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Chocolates Produced with Unroasted and Roasted Cocoa Beans: A Comparative Study of the Preservation of Bioactive Compounds

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The aim of the present study was to evaluate the effect of roasting and cocoa mass content on the composition of chocolates. Chocolate formulations were developed (40, 50, 60 and 70% cocoa solids) using unroasted and roasted cocoa beans, and analyses were performed: physicochemical, chemical composition, antioxidant capacity, procyanidin content, total phenolic compounds, quantification of theobromine, caffeine, catechin and epicatechin by high performance liquid chromatography (HPLC) and spectroscopic profile by mid-infrared spectroscopy (MIR). The results revealed that higher values of total phenolics, epicatechin and procyanidins, were obtained in chocolates produced with unroasted cocoa beans, with a significant difference in relation to those with roasted cocoa beans. It was observed that increasing the percentage of cocoa in the formulations caused an increase in the content of bioactive compounds. The use of MIR in the differentiation of chocolate samples produced with roasted and unroasted cocoa beans obtained a positive result. This technique associated with principal component analysis (PCA) enabled the separation of chocolates according to the type of cocoa bean (roasted and unroasted) used in production, confirming its importance for a quick evaluation of the parameters.

Keywords*:* antioxidant activity, cocoa beans, phenolic compounds, principal component

Introduction

Cocoa is a rich source of bioactive compounds such as polyphenols (epicatechin, catechin, procyanidins and anthocyanins) that have a high antioxidant capacity, which promotes the neutralization of free radicals in the human body. In cocoa, methylxanthines (theobromine and caffeine) can also be found, which can cause a variety of desirable effects such as mood improvement, muscle relaxant activity, protection to cognitive activities, among others. However, the bioavailability of these compounds may vary according to the variety of cocoa, as well as climatic conditions, region, cocoa processing stages and stages of production of its derivatives.[1-3]

Several products are obtained from cocoa, including chocolate, which is an emulsion containing a semi-solid dispersion of fine cocoa particles, sugar and milk involved in a continuous phase of cocoa butter and milk fat.[4]

Chocolate contains lipids, proteins, carbohydrates, minerals and vitamins. It has great potential in promoting health, minimizing the impact of free radicals on the human body, reducing stress and depression. Besides that, it protects against cardiovascular disease, due to the presence of biologically active compounds that improve the functioning of blood vessels by reducing blood pressure.[5-7]

The content of these compounds in chocolate can vary according to the raw material, formulation and processing conditions. During production, the industry receives the fermented and dried cocoa beans to start the roasting process. In the roasting, some of the product's quality characteristics are developed, and as it normally occurs at high temperatures, there may be a reduction in the concentration of bioactive compounds. This stage also involves the reduction of moisture and undesirable volatile acids, production of aromatic compounds, development of the characteristic chocolate flavor and color changes.[4,8]

Kitani *et al.*[9] studied commercial bean-to-bar (raw bean and roasted nib) samples and chocolates from different cocoa origins to investigate the effect of processing on

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composition, and observed influence of the manufacturing process higher than the difference in cocoa production area, with the influence of fermentation and roasting on component profiling. Żyżelewicz *et al.*[10] evaluated chocolates with 75% cocoa mass made with unroasted cocoa beans and observed high moisture, acidity, viscosity and lower hardness when compared to chocolates made with roasted cocoa beans.

This information indicates that the elimination of the roasting step, using minimal processing of cocoa beans, influences the composition and characteristics of the chocolates produced from these unroasted cocoa beans, with products tending to have a more intense flavor. Therefore, the elimination of the roasting step, combined with the use of a higher concentration of cocoa mass in the chocolate formulation, presents itself as an alternative for the preservation of bioactive compounds in the final product.

The quantification of bioactive compounds present in cocoa and chocolate are mainly determined by high performance liquid chromatography (HPLC), contributing to the understanding of the effects of processing traditional chocolate and chocolate with unroasted cocoa beans, allowing the analysis of the level of degradation of these compounds after roasting.[11] More recently, mid-infrared spectroscopy (MIR) associated with chemometric techniques has been used to assess the quality of cocoa beans and chocolate. This technique has been used to confirm the identity of compounds, to monitor the geographical origin and to determine the main components of foods, evaluating qualitative aspects, as well as to obtain quality certification. In chocolates, the use of the MIR to identify the cocoa solids content and possible adulterations proved to be efficient.[12,13]

In this context, this work evaluated the effect of roasting and cocoa bean content on the physicochemical properties and bioactive compounds of produced chocolates.

Experimental

Obtaining cocoa beans and roasting

The cocoa beans of the Forasteiro species were purchased from producers in different regions of the state of Bahia, Brazil: Baixo Sul, Litoral Sul, Médio Rio de Contas and Vale do Jiquiriçá, generating 25 kg of a blend.

Preliminary tests were carried out to define the roasting parameters to be used. The roasting temperature can vary from 90 to 170 °C, and above this temperature excessive roasting can occur, leading to burning and a significant change in the aroma and flavor of the almonds. From this range, the following temperatures were chosen for the test: without roasting (control), 110, 130 and 150 °C, for 50 min, according to recommendations de Żyżelewicz *et al.*[10] Mid-infrared (MIR) (ca. 4000-400 cm^{-1}) was used for the analysis of cocoa beans, a fast, sensitive and robust technique, which allows for possible differences in the composition of roasted cocoa beans to be observed in different temperatures. The spectra of the samples were obtained by means of Fourier transform infrared spectroscopy using attenuated total reflection (FTIR-ATR) in a Cary 630 FTIR equipment (Agilent Technologies Inc., Santa Clara, CA, USA).

The blend was divided into three batches, each weighing approximately 7.5 kg, corresponding to the repetitions. Each of these batches was subdivided into two parts (approximately 3.75 kg), where one of them was roasted at 110 °C for 50 min, in Oven Vipinho 0448 (Perfecta Curitiba, Curitiba, Brazil), and the other remained unroasted. Afterwards, the cocoa beans were peeled and crushed in a Compact Processor model RI7620 (Philips Walita, São Paulo, Brazil), to obtain the nibs and subsequent production of the chocolates.

