1 Introduction

Cocoa (*Theobroma cacao* L.) is the main ingredient for the manufacture of chocolate. Contrary to earlier reports, *T. cacao* was first domesticated in South America, 1,500 years before its cultivation began in Central America and Mesoamerica. Recently, three independent lines of archeological evidence (cacao starch grains pottery, absorbed theobromine, and ancient DNA) demonstrated that the upper Amazon region is the oldest center of cacao initial use so far identified (Zarrillo et al., 2018). Iconographic representations of cocoa pods in ancient pre-Columbian ceramics found in Ecuador and Peru support this.

The upper Amazon is also considered the center of genetic diversity of *T. cacao*. Cocoa consists of 10 genetic clusters, of which six can be found in Peru (Motamayor et al., 2008).

According to the International Cocoa Organization (2019), Peru is a producer and an exporter of fine-flavor cocoa varieties, in high demand for the fine chocolate market. There are many varieties of native cocoa to offer in response. As part of an ongoing project elaborating the sensory map of Peruvian cocoa, this study aimed to determine the main chemical and sensory characteristics of native cocoa varieties from the Bagua and Quillabamba regions and of the chocolates formulated with them.

2 Materials and methods

2.1 Material and postharvest

Quillabamba (Cusco) and Bagua (Amazonas) were chosen as study locations. Ripened, healthy native cocoa pods were selected. The pods were from one plot of one cocoa farmer within the zone. The fresh cocoa beans were transported in 20-L buckets to a postharvest facility. There, fermentation was carried out in wooden boxes of 200 kg capacity, covered with banana leaves and jute bags. Parameters such as pH of the beans and fermentation mass temperature were measured daily.

The end of fermentation was noted according to pH measurements and results of the cut test. The samples from Quillabamba differ from those of Bagua mainly by having a higher fat and lower theobromine content. The main sensory attributes detected for Bagua samples that differ from those of Quillabamba were fruity, acidic, astringent, and bitter notes.
2.2 Cocoa mass and chocolate processing

Cocoa beans were processed at La Ibérica chocolate company (Arequipa, Peru). The beans were sorted and roasted in 30-kg capacity roaster. Roasting temperature and time were set according to results of a sensory analysis of the raw beans. Cocoa beans from Bagua were roasted at 110-120 °C for 9 min, and cocoa beans from Quillabamba were roasted at 118-122 °C for 11 min. Then the beans were cracked out and winnowed to obtain nibs, which were then ground and refined to create a fine cocoa mass. For chocolate processing, the mass was conched for 48 h. Sugar and cocoa butter were used to make the chocolates with 70% cocoa solids, and lecithin was added to a formulation that had 52% cocoa solids. The samples were molded into 100-g bars.

2.3 Proximate analysis

Proximate analyses of samples were performed according to the Association of Official Analytical Chemists methods (Association of Official Analytical Chemists, 2010). Moisture contents of the samples were determined during desiccation at 105°C until a constant weight was reached. Total fat was extracted with hexane by the Soxhlet method. Nitrogen content estimated by the Kjeldahl method was converted to protein content using a conversion factor of 6.25. Total fiber was analyzed by acid base extraction, and the ash content was determined by incineration in a muffle furnace at 550 °C. The carbohydrates content was calculated with a difference method.

2.4 Fatty acid profile

Deriving a profile of fatty acids began with esterification of 100 mg of the sample with 2 N KOH methanolic solution (Ichihara et al., 1996). The methyl esters of the fatty acids were separated using an Agilent DB-5ms capillary column, with dimensions 60 m, 0.25 mm, and 0.25 μm. Samples were then analyzed by capillary gas chromatography (Agilent 7890) operated under the following conditions: detector temperature 230 °C; injector temperature 250 °C; split ratio 200:1; furnace temperature 100 °C, 100-190°C (at 20 °C/min), 190-230 °C (at 3 °C/min), 36 min. The carrier gas used was helium (1 mL/min), and a 5-μL aliquot of each sample was injected into the apparatus. Fatty acids were determined in the results by comparing the peak retention times with the respective fatty acid standards.

2.5 Theobromine and caffeine

Using 0.2 g of defatted cocoa paste, extraction proceeded with ultrapure water for 30 min under reflux. The extract was centrifuged for 5 min at 5000 rpm, and the supernatant was brought to volume in a 50-mL volumetric flask. Next, 2 mL of the aqueous solution was placed inside a previously conditioned Sep-Pak C18 filter and the sample was eluted with 10 mL of chloroform. The solvent was evaporated, and the residue remaining was dissolved in 5 mL of ultrapure water and transferred to a vial for injection into a high-performance liquid chromatograph with diode array detection (HPLC-DAD). The elution system used acetonitrile–water (20:80), isocratic, with a running time of 8 min, injection volume of 20 μL, and flow of 1.2 mL/min (Pura Naik, 2001).

