another one. In addition, there are two isolated samples (S4 and S5).

Therefore, there are several intrinsic and extrinsic factors that affect the quality and quantity of chemical compounds in cocoa and, consequently, in its derivatives. Therefore, the registered differences could be explained, at least in part, by the interaction of several factors such as genetic, physiological, agronomic, and environmental factors (microclimate).

Figure 5. PCA considering the sum of saturated (SFA), monounsaturated (MFA), and polyunsaturated fatty acids (PFA)

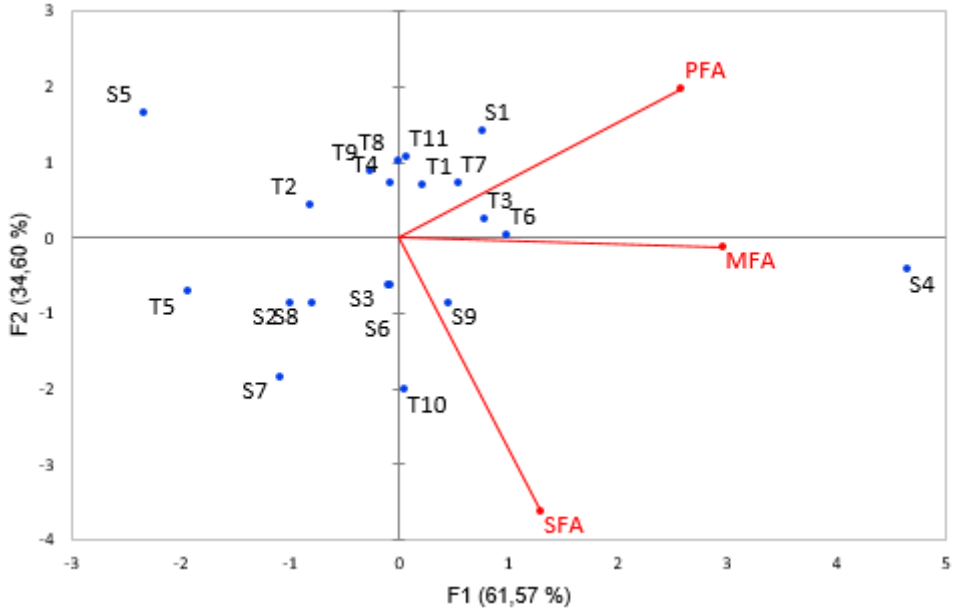
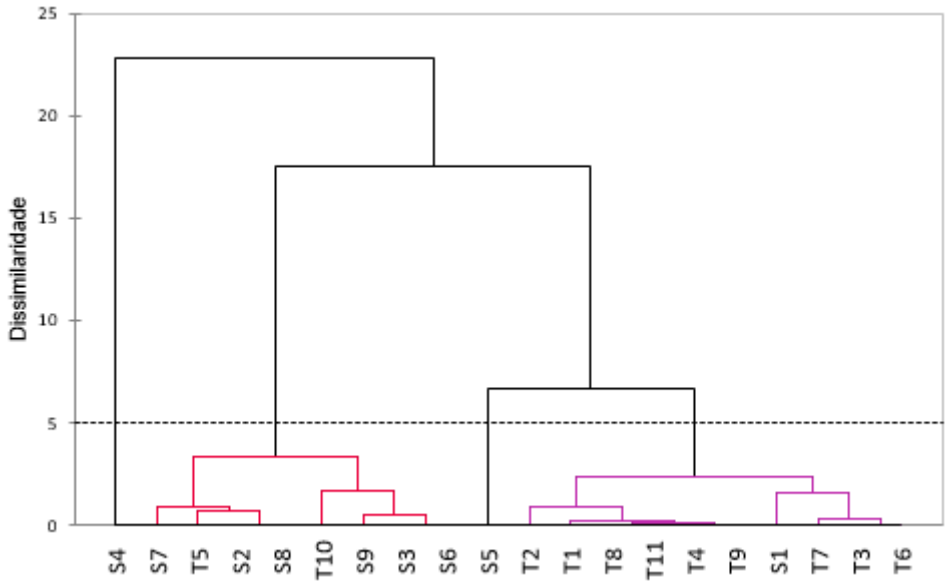


Figure 6. Dendogram (HCA) representing the dissimilarity between different cocoa samples according to the level of saturation of fatty acids and harvest time



4 CONCLUSIONS

The multivariate analysis enabled the separation of the cocoa samples according to the chemical composition, separating the groups according to the microregion where they were produced. On the other hand, the analysis of the fatty acids profile allowed the separation between harvest periods, showing that the cocoa harvested in the main season had a greater influence of saturated fatty acids, while in the early season the unsaturated fatty acids were more important. Thus, multivariate techniques were able to discriminate

the different types of cocoa according to their chemical profile, enabling the investigation of other parameters, in order to provide a better cocoa classification, therefore contributing to the chemical characterization of this product and collaborating to the creation of an origin denomination for the cocoa produced in Southern Bahia.

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