Chocolate production

The chocolates were processed using unroasted and roasted cocoa beans, and for each type of cocoa beans, different concentrations of cocoa mass were used (40, 50, 60 and 70%), totaling eight formulations, all with 3 replicates.

The formulations were composed of fixed amounts of cocoa butter (6%), powdered milk (7%) and soy lecithin (0.4%), varying the amounts of cocoa nibs and sugar, to reach the desired solids content of cocoa. For the 40, 50, 60 and 70% formulations, 34, 44, 54 and 64% of cocoa nibs were added, and 52.6, 42.6, 32.6 and 22.6% sugar, respectively.

Cocoa nibs obtained from cocoa beans, refined sugar (União, Araquari, Brazil) and powdered milk (Nestlé Brazil Ltda., Ituiutaba, MG, Brazil) were transferred to a multifunction equipment (Melanger, Spectra 11, Coimbatore, TN, India) with 1.5 kg of chocolate being produced for each formulation. In the equipment, the operations of mixing the ingredients, refining and conching were carried out, where the mass of each formulation remained for a standardized time of 24 h. After 2 h of refining the dough, half of the cocoa butter (Barry Callebaut, Ilhéus, BA, Brazil) was added to the formulations and the remainder after 10 h. Soy lecithin (Grings and Filhos Ltda., São Paulo, Brazil) was added approximately 2 h before the completion of conching.[13]

The shelled mass at 60 °C was conducted to the tempering process, in a Mini Chocomachine temperer

(Finamac, São Paulo, Brazil), where it remained for 40 min under constant agitation at 45 °C and then cooled to 28 °C. This mass obtained from tempering was transferred to polycarbonate molds for molding the chocolates and subjected to vibration. The molds with chocolates were cooled at 5 °C for 12 h. After cooling, the chocolates were wrapped in laminated paper (Cromus Embalagens Ind. e Com. Ltda., São Paulo, Brazil) and stored at a temperature of 15 °C until the moment of analysis.

Chemical composition, physicochemical characterization and determination of bioactive compounds in cocoa beans and chocolates

Physicochemical parameters, chemical composition and bioactive compounds of unroasted and roasted cocoa beans and chocolates were determined.

Physicochemical analysis and chemical composition

The determination of pH and titratable acidity values (AOAC 970.21), moisture (AOAC 931.04), fat (AOAC 963.15), protein (AOAC 970.22), ash (AOAC 972.15) were carried out in triplicate, according to Association of Official Analytical Chemists (AOAC).[14] To determine the reducing sugar and total sugar content, the DNS method (3,5-dinitrosalicylic acid) (Sigma-Aldrich, St. Louis, MO, USA) was performed using the methodology of Cecchi.[15] Solutions of glucose (0, 0.2, 0.4, 0.6, 0.8 and 1.0 g L^{-1}) were used to prepare a standard curve.

Quantification of theobromine, caffeine, catechin and epicatechin contents by high performance liquid chromatography (HPLC)

For the quantification of theobromine, caffeine, catechin and epicatechin of cocoa beans (without roasting and roasting) and elaborated chocolates, HPLC was used. This analysis was performed according to the method used by Jolic *et al.*[16] Sample extracts were separated in reverse phase-liquid chromatography (RP-LC) column (Zorbax SB-C18, 4.6 mm ID (internal diameter) × 250 mm, 5 µm and Zorbax SB-C 18 guard column, 4.6 mm ID × 12.5 mm, 5 µm) using an HP Agilent 1260 Infinitty II system. The mobile phase consisted of 2.5% acetic acid (Vetec, Rio de Janeiro, Brazil) (solvent A) and acetonitrile (Dinâmica Química Contemporânea Ltda., São Paulo, Brazil) (solvent B) at a flow rate of 1 mL min^{-1}. 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. Chromatograms were recorded at 274 and 280 nm. The identification of compounds was obtained by comparing their UV spectra and retention times of the separate peaks with those of the standards. The phenolic compounds identified were quantified by the external standard method, the quantification is based on the peak area. Standard calibration curves were prepared by diluting (−)-epicatechin, (+)-catechin, caffeine, and theobromine standards (Sigma-Aldrich, Saint Louis, Missouri, USA) in an extractor solution (85% H_2O, acidified with 0.3% acetic acid and 15% methanol) (Vetec, Rio de Janeiro, Brazil). For each pattern, a curve consisting of eight points (1, 2, 4, 8, 16, 32, 64 and 128 µg mL^{-1}) was obtained.

For the preparation of the samples of unroasted and roasted cocoa beans and chocolates, approximately 0.02 g of each sample was weighed, 5 mL of the same extractant solution was added, and this mixture was taken under agitation for 15 min. Then, the samples were taken to the thermostatic bath (Tecnal, Te-2005, Piracicaba, Brazil) at 60 °C for 10 min and centrifuged (SP Labor, Sp-701, Presidente Prudente, Brazil) at 3000 g for 15 min for separation. The extract was filtered with sterile filters (Filtrilo, Colombo, Paraná, Brazil) of 0.22 µm.

Determination of total phenolic compounds

The content of total phenolic compounds in cocoa beans (unroasting and roasting) and chocolates was determined by spectrophotometry according to the Folin-Ciocalteau method adapted from Lee *et al.*[17] For the preparation of the extracts, 0.2 g of each sample was weighed and 10 mL of distilled water at 40 °C (chocolate) and 100 °C (cocoa beans) were added to dilute the samples. 0.5 mL was transferred to a 10 mL volumetric flask, and then transferred to test tubes, adding 2.5 mL of 10% Folin-Ciocalteau reagent (Dinâmica Química Contemporânea Ltda., São Paulo, Brazil). The mixture remained at rest for 8 min and after this period 2 mL of 4% sodium carbonate (Dinâmica Química Contemporânea Ltda., São Paulo, Brazil) was added. After incubation for 2 h at 25 °C and protected from light, the absorbance was measured in a spectrophotometer (Quimis, model Q898UV2, São Paulo, Brazil) at 773 nm (chocolate) and 740 nm (cocoa beans). The results of the total phenolic compounds were expressed as epicatechin equivalents (mg EAG g sample).