2.6 Total phenolics and flavonoids

The total phenolics content (TPC) was determined using the Folin–Ciocalteu method according to Yazdizadeh Shotorbani et al. (2013), with some modifications. A 0.5-g defatted sample was extracted three times with 5 mL 80% ethanol. After filtration, the extract was made up to a final volume of 25 mL in a volumetric flask. Fifty-microliter aliquots of sample extract were mixed with 1 mL Folin–Ciocalteu (1/10) reagent. The mixture was incubated for 2 min, followed by the addition of 1 mL of Na2CO3 (7.5%) solution, and then was incubated in the dark at room temperature for 15 min. The absorbance of the reaction mixture was measured at 760 nm with a spectrophotometer. TPC values of samples were calculated by means of a gallic acid calibration curve. TPC was expressed as grams of gallic acid equivalents per 100 g of sample.

Flavonoid content was determined using the method of Ivanova et al. (2010), with some modifications. One gram of sample was extracted with 20 mL 80% methanol. After filtration, the extract was made up to a final volume of 25 mL in a volumetric flask. A 2-ml extract sample was mixed with 1.5 ml distilled water and 0.15 mL NaNO2 solution (0.05%). After 5 min, 0.15 mL of 0.1% AlCl3 solution was added, and 6 min later, 1 mL of NaOH (1 M) was also added to the mixture. The total volume of the solution was made up to 5 ml with distilled water, and the absorbance was measured at 510 nm in a spectrophotometer. A calibration curve was constructed with catechin, and the total flavonoids concentrations were expressed as catechin equivalents per 100 g of sample (g CAT/100 g sample).

2.7 Antioxidant activity

The antioxidant activity was estimated according to the method of Othman et al. (2007). First, 0.5 g of defatted sample was extracted three times with 5 mL 80% ethanol. After filtration, the extract was made up to a final volume of 25 mL in a volumetric flask. An aliquot of cocoa extract (50 μL, 0.063-0.63 mg/mL in 80% ethanol) was mixed with 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH). The mixture was left to stand for 30 min in the dark at room temperature. Absorbance at 517 nm was measured in a spectrophotometer using ethanol (80%) as a blank control. The DPPH radical scavenging activity of each sample was calculated according to the following Formula 1:

\[
\text{DPPH inhibition} = \frac{100 \times (\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} 
\]

The EC50 value (concentration of sample extract that decreases the initial DPPH radical concentration by 50%) was calculated by plotting the curve of DPPH inhibition (%) against the concentrations of extract samples.

2.8 Sensory evaluation

Sensory evaluation of the samples was carried out by a semi-trained panel of cocoa and chocolate testers. The panel consisted of 8 persons, ages 25 to 60 years. First, following ISO guidelines, different sensory tests (basic taste recognition and threshold) were carried out for the selection of the panelists. Then, discriminative and descriptive tests were used to
familiarize the panelists with the sensory analysis vocabulary; panel performance was evaluated in terms of repeatability and reproducibility. Cocoa liquor sensory evaluation guidelines were taken from Sukha et al. (2008). Approximately 2 ± 0.10 g of a sample was portioned out into a plastic container. To avoid code similitude and positioning effects, random three-digit numbers were generated to label the samples. Once the samples were portioned out, the containers were covered with lids and the contents were melted in a convection oven at 50 °C for 15 min. Samples were tasted in triplicate, and a maximum of six samples was evaluated per session. Warm water and water crackers were used to cleanse the palate before and between samples. The sensory sessions took place in an odor-free, spacious environment with adequate lighting. To determine the sensory profile of samples, quantitative descriptive analysis was used to identify and quantify the intensity of the flavor attributes using a 10-point scale (0 = absent; 1 = just a trace; 2 = present in the sample at low intensity; 3 to 5 = clearly characterizing the sample; 6 to 8 = dominant characterization of the sample; 9 to 10 = maximum, overpowers other flavor attributes), according to the glossary of terms and scale legend of the Cocoa of Excellence Programme (CoEx, 2019). Three attributes of aroma (cocoa/chocolate, fruity, and floral) and eight attributes of flavor (cocoa/chocolate, acid, sweet, bitter, astringent, nutty, fruity, and floral) were evaluated.

