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Colour, fatty acids, bioactive compounds, and total antioxidant capacity in commercial cocoa beans (*Theobroma cacao* L.)

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Abstract: Cocoa bean is a resource with great level of bioactive components that have shown potential beneficial effects on health, in addition to being the main ingredient in the chocolate industry. This study evaluated the total antioxidant capacity (quencher-DPPH$^{\circ}$), polyphenols, fatty acid profile, and chromatic parameters of Peruvian commercial cocoa beans. The different analytes were quantified using UV-Vis absorption spectroscopy, gas chromatography-flame ionization detection (GC/FID) and liquid chromatography coupled with diode array detector (HPLC-DAD). Results showed that the lightness (L^*) and the hue angle (h_{ab}) were the greatest variation in both cocoa kernel and cocoa powder. The main fatty acids were oleic, stearic, and palmitic (respective averages of 34.48±1.49, 31.81±1.51 and 30.01±0.89%). Theobromine (9.79 - 12.95 mg/g), catechin (3.90 - 18.22 mg/g) and epicatechin (6.15 - 13.09 mg/g) represented the major bioactives. Also, hybrid cultivars (Hy1, Hy2, Hy3, Hy4, Hy5, and Hy6) provided the highest content in polyphenols, flavonoids, and flavanols, also resulting in the highest total antioxidant capacity.

Keywords: Commercial cocoa beans; chromatic parameters; oil; flavanols; Q-DPPH$^{\circ}$

1. Introduction

In America, the cocoa production is important, especially in countries such as Ecuador, Brazil, Peru, Colombia, Dominican Republic, and Mexico. In Peru, cocoa is essentially an export product (López Cuadra, Cunias Rodríguez, & Carrasco Vega, 2020),

so in 2015 it reached a value of US$ 267 million, while in 2019 it was US$ 294 million in exports of cocoa beans and their derivatives. In Peru, cacao is distributed in four genetic groups: Trinitario (located mainly in Junín), Amazonian Forastero (produced mainly in Cusco and Ayacucho), CCN 51 (located mainly in San Martín and Cusco) and Criollo + Natives (particularly in Cusco, Amazonas, and Cajamarca) (López Cuadra et al., 2020). On the other hand, Peru is considered one of the producing countries of fine aroma cocoa. For example, the "Criollo cocoa is of high value and is a fine cocoa used to produce high-quality chocolates" (Castro-Alayo, Idrogo-Vásquez, Siche, & Cardenas-Toro, 2019). The cocoa production in Peru, from 2015 to 2019 ranged from 105 to 134 thousand tonnes (Foresight, 2020).

Cocoa beans are fermented by various yeasts, lactic acid bacteria and acetic acid bacteria (De Vuyst & Weckx, 2016) and subsequently sun-dried or artificially. The cocoa is an important commodity in the world economy and essential for the chocolate confectionery products, chocolate-covered foods (e.g., chocolate-dipped cookies, coffee beans, peanut, sacha inchi seed, strawberries, blueberries, bananas, citrus peel), and other foodstuff containing cocoa powder (e.g., chocolate flavored drinks, flakes, cakes, mousse, biscuits, ice cream).

Bioactive compounds in cocoa beans are occurring naturally or synthesized during of the technological process and are responsible for sweet, bitter, acid, and astringent taste. In cocoa beans, polyphenol compounds are around 12-18% of total constituents. The main classes of phytochemicals detected in cocoa beans are phenolic acid derivatives, flavonoids, amino acid derivatives and other polar compounds (Cadíz-Gurrea et al., 2020). These phytochemicals have shown multiple benefits, including antioxidant

potential, prevention of type 2 diabetes mellitus, antimicrobial activity, reduced risk of cardiovascular diseases and lower blood pressure, and antimicrobial activity (Oracz & Nebesny, 2016; Ramos, Martín, & Goya, 2017; Todorovic, Milenkovic, Vidovic, Todorovic, & Sobajic, 2017, Ludovici et al., 2017; Dugo, Tripodo, Santi, & Fanali, 2018).

Cocoa butter is a vegetable fat found in cocoa beans, whose fat percentage ranges between 40 to 50%. Cocoa butter is an important ingredient in product development in the chocolate and other confectionery industries. Besides, the cocoa and cocoa-derived products contain large quantities of polyphenols, especially flavonoids. The main flavanol in cocoa beans is (-)-epicatechin (Peláez, Bardón, & Camasca, 2016) which is found around 35% of the total of this polyphenol class. Findings suggest that the polyphenols, epicatechin and flavanols are strongly dependent on several factors including, geographical origin, cultivars, environmental factors, altitude, ripeness degree and processing operations (Oracz, Żyżelewicz, & Nebesny, 2014; Urbańska, Derewiaka, Lenart, & Kowalska, 2019). Polyphenols from cocoa beans have been reported in various investigations as bioactive constituents with antioxidant properties (Oracz & Nebesny, 2016). Several methods have been used to evaluate the antioxidant activity of cocoa beans and their derivative products, such as 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\circ}$), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) (Cádiz-Gurrea et al., 2014) and Fourier transform infrared spectroscopy (FTIR) (Batista, de Andrade, Ramos, Dias, & Schwana, 2016).

Although there is a large number of studies on the chemical composition and quality parameters of cocoa beans, there are still few studies on the bioactive components (methylxanthines, fatty acids and phenolic compounds) and other measurements in the

Peruvian cocoa of different genotypes. According to the "Catálogo de cultivares de cacao del Perú" (García Carrión, 2016) in the Peruvian Amazon there are different cocoa populations: Trinitarian, Forastero, Criollo, and Nacional, Miscellaneous, Huallaga, Ucayali-Urubamba, Marañón, Native, and hybrid selections.

The aim of this research was to characterize the phenolic profile, fatty acid composition, and evaluate antioxidant capacity. Furthermore, usual chromatic colour parameters and others chemical quality parameters in Peruvian commercial cocoa beans. Therefore, the information generated in this study provides relevant data to expand what is already known or to expand the discussion in future studies.