Determination of antioxidant capacity-radical scavenging test (DPPH)

The determination of the antioxidant capacity by the radical scavenging test of samples of cocoa beans (unroasting and roasting) and chocolates was carried

out using 2,2-diphenyl-1-picrylhydrazilyl (DPPH) by the method of Molyneux.[18] The same extract was used for this analysis as for the total phenolics. 100 μL of the extract of the samples were transferred to test tubes containing 3.9 mL of the ethanolic solution of the DPPH radical (0.004%) (Vetec, Rio de Janeiro, Brazil). The mixtures were stored away from light for 30 min at room temperature (± 25 °C). The free radical scavenging capacity was then evaluated by measuring the absorbance at 517 nm in a spectrophotometer (Quimis, model Q898UV2, Diadema, São Paulo, Brazil).

Determination of *in vitro* antioxidant capacity by the co-oxidation method of the β-carotene:linoleic acid (BCAL) system

The analysis method used for antioxidant evaluation by the β-carotene:linoleic acid system in the samples was described by Miller.[19] For this analysis, a β-carotene reactive mixture (Sigma-Aldrich, St. Louis, MO, USA) was prepared, presenting absorbance between 0.6 and 0.7 at 470 nm, measured in a spectrophotometer (Quimis, model Q898UV2, São Paulo, Brazil). Another reactive mixture was prepared in a similar way with the exception of the addition of β-carotene solution for the blank. For the preparation of the extracts of the almond and chocolate samples, 0.2 g of each sample was weighed and diluted in 10 mL of distilled water. Then, aliquots of 5 mL of the emulsion were mixed with 0.5 mL of the diluted extracts and the absorbances were read immediately at 470 nm, performed in triplicate. After the first reading, the tubes were incubated at 50 °C to favor the oxidation reaction, and at fifteen-minute intervals until 120 min were completed, the reading was repeated. The results were expressed as percentage of oxidation inhibition.

Determination of condensed tannins-vanillin method

The determination of condensed tannins was carried out using the vanillin method and the methodology of Tiitto-Julkunem[20] was followed. The results were expressed in mg of catechin *per* 100 g of sample on a dry basis.

Mid-infrared (MIR) spectroscopy analysis

The spectra of the samples were obtained by Fourier transform infrared spectroscopy through attenuated total reflection (FTIR-ATR) in a Cary 630 FTIR equipment (Agilent Technologies Inc., Santa Clara, CA, USA). Before each scan, a background spectrum reading was performed. Then, 0.5 g of the samples were placed individually on the accessory compartment where the rays in the infrared range (diamond crystal) hit, obtaining the spectra in the absorbance mode. During the analysis, the room temperature was maintained at around 18 °C. All repetitions of each sample were evaluated in the spectral region with a wavenumber from 4000 to 600 cm^{-1}. The maximum absorbances were used as variables for statistical analysis and subsequent comparison of their spectroscopic profile. Three measures were obtained for each sample and the average of these spectra was used for the statistical analysis.

Statistical analysis

The results of the analytical measures to evaluate the effect of roasting cocoa beans were subjected to analysis of variance and *F* test at 5% significance. The results of the analytical measures of the chocolates to evaluate the effect of roasting and the concentration of cocoa mass were submitted to analysis of variance and regression at 5% significance. For the analysis of the spectra obtained by MIR, after the identification of the main peaks (maximal absorbance), they were related to the functional groups with literature data and the spectra obtained were organized in numerical data sets and the principal component analysis (PCA) was performed. In PCA a new coordinate system was created with orthogonal axes, giving rise to the principal components (PC). The criterion for choosing the number of principal components was one of the interpretable factors that determines that number, which together retain a proportion of more than 70% of the variance. All analyses were performed using the Statistical Analysis System (SAS) statistical software program, Student version 9.1.[21]

Results and Discussion

Preliminary test for defining the roasting temperature

The maximum absorbance obtained in the MIR of the samples of cocoa beans roasted at different temperatures was associated with their wavenumber range and was used as variables for the statistical analysis. With this test, it was found that cocoa beans roasted at 110 °C had characteristics closer to unroasted cocoa beans. Therefore, because it is a milder temperature, with less loss of phenolic compounds, the temperature of 110 °C was chosen to be used in the roasting of cocoa beans for later production of chocolates.

Chemical composition, physicochemical parameters, content of bioactive compounds and antioxidant activity of cocoa beans and chocolates

The results found for the analysis of unroasted and roasted cocoa beans are presented in Table 1.

No significant differences ($P < 0.05$) were observed between samples of unroasted and roasted cocoa beans for ash and total sugar content. In the variables that showed differences, all mean results for unroasted cocoa beans were higher. The reduction of water and volatile acids present in cocoa beans during the roasting process can be explained by the volatility of these components, at higher temperatures such as that used in the roasting process. This acidity was reduced by half, a very important technological parameter that can directly impact the acceptance of the cocoa-derived product. Leite *et al.*,[22] in an analysis of the composition of unroasted cocoa beans, found values close to those of the present study, where the moisture value was 6.95%, pH of 5.2 and total acidity of 8.76 meqNaOH *per* 100 g.

The decrease in protein and reduced sugar content in roasted cocoa beans can be explained by the Maillard reaction that starts when the food is subjected to high temperatures, with the condensation of the reducing sugar carbonyl group with the amino group free of amino acids, peptides or proteins. In the intermediate phase of the Maillard reaction, the degradation of ketosamines occurs, in which the reducing power is developed, causing a decrease in pH, which was observed.[23]

The antioxidant activity (DPPH and BCAL) and the catechin content showed no significant difference ($P < 0.05$) between unroasted and roasted cocoa beans (Table 1). As for the other parameters analyzed, the content of total phenolics, theobromine, caffeine, epicatechin and condensed tannins, there was a significant difference ($P > 0.05$) with a decrease in values after roasting.