2.9 Data analysis

One-way analysis of variance and Tukey's post hoc test ($p \leq 0.05$) were used to assess significant differences among samples groups in terms of sensory and chemical data. The statistical analysis was carried out using multiple factorial analysis (MFA) with the aid of R statistical software using the packages FactorMineR and Factoextra (Kassambara, 2017). The sensory data and the chemical data were analyzed separately.

3 Results and discussion

Table 1 shows the results of the proximate chemical analysis carried out on samples of cocoa pastes and chocolates from Bagua and Quillabamba. A slightly higher content of fats (58.1%) and proteins (13.1%) was observed for the cocoa paste from Quillabamba. Also, the chocolates made from cocoa paste from Quillabamba had higher fat content than the ones from Bagua.

In Brazil, de Melo et al. (2020) selected 9 varieties of cocoa to make 70% chocolates. These samples, compared to the 70% Peruvian chocolates, had lower fat (34.8-41.8%), similar protein (7.9-9.1%) and higher carbohydrate contents (46.4-54.1%). On the other hand, a 70% Brazilian chocolate prepared by Milagres et al. (2020) contained similar amount of proteins (8.8%), but lower fat (29.4%) and higher carbohydrates (57.8%) percentages. These differences may be due to the variety and origin of cocoa studied and the chocolate recipe.

Fatty acid profiles of cocoa pastes from both regions were characterized by a high content of saturated fatty acids (palmitic and stearic acid), followed by the monounsaturated oleic acid. This same profile of fatty acids was maintained in the derived products (chocolate 52% and 70%). Cocoa paste from Quillabamba had a higher percentage of palmitic acid than cocoa paste from Bagua (31.6% and 23.5%, respectively), but the opposite was observed for the presence of stearic acid (27.3% and 36.4%, respectively). The total amount of saturated fatty acids in samples from both regions was high (60%); however, 27.3-36.4% of that amount corresponded to stearic acid, a fatty acid considered less cholesterogenic than other saturated acids because it is more rapidly converted to oleic acid (Denke & Grundy, 1991).

Table 1. Proximate analysis and fatty acid profiles of cocoa pastes and chocolates.

<table>
<thead>
<tr>
<th>Proximate analysis (%) dry weight basis</th>
<th>QUILLABAMBA</th>
<th>BAGUA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cocoa paste</td>
<td>Chocolate 52%</td>
</tr>
<tr>
<td>Fat</td>
<td>58.1 ± 1.1</td>
<td>34.4 ± 0.5</td>
</tr>
<tr>
<td>Ash</td>
<td>2.8 ± 0.0</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Protein</td>
<td>13.2 ± 0.3</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>Fiber</td>
<td>1.6 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>24.3 ± 0.7</td>
<td>57.6 ± 0.3</td>
</tr>
<tr>
<td>Fatty acids (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturated</td>
<td>59.9 ± 0.9</td>
<td>61.80</td>
</tr>
<tr>
<td>Myristic C14:0</td>
<td>0.06±b</td>
<td>0.05±b</td>
</tr>
<tr>
<td>Palmitic C16:0</td>
<td>31.63±</td>
<td>26.77±</td>
</tr>
<tr>
<td>Margaric C17:0</td>
<td>0.13±</td>
<td>0.31±</td>
</tr>
<tr>
<td>Steric C18:0</td>
<td>27.32±</td>
<td>33.72±</td>
</tr>
<tr>
<td>Arachidic C20:0</td>
<td>0.77±a</td>
<td>0.95±</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>37.34±</td>
<td>35.59±</td>
</tr>
<tr>
<td>Palmitoleic C16:1</td>
<td>0.20±d</td>
<td>0.21±d</td>
</tr>
<tr>
<td>Oleic acid C18:1</td>
<td>36.66±</td>
<td>34.87±</td>
</tr>
<tr>
<td>Octadecenoic C18:1</td>
<td>0.48±</td>
<td>0.51±</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>2.30±</td>
<td>2.61±b</td>
</tr>
<tr>
<td>Linoleic acid C18:2</td>
<td>2.30±</td>
<td>2.61±b</td>
</tr>
</tbody>
</table>

Values are shown as means ± standard deviation. Means followed by the same letter within each row are not significantly different at $p < 0.05$ (Tukey's post hoc test).
The theobromine contents of cocoa pastes and chocolates from Quillabamba were lower than those found in cocoa pastes and chocolates from the Bagua region. The ratio of theobromine to caffeine (T/C) is considered to be associated with the quality of cacao and with the genotype. T/C values <2 correspond to the criollo type, values between 2 and 6 correspond to the trinitario type, values of 6 to 8 are considered miscellaneous, and from values 8 upward correspond to the forastero type (Davrieux et al., 2005; Osorio-Guarín et al., 2017). Cocoa from Bagua, with a T/C ratio of 4.5, would be labeled as a trinitario type, while cocoa from Quillabamba showed a T/C ratio of 1.2, typical of a criollo type. The contents of polyphenols and of total flavonoids in the Quillabamba samples were relatively higher than those of the Bagua samples, although the antioxidant activity, determined by the DPPH test, appeared to be similar in material from both regions (Table 2).

