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Biochemical compounds and structure evaluation of cocoa liquors from different origins and their derivative chocolates

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Abstract

Cocoa (*Theobroma cacao* L.) varieties have distinct characteristics that interfere with the quality of the chocolate produced. Cocoa liquors from Brazil and Peru were analyzed for bioactive compounds and physicochemical values, and dark chocolates were produced, evaluating their physical and physicochemical properties. Brazilian organic liquor showed higher phenolic content (4,403.63 mg GAE/100 g sample) and antioxidant activity (143.56 μg Trolox equivalent/g sample). Peruvian liquor cocoa butter has more palmitic acid (29.30%) and less stearic acid (32.92%), in the lower limit to oleic acid (32.92%) and the upper limit to linoleic acid (3.73%), and showed a higher content of saturated fatty acids (63.35%) and a lower content of monounsaturated acids (32.92%). Cocoa butter from organic liquor presented lower content of palmitic acid (24.01%), average stearic (34.71%) and oleic acids (35.81%), and higher content of linoleic acid (4.32%) and polyunsaturated acids (4.32%). Peruvian liquor showed higher melting point. Brazilian alkalized, organic liquors, and chocolates melting points showed no statistical difference ($p > 0.05$). The caramelization and carbonization points of all liquors did not show statistical differences ($p > 0.05$), as well as the caramelization point of all chocolates. The carbonization point was different ($p < 0.05$) for all chocolates. The chocolates were stable in terms of structure during storage, demonstrating the suitability for industrial production.

Keywords: biocompounds; rheology; thermal analysis; texture; nutrition.

Practical Application: This work has practical importance in comparing bioactive compounds and physicochemical properties of some types of national cocoa liquors and Peruvian cocoa liquor, helping the industry and users when choosing which type offers the best nutritional delivery.

1 INTRODUCTION

The pulp is obtained from whole cocoa, an ingredient in the manufacture of foods such as jellies, juices, and yogurts; the cocoa beans and the husk can be used for the production of organic fertilizer. Cocoa liquor is the paste obtained from ground, roasted, peeled, and fermented cocoa beans (commonly called "nibs") and consists of defatted cocoa solids and cocoa butter. The processing of alkalized liquor is carried out in a different way from the organic one, which produces variations in its characteristics and consequently in the derived chocolates. Chocolates made with alkalized liquor can be darker than those made with organic liquor. Much has been focused on bean-to-bar processing to improve the quality of chocolate produced with understanding and monitoring of the entire production chain (Lannes, 2017).

Cocoa is a natural source of phenolic compounds, substances that act as antioxidants and are capable of reducing or delaying oxidative damage from free radicals. Among these compounds, the tannins (procyanidins) and flavonoids (epicatechin and catechin flavanols) stand out.

In foods such as chocolate and red wine, the flavonoids most commonly found are the flavanols (flavan-3-ols) (-)-epicatechin and (+)-catechin monomers, which can condense to form condensed tannins (or proanthocyanidins) called procyanidins. They have beneficial properties such as cardioprotection, anti-inflammation, and protection for the endothelium (Bühler AG, 2011; Lannes, 2017), in addition to being associated with the development of color and flavor in chocolate (Zoumas et al., 2000).

The flavonoid content in chocolate can be reduced if milk or some protein is added to it, due to the formation of complexes between polyphenols and proteins. In addition, like the content of other phenolic compounds, these levels are sensitive to high temperatures (Richter & Lannes, 2007) and dependent on the pre- and post-harvest conditions of the fruits and grains (Lannes, 2017) (in addition to their intrinsic factors). In this way, the production chain must take into account several precautions to preserve the natural nutritional properties of cocoa in the final products.

Due to its high fat content and incomparable aroma, Peruvian cocoa has high chances of becoming a first choice product among world markets and consumers (Peru, 2007). Peru has its own native cocoa varieties that make possible the growing prominence of Peruvian cocoa exports. The country relies on

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its national identity, biodiversity, and ancestral traditions in which quality and high quantity cocoa production was possible, boosting the social and economic growth of the population (De Pereny, 2015). It is speculated that Peruvian cocoa has a market both for its seeds and butter, as well as for cocoa liquor/paste, bark, and even the fruit in its entirety (Peru, 2007). Major research interests and emerging technologies are helpful for the origin differentiation of cocoa quality (Hernandez & Granado, 2021). Jeri et al. (2023) observed the morphological and sensory attributes of native cocoa in the Bagua Province, Peru, and found the characteristics of the bean were predominantly sweet.

The relevance of this study was to verify the existing differences between cocoa liquors from different origins in relation to their biocompounds and application in dark chocolate formulation. Knowing how to work with chocolate formulations and identifying their best characteristics for consumption were the challenges.

The objective of this work was to produce dark chocolate with cocoa from different origins and to evaluate the physical and physicochemical properties and phenolic compounds in order to differentiate their characteristics.

2 MATERIALS AND METHODS

2.1 Materials

Brazilian liquors, alkalinized and natural (Cargill, Bahia, Brazil) and organic (IBC-Indústria Brasileira de Cacau, Rio das Pedras-SP, Brazil), and Peruvian — Trinitário variety (Puno, Peru), were evaluated. Chocolates (57% bitter) were produced with Brazilian liquors (alkalinized and organic) and Peruvian liquor, added with deodorized cocoa butter (Cargill, Bahia, Brazil), refined sugar (União, São Paulo, Brazil), soy lecithin (Tradal, São Paulo, Brazil), and powdered vanillin flavor (Mix, São Paulo, Brazil).

2.2 Methods

2.2.1 Chocolate preparing

Chocolates (57% cocoa) were produced with Brazilian liquors (alkalinized and organic) and Peruvian liquor. Physicochemical and antioxidant compound tests were carried out in the liquors and in the formulated chocolates, as well as tests to verify the structure of the chocolates (thermal, rheological, and texture analyses).

Universal equipment WA-FA 20, series 2872 ball mill (Mazzetti, Italy), with the potential to process 10 kg, the production of standard chocolate, a batch of 3 kg, was processed for 2 h at 45°C. Three batches of chocolates were produced in duplicate. Following the proportions presented in Table 1, the base formulation was used for chocolate with Brazilian alkalized liquor and the recalculated one for the others.

The tempering of the chocolates was lead in the Temperchoco-Universal (Varimaq Equipamentos, Brazil). Soon after pre-crystallization, the chocolates were shaped in polycarbonate molds with a bar mold, cooled at 6°C for 30 min. Then, they were demoulded and dehumidified at room temperature (20–25°C) for 24 h. The following day, the bars were packed with aluminum foil and kept in climatic chambers at 20°C, humidity 60%.