These results indicate that the roasting process of these cocoa beans, even when using a mild temperature, influenced their characteristics, both in their composition and physicochemical parameters, as well as for bioactive compounds. These changes in the levels of phenolic compounds can be explained due to the various reactions that occur at this stage, as is the example of the non-enzymatic oxidation of these compounds to *o*-quinones, which may result in the condensation of oxidized compounds with other polyphenols, proteins and polysaccharides into substances highly polymerized. These reactions degrade and form new compounds, giving rise to the characteristic aroma and flavor of chocolate.[10]

According to Kothe *et al.*,[24] in addition to the occurrence of the monomer epimerization reaction

Table 1. Mean values and standard deviations of physicochemical, composition, bioactive compounds content and antioxidant activity of samples of unroasted and roasted cocoa beans

Variable	Cocoa beans	
	Unroasted	Roasted
Physicochemical and composition		
pH	5.15 ± 0.02[a]	5.05 ± 0.01[b]
Acidity / (meqNaOH *per* 100 g)	12.05 ± 1.07[a]	5.72 ± 1.73[b]
Moisture / %	6.29 ± 0.15[a]	3.50 ± 0.10[b]
Ash / %	2.67 ± 0.08[a]	2.61 ± 0.07[a]
Fat / %	42.21 ± 1.38[a]	34.21 ± 1.11[b]
Protein / %	14.36 ± 0.34[a]	13.55 ± 0.12[b]
Total sugar / %	12.39 ± 2.95[a]	13.89 ± 1.19[a]
Reducing sugar / %	1.12 ± 0.062[a]	0.81 ± 0.063[b]
Bioactive compounds and antioxidant activity		
Total phenolics / (mg *per* 100 g)	450.77 ± 11.22[a]	398.47 ± 12.44[b]
Theobromine / (mg *per* 100 g)	3249.36 ± 128.87[a]	2433.37 ± 177.69[b]
Caffeine / (mg *per* 100 g)	272.55 ± 11.10[a]	224.18 ± 4.77[b]
Catechin / (mg *per* 100 g)	34.47 ± 0.82[a]	38.43 ± 2.78[a]
Epicatechin / (mg *per* 100 g)	466.51 ± 36.63[a]	292.58 ± 17.78[b]
Condensed tannins / (mg *per* 100 g)	9179.2 ± 366.66[a]	7072.1 ± 496.18[b]
DPPH / %	44.98 ± 4.65[a]	44.35 ± 0.15[a]
BCAL / %	91.96 ± 3.65[a]	90.58 ± 2.62[a]

[a,b]Results followed by the same letter in line do not differ ($P < 0.05$) by the *F* test. DPPH: 2,2-diphenyl-1-picrylhydrazilyl; BCAL: β-carotene:linoleic acid.

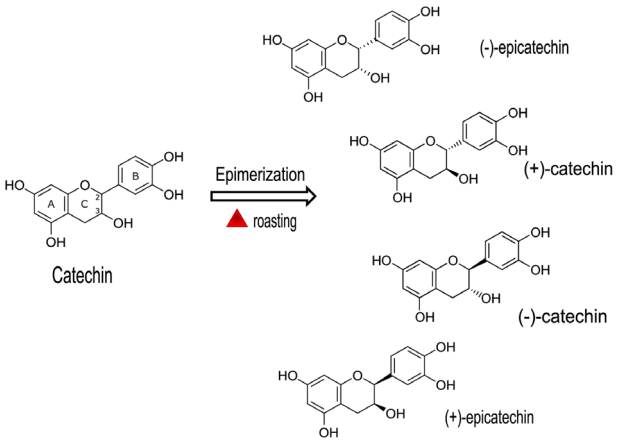

Figure 1. Compounds formed by the epimerization reaction of catechin during roasting.

during the roasting process of cocoa beans (Figure 1), the epimerization of procyanidin dimers (condensed tannins) also occurs, resulting in a reduction in the content found. These procyanidins are formed by catechin and epicatechin molecules, making them also susceptible to the epimerization reaction during roasting, concluding that the higher the roasting temperature, the more accelerated the epimerization, influencing the flavanol profile.

It is believed that the main process that promotes changes in the roasting stage is the Maillard reaction. In the intermediate phase of this reaction, the decomposition of ketosamines occurs, forming unsaturated α-dicarbonyl compounds or reductones. These compounds have reducing power and act as antioxidants.[25] This may explain why even with the loss of phenolics in roasted cocoa beans they maintain the same antioxidant activity as raw cocoa beans.

Despite finding a significant difference for the chemical and physical-chemical composition of cocoa beans, the same was not observed for the composition of chocolates, where there was no significant difference ($P > 0.05$) for the type of beans used (Table 2), that is, there was no difference between chocolates produced with unroasted and roasted cocoa beans in relation to the analysis of composition and physicochemical parameters in all analyzed formulations, being possible to affirm that the roasting process did not influence these variables. Influences on this process were verified in the levels of bioactive compounds that will be shown later in Figure 2.

The Maillard reaction will also occur during the refining and shelling process, where the mixture is subjected to a temperature between 60 and 70 °C for 24 h. This binomial time and temperature can be enough for the unroasted sample to achieve the same effects as the roasted samples. This result could also have been achieved by the addition of other ingredients (sugar, milk powder, for example) in the production of chocolate, where these differences in the type of cocoa beans were reduced by the composition of these ingredients. These possibilities make the removal of the roasting stage an interesting option, being an economy for the industry since it does not influence the chemical composition of chocolates.

