Chemical composition and fatty acids profile of chocolates produced with different cocoa (*Theobroma cacao L.*) cultivars

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Abstract

The objective of the present study was to characterize the physicochemical composition and to quantify the fatty acids of chocolates produced from different cocoa varieties (*Theobroma cacao L.)* cultivated in the Southern Region of Bahia. Nine samples of monovarietal chocolates were evaluated, according to their physical, thermal and fatty acid profile. The results were subjected to tests of variance and multivariate statistics. The chocolates PH16 and TSH1188 presented higher melting point. It was possible to identify a linear correlation between the fat content of the chocolates and the break force parameter. The chocolates showed higher concentration of saturated fatty acids, mainly palmitic acid (16:0) and stearic acid (18:0), followed by the unsaturated fatty acids among which oleic acid (18:1n-9) and linoleic acid (18:6) were predominant. The principal component analysis allowed to discriminate the different varieties of chocolate according to the profile of fatty acids. The thermogravimetric analysis of the samples showed a similar profile. The differences found in the samples of chocolates were attributed to factors such as the fruit maturation process, the cocoa variety and to which they were subjected during processing.

Keywords: monovarietal chocolate; cocoa solids; thermogravimetry.

Practical Application: The study show differences in chocolates obtained with nine cocoa cultivars produced in southern Bahia, being a source of information to characterize and improve the quality of monovarietal chocolates.

1 Introduction

Chocolate is a product obtained from cocoa beans, the fruit of the cocoa tree (*Theobroma cacao* L.) that grows in Central and South America and West Africa (Rusconi & Conti, 2010). The Southern Region of Bahia state is the second major cocoa producing region in Brazil. Until 1989, this region was free from the disease known as "Witches' Broom", caused by the fungus *Moniliophthoraperniciosa*, and responsible for high losses reaching 100% decrease on the production of cocoa in this region (Mandarino & Gomes, 2009). One of the alternatives to control the occurrence of the "witches broom" disease was the cultivation of resistant and high productivity varieties, developed by the cocoa tree genetic improvement program (Leite et al., 2013).

Good quality cocoa beans are essential to obtention and commercialization of chocolates with better attributes. The chocolate composition varies across the world due to cultural differences and legislation. The content of each nutrient depends, amongst other factors, on the percentage of cocoa. Carbohydrates mainly represented as sugars, have a total content up to 45%, and fat, a total content up to 30% according to National Nutrient Database for Standard Reference (US Department of Agriculture, 2010). The composition can also be influenced by the genetic variety of the cocoa beans that were used for the chocolate production, cultivation conditions and post-harvest techniques (Aprotosoaie et al., 2016; Carneiro et al., 2011).

The cocoa butter is one the most important ingredients during the chocolate formulation. Economically, it is the most expensive ingredient, corresponding to one third of the final product's price (Minim & Cecchi, 1998). The fat component in chocolate constitutes the continuous phase, that affects a many parameters during processing, storage and consumption. Cocoa butter by itself shows many good and interesting properties, as well as major limitations (Kerti, 1998). The amount of cocoa butter and the fatty acid profiles in chocolate depend on the growth conditions of the cocoa beans. In cocoa butter, fatty acids are found as triacylglycerols, most of which are palmitic and stearic acids (Segall et al., 2005; Simoneau et al., 1999). The structure of these acids directly affects the way the chocolate behaves in the manufacturing process and in the characteristics of the final product, such as texture, viscosity, melting behavior and flavor (Afoakwa, 2010). So, it is necessary to evaluate the physicochemical characteristics of these chocolates, pointing to verify the impact of each factor over the quality and preservation of the final product. The present study aimed to determinate the physicochemical proprieties and to analyze the fatty acid profile in chocolate samples produced from different varieties of cocoa trees (*Theobroma cacao* L.) cultivated on the Southern Region of Bahia state, Brazil.

2 Material and methods

2.1 Samples

Nine monovarietal chocolate samples were analyzed containing 70% of cocoa solids, produced from different varieties, denominated as PH16, SR162, TSH1188, CEPEC2002, CCN51, Pára-parazinho (Pára), Ipiranga I (Ipi.I), BN34 and PS1319. These cocoa varieties were selected for having specifics characteristics from the cocoa producing regions where they were cultivated. The chocolates were produced in Ilhéus, Southern Region of Bahia (coordinates: 39°11'57.69"W, 14°43'11.63"S). The 70% chocolate formulation was prepared utilizing: cocoa mass (66%), cocoa butter (4.0%), sugar (26%), and lecithin (0.4%).