2.2.2 Determination of fatty acids

The method used for lipid extraction and derivatization (AOAC, 2002) was the theoretical response factor of the flame ionization detector (FID) of the AOCS Ce 1h-05 method. The analysis conditions are as follows: GC 17 A Shimadzu/ Class GC 10 gas chromatograph; chromatographic column of fused silica SP-2560 (biscyanopropyl polysiloxane) of 100 m and 0.25 mm of i.d.; column temperature programming: isothermal at 140°C for 5 min and then heating at 4°C/min up to 240°C, remaining at this temperature for 30 min; vaporizer temperature: 250°C; detector temperature: 260°C; carrier gas: helium (1 mL/min); and sample split ratio: 1/100.

2.2.3 Extract preparation

The extract was prepared following the methodology described by Genovese and Lannes (2009), in which, using a ultra turrax (Marconi, Brazil), 0.5 g of the sample was added to 20 mL of methanol/water in the ratio 70:30 for 1 min at speed 4 and ice bath. The extract was filtered using filter paper and the resulting extract was stored in amber glass.

2.2.4 Total phenolics determination

From extracts in methanol-water solution (70:30) of liquors and chocolates, a 0.25 mL aliquot of the extract obtained was mixed with 0.25 mL of the Folin-Ciocalteu reagent and 2 mL of distillated water. After 3 min at room temperature, 0.25 mL of a saturated sodium carbonate (Na_2CO_3) solution was added, and the mixture was placed at 37°C in a water bath for 30 min. The absorbance was measured at 750 nm using a model Ultrospec 2000 UV/visible spectrophotometer (Amersham Biosciences, Cambridge, U.K.). Gallic acid was used as the reference standard, and the results were expressed as mg of gallic acid equivalent values for total phenolic content (mg GAE/g sample). Five dilutions of gallic acid were prepared as a standard solution, and determinations were performed in triplicate and with a blank (without gallic acid). The absorbance value was obtained from the complex formed between the flavonoid and aluminum of the Folin-Ciocalteu reagent (which turned the solution yellow). The analysis was performed according to Genovese and Lannes (2009) and Singleton et al. (1999), with some modifications.

Table 1. Dark chocolate (57%) formulations.

Ingredients	Based formulation (%)	Recalculated formulation (%)
Brazilian alkalized cocoa liquor	47.0	-
Brazilian organic cocoa powder	-	42.0
Trinitario Peruvian cocoa liquor	-	42.0
Refined sugar	42.3	42.3
Deodorized cocoa butter	10.0	15.0
Soy lecithin	0.5	0.5
Vanillin powder flavor	0.2	0.2

2.2.5 DPPH radical scavenging capacity

From extracts in methanol-water solution (70:30) of liquors and chocolates, the values of antioxidant capacity per DPPH radical (μmol Trolox equivalent/g product) were obtained. Trolox is a water-soluble analog of vitamin E and was used as a standard solution, with seven dilutions for the construction of its calibration curve. The anti-radical capacity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method according to the method of Brand-Williams et al. (1995) with some modifications. A 50 μL aliquot of the extract previously diluted and 250 μL of DPPH (0.5 mM) was mixed, and after 20 min the absorbance was measured at 517 nm using a Microplate Spectrophotometer (Benchmark Plus, BioRad, Hercules, CA). The control consisted of a methanolic solution of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at different concentrations. The decrease in absorbance caused by the sample, compared to a blank (50 μL methanol), was related to that of the control. The DPPH scavenging capacity was expressed as μmol Trolox equivalent/g sample in fresh weight (FW). All determinations were performed in triplicate (Genovese & Lannes, 2009).

2.2.6 Ferric reducing antioxidant power

FRAP was measured from extracts in methanol-water solution (70:30) of liquors and chocolates (in µmol ferrous sulfate/100 g) and followed the EMBRAPA methodology (Rufino et al., 2006).

2.2.7 Oxygen radical absorbance capacity

The ORAC assay was conducted on an automated microplate reader (Synergy HT, BIOTEK, Vermont, USA) with 2,2′-azobios(2-amidinopropane) dihydrochloride (AAPH) used as the peroxyl radical generator, Trolox as the standard, and fluorescein as the fluorescent probe (Prior et al., 2003). Final ORAC values were calculated using the net area under the fluorescence decay curves.

2.2.8 Physicochemical determinations

The physicochemical evaluations followed the methodologies of the Association of Official Analytical Chemists (AOAC, 2000) for moisture, ash, proteins, water activity, and pH. Hartman and Lago (1973) and Schetty et al. (1969) performed the base studies for lipid analysis. Analyses were performed in triplicate. The carbohydrate content was obtained by difference. The water activity was measured in a LabMaster Aw equipment (Novasina, Switzerland) using 1.5 g of sample in each determination plate. The pH was determined using a digital potentiometer (Quimis, Diadema, São Paulo, Brazil).

2.2.9 Temper index

The determination of the tempering index (TI) of the chocolates was carried out in a Multitherm TC Tempermeter (Buhler, Uzwil, Switzerland) for the purposes of the texture test (Bühler AG, 2011).

2.2.10 Rheology

The rheology test was carried out in triplicate in a Haake Mars II rheometer (Thermo Scientific, Karlsruhe, Germany) using the Rheowin software, using a cone plate sensor (C35/1 Ti polished) with a gap of 0.024 mm, at 40°C after 10 min of rest and preconditioning of the sample at 55°C for 75 min, based on the official method of the IOCCC. The rotational assay was conducted at a controlled rate in three steps: 0.00–65.00 s−1, t = 180 s; 65.00 s−1, t = 60 s; 65.00–0.00 s−1, t = 180 s.

The amount of sample was sufficient to fill the space between the plates. Data regarding plastic viscosity and yield stress were adjusted to the Casson equation.

2.2.11 Particle size

The particle size of liquors and chocolates was determined with the aid of a Digimatic Mitutoyo (Aurora, Illinois, USA) digital micrometer and paraffin. For more concise verification of the values obtained, each measurement was repeated at least three more times.

2.2.12 Color

The determination of the color spectrum of liqueurs and chocolates was performed with a ColorQuest XE spectrophotometer (HunterLab, USA). The data were analyzed using the EasyMatch QC software (HunterLab, USA). The color parameters L*, a*, and b*, the whiteness index (WI), Equation 1, were obtained in triplicate.

$$WI = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{0.5} \quad (1)$$

2.2.13 Differential scanning calorimetry

Thermal analysis was carried out in a DSC series equipment (Instrument Specialists Incorporated, Twin Lakes, WI, USA), calibrated with an aluminum pan as a reference. Notably, 3.0–6.0 mg of samples were weighed in other aluminum pans, which were later sealed with a lid and heated at a rate of 20°C min^{-1} from 15 to 250°C, in a nitrogen atmosphere (N_2). With the aid of the Acquire program (Instrument Specialists Inc., USA), the heating data were obtained and analyzed by the Infinity Pro-Thermal Analysis software (Instrument Specialists Incorporated, Twin Lakes, WI, USA), which generated the heating curves. It was indicated that the values of melting, caramelization, and carbonization peaks were in triplicate. After calibrating the equipment at a scan rate of 20°C min^{-1} using a sealed aluminum pan with a cap as a reference, samples of approximately 5 mg were loaded into 40 μL pans, sealed with a lid, and heated at 20°C min^{-1} from 15 to 250°C in a nitrogen atmosphere (N_2).