For the concentration of cocoa mass in the chocolates, significant differences ($P < 0.05$) were observed in the composition and in the physicochemical parameters and a linear model was fitted. Table 3 describes the minimum and

Table 2. Mean values and standard deviations of physicochemical and composition of different chocolate formulations (40, 50, 60 and 70%) produced with roasted and unroasted cocoa beans

Parameter	Chocolate	Formulation			
		40%	50%	60%	70%
pH	unroasted	6.24 ± 0.17[a]	6.13 ± 0.10[a]	6.02 ± 0.15[a]	5.98 ± 0.09[a]
	roasted	6.21 ± 0.06[a]	6.06 ± 0.03[a]	5.95 ± 0.02[a]	5.84 ± 0.13[a]
Acidity / (meqNaOH *per* 100 g)	unroasted	4.51 ± 2.24[a]	3.89 ± 0.28[a]	4.92 ± 0.03[a]	5.51 ± 0.90[a]
	roasted	3.07 ± 0.35[a]	4.07 ± 0.27[a]	4.99 ± 0.17[a]	5.79 ± 0.23[a]
Moisture / %	unroasted	1.19 ± 0.16[a]	1.66 ± 0.06[a]	1.97 ± 0.03[a]	2.67 ± 0.17[a]
	roasted	1.04 ± 0.16[a]	1.37 ± 0.25[a]	1.88 ± 0.22[a]	2.24 ± 0.28[a]
Ash / %	unroasted	1.32 ± 0.06[a]	1.56 ± 0.03[a]	1.81 ± 0.05[a]	2.05 ± 0.05[a]
	roasted	1.32 ± 0.05[a]	1.57 ± 0.04[a]	1.83 ± 0.01[a]	2.14 ± 0.06[a]
Fat / %	unroasted	32.33 ± 0.79[a]	32.51 ± 0.89[a]	33.15 ± 0.89[a]	32.63 ± 1.16[a]
	roasted	32.46 ± 1.22[a]	32.32 ± 1.14[a]	31.39 ± 0.92[a]	31.83 ± 0.52[a]
Protein / %	unroasted	7.66 ± 0.15[a]	9.17 ± 0.27[a]	10.73 ± 0.14[a]	12.54 ± 0.10[a]
	roasted	7.66 ± 1.11[a]	9.22 ± 0.26[a]	10.98 ± 0.33[a]	12.70 ± 0.14[a]
Total sugar / %	unroasted	68.85 ± 2.02[a]	53.75 ± 2.27[a]	48.37 ± 3.66[a]	34.87 ± 1.11[a]
	roasted	69.90 ± 4.31[a]	53.23 ± 2.48[a]	44.36 ± 3.86[a]	34.86 ± 4.18[a]
Reducing sugar / %	unroasted	3.16 ± 0.37[a]	3.97 ± 0.26[a]	4.02 ± 0.34[a]	4.94 ± 0.67[a]
	roasted	3.18 ± 0.72[a]	3.51 ± 0.62[a]	4.00 ± 0.21[a]	4.71 ± 0.22[a]

[a]Results followed by the same letter in column do not differ ($P < 0.05$) by the *F* test.

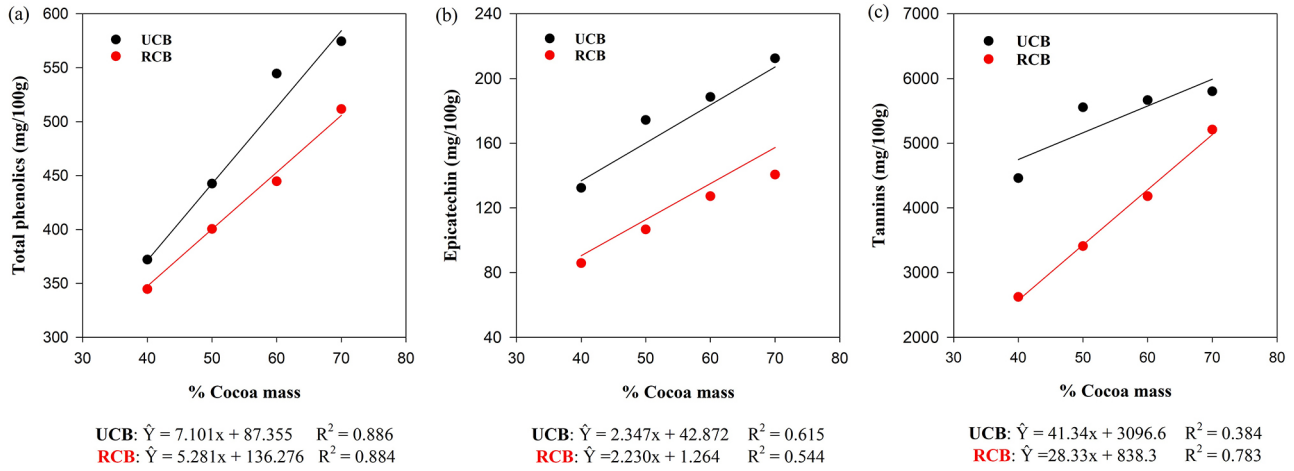

Figure 2. Effect of cocoa mass concentration in chocolates made with unroasted and roasted cocoa beans (a) total phenolic compounds, (b) epicatechin and (c) tannins. Fitted model and coefficient of determination (R^2). Experimental data (•) and model data (—). UCB: unroasted cocoa beans; RCB: roasted cocoa beans.

maximum values, the equations obtained by the regression analysis and the coefficient of determination (R^2) observed for the chocolates. We chose to adjust a single equation of these parameters for the chocolate samples with roasted and unroasted cocoa beans, as there was no significant effect for roasting.

There was an effect of the different percentages of cocoa mass in the chocolates for the acidity, moisture, ash, fat, protein and reducing sugar contents with a linear increasing behavior, that is, the high the cocoa mass in the formulations, the high these variables. For pH and total sugar values, a linear decreasing behavior was observed, with a reduction as the cocoa mass increased.

The behavior of these variables, regardless of the type of roast, can be explained by the formulations used. As the percentage of cocoa mass in production increased, less sugar was added and, consequently, there was an increase in the values of the other components present, especially cocoa beans.

The data obtained by HPLC of bioactive compounds of interest in chocolates were subjected to analysis of variance and regression, observing a significant difference ($P < 0.05$) between chocolates produced with roasted and unroasted cocoa beans in relation to the parameters of phenolic compounds total, epicatechin and condensed tannins for all formulations analyzed (Figure 2). It was not possible to quantify the catechin in any of the chocolates produced, and it can be considered that the small amount of catechin present in cocoa beans both roasted and unroasted was lost due to the binomial time/temperature, agitation, refining and conching process during the chocolate production process.