2.2 Chemical analysis

Three samples of each variety was analyzed in triplicate for moisture, ash, protein, total fat, melting point, texture, thermal profile and fatty acids content. The total carbohydrate content was estimated by difference.

The moisture and ash contents in chocolate were determined by the gravimetric method, drying 3 g of sample at 105 ± 2 °C until constant weight in electric oven. Ash contents were determined by using a muffle furnace at 550–600 °C for 4 huntil constant weight. The crude nitrogen content was determined by the Kjeldahl method (Cunniff, 1998) and calculated using 6.25 as the conversion factor (Protein = Nitrogen *6.25), with chloroform and methanol mixture (2:1 v/v), as described by Bligh & Dyer (1959). The chloroform lipid extract was submitted to 105 °C in oven, model (TE 3941 - Tecnal), for complete solvent evaporation and lipid final weight was measured. The carbohydrates were calculated by subtraction: 100 – (% moisture + % ashes + % crude nitrogen + % total lipids).

2.3 Melting point

Melting point determination was performed on a Tecnopon-PFMII metering apparatus. The heating rate used was 1 °C.min^{-1}, in the temperature range of 23 – 40 °C.

2.4 Texture measurements

The determination of the parameters of analysis was according to the conditions proposed by Afoakwa et al. (2008). Hardness of chocolate was measured using the Texture Analyzer with a penetration probe (needle SMS/P6). Eight replicates were made at a pre-test speed 1.0 mm/s, test speed 2.0 mm/s and post-test speed 10.0 mm/s, 5.0 mm compression distance, 5.1 g trigger force and 5.00 s duration. A 8 mm diameter double punch plate was used. The breaking force was expressed in Newtons (N). Conversion of mean values into hardness data was performed by XT.RA Dimension, Exponent 32 software (Stable Micro Systems).

2.5 Thermal analysis

The determination of the analysis parameters was according to the conditions proposed by Materazzi et al. (2014). The analyzes were performed using a Perkin Elmer® Thermogravimetric analyzer, Pyres 1 TGA, previously calibrated. Measurements were made under a nitrogen atmosphere, 10 mL.min^{-1}. The samples were placed in alumina containers. The mass of the sample loaded in the thermobalance was 10 mg. Measurements were taken in a temperature range from 25-700 °C, TG curves were obtained for temperature dependence of mass loss and first derivative data (DTG) were calculated. All analyzes were completed in triplicate. Measurements were also made on cocoa butter under the same conditions mentioned above, in order to assess the nature of the events that were found in the chocolates.

2.6 Preparation of Fatty Acid Methyl Esters (FAMEs)

FAMEs were prepared according to the AOAC official method 948.22 (Association of Official Analytical Chemists, 1995). For each sample, the process was perfomed in triplicate. The FAMEs were obtained from the lipid fractions after alkaline hydrolysis (NaOH/methanol 0.5 mol^{-1}), followed by methylation with boron trifluoride 12% in methanol. Samples were extracted with isooctane and washed with saturated sodium chloride solution, 1 mL of the organic phase was purified though a micro column filled with anhydrous sodium sulphate and then eluted with 2 mL of isooctane. Finally, the eluate was evaporated to dryness under N$_2$ flow in an amber bottle containing 1 mg of internal standard (C23:0 - T9900; Sigma Aldrich®) and reconstituted with 1 mL of isooctane for the chromatographic analysis.

2.7 Chromatographic conditions

Individual fatty acids were determined by Gas Chromatography (Perkin Elmer Clarus 680) coupled with flame ionization detector, according to the method proposed by Souza et al. (2017) using a DB-FFAP capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness). The analysis parameters were: inlet temperature at 250 °C; detector temperature at 280 °C; column program temperature at 150 °C for 16 min., 2 °C.min^{-1} until 180 °C, maintaining that temperature for 25 minutes; then raising 5 °C.min^{-1} until 210 °C, maintaining this temperature for 25 minutes. Helium was used as carrier gas at 1.0 mL.min^{-1} rate. Nitrogen was used as make-up gas at 30 mL.min^{-1} rate. The flame gases were hydrogen (30 mL.min^{-1}) and synthetic air (300 mL.min^{-1}). The injections were made in triplicate in *split* (1:90) mode, and the injection volume was 1 μL. The FAMEs identification was performed by comparison of the retention time of the peaks presented by the samples and the retention time of standard mix (189-19, Sigma, EUA). For the fatty acids quantification, internal standard was added to the extracted FAMEs (C23:0 Sigma, EUA). The calculations were made following the Equation 1 and expressed in mg/g of total lipids.