2.2.14 Texture

The texture was measured according to cracking tests of chocolate bars in a TA-XT2 texturometer (Stable Micro Systems, Godalming, UK). Each test was carried out with four replicates in an environment at 25°C. The HDP/3PB probe was used, and

the parameters such as sensitivity (trigger force) of 0.05 N; pre-test, test, and post-test speeds of 2.0 mm s^{-1}; force in compression return to the start model. The "Texture Expert Exceed" software, version 2.64 (Stable Micro System, Godalming, UK), was used to collect and process the data obtained in these three assays.

2.2.15 Statistical analysis

The collected data were computed in Microsoft Excel spreadsheets (Microsoft, USA) and the Minitab 17 Statistical software (Minitab Inc., State College, Pennsylvania, USA) were submitted to one-way statistical analysis of variance (ANOVA), followed by a comparison multiple through the Tukey's test ($p < 0.05$). Means, followed by the same letters, do not differ significantly from each other, according to Tukey's test at the 5% probability level.

3 RESULTS AND DISCUSSION

3.1 Temper index

The melted chocolates were manually tempered for this determination. The TIs found were chocolate with alkaline Brazilian liquor (2.00), chocolate with Peruvian liquor (2.41), and chocolate with Brazilian organic liquor (2.95). The tempering of a melted chocolate ideally marks a TI (or chocolate tempering unit - CTU) in the range of 4.00–6.80 (Afoakwa, 2016); values below characterize no tempering, in which it is easier for the fat crystals to disarrange and assume unstable polymorphic forms; values above characterize an over-tempered chocolate. Excessive tempering adds to the increased hardness, tackiness, darkening, and reduced gloss of product surfaces. In contrast, imperfect tempering induces the appearance of quality defects in color, texture, and surface gloss — the "blooms" ("fat bloom" and "sugar bloom"). In this, it is observed that the chocolates were not fully tempered properly as they should, due to the manual process, and thus have a greater predisposition to suffer some of these structural destabilizations.

3.2 Physicochemical results

The production of chocolate through a ball mill provides advantages when compared to the conventional technique (using refiner rolls), combining conching and refining in a single step, saving time and energy during production. In addition, it is a more hygienic and safer method since it reduces the rate of exposure of food to the environment and prevents exogenous contamination. However, ball mills require higher fat contents in the formulations; consequently, it becomes more difficult to remove moisture and volatile compounds that are usually eliminated during classic conching. In this way, the moisture content of the chocolates produced may be slightly higher than expected (Agibert, Lannes, 2018; Carvalho & Lannes, 2018; Lorenzo et al., 2022; Saputro et al., 2019).

Forte et al. (2023) evaluated cocoa beans' quality profile by NIR spectrometers and found it to be a valuable solution, monitoring ash, shell, fat, protein, total polyphenols, fermentation index, titratable acidity, and pH.

Typically, without affecting the flowability, melted chocolate has a moisture content of 0.50–1.50%, mainly in cocoa solids (Afoakwa, 2016). The seeds gain more moisture as they undergo longer fermentation times. Thus, exacerbated humidity is harmful to the stability and shelf life of chocolates produced with these seeds by influencing their viscosity as well as water activity. The high moisture content in chocolate aggregates the sugar particles, intensifies the interactions between them, forming lumps. These lumps can bind together and accumulate on the surface of the chocolate, thus increasing the chances of sugar bloom, increasing friction, and apparent viscosity. It can be seen from Table 2 that the Peruvian liquor already had a higher moisture content, which can give its derivatives a moisture content above normal. This may have been caused by the inadequate conservation of the liquor, being it exposed to the humidity of the refrigerator without having been properly packed or exposed to room temperature for a long time, or by the eventual manipulation of the chocolate with wet equipment or molds. Chocolates generally have lower moisture contents than liqueurs, as they have been reduced during the conching process (Ndife et al., 2013). In addition to the above factors, tempering, when poorly conducted, can damage the chocolate, giving it a slightly more moisture.

The amount of ash is directly proportional to the content of non-organic compounds, or specifically the minerals in the food. The inorganic nutritional profile varies according to the genetic type of the species and transformation processes such as fermentation. The ash content is higher in liqueurs than in their respective chocolates, generally varying between the ranges of 3.00–6.00 and 1.00–2.00%, respectively.

The lipid content in liquors and chocolates is provided by cocoa butter and varies based on the type of chocolate as well as the type and form of cocoa/liquor used. The Brazilian organic liquor, in powder form, showed a much lower fat content than the others due to most of the butter being removed from cocoa solids (Borchers et al., 2000), which explains the need for extra addition during the production of chocolate. In contrast, the Peruvian liquor already contained a considerable amount of butter inherent in the fruit itself; however, in view of the formulation used, it was still necessary to add extra fat to create compatibility and miscibility between the ingredients, and thus it can be noted that the content of fat in their chocolates was higher (Table 3). Chocolates with high viscosity and more pasty

Table 2. Physicochemical composition of the Brazilian alkalized, Peruvian, and Brazilian organic liquors*.

	Brazilian alkalized liquor	Peruvian liquor	Brazilian organic liquor
Humidity (%)	1.42 ± 0.04^{C}	3.96 ± 0.48^{A}	3.05 ± 0.00^{B}
Ash (%)	5.06 ± 0.02^{B}	$3{,}51 \pm 0.01^{C}$	5.60 ± 0.07^{A}
Lipids (%)	52.12 ± 0.35^{A}	50.48 ± 0.94^{B}	14.78 ± 0.15^{C}
Proteins (%)	11.36 ± 0.33^{C}	15.21 ± 1.04^{B}	17.59 ± 0.09^{A}
Carbohydrates (%)	30.04^{B}	26.84^{B}	58.98^{A}
Energy (kcal/100 g)	634.68^{A}	622.52^{B}	439.30^{C}

*Data on the same line that do not share the same capital letter are significantly different ($p < 0.05$) in Tukey's test. Results are expressed in mean of triplicate ± SD.

presentation require longer melting time, possibly because of their composition, distribution and size of solid particles. In general, liqueurs have a lipid content of around 48–58% while dark chocolates can range from 25 to 35% (Afoakwa et al., 2009). Liquor/cocoa powder may contain variations between 10 and 24% depending on the type and performance of fat extraction (Talbot, 2012). Fat influences several aspects of chocolate, including the amount of carbohydrates.

According to Tables 2 and 3, liquor has a lower amount of carbohydrates compared to dark bitter chocolate, because chocolate contains sugar in the formulation.

In analysis of chocolate proteins, in general, studies indicate values between 5–8%. The three batches of chocolates, as indicated in Table 4, presented a content consistent with the literature. Fermented cocoa liquors usually contain about 15–22% of protein by dry weight (Borchers et al., 2000). The values in chocolates are lower than those presented in liqueurs, due to the dilution of their content in the mixture with the other ingredients.