In our study, multiple factor analysis (MFA) was used to analyze the chemical and sensory variables of cocoa pastes and chocolates and to identify groups of variables linked to a given sample. MFA is based on a weighted PCA analysis of the tables of variables classified by groups and consists of the following stages: (1) To each group of variables, a point cloud is associated by means of a principle component analysis (PCA) of each group of variables; (2) then a global PCA of all groups of variables is performed; and (3) subsequently, each group of variables is averaged using the inverse of the first own value obtained from the PCA of the global table. This process maintains the structure of each table and balances the influence of each group by obtaining a value of inertia for each group equal to 1.

Figure 1 shows a point cloud of individuals and the correlation circle from the MFA analysis for the chemical variables. The analysis extracted the most significant variables with minimal loss of information. The results of MFA show two selected components that explain more than 65% of the total variation in the data set. The first dimension (Dim1) explains 39.7% of variation, and the second dimension (Dim2) explains 25.9% of variation.

Table 2. Theobromine, caffeine, total phenolics, flavonoids, and antioxidant activities of cocoa pastes and chocolates.

<table>
<thead>
<tr>
<th></th>
<th>QUILLABAMBA</th>
<th>BAGUA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cocoa paste</td>
<td>Chocolate 52%</td>
</tr>
<tr>
<td>Theobromine (T) (mg/100 g)</td>
<td>214.3 ± 4.0d</td>
<td>187.4 ± 4.8e</td>
</tr>
<tr>
<td>Caffeine (C) (mg/100 g)</td>
<td>172.2 ± 3.6b</td>
<td>117.0 ± 2.4e</td>
</tr>
<tr>
<td>Ratio T/C</td>
<td>1.2</td>
<td>1.6</td>
</tr>
<tr>
<td>TP (g GA/100 g)</td>
<td>1.8 ± 0.1a</td>
<td>1.1 ± 0.1b</td>
</tr>
<tr>
<td>TF (g CAT/100 g)</td>
<td>1.8 ± 0.1a</td>
<td>1.0 ± 0.0e</td>
</tr>
<tr>
<td>DPPH (µg extract/ml)</td>
<td>20.2 ± 0.7c</td>
<td>245.0 ± 10.8h</td>
</tr>
</tbody>
</table>

Values are shown as means ± standard deviation. Means followed by the same letter within each row are not significantly different at p < 0.05 (Tukey’s post hoc test). TP: total phenolics; TF: total flavonoids; GA: gallic acid; CAT: catechin.

Figure 1. Multiple factorial analysis (MFA) of chemical analysis data. Left: Point cloud of individuals. The six samples (PB, C70-B, C52-B, PQ, C70-Q, C52-Q) are made up of six clusters (four data points and a barycenter point for each sample). They are separated from each other with their respective ellipses of confidence (95% confidence level) and have different contributions to the dimensions Dim1 and Dim2. Right: Correlation circle of quantitative variables. The gray-scale gradient indicates the value of Cos2 (square or square cosine of the coordinate) that represents the quality of the representation of the variable on the factor map. A high cos2 indicates a good representation of the variable on the main components. In this case, the variable is placed near the circumference of the correlation circle. P-Q: Cocoa paste from Quillabamba. C52-Q: The 52% chocolate from Quillabamba. C70-Q: The 70% chocolate from Quillabamba. P-B: Cocoa paste from Bagua. C52-B: The 52% chocolate from Bagua. C70-B: The 70% chocolate from Bagua. sd = standard deviation, sdq = sum of the squared deviations, which are illustrative.
According to the point cloud of individuals, the samples of the Quillabamba region were chemically different from those of Bagua. On the right side of Dim1 is the cocoa paste from Quillabamba (P-Q), which is characterized by its contents of ash (corr = 0.969, cos² = 0.940), fats (corr = 0.962, cos² = 0.925), proteins (corr = 0.956, cos² = 0.914), oleic acid (corr = 0.693, cos² = 0.481), and total flavonoids (corr = 0.659, cos² = 0.434).