2. Materials and Methods

2.1. Chemicals

The chemical reagents used in this study were Folin-Ciocalteu reagent, sodium carbonate, aluminum chloride hexahydrate, *p*-dimethylaminocinnamaldehyde (DMACA), 2,2-Diphenyl-1-picrylhydrazyl, trifluoroacetic acid and acetonitrile for HPLC. The standards of gallic acid, rutin (quercetin-3-O-rutinoside), (+)-catechin hydrate, (-)-epicatechin, theobromine, caffeic acid were purchased from Merck KGaA (Sigma-Aldrich), Darmstadt, Germany. Ethanol, hydrochloric acid 36% and methanol were from Merck Peruana S.A, Lima, Peru.

2.2. Sample and sample treatment

Commercial samples of fermented and dried cocoa beans were obtained in Lima-Peru cocoa stores. Samples numbers and some morphological characteristics are described in Table 1. Approximately 1500 g of each sample were previously selected. Subsequently, the husk was removed manually from the cocoa beans. The cocoa powder

was obtained by grinding (IKA® A11, Staufen, Germany) (appr. 4 g of sample at maximum speed for 8 seconds). Cocoa powder was defatted with n-hexane in a Soxhlet extractor (E-816 SOX, BÜCHI Labortechnik AG, Flawil, Switzerland). The samples were vacuum packed and stored at -20°C until the analyses.

2.3. Colour measurements

The measurement of kernel and powder cocoa color was by image analysis. Image acquisition was obtained using a digital camera (Canon, Power Shot SX60 HS, Tokyo, Japan). The chromatic coordinates were obtained following the methodology described in previous works (Best et al., 2020). The *L**, *a**, and *b** values were used to calculate the hue angle and chroma of kernel and powder cocoa. In the case of cocoa powder, color measurements were carried out before fat removal.

2.4. Extraction and fatty acids analysis by GC-FID

Two grams of grounded cacao beans were mixed with 10 mL of petroleum ether in a 25 mL glass flat-bottom flask with ground joint. Ultrasonic extraction was performed with an ultrasound bath (Branson Ultrasonics Co, USA) with 40 kHz of frequency and 15 min of extraction time at 30°C. After extraction, the suspension was filtered through Whatman glass microfiber thimble, and the solvent removed at room temperature in laboratory hood (Labconco Corporation, Kansas City, MO). Approximately 50 mg of cocoa butter were dissolved in 1.5 mL hexane and then transesterified using 300 µL 2 N methanolic potassium hydroxide solution. After vigorous shaking and centrifugation, the upper phase (methyl ester fatty acids) was transferred to a 250 µL vial insert micro conical glass for analyses. GC analysis were carried out using a PerkinElmer Clarus® 690 gas chromatograph (PerkinElmer, Shelton, CT) equipped with a SP™-2380 fused silica

154 capillary column (60 m x 0.25 mm i.d.: 0.2 μm film thickness, Supelco®) and a flame
155 ionization detector (FID). The temperature of the injector, detector and the oven
156 temperature program were similar to those described by Ramos-Escudero et al. (2019).
157 Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injection volume
158 was 1 μL. The results of the fatty acid composition were expressed in relative percentage.
159
160 2.5. Polyphenol extraction
161 Approximately 0.5 g of defatted sample was placed in a 15.0-mL conical plastic tube.
162 Then 5.0-mL of an 80% ethanol solution was added, and the mixture was stirred for 2
163 hours. Subsequently, the mixture was placed in an ultrasonic bath 1800 (Branson
164 Ultrasonics Co, USA) at 40 kHz of frequency and 30 min of extraction time at 25°C (end
165 point < 35°C).
166
167 2.6. Determination of flavanol contents
168 The colorimetric *p*-dimethylamino-cinnamaldehyde (DMACA) method was used for
169 determination of flavanol contents (Gallego et al., 2018). In a 2.0-mL microcentrifuge
170 tube, 20 μL of defatted sample extract, 150 μL of ethanol solution (80%) and 900 μL of
171 DMACA solution (0.1% in 1 N HCl in ethanol) were mixture and vortexed vigorously
172 using a MX-S vortex at maximum speed for 10 min. The absorbance was read at 640 nm
173 in an Orion AquaMate 8100 Uv-Visible spectrophotometer (Thermo Scientific, Waltham,
174 MA, USA). Flavanol content was calculated using catechin calibration curve (y=0.059x-
175 0.0376; R^2=0.9972). The results were expressed as mg of catechin equivalents per g of
176 sample (mg CE/g).
177
178 2.7. Determination of flavonoid contents

The content of flavonoids were determined by the method described by Ramos-Escudero et al. (2012) with some modifications. In a 2.0-mL microcentrifuge tube whereby 10 µL of defatted sample extract was mixed with 1000 µL of distilled water, 100 µL of aluminum chloride (2% in 5% ethanolic solution of acetic acid) and 75 µL of sodium nitrite was added. The mixture was vortexed vigorously using a SBS vortex at maximum speed for a few seconds and the mixture was allowed to react at room temperature for 30 minutes; after this time the absorbance was read at 415 nm. Flavonoid contents were calculated using rutin calibration curve ($y=0.0058x+0.0266$; $R^2=0.9998$). The results were expressed as mg of rutin equivalents per g of sample (mg RE/g).

2.8. Determination of polyphenol contents

Total phenolic content was determined using Folin-Ciocalteu method described by Plaza et al. (2017) with some modifications. Briefly, 10 µL of defatted sample extract was mixed with 3.0 mL of distilled water and 750 µL of 0.2N Folin-Ciocalteu reagent. After 5 min, 750 µL of 7.5% (w/v) sodium carbonate was added. The reaction was developed for 2 hours at room temperature; after this time, the absorbance was read at 760 nm. Polyphenol contents were calculated using gallic acid calibration curve ($y=0.0058x+0.0266$; $R^2=0.9998$). The results were expressed as mg of gallic acid equivalents per mg of sample (mg GAE/g).