Water activity is an important parameter to assess the safety of a food, whose value can vary between 0 and 1, and which is related to the amount of free water available in the food. This free water can be used for the metabolism of microorganisms and for carrying out chemical reactions. Thus, it is essential for the food system that this parameter is preferably kept at low values, subject to microbiological growth control. The water activity value for chocolates should be between 0.5 and 0.6 (Lannes, 1997). As observed in Tables 4 and 5, both liquors and dark chocolates indicated water activity values below 0.55, being conducive to safe use and consumption.

The pH of chocolate near neutrality is due to the liquor's inherent less acidity pH. Organic liquor has a pH close to that indicated in its certificate of analysis. Chocolates made with non-alkalinized liquors have more sour and bitter notes, in contrast to chocolates made with alkalized liquors, which have a less astringent taste. The use of alkalized liquor makes it possible to reduce the time for conching, since many volatile compounds have already been eliminated during the processing of this liquor. The reduction in processing time may interfere with the content of phenolic agents, which is expected to be in greater amounts than in longer processing times (Beckett, 2009).

Table 3. Physicochemical composition of the chocolates produced with Brazilian alkalized, Peruvian, and Brazilian organic liquors*.

	Brazilian alkalized liquor chocolate	Peruvian liquor chocolate	Brazilian organic liquor chocolate
Humidity (%)	1.52 ± 0.04^{AB}	1.69 ± 0.10^{A}	1.49 ± 0.07^{B}
Ash (%)	1.23 ± 0.03^{C}	1.57 ± 0.03^{B}	1.89 ± 0.01^{A}
Lipids (%)	32.79 ± 2.6^{B}	37.91 ± 1.65^{A}	36.37 ± 0.21^{AB}
Proteins (%)	8.52 ± 0.69^{A}	5.93 ± 0.08^{B}	7.78 ± 0.22^{A}
Carbohydrates (%)	55.94^{A}	52.90^{A}	52.47^{A}
Energy (kcal/100 g)	552.95^{B}	576.51^{A}	568.33^{AB}

*Data on the same line that do not share the same capital letter are significantly different ($p < 0.05$) in Tukey's test. Results are expressed in mean of triplicate ± SD.

Table 4. Water activity and pH of the Brazilian alkalized, Peruvian, and Brazilian organic liquors*.

	Brazilian alkalized liquor	Peruvian liquor	Brazilian organic liquor
Water activity	0.290 ± 0.008^{C}	0.512 ± 0.007^{A}	0.371 ± 0.01^{B}
pH	6.88 ± 0.03^{A}	6.44 ± 0.03^{B}	5.24 ± 0.03^{C}

*Data on the same line that do not share the same capital letter are significantly different ($p < 0.05$) in Tukey's test. Results are expressed as the mean of triplicates ± SD.

3.3 Fatty acid composition

The fatty acid contents are presented in percentage of the total fatty acids and represent the average of three determinations (Table 6).

According to Lipp and Anklam (1998), who analyzed cocoa butter from some countries (Ecuador, Brazil, Ghana, Ivory Coast, Malaysia, and Java), the fatty acid content may vary among countries as palmitic acid (24.1–25.8%), stearic acid (33.3–37.6%), oleic acid (32.7–36.5%), linoleic acid (2.6–3.5%), linolenic acid (0.1–0.2%), arachidic acid (1.0–1.2%), and behenic acid (0.1–0.2%).

Our findings, as shown in Table 6, show that Peruvian liquor cocoa butter has more palmitic acid and less stearic acid, in the lower limit to oleic acid and upper limit to linoleic acid. Peruvian liquor showed a higher content of saturated fatty acids and lower content of monounsaturated acids. Cocoa butter from organic liquor presented lower content of palmitic acid, average stearic and oleic acids, and higher content of linoleic acid and polyunsaturated acids.

3.4 Analysis of antioxidant capacity

Dark chocolates are expensive for their content of bioactive compounds. Chocolate flavonoids are thermolabile and are therefore destroyed during the thermal processes and other common conditions of the production chain, from the cocoa harvest to the manufacture of the product (Lannes, 2017; Richter & Lannes, 2007). They were also reduced with the passage of time and by the industrial treatments. To compare the contents between Peruvian and Brazilian organic liquors, two main considerations should be taken into account: the Peruvian liquor was stored in a climatic chamber for a short time and under refrigeration for a long time than the organic liquor, which remained preserved in a freezer and the analysis of the antioxidants was carried out after a long time from the production of chocolates with Peruvian liquor (and Brazilian alkalized), while for chocolates with organic liquor they were in a shorter and

Table 5. Water activity and pH of the chocolates produced with Brazilian alkalized, Peruvian, and Brazilian organic liquors*.

	Brazilian alkalized liquor chocolate	Peruvian liquor chocolate	Brazilian organic liquor chocolate
Water activity	0.414 ± 0.017^{A}	0.388 ± 0.017^{AB}	0.377 ± 0.004^{B}
pH	7.11 ± 0.04^{A}	6.25 ± 0.06^{B}	5.53 ± 0.03^{C}

*Data on the same line that do not share the same capital letter are significantly different ($p < 0.05$) in Tukey's test. Results are expressed as the mean of triplicates ± SD.

more immediate period of time. This will influence the content of the antioxidants portrayed, in which Peruvian liquor may prove to be more advantageous than the others.

3.5 Total phenolics determination

As shown in Tables 7 and 8, Peruvian and organic liquors are naturally good sources of antioxidants, which lose about half of them along the production chain, considering the content in their chocolate. Its content is greater than that of Brazilian alkalized liquor. Despite the loss due to alkalization, Brazilian alkalized liquor can reach between 7.8 and 13.8 mg of phenolic content per gram of product.

3.6 Antioxidant capacity by DPPH

DPPH is a free radical molecule that is relatively stable and less reactive due to the effect of resonance and electron-withdrawing groups in its structure. In this case, DPPH will act as a scavenger of other free radicals capable of stabilizing it and forming DPPH-H. A reduction reaction will occur in which a lone pair of electrons can capture a proton and in which the color of the solution changes from violet to yellow.

The Peruvian liquor visually acquired a darker color than the others during absorbance readings, which presupposes its greater antioxidant power to capture free radicals and form stable DPPH-H with greater efficiency. The loss of antioxidant capacity by the chocolate production process is observed in Table 9.

Carvalho and Lannes (2018) found 74.22 ± 4.10 mg/g GAE and 278.75 ± 3.98% Trolox in milk chocolate.

Grassia et al. (2019) studied the nutritional profile of three cocoa bean samples of different origins (Peru, Ecuador, and Ghana) and cocoa products from the Criollo variety from 70 to 100% solids content. Modicana chocolate (70% solid cocoa) showed the highest content of epicatechin (6.5 mg/g), with a reduction of about 36% compared with raw beans.