Flavonoids, catechin, epicatechin and procyanidins (condensed tannins), are compounds considered to be

Table 3. Composition and physicochemical parameters of different chocolate formulations produced from unroasted and roasted cocoa beans (minimum values, maximum values, adjusted regression equations and coefficients of determination (R^2))

Variable	Chocolates Unroasted cocoa beans/Roasted		
	Min.-Max.	Estimated equations	R^2
pH	5.83-6.35	$\hat{Y} = -69.2614x + 474.2623$	0.7330
Acidity[a] / (meqNaOH *per* 100 g)	3.07-6.03	$\hat{Y} = 10.9431x + 5.1631$	0.9812
Moisture / %	1.10-2.50	$\hat{Y} = 21.8999x + 16.6157$	0.9710
Ash / %	1.30-2.13	$\hat{Y} = 38.4625x - 10.3852$	0.9956
Fat / %	29.77-47.43	$\hat{Y} = 0.47703x + 13.1655$	0.8400
Protein / %	7.00-12.63	$\hat{Y} = 5.9132x - 4.6250$	0.9778
Total sugar / %	32.03-72.18	$\hat{Y} = -0.8570x + 98.7275$	0.9484
Reducing sugar / %	2.61-5.30	$\hat{Y} = 14.7599x - 3.1068$	0.7695

[a]Milliequivalents of NaOH solution (0.1 mol L^{-1}) *per* 100 g of product. $\hat{Y}$: response variable, x: values obtained in the analytical measures to generate the response variable.

thermally unstable,[24] which may explain the results found, since part of these compounds is degraded when subjected to higher temperatures. Chocolates produced from roasted cocoa beans were characterized by a lower content of epicatechin and condensed tannins compared to those produced with unroasted cocoa beans, leading to a reduction in the content of total phenolic compounds.

Mudenuti *et al.*[11] in a study with chocolates made from unroasted and roasted cocoa beans (100 °C) observed that chocolates produced with unroasted cocoa beans were characterized by a higher polyphenol content compared to chocolates made with roasted cocoa beans.

One of the most valued properties of chocolates is their antioxidant activity. The analyses showed that antioxidant activity (DPPH and BCAL), theobromine and caffeine contents were not influenced ($P > 0.05$) by roasting in any of the formulations (Figure 3).

The results showed that even with the reduction of the content of phenolic compounds in the chocolates produced, the values of antioxidant activity were not affected, a similar behavior observed for the results in Table 1 for cocoa beans (unroasted and roasted). Due to these aspects, it is not necessary to carry out the roasting step, leading to a reduction in production costs.

The effect of different percentages of cocoa mass on the content of total phenolic compounds, antioxidant activity (DPPH and BCAL), epicatechin and methylxanthines (theobromine and caffeine) of chocolate formulations was evaluated using regression analysis. The behavior of this analysis is also represented in the graphs in Figures 2 and 3.

All parameters showed an increasing linear behavior in relation to the increase in cocoa mass, that is, the high the cocoa mass content in the chocolates, the high antioxidant activity (DPPH and BCAL) and phenolic compounds,

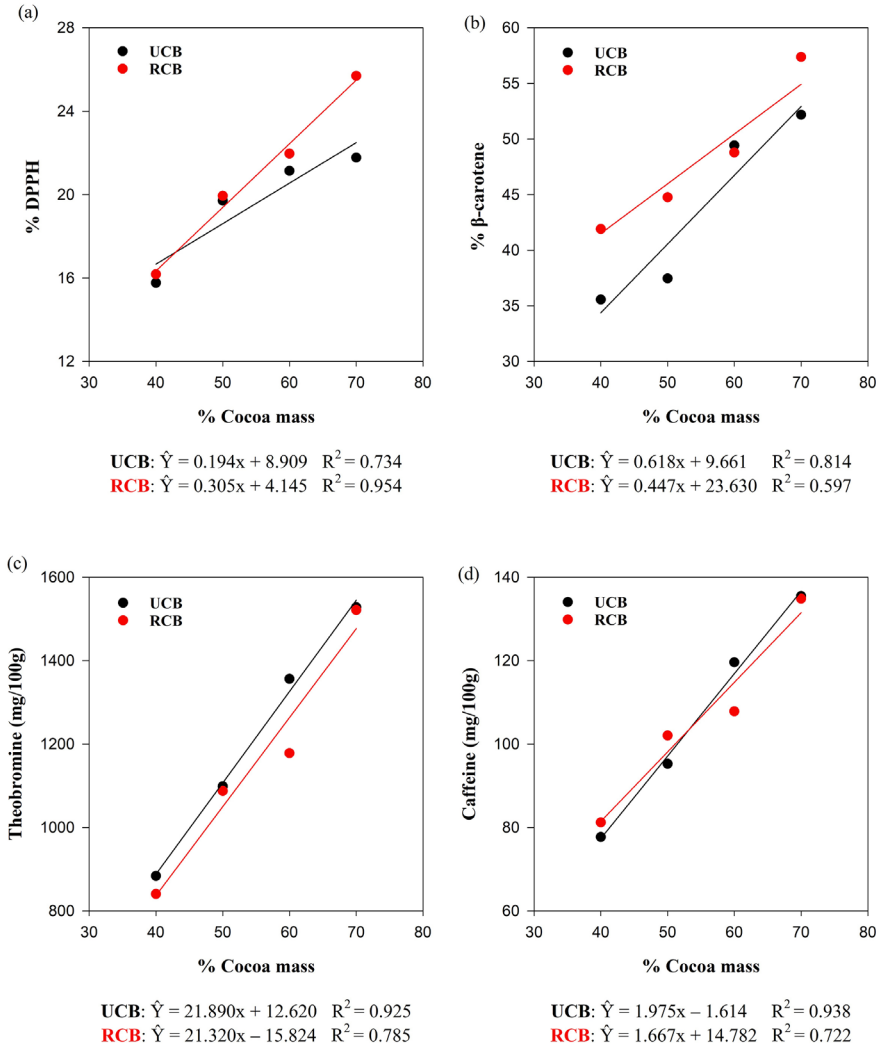

Figure 3. Effect of cocoa mass concentration in chocolates made with unroasted and roasted cocoa beans (a) DPPH, (b) BCAL, (c) theobromine and (d) caffeine, fitted model and coefficient of determination (R^2). Experimental data (•) and model data (—). UCB: unroasted cocoa beans; RCB: roasted cocoa beans.

theobromine, caffeine, epicatechin and condensed tannins.