$$Concentration\ (mg/g) = \frac{A_{AG}\ x\ M_{PI}\ x\ F}{A_{PI}\ x\ M\ x\ F_{AG}}\ x\ 1000 \qquad (1)$$

A_{AG} = FAME's peak area on sample chromatogram;

M_{PI} = Internal standard mass added to the sample in milligrams;

F = Response correction factor of each FAME on FID in relation to C23:0;

A_{PI} = Internal Standard area on sample chromatogram;

M = Sample mass in milligrams;

F_{AG} = Conversion factor from FAME to fatty acid.

2.8 Statistical analysis

A one-way ANOVA was used to study the effect of cocoa origin on chemical composition and fatty acid profile of samples of monovarietal chocolates. And the means were compared using the Tukey Test at 95% confidence level. Principal Component Analysis (PCA) was used to summarize the information in some key components and was calculated using the correlation matrix. Statistical analyzes were performed using Minitab software v. 17. The data obtained from the TG and DTG curves were plotted using the Origin v. 8.

3 Results and discussion

3.1 Chemical composition

Table 1 show the chemical composition of different monovarietal chocolates. For all samples, carbohydrates were the main nutrient (>46%), followed by fat (>34%) and proteins (7.87 – 9.14%). Ash and moisture contents were less than 6%. The composition of the chocolate changes depending on the variety of the cocoa that was used for the samples processing.

No significant differences were observed in protein contents (p>0.05) among the samples of varieties BN34, PH16 and TSH1188, but significant difference was found when compared to the sample Ipiranga I, that possess the lower protein content (7.87%). The samples CCN51 and CEPEC2002 also presented no difference (p>0.05) and having intermediary protein values between 8.79 and 8.69%.

A higher protein percentage may provide a reasonable number of peptides and some amino acids as histidine, methionine and cysteine that present antioxidant activity. The variation in protein concentration might be related to the nitrogen content in the soil were the cocoa tree was cultivated, as such the genetic characteristics of the cocoa variety used in chocolate processing (Jalil & Ismail, 2008).

For the total lipids content, the samples TSH1188, SR162, BN34, CEPEC2002 and Ipiranga I showed no significant difference among them, but differed (p<0.05) in relation to the PH16 sample. Samples of Pará-Parazinho, PS1319, CCN51 and PH16 varieties did not differ among them (p>0.05), presenting values between 34.83 and 39.70%, being lower than those

found in samples TSH1188, SR162, BN34, CEPEC2002 and Ipiranga I. The values found for total fat were similar to those obtained by Leite et al. (2013) by studying the differences in fat composition of single-grain chocolates containing 70% of cocoa solids. The variation of the samples lipid content may be related to factors such as the process of maturation of the cocoa fruit, genetic origin and the conditions to which they were subjected during processing (Quast et al., 2007).

The ash content ranging between 1.50 and 1.89%, the chocolates from PH16 and Ipiranga I varieties showed higher values (1.86 and 1.89%) and did not show significant difference when compared to each other (p>0.05), but they differ from Pará-Parazinho, CEPEC2002, PS1319, BN34, CCN51, TSH1188 and SR162 samples. Genovese & Lannes (2009)sugest that chocolates with higher ash content may present a higher mineral concentration.

The mean moisture values were between 0.90 and 1.40% this range is lower than that described by Genovese & Lannes (2009) that was from 1.93 to 3.05% for 70% cocoa solids chocolates. Moisture content was similar in SR162 and CEPEC2002 samples. The samples CCN51 and Pará-parazinho presented a higher moisture content and differ significantly of all samples (p<0.05). Moisture can interfere on sensorial attributes of melting and texture.

The carbohydrate content ranged from 46.39 to 53.36%, the PH16 and PS1319 samples did not differ from each other (p>0.05). These variations can be attributed to the fact that since carbohydrates were calculated by difference, a low protein and lipid content will give a high carbohydrate content (Padilla et al., 2000).