3.7 Antioxidant capacity by ORAC

ORAC is a standardized method for determining the antioxidant capacity of a substance. This is based on the inhibition of the peroxyl radical-induced oxidation initiated by the thermal decomposition of azo compounds such as 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH). The application of the ORAC-FL assay uses Trolox® (a water-soluble analog of vitamin E) as a standard by which all other antioxidant compounds are compared.

Table 7. Determination of total phenolics in 70% aqueous methanol - dark chocolate samples*.

Sample	Total phenolics (mg GAE/100 g sample)
Chocolate with Peruvian liquor	776 ± 0.05^{D}
Chocolate with Brazilian alkalized liquor	510 ± 0.08^{E}
Chocolate with Brazilian organic liquor	863 ± 0.11^{CD}

*Different letters in the same column indicate a significant difference ($p < 0.05$) in Tukey's test; Results are expressed as the mean of triplicates ± SD.

Table 8. Determination of total phenolics in 70% aqueous methanol. (1). Brazilian alkalized liquor, (2). Natural Brazilian alkalized liquor, (3). Brazilian organic liquor, and (4). Peruvian cocoa liquor*.

Sample	Total phenolics (mg GAE/100 g sample)
1	2,294.50 ± 133.86
2	3,456.68 ± 70.87
3	4,403.63 ± 208.16
4	3,673.51 ± 160.51

*Results are expressed as the mean of triplicates ± SD.

Table 9. Determination of antioxidant activity by DPPH radical scavenging in μg Trolox equivalent/g of cocoa liquor sample*.

Sample	μg Trolox equivalent/g of sample
Peruvian cocoa liquor	116.90 ± 2.33^{B}
Brazilian alkalized cocoa liquor	54.86 ± 0.98^{CD}
Brazilian natural cocoa liquor	76.97 ± 0.33^{C}
Brazilian organic cocoa liquor	143.56 ± 1.33^{A}
Chocolate with Peruvian liquor	48.84 ± 0.42^{DE}
Chocolate with Brazilian alkalized liquor	30.62 ± 0.25^{E}
Chocolate with Brazilian organic liquor	70.52 ± 0.31^{C}

*Different letters in the same column indicate a significant difference ($p < 0.05$) in Tukey's test. Results are expressed as the mean of triplicates ± SD.

Table 6. Fatty acid contents in the fat phase (%) of the (1). Brazilian alkalized liquor, (2). natural Brazilian alkalized liquor, (3). Brazilian organic liquor, and (4). Peruvian cocoa liquor*.

	Fatty acid	Sample 1	Sample 2	Sample 3	Sample 4
14 : 0	Myristic	—	—	—	—
16 : 0	Palmitic	25.65 ± 0.08	25.05 ± 0.19	24.01 ± 0.50	29.30 ± 0.05
17 : 0	Margaric	—	—	—	—
17: 1	Heptadecenoic	—	—	—	—
18 : 0	Stearic	34.78 ± 0.02	34.31 ± 0.10	34.71 ± 0.36	32.92 ± 0.27
18 : 1 (n-9)	Oleic	35.15 ± 0.01	35.98 ± 0.12	35.81 ± 0.28	32.92 ± 0.18
18 : 2 (n-6)	Linoleic	3.38 ± 0.06	3.58 ± 0.10	4.32 ± 0.81	3.73 ± 0.32
20 : 0	Arachidonic	1.04 ± 0.04	1.08 ± 0.08	1.15 ± 0.19	1.13 ± 0.11
22 : 0	Benzoic	—	—	—	—
	Saturated	61.47 ± 0.07	60.44 ± 0.22	59.87 ± 0.62	63.35 ± 0.15
Total	Monounsaturated	35.15 ± 0.01	35.98 ± 0.12	35.81 ± 0.28	32.92 ± 0.18
	Polyunsaturated	3.38 ± 0.06	3.58 ± 0.10	4.32 ± 0.81	3.73 ± 0.32

*Results are expressed as the mean of triplicates ± SD.

As we can observe in Tables 9 and 10, it is not easy to compare the results of two methods, namely, DPPH and ORAC. Peruvian liquor showed higher antioxidant activity, and Brazilian alkalized liquor showed smaller antioxidant activity by the ORAC method.

3.8 Antioxidant capacity by FRAP

FRAP analysis verifies the antioxidant capacity through free radical scavenging in the reduction of Fe^{3+} ion to Fe^{2+} ion. When this reduction occurs, a change is observed in the tone of the reaction mixture, from light purplish to intense purplish. Visually, it is expected that the antioxidant potential will be greater, the more intense the staining and the greater the absorbance.

According to Table 11, it is observed that the high values of ferrous sulfate that reacted and underwent reduction of ferrous to ferric ion in liquors are consistent with the fact that liquor has greater antioxidant capacity than a chocolate derivative, since liquor does not suffer industrial processing as chocolate. The chocolates produced with Peruvian and organic liquors showed great activity, with a subtle difference that favors the organic liquor. It is assumed that it has greater activity and chance of keeping its antioxidants in the product. However, if it is noted that Peruvian liquor has a strong aromatic flavor, in addition to considerations of differences in conservation and analysis time after chocolate manufacture, then it is speculated that it may lose in qualitative terms to Peruvian liquor. Even so, they showed no significant differences ($p > 0.05$) in Tukey's test, but the respective chocolates differed ($p < 0.05$) from each other.

3.9 Color analysis: shelf life

The three batches of chocolates have a gloss on their surfaces; additionally, a subtle difference can be visually noticed between the chocolate bars in relation to color. Chocolates produced with alkalized liquors are darker than organic because of the alkalization process.

The WI is a parameter used to measure the colorimetric properties in chocolate, affected by the loss of brightness and appearance with the formation of whitish spots characteristic of the appearance of "fat bloom". The WI was obtained through Equation 2 by correlating the values of L*, a*, and b* (Table 12):

$$WI = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{0.5} \quad (2)$$

The L* parameter refers to luminosity and can vary from 0 to 100, and the closer the values approach 100, the lighter the food is considered. The chromatic coordinates a* and b* measure color dimensions. The a* is associated with the green-red axis; positive values of a* indicate samples with predominantly red hues, while negative values indicate more greenish samples. The b* is associated with the blue-yellow axis; (+) b* indicates more yellowish samples, and (-) b* indicates samples with predominant chromatic regions of blue.

According to Table 12, the chocolates had brightness parameters suitable for dark chocolate (without the addition of milk), given the L* values presented. Considering the positive values of a* and b*, it can be said that the chocolates have predominant shades of red and yellow, respectively. The WI remained between 20 and 40, being suitable for dark chocolate with stability at 20°C resistant to the formation of "fat bloom". Tables 13–16 show the results obtained in the evolution of chocolates in terms of color analysis.

Table 10. Determination of the antioxidant capacity of samples by ORAC method: (1). Brazilian alkalized liquor, (2). Natural Brazilian alkalized liquor, (3). Brazilian organic liquor, and (4). Peruvian cocoa liquor*.