2.9. Extraction and analysis of theobromine and phenols by HPLC-DAD

For the characterization of phenols, an extract of a defatted sample of cocoa was made by a method previously used with cacao cotyledon in which the methanol-acetone at pH 3 was employed (Hernández-Hernández, Viera-Alcaide, Morales-Sillero, Fernández-Bolaños, & Rodríguez-Gutiérrez, 2018).

The quantification of phenols and the analysis conditions were developed using an optimized method (Hernández-Hernández et al., 2018). The analysis was performed with Varian Prostar HPLC system equipped with a diode array detector and the software used to manage chromatographic separations was Varian Star Workstation version 6.41. The system was equipped with a Rheodyne injection with a 20-µL loop, and a C18 column (Biphenyl 100 Å, 250 mm x 4.6 mm i.d., 5.0 µm particle size; Kinetex®) at a flow rate of 1.0 mL/min. The mobile phase was 0.01 % trichloroacetic acid in water (A) and acetonitrile (B), using the following gradient over a total run time of 55 min: 95 % A and 5% of B initially, 75 % A and 25% of B in 30 min, 50 % A and 50 % of B in 45 min, 0 % A and 100 % of B in 47 min, 75 % A and 25 % of B in 50 min, and 95 % A and 5% of B in 52 min until the end of the run (Hernández-Hernández et al., 2019). Chromatograms were acquired at 254, 280, and 340 nm. Quantification was carried out by integration of the peaks at different wavelengths with reference to calibrations made using external standards: theobromine (280 nm), (-)-epicatechin (280 nm), (+)-catechin (280 nm) and caffeic acid (340 nm).

2.10. QUENCHER-DPPH° assay

The QUENCHER-DPPH° assay with some modification was used to assess the total antioxidant capacity (TAC) of the commercial cacao beans (Alvites-Misajel, García-Gutiérrez, Miranda-Rodríguez, & Ramos-Escudero, 2019). Briefly, 5 mg of grounded cacao beans were weighed and placed in a 15.0 mL conical plastic tube, and the reaction was started by adding 3 mL of DPPH° solution (100 µmol/L in ethanol/water 80:20 v/v). The reaction was carried out at room temperature under agitation using a LP vortex mixer (Thermo Scientific, Waltham, MA, USA) at 3000 rpm for 5 min. The reaction continued

under an ultrasound device with a frequency of 40 MHz and temperature of 25°C for 5 min. Finally, 1.0 mL of the reaction was centrifuged at 10000 x g for 5 min in micro-centrifuge 5418R (Eppendorf AG, Hamburg, Deutschland). The supernatant (700 µL) was put into a plastic disposable cell and the absorbance was read at 515 nm in a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA). The result of the total antioxidant capacity was expressed as mmol equivalent trolox/g sample (mmol TE/g).

2.11. Statistical analysis

All analyses were conducted in triplicate, the results were expressed as mean values±standard deviation (SD). An analysis of variance (ANOVA) was carried out for all experimental runs with post hoc Tukey (Honestly Significant Difference) test at a p-value is less than alpha=0.05 among means. Pearson's product moment correlations were also carried out, with a 95% confidence level, the correlation coefficients (r) were considered as statistically significant for p-value less than alpha=0.05. A heatmap was performed using the quantities of phenolic compounds, antioxidant activity, fatty acids, and chromatic parameters to identify the distribution of the Peruvian commercial cocoa beans. The ANOVA, Pearson's correlations and heatmap were performed using a trial version of the GraphPad Prism version 9.0.0 software, LLC (San Diego, CA).

3. Results and Discussion

3.1. Colour intensities of kernel and powder cocoa

The colour parameters of kernel and powder cocoa are displayed in Tables 1. The *L** values in cocoa kernel ranged between 16.82-44.46 units, being the Blanco (BLA) and Chuncho (CHU) cultivars the ones that showed the lowest values, while the hybrid

cultivars (Hy1, Hy2, Hy3, Hy4, Hy5 and Hy6) exhibited values ranging from 33.74-44.46 units. When all the cocoa kernel samples were considered, it was observed that the hue angle values were in the range of 19.50°-42.88° units. While the chroma values varied from 3.04-17.21 units. The colour coordinate that takes positive values for *a** and *b**, correspond to brown pigments that were observed in cocoa beans of Forastero cultivar (Żyżelewicz, Krysiak, Nebesny, & Budryn, 2014). Taking into consideration *a** it was observed that the cocoa kernel was within the range from 2.38-14.78 units. While the chromatic parameter *b** was found between 1.23-8.47 units. Żyżelewicz et al. (2014) reported that the cocoa beans of Forastero cultivar from Togo showed the following chromatic values (L^* = 34.69; a^* = 5.52; b^* = 2.68). According to Hartuti et al., (2019) the chromatic parameters of fermented dried cocoa beans at different temperatures and times showed the following values (L^* = 31.64-48.56; a^* = 11.06-20.54; b^* = 8.65-18.76; C^*_{ab} = 16.50 -27.64; h_{ab} = 30.01°-51.73°).

As shown in Table 1, the lightness increased in cocoa powder between 49.89-63.48 units, compared to cocoa kernel samples. Likewise, the chroma and hue angle exhibited values ranging from 12.59-19.62 and 37.98°-64.52° respectively. While the chromatic parameters of *a** and *b** showed values of 5.26-13.94 and 10.58-16.10 units, respectively. According to Septianti et al. (2020) the colour measurement of cocoa powder before fat removal shows lightness values between 55.70-63.53 units, while the chromatic parameters of *a** and *b** range between 3.77-9.13 and 6.67-11.40 units, respectively.

In general, the colour in the cocoa kernel comes from the content of phenolic compounds and anthocyanins. In the cocoa kernel, a set of biotransformation's occur during the fermentation and drying process that leads to the formation of water-insoluble

brown or brown-violet phlobaphenes (Belitz, Grosch, & Schieberle, 2009; Krysiak, Adamski, & Żyżelewicz, 2013), which transmit the chromatic characteristics in the fermented cocoa kernel. On the other hand, Septianti et al. (2020) reported that dark color produced in cocoa powder is due to the presence of fat content.