Table 2 presents the results of mechanical assays for determination of melting point and hardness of the different monovarietal chocolate samples.

Mean values of melting point ranged from 28 to 31.60 °C (p <0.05). PH16 and TSH1188 samples presented the highest melting point of 31.60 and 31.43 °C, respectively, when compared to the other samples. According to Stapley et al. (1999), in studies conducted with chocolate tempering, samples with higher melting points are associated with more stable polymorphic forms for properly tempered chocolates. The melting point depends on its chemical composition, polymorphic behavior and the crystallization conditions to which samples were subjected (Quast et al., 2011).

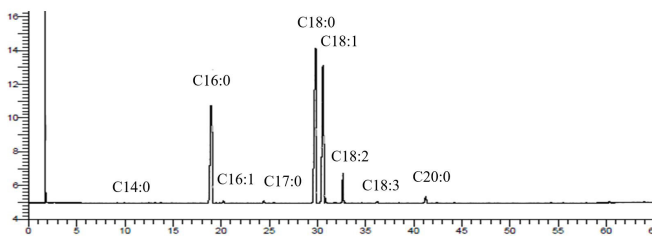**Table 1**. Chemical composition of chocolate samples.

Samples	Protein (%)	Lipids (%)	Ashes (%)	Moisture (%)	Carbohydrates (%)*
SR162	8.42 ± 0.19abc	38.40 ± 0.18abc	1.52 ± 0.02ef	0.90 ± 0.04e	50.83 ± 0.26abc
BN34	9.14 ± 0.49a	38.28 ± 0.78abc	1.64 ± 0.03cd	1.14 ± 0.02bc	49.92 ± 0.63bc
PH16	8.93 ± 0.02a	34.83 ± 0.59c	1.86 ± 0.02a	1.10 ± 0.08cd	53.36 ± 0.70a
CEPEC	8.69 ± 0.32ab	37.80 ± 1.03abc	1.50 ± 0.01f	0.91 ± 0.01de	51.04 ± 1.34abc
TSH1188	8.99 ± 0.25a	41.82 ± 0.96a	1.58 ± 0.02de	1.06 ± 0.06cde	46.39 ± 0.66d
Ipiranga I	7.87 ± 0.06c	39.70 ± 3.65ab	1.89 ± 0.02a	1.29 ± 0.07ab	49.25 ± 3.70cd
CCN51	8.79 ± 0.12ab	35.98 ± 0.82bc	1.76 ± 0.04b	1.40 ± 0.03a	51.99 ± 0.94ab
PS1319	8.04 ± 0.37bc	35.21 ± 0.67bc	1.74 ± 0.02b	1.15 ± 0.07bc	54.07 ± 1.00a
Pará	8.99 ± 0.25a	36.21 ± 0.91bc	1.69 ± 0.05bc	1.32 ± 0.03a	51.75 ± 0.60abc

*Results given as mean ± standard deviation of triplicate analyses of nine different samples. Different letters in the same column indicate a significant difference by Tukey test at (p <0.05) confidence level.

Table 2. Mean values and standard deviation, melting point and hardness for samples of chocolates.

Samples	Melting point (°C)	Hardness (N)
SR162	28.50 ± 0.50[de]	82.48 ± 10.9[ab]
BN34	28.33 ± 0.58[de]	71.98 ± 3.40[ab]
PH16	31.60 ± 0.58[a]	76.82 ± 21.8[ab]
CEPEC2002	28.00 ± 0.00[e]	83.38 ± 5.71[ab]
TSH1188	31.43 ± 0.32[a]	98.54 ± 7.95[a]
Ipiranga I	31.26 ± 0.46[ab]	89.08 ± 3.55[ab]
CCN51	29.66 ± 0.58[cd]	88.33 ± 9.30[ab]
PS1319	29.26 ± 0.25[cde]	65.63 ± 4.77[b]
Pará-Parazinho	30.00 ± 0.00[bc]	74.25 ± 9.17[ab]

Results given as mean ± standard deviation of triplicate analyses of nine different samples. Different letters in the same colum indicate a significant diference by Tukey test at (p <0.05) confidence.

Figure 1. Characteristic GC–FID chromatogram of the FAMEs from sample of chocolate BN34.