Sample	μmol Trolox equivalent/g sample	mmol Trolox equivalent/100 g sample
1	687.71 ± 103.04	68.77 ± 8.41
2	504.84 ± 57.58	50.40 ± 4.74
3	629.99 ± 58.09	62.99 ± 4.7
4	345.4 ± 35.49	34.54 ± 2.9

*Results are expressed as the mean of triplicates ± SD.

Table 11. Comparative table with antioxidant capacity of hydromethanolic extracts from liquor and dark chocolate samples for FRAP analysis*.

Sample	(μg ferrous sulfate equivalent/g sample)
Peruvian cocoa liquor	302.17 ± 0.49^{A}
Brazilian alkalized cocoa liquor	183.1 ± 0.33^{B}
Brazilian organic cocoa liquor	302.8 ± 0.38^{A}
Chocolate with Peruvian liquor	137.49 ± 0.25^{C}
Chocolate with Brazilian alkalized liquor	92 ± 0.11^{D}
Chocolate with Brazilian organic liquor	167.92 ± 0.15^{B}

*Different letters in the same column indicate a significant difference ($p < 0.05$) in Tukey's test. Results are expressed as the mean of triplicates ± SD.

Table 12. Comparative table of color analysis between chocolates with Brazilian alkalized, organic liquors, and Peruvian Brazilian liquor*.

	Brazilian alkalized liquor chocolate	Peruvian liquor chocolate	Brazilian organic liquor chocolate
L*	24.89 ± 0.71^{A}	25.78 ± 0.79^{A}	25.16 ± 0.59^{A}
a*	4.20 ± 0.59^{A}	4.35 ± 0.49^{A}	4.00 ± 0.22^{A}
b*	10.45 ± 0.59^{A}	10.80 ± 0.71^{A}	9.88 ± 0.35^{A}
WI	24.59 ± 0.62^{A}	25.32 ± 0.88^{A}	24.40 ± 0.57^{A}

*Data on the same line that do not share the same letter are significantly different ($p < 0.05$) in Tukey's test.

Table 13. Structural evaluation of the produced dark chocolate with respect to the L* parameter of the color analysis*.

L*	Brazilian alkalized liquor chocolate	Peruvian liquor chocolate	Brazilian organic liquor chocolate
1 day	24.13 ± 0.58^{A}	25.44 ± 1.04^{A}	24.23 ± 0.11^{A}
7 days	24.10 ± 0.35^{B}	25.78 ± 0.95^{A}	24.13 ± 0.35^{B}
14 days	24.41 ± 0.03^{B}	26.69 ± 0.34^{A}	24.74 ± 0.53^{B}
21 days	25.98 ± 0.04^{AB}	27.06 ± 1.02^{A}	25.11 ± 0.28^{B}
28 days	$24{,}68 \pm 1.3^{A}$	26.73 ± 1.27^{A}	25.60 ± 0.63^{A}
35 days	25.52 ± 0.25^{A}	26.35 ± 1.53^{A}	25.86 ± 0.28^{A}
49 days	25.55 ± 0.08^{A}	25.01 ± 0.66^{A}	25.45 ± 0.61^{A}
63 days	25.87 ± 0.13^{A}	25.51 ± 0.42^{A}	25.39 ± 0.56^{A}
77 days	25.33 ± 0.27^{A}	25.13 ± 0.32^{A}	25.87 ± 0.43^{A}
91 days	24.87 ± 0.9^{A}	24.83 ± 0.86^{A}	25.22 ± 0.78^{A}
105 days	25.38 ± 0.79^{A}	25.09 ± 0.61^{A}	25.15 ± 0.31^{A}

*Data on the same line that do not share the same letter are significantly different ($p < 0.05$) in Tukey's test. Results are expressed as the mean of triplicates ± SD.

In the first month, the chocolates showed no significant visual difference in terms of color. However, some fluctuations in the values are observed. Around days 21 to 63, notifications of "fat bloom" and "sugar bloom" in some chocolate bars were observed (Tables 13–16). The values b* were higher than a* because of the chocolate color.

Practically no significant differences were found ($p > 0.05$) between the samples over the 105 days of the study for the color analysis, both for spaces a*, b*, and L* and for the WI (Tables 13–16).

Although the "fat bloom" does not pose a risk to the health of the consumer, being only disruption and being able to return to normal after melting and a new tempering, the product has an unattractive appearance and changes in the texture of the chocolate, making the product unacceptable for sale and consumption. Products that have suffered "sugar bloom", can occur because of poor storage conditions in which humidity fluctuations have occurred, causing whitening of the surface of the chocolate.

Table 14. Structural evaluation of the produced dark chocolate with respect to the a* parameter of the color analysis*.

a*	Brazilian alkalized liquor chocolate	Peruvian liquor chocolate	Brazilian organic liquor chocolate
1 day	4.43 ± 0.32^{A}	4.29 ± 0.11^{A}	4.10 ± 0.22^{A}
7 days	4.15 ± 0.33^{A}	4.39 ± 0.27^{A}	4.28 ± 0.13^{A}
14 days	4.35 ± 0.29^{A}	4.36 ± 0.34^{A}	4.05 ± 0.18^{A}
21 days	4.85 ± 0.39^{A}	4.54 ± 0.40^{A}	4.27 ± 0.18^{A}
28 days	4.90 ± 0.44^{A}	4.36 ± 0.34^{AB}	3.78 ± 0.27^{B}
35 days	4.25 ± 0.29^{B}	5.09 ± 0.35^{A}	4.11 ± 0.23^{B}
49 days	4.74 ± 0.43^{A}	4.71 ± 0.40^{A}	3.97 ± 0.25^{A}
63 days	4.46 ± 0.21^{A}	4.88 ± 0.55^{A}	4.20 ± 0.29^{A}
77 days	3.53 ± 0.43^{A}	4.25 ± 0.20^{A}	3.74 ± 0.17^{A}
91 days	3.24 ± 0.46^{A}	3.45 ± 0.18^{A}	3.80 ± 0.21^{A}
105 days	3.31 ± 0.57^{A}	3.57 ± 0.35^{A}	3.67 ± 0.13^{A}

*Data on the same line that do not share the same letter are significantly different ($p < 0.05$) in Tukey's test. Results are expressed as the mean of triplicates ± SD.