On the other hand, the 70% chocolate from Quillabamba (C70-Q) correlated with Dim2(+), and it is characterized by its contents of palmitic (corr = 0.876, cos² = 0.768) and araquidic (corr = 0.588, cos² = 0.346) acids.

The 52% chocolates from Quillabamba and Bagua (C52-Q and C52-B, respectively) were correlated with Dim1(–) and are characterized by their contents of DPPH (corr = –0.974, cos² = 0.949), carbohydrates (corr = –0.973, cos² = 0.947), margaric acid (corr = 0.814, cos² = 0.663), and linoleic acid (corr = –0.648, cos² = 0.421). Cocoa paste (P-B) and 70% chocolate (C70-B) from Bagua correlate strongly with Dim 2(–); these samples correspond in the correlation circle with the variables theobromine (corr = –0.948, cos² = 0.888), stearic acid (corr = –0.883, cos² = 0.780), and palmitoleic acid (corr = 0.741, cos² = 0.550).

In terms of aroma, Bagua and Quillabamba samples are characterized by their cocoa/chocolate and fruity notes. Figure 2 shows the main flavor attributes detected by the sensory panel for cocoa pastes and chocolates from the two regions.

According to the research of Oberrauter et al. (2018), dark origin chocolates evidenced a dominance of bitterness independent of cocoa content, with a later perception of astringency after swallowing, compared to the dominance of sweetness in non-origin chocolates. For the Quillabamba and Bagua samples, a lower cocoa content was related to less perception of acidity, bitterness, and astringency notes, and to an increase in perceived sweetness. A similar perception of the intensity of cocoa/chocolate notes regardless of the percentage of cocoa was particularly identified among the Quillabamba samples. Furthermore, the cocoa paste from Bagua was scored low for sweetness and the cocoa content of the dark chocolates did not make a difference in the perception of this attribute. These differences in the flavor profiles of the Bagua and Quillabamba samples demonstrate that the dominance of flavor attributes is strongly related to the cocoa origin and cocoa content (Oberrauter et al., 2018). Nutty and fruity flavor notes were also perceived in both origin samples. Addition of sugar did not diminish the perception and intensity of those specific attributes. Some additional comments from the panel included detection of honey notes and ripe fruit notes for Quillabamba and Bagua, respectively.

Figure 3 shows a point cloud of individuals and the correlation circle of the MFA analysis for the sensory analysis variables. This analysis extracted the most significant variables with minimum loss of information. The results of the MFA show two selected components that explain more than 48% of the total variation in the data set. The first dimension (Dim1) explains 27.5% of variation, and the second dimension (Dim 2) explains 20.9%.

There are some sensory differences among cocoa pastes and chocolates from the Quillabamba and Bagua regions. Sample P-B correlates with Dim2 (closest to the y-axis). In parallel, in the correlation circle it can be seen that cocoa paste P-B is

Figure 2. Flavor attributes of cocoa pastes and chocolates from Quillabamba and Bagua.
Characterized by its bitter (corr = 0.829, cos2 = 0.689), acid (corr = 0.806, cos2 = 0.650), floral (corr = 0.576, cos2 = 0.332), and astringent (corr = 0.527, cos2 = 0.278) notes. Cocoa paste P-Q and chocolates C70-Q and C52-Q correlate better with Dim1. In the correlation circle, P-Q, C70-Q, and C52-Q are characterized by cocoa/chocolate (aroma) (corr = 0.794, cos2 = 0.630), cocoa/chocolate (taste) (corr = 0.712, cos2 = 0.507), and nutty (corr = 0.606, cos2 = 0.367) notes. In contrast, chocolate C70-B is characterized by fruity (taste) (corr = −0.623, cos2 = 0.388) and fruity (aroma) (corr = −0.571, cos2 = 0.326) notes. Finally, chocolate C52B differs from chocolate C52Q by its sweet note (corr = −0.728, cos2 = 0.530).

Aguilar-Villa et al. (2020) demonstrated that by using bulking agents (maltitol polydextrose or inulin), in combination with a non-caloric sweetener (stevia), it is possible to make sugar-free milk chocolates without losing their sensory and rheological properties. The use of inulin and stevia would be advisable for making Bagua and Quillabamba chocolates with lower caloric content, while preserving their own organoleptic qualities.

4 Conclusions

According to the MFA analysis, cocoa pastes and chocolate formulations (52% and 70%) from the two regions studied differ in terms of their chemical and sensory characteristics. The Quillabamba samples differ from those in Bagua mainly because of their higher fat and lower theobromine content. The main sensory attributes detected for the Bagua samples that differed from those of Quillabamba were their fruity, acidic, astringent, and bitter notes.

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References