Lohman & Hartel (1994) when determining melting point in dark chocolates found average values of melting point between 32.6 and 33.7 °C. These results are similar to those presented in this study. Leite et al. (2013) determined melting point between 29.63 and 30.43 °C for chocolates produced from witch's broom resistant cocoa, being the values close to those found in the present study. Low melting point influences the texture of the final product, making the chocolate inconsistent and unstable. According to Melo & Chitarra (1999) the main problem for the chocolate conservation is related to its high fat content and its low melting point.

The hardness of the chocolates evaluated presented a significant difference (p>0.05). Hardness is one of the evaluated texture parameters, which confers the product firmness, being a characteristic instrumentally defined as the force required to cause a certain deformation in the sample (Szczesniak, 2002). The results ranged from 98.54 N to 65.63 N, being the TSH1188 the one with highest value. So, the energy required to snap these samples were higher than the others. BN34 and PS1319 samples demand less energy since they presented results of 71.98 N and 65.63, respectively. The results ranged from 98.54 N to 65.63 N, being the TSH1188 the one with highest value. So, the energy required to snap these samples was higher than the others. BN34 and PS1319 samples demand less energy since they presented results of 71.98 N and 65.63, respectively. In order to evaluate the relationship between fat content and the hardness of the different chocolates, Pearson's correlation coefficient was calculated, and the results were moderately high

and statistically significant (r=0.72, p=0.028). Carneiro et al. (2011) when evaluating the texture parameters in chocolate bars, also verified a significant correlation (r= 0.71, p=0.005) between fat content and breaking strength. According to Afoakwa et al. (2007), the observed differences may be related to formulation, manufacturing techniques, tempering, polymorphism (stability of fats) and cooling temperature controls, impacting on the final texture (hardness) of chocolate. Keogh et al. (2002) concluded that hardness is a good indicator of good tempering, or degree to which a stable fat network has been formed.

3.2 Fatty acids profile

Fatty acids from the different chocolate samples were identified and quantified using GC–FID analysis, showing the separation of fifteen different FAMEs, as illustrated in a typical chromatogram in Figure 1.

The fatty acids detected in the chocolates were C14:0 (myristic acid), C16:0 (palmitic acid), C16:1 (palmitoleic acid), C17:0 (margaric acid), C18:0 (stearic acid), C18:1n9 (oleic acid), C18:2n6 (linoleic acid), C18:3n3 (linolenic acid), C20:0 (arachidic acid) and C22:0 (behenic acid), sorted by order of retention time. Table 3 shows the average total fatty acids content of chocolates. The effect of the cocoa variety used in chocolate processing on the fatty acid profile had a significant impact only on the following fatty acids: C16:0 (p=0.008), C17:0 (p=0.016), C18:0 (p=0.002), C18:1n9 (p=0.005), C18:2n6 (p=0.002), C18:3n3 (p=0.001) and C20:0 (p= 0.009). These results confirmed that the cocoa variety influenced the composition of fatty acids, as pointed out by Lipp & Anklam (1998) and Torres-Moreno et al. (2015). Despite the mentioned differences, the fatty acids profile was very similar in all the chocolate samples. Quantitatively C16:0 (>23.91%), C18:0 (>30.25%) and C18:n1 (>32.24%) were the most important fatty acids for all the chocolate samples. The results are in accordance with the results obtained by other authors, such as Leite et al. (2013), Menezes et al. (2016), Torres-Moreno et al. (2015) and Suzuki et al. (2011), who reported that C16:0, C18:0 and C18:n1 were the most important fatty acids in chocolates with high cocoa content. The results of ANOVA, as show in Table 3, indicate that the samples CEPEC2002 and PS1319 presented a lower content of fatty acids. BN34 and TSH1188 samples had a significantly higher content of C16:0 (29.02 and 28.83%). On the other hand, SR162 and Pará-Parazinho had a higher content of C18:0 (34.26 and 35.18%) and C18:n1 (34.35 and 35.41%). Ipiranga I and CEPEC2002 had a higher content of C18:1 (34.82 and 34.09%) and a lower C18:0 content (32.22 and 32.56%). When considering the proportion of saturated/unsaturated fatty acids, differences were found among the different types of chocolates (p<0.05). Therefore, these results indicate that the chocolates of Pará-parazinho and Ipiranga I varieties seem to have a healthier fatty acids profile, since it contains more unsaturated fatty acids (39.43 and 38.78%) and less saturated fatty acids (60.56 and 61.21%) than the other chocolates. According to Torres-Moreno et al. (2015), the prevalence of saturated fatty acids on unsaturated fatty acids is considered to be negatively from a nutritional point of view. Among the unsaturated fatty acids, oleic acid was the most common in the samples. Oleic acid is considered to be responsible for lowering the LDL-cholesterol levels. Much scientific evidence has shown that daily consumption of the

Table 3. Profile of fatty acids monovarietal chocolates containing 70% cocoa in mg of fatty acids/g of lipids.