Table 15. Structural evaluation of the produced dark chocolate with respect to the b* parameter of the color analysis*.

b*	Brazilian alkalized liquor chocolate	Peruvian liquor chocolate	Brazilian organic liquor chocolate
1 day	9.49 ± 0.55^{A}	10.09 ± 0.01^{A}	9.20 ± 0.44^{A}
7 days	10.42 ± 0.24^{AB}	10.53 ± 0.01^{A}	10.14 ± 0.03^{B}
14 days	10.61 ± 0.25^{A}	10.69 ± 0.16^{A}	9.94 ± 0.48^{A}
21 days	11.14 ± 0.40^{A}	11.46 ± 0.36^{A}	10.21 ± 0.22^{B}
28 days	11.10 ± 0.65^{A}	11.71 ± 1.02^{A}	9.89 ± 0.41^{A}
35 days	10.11 ± 0.29^{B}	11.98 ± 0.89^{A}	9.90 ± 0.32^{B}
49 days	10.95 ± 0.83^{A}	10.64 ± 0.38^{A}	9.77 ± 0.25^{A}
63 days	11.21 ± 0.21^{AB}	11.33 ± 0.44^{A}	10.33 ± 0.37^{B}
77 days	9.95 ± 0.55^{A}	10.39 ± 0.38^{A}	10.16 ± 0.21^{A}
91 days	10.15 ± 0.26^{A}	9.85 ± 0.15^{A}	9.35 ± 0.74^{A}
105 days	9.85 ± 0.33^{A}	10.15 ± 0.34^{A}	9.77 ± 0.37^{A}

*Data on the same line that do not share the same letter are significantly different ($p < 0.05$) in Tukey's test. Results are expressed as the mean of triplicates ± SD.

Table 16. Structural evaluation of the produced dark chocolate with respect to the WI parameter of the color analysis*.

WI	Brazilian alkalized liquor chocolate	Peruvian liquor chocolate	Brazilian organic liquor chocolate
1 day	23.41 ± 0.49^{A}	24.64 ± 1.72^{A}	23.56 ± 0.12^{A}
7 days	24.64 ± 1.65^{A}	24.91 ± 0.73^{A}	23.34 ± 0.34^{A}
14 days	24.12 ± 1.16^{A}	25.79 ± 0.33^{A}	23.97 ± 0.50^{A}
21 days	24.74 ± 0.84^{B}	26.67 ± 0.98^{A}	24.30 ± 0.25^{B}
28 days	23.63 ± 1.13^{B}	26.56 ± 1.06^{A}	24.85 ± 0.57^{AB}
35 days	24.72 ± 0.22^{A}	26.50 ± 1.34^{A}	25.09 ± 0.26^{A}
49 days	24.89 ± 0.3^{A}	24.64 ± 0.61^{A}	24.71 ± 0.61^{A}
63 days	24.74 ± 1.13^{A}	24.92 ± 0.48^{A}	24.56 ± 0.54^{A}
77 days	25.04 ± 0.5^{A}	24.58 ± 0.3^{A}	25.08 ± 0.43^{A}
91 days	25.35 ± 1.56^{A}	24.71 ± 0.61^{A}	24.53 ± 0.77^{A}
105 days	25.23 ± 0.77^{A}	24.59 ± 0.85^{A}	24.42 ± 0.29^{A}

*Data on the same line that do not share the same letter are significantly different ($p < 0.05$) in Tukey's test. Results are expressed as the mean of triplicates ± SD.

3.10 Rheology

Molten cocoa butter exhibits low viscosity and Newtonian fluid rheological behavior, while melted cocoa liquor exhibits pseudoplastic behavior due to solid particles dispersed in the lipid medium. Melted chocolate rheological behavior is dependent on water content, emulsifier (in this case, lecithin), fat content, granulometry, chocolate formulation (whether it is with or without milk), and temperature. Its viscosity decreases with the addition of fat and an emulsifier but increases with the amount of water (Lannes, 1997).

The incorporation of cocoa butter into the liquor allows for a decrease in viscosity by dispersing the solid particles in the mixture (Table 17), but the viscosity in the chocolate does not drop too much due to the presence of sugar, which adds more particles and maintains the yield strength (shear stress). Both tension and viscosity reach higher values in the presence of fewer solids dispersed in the butter, due to the ease of movement and greater interaction between the component particles.

Thixotropy is a parameter used to measure time-dependent rheological behavior and is related to the change in the structure of the material over a period of time. Through it, it is possible to analyze how much shear stress and viscosity, at a given shear rate, the melted chocolate can present and alternate during a certain period.

Casson's model ($r > 0.98$) was used to adjust the values found in the chocolate analysis, obtaining Casson's initial shear stress, Casson's plastic viscosity, thixotropy, and texture. For each purpose of chocolate use, its viscosity and shear stress values must be set and prior planning of the formulation in order to reach the parameters is necessary. The parameters are different for each industry.

Table 17. Particle size of Brazilian organic, Brazilian alkalized, and Peruvian liquors*.

	Brazilian alkalized liquor	Peruvian liquor	Brazilian organic liquor
Particle size (mm)	0.042 ± 0.005^{B}	0.432 ± 0.148^{A}	0.019 ± 0.03^{B}

*Data on the same line that do not share the same letter are significantly different ($p < 0.05$) in Tukey's test. Results are expressed as the mean of triplicates ± SD.

According to Table 18, the Casson viscosity remained between 1.3 and 2.5 Pa s, and the initial stress was close to the values of 5 and 9 Pa. Although these intervals are described as parameters for semisweet and non-bar chocolate coatings (Lannes, 1997), the chocolates produced maintained remarkable stability for a considerable period of time (about 3 months) for the purpose of bar chocolate since they approached industry standard values. Like chocolate bars, they can have a higher shear stress than the icing, usually because they are more viscous and creamier. Small differences in values may be due to variations in temperature and storage time.

Fat has a proportionally greater effect on plastic viscosity than tension and is also related to the melting point of chocolate. The particle size influences the rheological values and melting point (Afoakwa et al., 2016) and the sensory properties, giving a smoother and creamy texture that can affect the colorimetric characteristics of the product. The values obtained for the particle size of chocolates are suitable for the ideal, keeping below 0.035 mm (Afoakwa et al., 2009). The values for chocolates were lower than for their liqueurs (Tables 17 and 18), due to the refining process, which reduces the size of the particles (Afoakwa et al., 2008). This also contributes to the better incorporation of fat and fluidity of the particles, attributing homogeneity to the melted chocolate.

3.11 Texture and shelf life

The texture of a food refers to its rheological properties, covering parameters such as hardness, adhesiveness, spreadability, and fracturability. Such characteristics can be perceived by the sense of touch, sight, and hearing. Storage time affects the texture of chocolates. The fat content directly influences the texture, which in turn is one of the indicators of stability and shelf-life.

The shelf-life of chocolate depends directly on the conditions in which it is stored as well as intrinsic factors such as water activity and pH. From a sensory point of view, the texture of the chocolate can be correlated with the shelf life, which decreases as the product softens under ambient conditions (it is not a softening due to the melting phenomenon but generally due to high moisture content and water activity). The lipid content, especially unsaturated fats, is susceptible to oxidation and reduces the shelf life of chocolate (Ndife et al., 2013). The chocolates produced showed a stable shelf life for a while with weekly control and verification (Table 19).