3.2. Fatty acid composition of cocoa butter

The cocoa butters obtained from Peruvian commercial cocoa beans were analyzed for their fatty acid compositions, the results are listed in Table 2. All fatty acids showed significant differences ($p < 0.05$) between the eight oil cultivars. All the samples showed very similar profiles of the main as well as the minor fatty acids. The distribution and the contents of the fatty acids varied as follows: palmitic acid (C16:0) (between 28.58-31.19%), stearic acid (C18:0) (between 28.83-33.44%), oleic acid (C18:1) (between 33.03-37.97%), linoleic acid (C18:2) (between 2.49-4.21%), and α-linolenic acid (C18:3) (between 0.21-0.31%). The cocoa butters obtained from Peruvian commercial cocoa beans from different cultivars showed high content of palmitic, stearic, and oleic acids. Torres-Moreno et al. (2015) reported that the content of fatty acids in unroasted cocoa beans was as follows: oleic acid (34.30-34.73%), stearic acid (33.75-36.40%), palmitic acid (25.02-27.61%), linoleic acid (2.02-2.43%), and α-linolenic acid (0.13-0.14%), in cocoa butter from Ecuador and Ghana.

When the general composition of the fatty acid profile is taken into account, the following order was observed: SFA > UFA > MUFA > PUFA (Table 2), these indexes are similar to those found by Torres-Moreno et al. (2015). The values of SFA ranging from 59.30-62.72%, the unsaturated fatty acids (ΣUFA = MUFA + PUFA) accounted between 37.28-40.70% of total fatty acids, MUFA between 33.03-37.97%, and PUFA

between 2.73-4.49%. When considering the ratio of UFA/SFA fatty acids, the cocoa butter obtained from Peruvian commercial cocoa beans presented a range of 0.59-0.69. Also, Stonehouse et al. (2020) obtained values of ΣSFA (64.5%), ΣMUFA (33.1%), ΣPUFA (2.5%) and UFA/SFA ratio of 0.55 in Malaysian cocoa beans. This ratio in other seed oils like pumpkin (*Cucurbita maxima*, var. Berrettina) was 3.7, and sesame between 5.2-6.0 (Montesano, Blasi, Simonetti, Santini, & Cossignani, 2018; Gharby et al., 2017). From the nutritional point of view, the consumption of cocoa butter increases the levels of C18:0 on serum lipid profile (Stonehouse et al., 2020). However, the processing of several foods and confectionary products uses cocoa butter as part of their formulations because its SFA are more stable to oxidation and contributes substantially to sensory properties.

3.3. Polyphenols contents and antioxidant capacity of cocoa beans

Table 3 presents polyphenols contents, and total antioxidant capacity (QUENCHER-DPPH$^{\circ}$) from Peruvian commercial cocoa beans. The total polyphenol content of the different cultivars of cocoa beans ranged between 19.85-33.39 mg GAE/g. When the whole set of samples was considered, the content of flavanols and flavonoids varied from 9.99-22.30 mg CE/g and from 13.78-35.93 mg RE/g, respectively. The total bioactive contents in the BLA and CHU cultivars was lower compared to the hybrid cultivars that showed the highest content of polyphenols, flavanols and flavonoids. Urbańska and Kowalska (2019) reported that the total phenolic content of cocoa beans of different cultivars and geographic origin (Colombia, Dominican Republic, Ecuador, Ghana, and Venezuela) varied from 10.34 to 37.66 mg/g. In this study, a sample from Peru showed a phenolic content of 27.78 mg/g. Oracz and Nebesny (2016) reported higher values of total polyphenols for Nacional, Trinitario and Forastero cultivars (140.53, 167.23 and 173.58

mg GAE/g, respectively), than hybrid clones such as UAF-Upper Amazon Forastero (105.18 mg GAE/g from Ghana) and Trinitario-UAF (129.37 mg GAE/g from Indonesia). In relation to the content of flavanols, Cádiz-Gurrea et al. (2020) reported for different cultivars between 19 to 130 mg CE/g dry extract. While Gu et al. (2013) reported that the flavonoid content for cocoa beans of different origin ranged from 3.50 to 12.62 mg epicatechin equivalents/g. The differences in the contents can be explained by several factors such as cocoa cultivars, geographical location, different maturity stages, post-harvest operations, fermentation and drying, extraction procedures and analytical methodologies used in the assessment (Urbańska and Kowalska, 2019; Rojas, García, Cerón, Ortiz, & Tarazona, 2020; Santander Muñoz, Rodríguez Cortina, Vaillant, & Escobar Parra, 2020; Plaza et al., 2017).

Total antioxidant capacity of the different cultivars was found between 103.38-372.11 mmol TE/g (Table 3). Di Mattia et al. (2017) reported an antioxidant activity of 240-490 mmol TE/g were found with the DPPH° assay. While Oracz and Nebesny (2016) reported that the antioxidant activity measured by the DPPH° test of the extractable fraction of cocoa cultivars of different geographical areas presented values from 323.81 to 1370.12 µmole TE/g of dry weight. The BLA and CHU cultivars showed lower TAC, while the hybrid cultivars showed higher values. The phenolic content of cocoa cultivars, which are associated with the antioxidant capacity, including the TAC/POLY ratio are around 4.73-11.83, and the correlation was $r = 0.9306$. A positive correlation similar to this study was found by Oracz and Nebesny (2016) between DPPH° antioxidant capacity and total phenolic content ($r = 0.968$; $p < 0.001$). Magrone et al. (2017) have reported that cocoa possess polyphenols as major constituents and is associated to beneficial effects.