AG	SR162	BN34	PH16	CEPEC2002	TSH1188	Ipiranga I	CCN51	PS1319	Pára
C14:0	0.73 ± 0.40[a]	0.60 ± 0.03[b]	0.56 ± 0.13[b]	ND	0.80 ± 0.04[a]	0.84 ± 0.25[a]	0.71 ± 0.02[a]	0.73 ± 0.10[a]	0.90 ± 0.07[a]
C16:0	221.25 ± 5.43[ab]	277.49 ± 14.32[a]	206.05 ± 3.90[ab]	191.98 ± 21.37[b]	274.39 ± 2.45[a]	217.65 ± 12.62[ab]	233.69 ± 34.02[ab]	187.82 ± 34.56[b]	203.78 ± 5.34[ab]
C16:1n7	1.83 ± 0.14[a]	2.24 ± 0.43[a]	1.63 ± 0.08[a]	1.42 ± 0.41[a]	2.43 ± 0.08[a]	1.54 ± 0.13[a]	2.06 ± 0.21[a]	1.37 ± 0.26[a]	1.64 ± 0.66[a]
C17:0	2.13 ± 0.10[a]	1.96 ± 0.48[ab]	1.38 ± 0.09[ab]	1.27 ± 0.35[ab]	2.11 ± 0.49[ab]	1.46 ± 0.02[ab]	2.23 ± 0.33[a]	0.98 ± 0.06[b]	1.51 ± 0.15[ab]
C18:0	289.45 ± 8.65[ab]	303.42 ± 23.49[a]	246.00 ± 12.15[abc]	218.16 ± 20.94[bc]	312.15 ± 1.85[a]	252.20 ± 13.08[abc]	280.57 ± 36.38[ab]	184.91 ± 29.30[c]	299.82 ± 16.43[ab]
C18:1n9	292.89 ± 6.80[abc]	327.30 ± 19.50[a]	248.85 ± 13.91[abc]	230.0 ± 24.70[bc]	321.59 ± 0.46[a]	275.30 ± 14.50[abc]	268.60 ± 31.70[abc]	207.40 ± 42.60[c]	304.30 ± 16.60[ab]
C18:2n6	26.10 ± 0.48[abc]	31.63 ± 2.05[a]	21.35 ± 0.86[bc]	19.72 ± 1.69[c]	28.65 ± 0.42[ab]	26.70 ± 1.39[abc]	26.96 ± 3.32[abc]	19.91 ± 3.39[c]	27.97 ± 1.80[ab]
C18:3n3	ND	1.70 ± 0.23[ab]	1.06 ± 0.25[b]	ND	ND	ND	1.03 ± 0.35[b]	1.32 ± 0.15[ab]	2.18 ± 0.03[a]
C20:0	8.81 ± 0.50[ab]	9.84 ± 0.25[a]	8.70 ± 0.40[ab]	7.35 ± 0.93[ab]	9.78 ± 0.08[a]	7.00 ± 0.89[ab]	8.93 ± 1.01[ab]	5.77 ± 1.63[b]	8.89 ± 0.16[ab]
C22:0	1.55 ± 0.56[a]	ND	1.39 ± 0.06[a]	ND	ND	ND	ND	0.99 ± 0.28[a]	1.14 ± 0.19[a]
S	523.92 ± 12.51[ab]	593.31 ± 38.58[a]	464.07 ± 7.74[ab]	414.29 ± 38.48[b]	599.22 ± 3.92[a]	479.14 ± 26.86[ab]	526.11 ± 71.76[ab]	381.06 ± 65.89[b]	521.11 ± 15.15[ab]
M	294.73 ± 6.65[abc]	329.56 ± 19.92[a]	250.48 ± 13.82[abc]	231.46 ± 25.12[bc]	324.23 ± 0.09[a]	276.82 ± 14.38[abc]	270.68 ± 31.86[abc]	208.80 ± 42.89[c]	308.75 ± 12.03[ab]
P	26.11 ± 0.49[abcd]	33.33 ± 1.82[a]	22.41 ± 0.61[bcd]	18.53 ± 1.68[d]	29.36 ± 1.43[ab]	26.70 ± 1.39[abcd]	27.99 ± 3.68[a]	21.23 ± 3.54[cd]	30.70 ± 0.99[a]
Σ	844.80 ± 19.7[ab]	956.20 ± 60.3[a]	737.00 ± 22.2[ab]	670.00 ± 70.4[b]	952.61 ± 2.86[a]	782.60 ± 42.6[ab]	824.80 ± 107.3[ab]	611.10 ± 112.4[b]	852.10 ± 40.0[ab]