In the present study, for the formulation of chocolate with a lower percentage of cocoa mass (40%) there was an epicatechin value of 132.35 mg *per* 100 g for chocolates made with unroasted cocoa beans and 85.81 mg *per* 100 g for chocolates made with roasted cocoa beans. These values are lower than those found by Żyżelewicz *et al.*[10] in their study with chocolates produced with unroasted and toasted cocoa beans, but with the same reduction behavior of this compound. For the caffeine content, the same authors found higher values in relation to the present study and a similar result to that study for the effect of roasting on this component.

Variations observed between values can be explained by differences in the composition of cocoa beans, which depend on the variety of cocoa used, degree of fruit maturation, place of cultivation, climatic conditions, its processing process and the variation in the method used for the quantification of these compounds.[3]

Assessing the levels of bioactive compounds present in unroasted cocoa beans (Table 1) with the maximum values found in chocolates produced with these beans (Figures 2 and 3), there was a reduction of approximately 50% of these values, revealing the influence of the production process on chocolates. In addition to having the content of these compounds reduced due to the addition of other ingredients, the temperature of the production process rose (approximately 55 °C) during the refining and shelling step with the presence of oxygen, increasing the oxidative activity of the polyphenols.

The more these compounds are preserved in cocoa beans, the better source of bioactive compounds chocolate will be. According to Żyżelewicz *et al.*[10] in all stages of the chocolate production process, cocoa bean polyphenols can undergo many changes, including polymerization, hydrolysis or reactions with proteins, and the Maillard reaction may also occur. Thus, if the roasting step is eliminated from this chocolate production process, a higher content of these bioactive compounds will be preserved.

Spectra obtained by mid infrared spectroscopy (MIR)

The spectra obtained by MIR showed absorption bands with similar characteristics for unroasted and roasted cocoa beans (Figure 4), this same profile was found by Villanueva *et al.*[26] Eighteen main absorption bands were identified referring to the vibrational modes of different functional groups present in the samples, associated with the wavenumber range of 3281.97-716.80 cm^{-1}, but with differences in the band intensities between the analyzed samples.

In Figure 4b are the spectra of the cocoa bean samples, revealing that after roasting there was a decrease in the intensity of the bands 1634 and 1544 cm^{-1} (C=C vibrations), 1379 cm^{-1} (CH_3 vibrations), 1248 cm^{-1} (CO vibrations), 1167 cm^{-1} (CH vibrations), all of them associated with the binding of the aromatic ring referring to the phenol group of phenolic compounds.[27,28]

The spectral characteristics of the chocolates allowed the identification of different vibrations of functional groups, associated with their composition, with 19 bands being obtained (maximal absorbances, referring to the vibration modes of different function groups present in the samples). It was observed that the chocolate samples

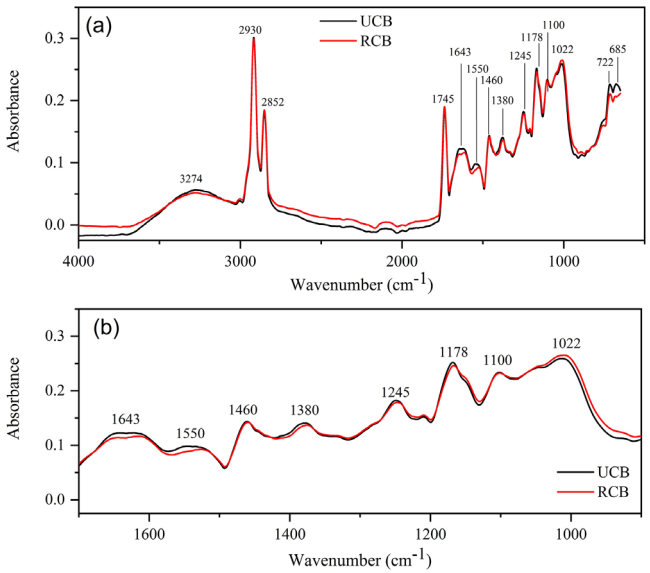

Figure 4. Spectra of unroasted cocoa beans (UCB) and roasted (RCB) obtained by medium infrared spectroscopy (MIR): (a) full spectrum; (b) expansion of the region from 1700-900 cm^{-1}.

showed similar behavior (Figure 5a), with the spectra differing only in the intensities of the bands.

Chocolates produced with unroasted cocoa beans showed higher intensity in all bands in the region between 3556-1104 cm^{-1}, when compared to chocolates produced with roasted cocoa beans. During the roasting process of cocoa beans, phenolic compounds are lost due to the temperature used for this process, which may have resulted in a decrease in the intensity of absorbance in the observed regions.[28]

In Figures 5b and 5c, the magnified spectra of the 1800-1500 and 1200-1140 cm^{-1} regions are shown, respectively. These selected regions belong to the group of phenolic compounds. In the 1800-1500 cm^{-1} region, there are 1734 cm^{-1} (CO vibrations), 1643 cm^{-1} (CC vibrations) and 1518 cm^{-1} (C=C vibrations) bands associated with an aromatic ring referring to phenolic compounds. In the 1200-1140 cm^{-1} region it was possible to identify the 1178 cm^{-1} band (CH vibration) which is associated with the phenol group.[12]

The 1080-980 cm^{-1} region corresponds to the vibrations O–H/C–O/C–C/C–O–C, which is associated with carbohydrates, noting that in this region the bands of the different chocolate formulations from unroasted and roasted cocoa beans overlap, probably due to the use of the same amount of sugar for the formulations.[13,28,29]

The spectra obtained from the chocolates were analyzed by PCA. The first two components explained 97.73% of the total variance for the formulations, in which PC1 explained 94.01% and PC2 3.72%. The data distribution was better represented by PC1, where the chocolates were separated according to the type of cocoa bean used in their production, forming two distinct groups, verified in the data dispersion (Figure 5d).