354 *3.4. Theobromine and phenols of cocoa beans*

355 The main best-known bioactive components in cocoa powder are theobromine,
356 catechin and epicatechin (Table 4). When all the samples are considered, the theobromine
357 content presented an average of 11.42 mg/g, being the hybrid cultivars (11.90 mg/g)
358 slightly higher than the CHU and BLA cultivars (9.98 mg/g). Previously, Hernández-
359 Hernández et al. (2018) reported the theobromine content of 26 genotypes of fermented
360 cotyledon of cocoa between 9.79 to 24.38 mg/g. While Peláez et al. (2016) reported
361 higher theobromine levels in cocoa samples from Peru (61.95 to 76.06 mg/g). Variation
362 in theobromine content in cocoa samples may be related to fermentation times. Febrianto
363 and Zhu (2020) have reported that theobromine and caffeine decrease between 30 and
364 34% respectively, after 240 h of fermentation. This decrease is important since it notably
365 improves the sensory properties of cocoa-derived products. In a recent study,
366 theobromine has been established as a natural component capable of reducing
367 overweight/obesity by regulation of lipid metabolism through inhibition of
368 phosphodiesterases type 4 (Jang et al., 2020). The concentrations of catechin in the cocoa
369 cultivars ranged from 3.90 to 18.22 mg/g. The hybrid cultivars showed higher content
370 (averaging 13.90 mg/g) than the CHU and BLA cultivars (averaging 4.09 mg/g). While
371 epicatechin was the third most important compound that presented a mean for all cocoa
372 cultivars of 7.97 mg/g. While the CHU cultivar contained higher epicatechin (13.09±1.26
373 mg/g) than the rest of the cultivars. Delgado-Ospina et al. (2020) reported that the
374 catechin and epicatechin contents in Colombian Criollo cocoa samples after fermentation
375 and drying ranged from 0.03 to 4.43 mg/g and 0.45 to 2.34 mg/g respectively. Moreover,
376 the results in genotypes of fermented cotyledon of cocoa for catechin was 0.42 to 6.02
377 mg/g, and for epicatechin was 6.16 to 51.57 mg/g. In this study, the epicatechin content
378 was higher than that of catechin (Hernández-Hernández et al. 2018). Similar results were

observed by Quelal-Vásconez et al. (2020) who reported that the epicatechin content (average ~1.63 mg/g) was higher than the catechin content (average ~0.78 mg/g) in cocoa powder from different origins. Febrianto and Zhu (2020) have reported that epicatechin and catechin decreased as the fermentation progressed. Therefore, these flavanols decreased by ~93 and ~85% of the original values, respectively, at the end of 10 days of fermentation. On the other hand, the presence of polyphenol oxidase enzyme during fermentation catalyzes the rapid decrease in the content of flavanols in cocoa beans. In this study, the epi/cat ratio was found between 0.38 to 3.06, while Delgado-Ospina et al. (2020) reported values for cocoa bean samples between 0.13 to 17.55. The epi/cat ratio is an indicator of processing of cocoa beans. Fernández-Romero et al. (2020) have reported that roasting affects this ratio due to epimerization of epicatechin.

Small quantities of three derivatives of epicatechin and caffeic acid are shown in Table 4. When the samples were considered together, the results were as follows: derivative I (averaging 1.80 mg/g), derivative II (averaging 1.16 mg/g), derivative III (averaging 0.78 mg/g), and caffeic acid (averaging 0.05 mg/g). CHU and BLA cultivars presented lower contents than that of the hybrid cultivars. These derivatives correspond to isomers of ethyl-linked epicatechin as well as several isomers of epicatechin-ethyl-procyanidin that have been identified as metabolites of the microorganisms during the fruit fermentation process (Fayeulle et al., 2018). These derivatives have been shown to contribute significantly to the antioxidant activity of cocoa (Hernández-Hernández, Fernández-Cabanás, Rodríguez-Gutiérrez, Bermúdez-Oria, & Morales-Sillero, 2021). According to Hernández-Hernández et al. (2018) reported values for epicatechin derivatives from traces to 4.83 mg/g. Other compounds such as procyanidin B1 and B2 dimers were also quantified at low concentrations compared to monomers (catechin and

404 epicatechin) in conventional cocoa powder and enriched cocoa powder. In a recent study,
405 catechin and epicatechin have demonstrated a potent antihyperglycemic activity, in
406 addition the results of this study have postulated as a new formulation compared to
407 conventional drugs (Mechchate et al., 2021).
408
409 *3.5. Correlations and heatmap*
410 Pearson product-moment correlation between the total antioxidant capacity (TAC)
411 and seven compound (catechin, derivative I, derivative II, derivative III, polyphenols,
412 flavanols and flavonoids) presented values of r = 0.605 - 0.931 (blue colors represent
413 stronger positive correlations) (Fig. 1). However, between the TAC *vs* theobromine (r =
414 0.279) and TAC *vs* caffeic acid (r = 0.271) showed a little correlation. In addition, a low
415 negative correlation was observed between the TAC *vs* epicatechin (r = - 0.423). Many
416 studies have shown a positive correlation between antioxidant activity and polyphenol
417 contents of edible food plants (Sombié et al., 2018; Cadíz-Gurrea et al., 2020). However,
418 the correlation between antioxidant activity and the different chemical constituents found
419 in food matrices have shown little to very high correlation. For example, Cadíz-Gurrea et
420 al. (2020) in cocoa bean samples found a positive correlation with catechin, procyanidins,
421 and various epicatechins, while with epigallocatechin the correlation was negative.
422 Another interesting correlation has been observed between *L* vs TAC* (r = 0.6131,
423 moderate correlation). In this regard, it can be indicated that when the *L** is higher, the
424 antioxidant activity is also higher. While the hue angle and TAC present a little negative
425 correlation (r = -0.1014). Cömert et al. (2020) have reported that the *L** coordinate could
426 not be a good indicative of the colour, unlike the hue angle that takes the chromatic
427 parameters *a** (red/green colour component) and *b** (blue/yellow colour component). The
428 colour of fruits, vegetables, tubers, roots, grains, leaves, and edible flowers reflects the

presence of different pigments. Some of the produced colors as: green (chlorophyll), yellow, orange (carotenoids), purple, mauve, blue, magenta, and crimson (anthocyanins), red, yellow, purple (betalains), pale-yellow, light-brown and brown (tannin) (Martín et al., 2012). In addition, these compounds have shown multiple beneficial effects on human health and contribute to antioxidant activity. Cömert et al. (2020) have reported that hue angle values above 180° have a high antioxidant capacity and those foods that present hue angle values between 20° and 180° have less antioxidant activity. In this study, it was founded that the cocoa cultivars presented a hue angle of 37° to 65°. However, despite the hue angle in these samples was low the antioxidant activity was much higher. In this regard, it should be noted that there is not always a positive correlation between the hue angle and the antioxidant activity. Such is the case of strawberry, red pepper, and red apple, which showed a hue angle of less than 20° (Cömert et al. (2020).