Means followed by different letters in the same line represent significant differences between them by the Tukey test (p <0.05); S = sum of saturated fatty acids; M = sum of monounsaturated fatty acids; P = sum of polyunsaturated fatty acids; ND = Not detected.

fatty acids C18:2n6 and C18:3n3 has protective effects in the areas of cardiovascular health, cancer and diabetes. In addition chocolate can be considered a source of these essential fatty acids (García-Lorda et al., 2003; Jiang et al., 2002).

To better understand the relationship between the samples and the identified variables, the principal component analysis method (PCA) was performed, which, by the direction and size of the vectors, indicates their contributions to the discrimination of samples of monovarietal chocolates. Figure 2 illustrates the scores of the major components, PC1 and PC2, which explained respectively 63.7% and 14.8% and demonstrated the variance stored in each main component expressed by the eigenvalues of the standardized matrix. It was verified in the present study that the two main components explained 78.5% of the total variance observed among the samples. Each sample was located near the vectors that describe with greater intensity its characteristics. Vectors close to each other, suggest attributes with statistically significant correlations. The PC1 (X-axis) is mainly related to the samples TSH1188, BN34, CCN51 and Ipiranga I, CEPEC2002, PH16 and PS1319 (negative direction), while PC2 (Y-axis) was discriminated by the Pará-Parazinho sample.

3.3 Thermogravimetric analysis

Thermogravimetric curves (TG) were determined and the first derivative (DTG) for all samples were calculated. In Figures 3 and 4, TG and DTG curves of different monovarietal chocolates and cocoa butter are presented at heating rate of 10 °C.min^{-1}.

The TG curves were similar for the different samples of monovarietal chocolates, except for CEPEC2002. The TG curves that characterize samples of chocolates can be divided into three stages. First stage ranged from 50 to 210 °C, second from 210 to 350 °C, and third from 350 to 700 °C. The DTG curve can be divided into five steps.

The first stage observed on DTG curves varied from 107 to 150 °C, it is characterized by an event caused by the dehydration of the samples that occurred due to the weakly bound water molecules evaporation. The type and degree of binding are reflected by the temperature of mass loss (Ducat et al., 2015). A mean mass loss of 0.74% was observed, being these results inferior to those obtained by the traditional gravimetric method for the determination of moisture (0.90 to 1.40%).

The second stage, was observed from 150 to 285 °C, with a mass loss of 18.10%. Iqbal et al. (2013) also found a similar weight loss (18-36%) in the temperature range of 225 – 325 °C. Ostrowska-Ligęza et al. (2018), evaluating the thermal stability of chocolates with high cocoa content at different stages of processing, found that the second event is correlated with the sugar decomposition on all the DTG curves. The third stage from 285 to 347 °C, with an average mass loss of 9.50%. Fourth, from 347 to 390 °C and the fifth from 390 to 512 °C. The fifth event was characterized by the largest mass loss (40.31%), it may be related to the cocoa butter decomposition (Figure 5). According to Materazzi et al. (2014) by the analysis of the starting materials (sugar, cocoa liquor, and cocoa butter), it is very easy to assign each thermic event that occurs in chocolate to the ones presented by each ingredient. Ostrowska-Ligęza et al. (2018) also related the final event to the degradation of cocoa butter (350 – 650 °C).
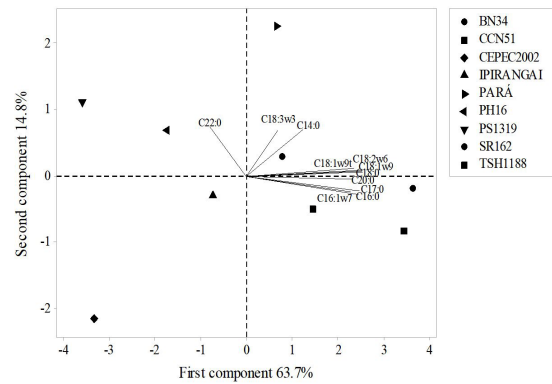