Chocolates produced with roasted cocoa beans, regardless of cocoa mass concentration, showed strong

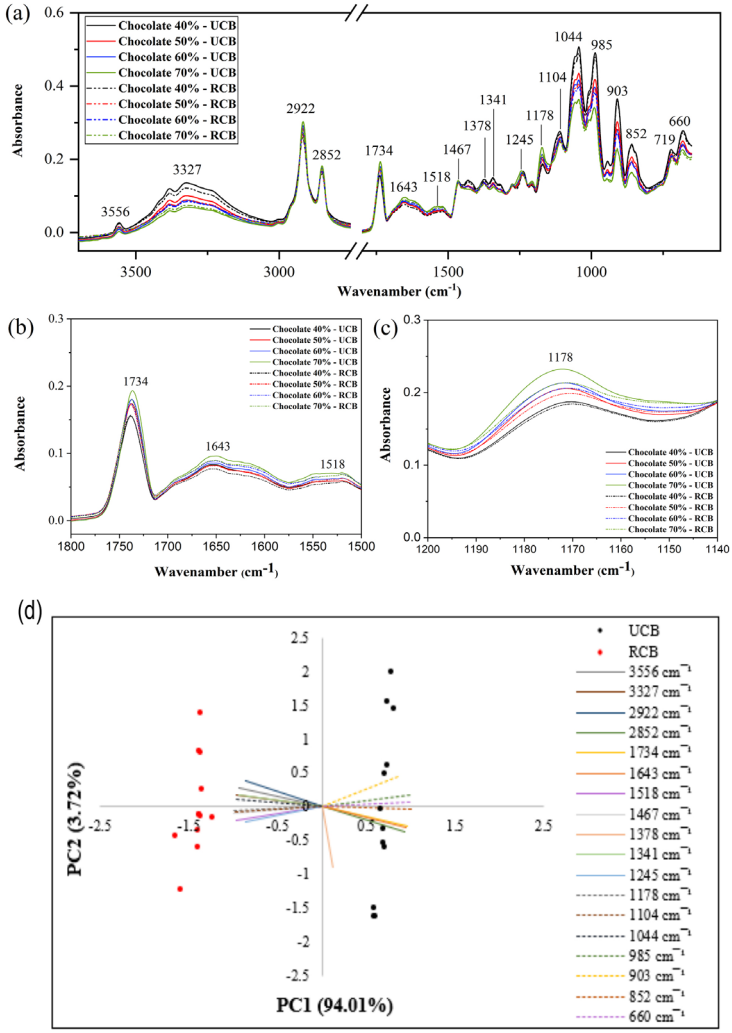

Figure 5. Spectra of chocolate formulations produced with unroasted cocoa beans (UCB) and roasted (RCB) obtained by medium infrared spectra: (a) full spectrum; (b) expansion of the region from 1800-1500 cm^{-1}; (c) expansion of the region from 1200-1140 cm^{-1}; (d) score graph of samples of chocolates produced from unroasted and roasted cocoa beans in relation to the principal components PC1 and PC2.

similarity, being grouped on the negative side of PC1, and chocolates produced with unroasted beans grouped on the opposite side. PC1 was negatively correlated with the wavenumbers 3327 cm^{-1} (OH), 2922 cm^{-1} (CH), 1518 cm^{-1} (C=C), 1467, 1341, 1245, 1178, 1104 and 1044 cm^{-1} (CH, C=O) associated with lipids, protein and phenolic compounds, positively with the wavenumbers 2852 cm^{-1} (CH), 1734 cm^{-1} (CO), 1643 cm^{-1} (C=O), 985, 903, 852 and 660 cm^{-1} (OH, CO, C=C/C-OC), associated with lipids, proteins, carbohydrates and phenolic compounds. PC2 was negatively correlated with the wavenumber 1378 cm^{-1} (CH) associated with lipids.

These results suggest that the use of MIR associated with a multivariate analysis is a good alternative to differentiate chocolate samples produced from unroasted and roasted cocoa beans, allowing a quick and reliable evaluation of the analyzed parameters. In addition to being a new, promising technique with few studies in the literature on its use for this purpose.

Conclusions

In the analyzed chocolates, there was no difference in composition, physicochemical parameters, antioxidant activity (DPPH and BCAL) and theobromine and caffeine contents in relation to the type of cocoa beans (unroasting or roasting) used in production.

For the contents of total phenolic compounds, epicatechin and procyanidins, there was a difference between chocolates produced with unroasted and roasted cocoa beans, where those produced with unroasted cocoa beans were characterized by higher values of these parameters compared to those produced with roasted cocoa beans.

The increase in cocoa mass in the production of chocolates affected its composition. As the percentage of cocoa mass increased, regardless of the type of bean used, the content of phenolic compounds and methylxanthines increased, making the formulation with 70% of cocoa mass the richest in relation to these compounds.

There was a significant loss after roasting of important compounds, such as total phenolics, epicatechin and tannins. However, the main functionality of these compounds was not changed in cocoa and chocolate, in both products there was no effect of the roasting treatment on the antioxidant activity. This shows that this step can be removed, avoiding nutritional losses, saving production time, saving the producer and without harming the health of the consumer, who will be consuming a product rich in polyphenols.

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Author Contributions

Rebeca R. V. Onelli was responsible for the formal analysis, data curation, investigation, methodology, project administration and writing original draft; Josane C. de Jesus, Lucas C. C. Reis and Isabel C. S. Alves for the data curation and investigation; Leandro S. Santos for the conceptualization, formal analysis and supervision; Sibelli P. B. Ferrão for the conceptualization, supervision, project administration and writing review and editing.

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