It is possible to verify (Table 19) that the texture of dark chocolates produced presented values close to those of the industry (hardness between 22.00 and 33.00 N) at 20°C (Lannes, 1997). The chocolate with Brazilian alkalized liquor showed greater softness compared to the other batches, whose values are close to the hardness of white chocolate (about 22.00 N). This can be explained by the formulation used, as well as by the process applied. On most days, there was no significant difference ($p > 0.05$) in the values of chocolates produced with alkalized Brazilian liquor and Peruvian liquor. The type of cocoa butter present in each liquor may have contributed to this result, as well as the particle size of the liquor since the formulations and the process were the same. Note that the texture of chocolate with organic liquor showed a firmer texture, with a value closer to that of milk or dark chocolate (about 30.00 N). Added to the previous analyses, it can be concluded that these dark chocolates elaborated are suitable for industrial production.

3.12 Thermal analysis

Cocoa butter imparts less fracturability and greater softness and spreadability to chocolate, it remains at an ideal value close to 37°C, which is a healthy body temperature and is consistent with the melting of chocolate in the mouth (Ostrowska-Ligeza et al., 2019).

Sugar is considered a body agent in chocolate, contributing to the sweetness, and the caramelization point is also due to its content in the formulation. The presence of sugar confers good texture and gloss properties (when related to the moisture content) but it also influences the carbonization point, which is also related to cocoa solids. The carbonization point determines the temperature at which the organic compounds in the chocolate are carbonized and evaporated, leaving the inorganic content (the minerals). The results obtained from the thermal analysis of liquors and chocolates are shown in Tables 20 and 21.

Table 18. Rheological parameters and particle size of chocolates made with Brazilian alkalized, Brazilian organic, and Peruvian liquors*.

	Brazilian alkalized liquor chocolate	Peruvian liquor chocolate	Brazilian organic liquor chocolate
Maximum particle size (mm)	0.025 ± 0.003^{A}	0.018 ± 0.001^{B}	0.015 ± 0.003^{B}
Casson shear stress (Pa)	7.02 ± 0.4^{A}	6.19 ± 0.6^{A}	4.27 ± 0.81^{B}
Casson's plastic viscosity (Pa·s)	2.01 ± 1.23^{A}	1.56 ± 0.21^{A}	1.66 ± 0.17^{A}
Thixotropy ($Pa \cdot s^{-1}$)	1131 ± 907^{A}	779 ± 232^{A}	718 ± 66^{A}
Texture (N)	20.95 ± 1.10^{B}	24.29 ± 2.39^{AB}	28.38 ± 1.11^{A}

*Data on the same line that do not share the same letter are significantly different ($p < 0.05$) in Tukey's test. Results are expressed as the mean of triplicates ± SD.

Table 19. Structural evaluation of chocolates produced in relation to texture (N) over days (shelf life)*.

Texture (N)	Brazilian alkalized liquor chocolate	Peruvian liquor chocolate	Brazilian organic liquor chocolate
1 day	21.06 ± 1.74^{B}	22.45 ± 0.57^{B}	28.68 ± 1.87^{A}
7 days	20.19 ± 1.05^{B}	20.63 ± 0.47^{B}	26.35 ± 0.99^{A}
14 days	19.52 ± 1.90^{B}	22.08 ± 1.53^{B}	28.73 ± 2.80^{A}
21 days	20.42 ± 0.43^{C}	25.24 ± 0.29^{B}	28.71 ± 2.03^{A}
28 days	19.87 ± 1.00^{B}	25.24 ± 0.13^{A}	29.69 ± 3.44^{A}
35 days	20.73 ± 0.35^{C}	$23,63 \pm 1.39^{B}$	27.97 ± 0.76^{A}
49 days	22.80 ± 1.05^{B}	29.32 ± 1.44^{A}	29.20 ± 0.43^{A}
63 days	20.42 ± 0.43^{B}	26.37 ± 3.71^{AB}	27.86 ± 1.99^{A}
77 days	22.94 ± 0.63^{A}	25.41 ± 2.38^{A}	27.05 ± 3.95^{A}
91 days	21.57 ± 1.91^{B}	22.78 ± 3.19^{B}	30.13 ± 1.84^{A}
105 days	21.00 ± 0.37^{B}	24.08 ± 1.07^{AB}	27.80 ± 2.38^{A}

*Data on the same line that do not share the same letter are significantly different ($p < 0.05$) in Tukey's test. Results are expressed as the mean of triplicates ± SD.

Table 20. Parameters of the thermal analysis (DSC) of the Brazilian alkalized, Brazilian organic, and Peruvian liquors*.

	Brazilian alkalized liquor	Peruvian liquor	Brazilian organic liquor
Melting point (°C)	28.2 ± 0.1^{B}	30.2 ± 0.2^{A}	28.3 ± 0.1^{B}
Caramelization point (°C)	177.5 ± 3.5^{A}	178.3 ± 0.8^{A}	176.3 ± 0.6^{A}
Carbonization point (°C)	219.8 ± 0.2^{A}	219.3 ± 0.5^{A}	219.6 ± 0.3^{A}

*Data on the same line that do not share the same letter are significantly different ($p < 0.05$) in Tukey's test. Results are expressed as the mean of triplicates ± SD.

Table 21. Parameters of the thermal analysis (DSC) of the chocolates produced with Brazilian alkalized, Brazilian organic, and Peruvian chocolates*.

	Brazilian alkalized liquor chocolate	Peruvian liquor chocolate	Brazilian organic liquor chocolate
Melting point (°C)	31.6 ± 0.6^{A}	32.4 ± 0.1^{A}	30.2 ± 0.1^{B}
Caramelization point (°C)	183.6 ± 3.2^{A}	182.8 ± 0.6^{A}	180.7 ± 0.9^{A}
Carbonization point (°C)	237.1 ± 0.3^{A}	224.6 ± 1.1^{B}	219.8 ± 0.2^{C}

*Data on the same line that do not share the same letter are significantly different ($p < 0.05$) in Tukey's test. Results are expressed as the mean of triplicates ± SD.

Brazilian alkalized and Brazilian organic liquors melting showed no statistical difference ($p > 0.05$). Caramelization and carbonization points of all liquors did not show statistical differences for all liquors.

Brazilian alkalized and Peruvian chocolates melting point showed no statistical difference ($p > 0.05$). Caramelization points of all chocolates did not show statistical difference ($p > 0.05$) for all chocolates. Carbonization points were different ($p < 0.05$) for all chocolates. Peruvian chocolates and liquor showed the same thermal behavior.

4 CONCLUSION

Peruvian and organic Brazilian cocoa liquors showed better antioxidant activity. Peruvian liquor showed higher melting point. Brazilian alkalized and Brazilian organic liquors and chocolates melting showed no statistical difference ($p > 0.05$). Caramelization and Carbonization points of all liquors did not show statistical differences ($p > 0.05$) for all liquors, as well as Caramelization point for all chocolates. Carbonization point was different ($p < 0.05$) for all chocolates. Peruvian chocolates and liquor showed the same thermal behavior.

It was possible to conclude that the comparative data obtained support the consumption of each type of cocoa. The chocolates were stable in terms of structure during storage, demonstrating suitability for industrial production.

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