On the other hand, for a better graphical display of the Peruvian commercial cocoa beans with respect to the distribution of the chemical components, a heatmap was plotted (Fig. 2). The heatmap chart describes the different cocoa cultivars through variations in colouring. The catechin content is more abundant in Hy4 and Hy2 cultivars, while the CHU cultivar is more abundant in epicatechin. Hybrid cultivars have a major content of total polyphenols, flavonoids and flavanols. Lastly, the Hy2 and Hy4 cultivars are the ones with the highest antioxidant activity.

4. Conclusions

Peruvian commercial cocoa beans have shown an interesting content of bioactive compounds and antioxidant potential. The chemical composition as well as the chromatic parameters are strongly influenced by various factors such as edaphoclimatic conditions,

fermentation, drying and roasting. Moreover, results of this study showed that the chromatic parameters, especially the *L** coordinate were greater dispersion for the cocoa kernel than for the cocoa powder. The main fatty acids showed the following order: C18:1ω9 oleic > C16:0 palmitic > C18:0 stearic. Furthermore, high amounts of theobromine, catechin and epicatechin were measured, and the epi/cat ratio ranged from 0.38 to 3.06. The TAC and the different analytes such as catechin, derivatives of epicatechin I, II and III, polyphenols, flavonoids and flavanols showed positive correlations. Hy2 and Hy4 hybrid cultivars are the ones with the highest bioactive content and total antioxidant capacity, moreover the *L** coordinate was higher in both samples and lastly the hue angle was lower in Hy2 than in Hy4.

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CRediT authorship contribution statement

Fernando Ramos-Escudero: Conceptualization, Methodology, Investigation, Formal analysis, Supervision, Writing - Original Draft. **Sandra Casimiro-Gonzales**: Conceptualization, Methodology, Investigation, Formal analysis. **África Fernández-Prior**: Investigation, Methodology, Formal analysis, Writing - Review & Editing. **Keidy Cancino Chávez**: Data curation, Visualization, Writing - Review & Editing. **José Gómez-Mendoza**: Data curation, Formal analysis, Writing - Original Draft. **Luciana de la Fuente-Carmelino**: Investigation, Funding acquisition, Project administration, Resources. **Ana María Muñoz**: Investigation, Visualization, Supervisión, Writing - Review & Editing, Resources.

Declaration of competing interest

None to declare.

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670 **Tables**

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672 **Table 1.** Some morphological parameters and colour parameters from commercial
673 cocoa beans

	Blanco	Chuncho	CCN 51 hybrid					
Abbreviation used in the text	BLA	CHU	Hy1	Hy2	Hy3	Hy4	Hy5	Hy6
Weight (g) [a]	121.85	43.94	89.83	75.13	82.19	78.04	67.35	74.76
Weight (%kernel)	86.19	80.92	88.91	87.78	87.94	86.99	90.66	88.94
Weight (%husk)	13.81	19.08	11.09	12.22	12.06	13.01	9.34	11.06
Shape in longitudinal section	elliptical, ovoid	oblong	oblong	oblong	oblong	oblong	oblong	oblong
Cotyledon colour	violet	purple	purple	purple	purple	purple	purple	purple
Colour of cocoa kernel								
*L**	17.51	16.82	37.75	44.46	42.35	33.74	35.49	43.34
*a**	4.11	5.22	6.40	7.77	5.28	14.78	2.38	4.93
*b**	3.29	2.31	3.08	4.37	1.96	8.47	1.23	2.38
Hue angle (h_{ab})	42.88	23.59	25.06	28.04	19.50	30.14	31.64	24.24
Chroma (C^*_{ab})	5.80	6.37	7.18	8.95	6.02	17.21	3.04	6.24
Nix sensor colour								
Colour of cocoa powder								
*L**	50.14	57.61	49.89	58.73	53.33	63.48	58.04	55.48
*a**	7.67	7.29	11.16	8.72	13.94	7.97	5.26	8.96
*b**	13.44	14.05	16.10	10.58	11.07	12.63	11.29	13.55
Hue angle (h_{ab})	60.08	62.88	55.18	50.45	37.98	57.18	64.52	55.90
Chroma (C^*_{ab})	15.57	15.94	19.62	13.82	18.00	15.00	12.59	16.36
Nix sensor colour								

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[a] Weight of 50 units of cocoa beans

Table 2. Fatty acid compositions of the cocoa butter (%)

%	BLA	CHU	Hy1	Hy2	Hy3	Hy4	Hy5	Hy6
C16:0	28.95±0.05 [cd]	30.47±0.06 [ab]	28.58±0.51 [d]	30.19±0.19 [abc]	29.62±0.32 [bcd]	30.27±0.29 [abc]	31.19±0.68 [a]	30.78±0.10 [ab]
C18:0	33.33±0.14 [a]	28.83±0.16 [c]	33.44±0.26 [a]	32.30±0.65 [ab]	32.20±0.55 [ab]	32.45±0.77 [ab]	30.83±0.51 [b]	31.14±0.06 [b]
C18:1	34.35±0.16 [b]	37.97±0.25 [a]	34.47±0.69 [b]	33.03±0.43 [b]	34.03±0.89 [b]	33.63±0.58 [b]	34.10±0.22 [b]	34.24±0.03 [d]
C18:2	3.07±0.03 [f]	2.49±0.03 [g]	3.30±0.09 [e]	4.21±0.04 [a]	3.92±0.03 [b]	3.44±0.09 [de]	3.67±0.06 [c]	3.62±0.01 [cd]
C18:3	0.31±0.00 [a]	0.24±0.00 [cd]	0.21±0.00 [d]	0.28±0.01 [b]	0.22±0.00 [d]	0.22±0.00 [ed]	0.21±0.00 [d]	0.22±0.00 [d]
SFA	62.27	59.30	62.02	62.49	61.83	62.72	62.02	61.92
MUFA	34.35	37.97	34.47	33.03	34.03	33.63	34.10	34.24
PUFA	3.38	2.73	3.50	4.49	4.14	3.66	3.88	3.84
UFA	37.73	40.70	37.98	37.51	38.17	37.28	37.98	38.08
UFA/SFA	0.61	0.69	0.61	0.60	0.62	0.59	0.61	0.61

Means in the same row with different superscript letters were significantly different by Tukey's honest significant difference test ($p < 0.05$).