Figure 2. Score and factorial load of Principal Component Analysis of fatty acid composition in monovarietal chocolates.
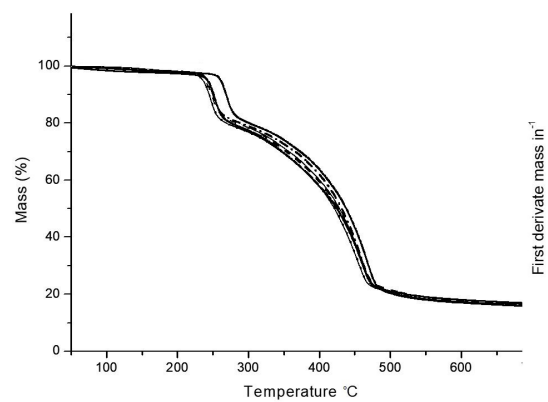

Figure 3. TG and DTG curves of monovarietal chocolates under nitrogen atmosphere at heating rate of 10 °C.min^{-1}.
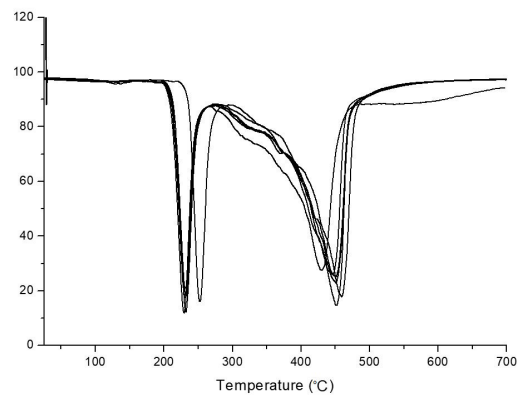

Figure 4. TG and DTG curves of monovarietal chocolates under nitrogen atmosphere at heating rate of 10 °C.min^{-1}.

The TG curve of cocoa butter under nitrogen atmosphere is characterized by only one step of decomposition (Figure 5). The average T$_{peak}$ temperature of the first derivative was observed about 426 °C. Materazzi et al. (2014) detected on TG curve for cocoa butter, one event in the range from 310 – 440 °C corresponding to its decomposition.
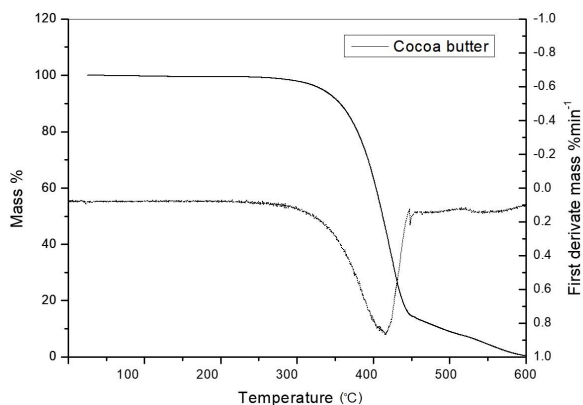
Figure 5. TG and DTG curves of butter of cocoa in nitrogen with heating rate 10 °C min⁻¹.

4 Conclusions

It was possible to differentiate monovarietal chocolates based on their physicochemical properties. A significant difference was found among the samples for all physical-chemical parameters that were evaluated. These differences can be attributed to processing conditions, environmental factors and genetic varieties of cocoa that were used for the preparation of chocolates. C16:0, C18:0, C18:1n9 and C18:2n6 were, quantitatively, the most important fatty acids in all of the studied samples. Pará-parazinho and Ipiranga I chocolates seem to have a healthier fatty acid profile as they contain more unsaturated fatty acids and less saturated fatty acids when compared to the other samples. The prevalence of saturated fatty acids on unsaturated fatty acids is considered to be negative from a nutritional point of view. From the analysis of main components (PCA), it was possible to discriminate the different chocolate varieties according to the fatty acids profile. No differences were observed in TG curves for the different monovarietal chocolates samples. It was possible to correlate the degradation of the cocoa butter with the last event of mass variation in the chocolate samples (350 – 650 °C).

Acknowledgements

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