Table 3. Polyphenol contents, and total antioxidant capacity from commercial cocoa beans

	Polyphenols (mg GAE/g)	Flavanols (mg CE/g)	Flavonoids (mg RE/g)	TAC (mmol TE/g)	TAC/POLY ratio
CHU	21.88±0.49 ^{e}	14.05±0.05 ^{f}	18.54±0.24 ^{e}	156.01±2.08 ^{e}	7.86
BLA	19.85±0.12 ^{f}	9.99±0.14 ^{g}	13.78±0.24 ^{f}	103.38±5.72 ^{f}	4.73
Hy1	28.46±1.03 ^{c}	21.20±0.02 ^{d}	28.78±0.99 ^{bc}	244.24±3.89 ^{b}	8.58
Hy2	33.39±0.57 ^{a}	21.62±0.02 ^{b}	35.93±0.13 ^{a}	368.40±1.03 ^{a}	11.03
Hy3	26.39±0.49 ^{d}	22.30±0.02 ^{a}	29.93±0.15 ^{b}	249.42±2.14 ^{b}	9.45
Hy4	31.46±0.34 ^{b}	21.36±0.13 ^{cd}	33.22±1.11 ^{a}	372.11±0.74 ^{a}	11.83
Hy5	22.21±0.39 ^{e}	20.31±0.02 ^{e}	24.82±2.09 ^{d}	172.99±2.69 ^{d}	7.79
Hy6	25.61±0.20 ^{d}	21.44±0.03 ^{bc}	26.68±1.22 ^{cd}	198.26±1.28 ^{c}	7.74

TAC, total antioxidant capacity; POLY, polyphenols. Means with different superscript letters in the columns were significantly different by Tukey's honest significant difference test ($p < 0.05$).

Table 4. Bioactive compounds from commercial cocoa beans

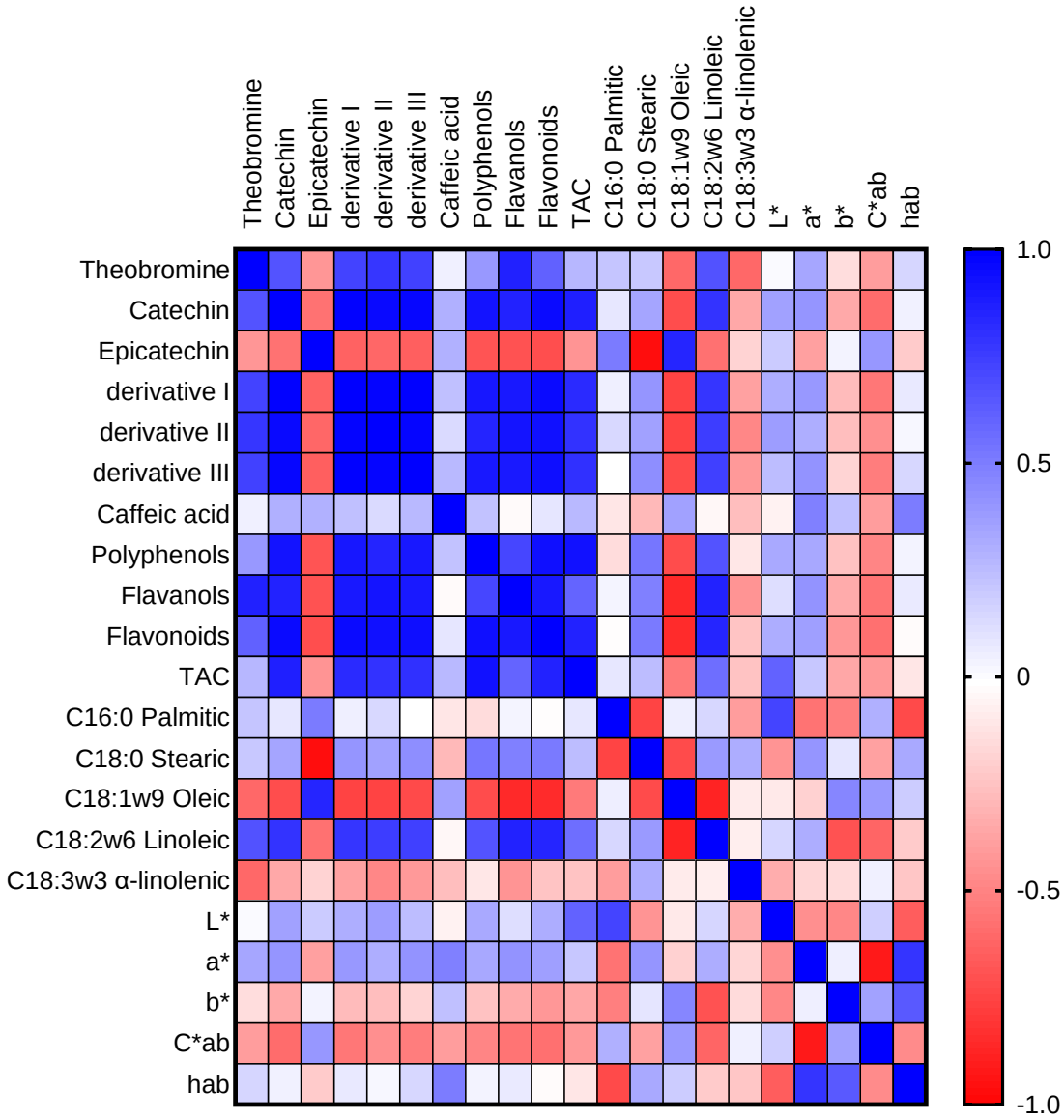
	BLA	CHU	Hy1	Hy2	Hy3	Hy4	Hy5	Hy6
Theobromine	9.79±0.80 ^{d}	10.17±0.42 ^{cd}	11.83±0.33 ^{b}	11.34±0.02 ^{bc}	11.99±0.36 ^{b}	11.43±0.09 ^{bc}	11.85±0.54 ^{b}	12.95±0.08 ^{a}
Catechin	4.28±0.26 ^{f}	3.90±0.59 ^{f}	12.81±0.11 ^{d}	18.22±0.01^{a}	13.76±0.02 ^{c}	15.84±0.13 ^{b}	8.72±0.26 ^{e}	14.05±0.07 ^{c}
Epicatechin	13.09±1.26 ^{a}	6.53±0.90 ^{c}	6.25±0.11 ^{c}	7.00±0.07 ^{b}	7.41±0.06 ^{b}	6.15±0.01 ^{c}	9.25±0.08 ^{b}	8.06±0.16 ^{b}
Derivative I	0.65±0.06 ^{g}	0.80±0.12 ^{f}	2.11±0.05 ^{cd}	2.62±0.03 ^{a}	2.06±0.06 ^{d}	2.45±0.01 ^{b}	1.47±0.01 ^{e}	2.27±0.04 ^{bc}
Derivative II	0.31±0.05 ^{e}	0.40±0.07 ^{e}	1.35±0.05 ^{c}	1.60±0.01 ^{ab}	1.29±0.09 ^{c}	1.73±0.02 ^{a}	1.07±0.00 ^{d}	1.55±0.03 ^{b}
Derivative III	0.23±0.01 ^{g}	0.29±0.05 ^{f}	1.02±0.03 ^{bc}	1.14±0.01 ^{a}	0.87±0.04 ^{d}	1.07±0.01 ^{ab}	0.61±0.01 ^{e}	1.00±0.02 ^{c}
Caffeic acid	0.08±0.01 ^{a}	0.01±0.00 ^{d}	0.07±0.01 ^{b}	0.07±0.00 ^{b}	0.06±0.00 ^{b}	0.03±0.00 ^{c}	0.01±0.00 ^{d}	0.06±0.00 ^{b}

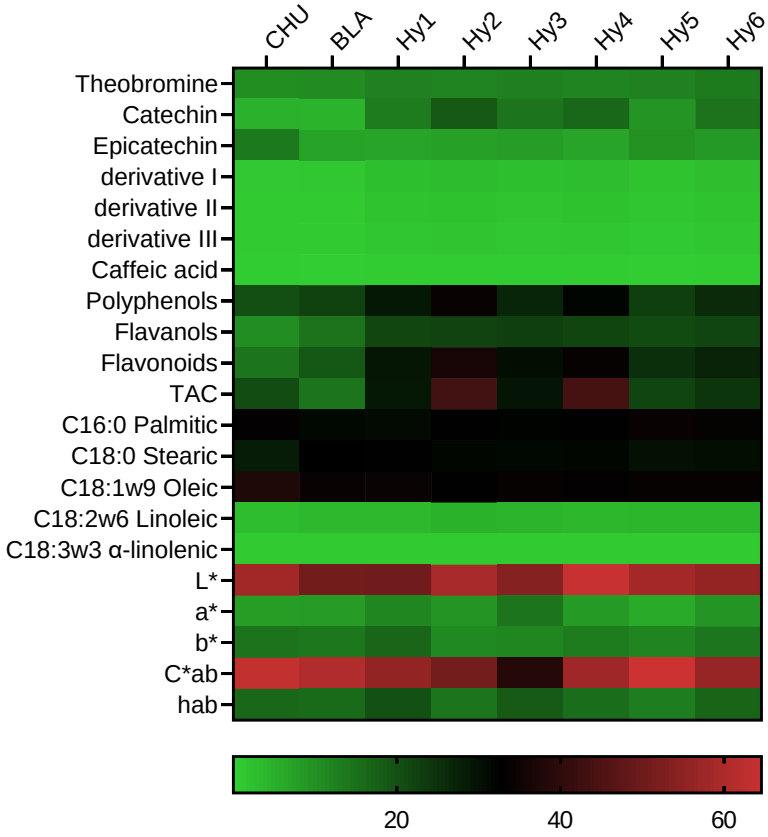
Means with different superscript letters in the rows were significantly different by Tukey's honest significant difference test ($p < 0.05$). The cocoa sample codes are displayed in Table 1.

Figures

Fig. 1. Heatmap showing Pearson product-moment correlation between the different variables analyzed. Darker blue colors represent stronger positive correlations.

Fig. 2. Heatmap chart describes the different Peruvian commercial cocoa beans through colour variations with respect to chromatic parameters and chemical compound distribution.


Theobromine
Catechin
Epicatechin
derivative I
derivative II
derivative III
Caffeic acid
Polyphenols
Flavanols
Flavonoids
TAC
C16:0 Palmitic
C18:0 Stearic
C18:1w9 Oleic
C18:2w6 Linoleic
C18:3w3 α-linolenic
L*
a*
b*
C*ab
hab
1.0
0.5
0
-0.5
-1.0


CHU
BLA
Hy1
Hy2
Hy3
Hy4
Hy5
Hy6
Theobromine
Catechin
Epicatechin
derivative I
derivative II
derivative III
Caffeic acid
Polyphenols
Flavanols
Flavonoids
TAC
C16:0 Palmitic
C18:0 Stearic
C18:1w9 Oleic
C18:2w6 Linoleic
C18:3w3 α-linolenic
L*
a*
b*
C*ab
hab
20
40
60

Graphical abstracts