Characterization of cocoa (*Theobroma cacao* L.) seeds and paste through physicochemical and functional analysis

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ABSTRACT

Objective: The objective of this study was to determine the functional properties and antioxidant capacity of the seeds and paste from five varieties of cocoa (*Theobroma cacao* L.).

Design/Methodology/Approach: Polyphenol content was quantified using spectrophotometry, measuring absorbance at wavelengths ranging from 500 to 760 nm over a defined period. Antioxidant capacity was assessed using the $DPPH^+$ (2,2-diphenyl-1-picrylhydrazyl) assay. A multivariate analysis was performed on the resulting data. Morphological characterization of the germplasm was conducted using 20 descriptors, which were subsequently analyzed through principal component analysis (PCA).

Results: The morphological descriptors most strongly associated with varietal differentiation, with a correlation coefficient $p>0.70$, were fruit length and fruit width. For physicochemical characterization, a one-way ANOVA revealed statistically significant differences among the varieties. This was further confirmed through a homogeneous group analysis at $\alpha=0.05$. Among the studied varieties, 'Rabo Lagarto' and 'Calabacillo' exhibited significant differences in polyphenol content compared to the others.

Limitations/Implications of the Study: This study is limited to cocoa varieties cultivated by smallholder producers in the localities of Miahuatlán and town C-11, within the municipalities of Cárdenas and Cunduacán, Tabasco.

Findings/Conclusions: The results demonstrate that the evaluated cocoa varieties can be distinctly characterized based on morphoagronomic traits, polyphenol content, and antioxidant capacity. These findings contribute valuable insights into the functional properties of cocoa and its derivatives.

Keywords: Cocoa, characterization, antioxidants.

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INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a plant native to the humid tropics of South America (Lanaud *et al.*, 2024). It belongs to the family Sterculiaceae and is naturally distributed in tropical regions characterized by warm and humid climates (Rangel-Fajardo *et al.*, 2012). The Amazon rainforest of Ecuador exhibits the greatest genotypic variability of

the species, where at least three cocoa phenotypes Landrace, Forastero, and Trinitario are found (Zarrillo *et al.*, 2018). According to SIAP (2022), cocoa production in Mexico is concentrated in three southern states: Tabasco (64%), Chiapas (35%), and Guerrero (1%). The economic significance of cocoa cultivation in this region lies in the distinctive and highly prized flavor of the beans, which sets them apart from those produced elsewhere in the world (Jiménez *et al.*, 2018). The chemical composition of cocoa beans and paste confers notable nutritional properties, particularly due to the presence of various antioxidant and anti-inflammatory compounds known as polyphenols, which provide multiple health benefits when consumed (Chacón, Mori, & Chavez, 2021; Rodríguez-Sánchez *et al.*, 2015). In recent years, the complexity of cocoa compounds has drawn significant attention due to the dynamic interactions between environmental conditions and product development. These factors are key drivers of variations in both the quality and nutritional value of cocoa (Jurado-Teixeira *et al.*, 2016). Polyphenols are classified into phenols, flavonoids, and procyanidins groups of secondary metabolites with a broad range of chemical structures (Rojo-Poveda *et al.*, 2020). Cocoa seeds and paste contain polyphenolic compounds such as flavanols (catechin and epicatechin), methylxanthines (theobromine and caffeine), and other bioactive molecules like phenylethylamine (PEA) (Morales *et al.*, 2012). Antioxidants are defined as substances that, at low concentrations, have the ability to reduce, delay, or prevent the oxidation of a substrate (López-Medina & Gil-Rivero, 2017). The antioxidant potential of cocoa polyphenols is attributed to their ability to inhibit oxidative cellular stress through modulation of redox reactions and their metal-chelating properties (Rossin *et al.*, 2021). These mechanisms offer potential health benefits, as they may help reduce the incidence of chronic degenerative diseases (Oliveira *et al.*, 2022). Cocoa contains both endogenous (enzymatic) and exogenous (non-enzymatic) antioxidants, which work by transforming free radicals into less reactive compounds (Chávez-Rivera & Ordoñez-Gómez, 2018). According to Ferreira de Oliveira *et al.* (2021), consumers are increasingly concerned with the quality of cocoa-derived products, especially regarding traceability and nutritional value including mineral content and antioxidants. Thus, understanding whether the type of cocoa consumed influences antioxidant content is of great importance. Such knowledge could enhance the valuation of certain varieties over others, depending on their intended use, and foster the development of niche markets. This presents opportunities for local agro-industries and contributes to preserving cocoa cultivation, especially in the face of displacement by more economically profitable crops such as sugarcane (Tadeo-Sánchez & Tolentino-Martínez, 2020). The aim of the present study was to characterize the seeds and paste of cocoa from Tabasco through physicochemical and functional analyses, in order to identify their phytochemical and functional properties.

MATERIALS AND METHODS

Study material

In the present study, five cocoa (*Theobroma cacao* L.) accessions were evaluated, corresponding to the varieties 'Amelonado', 'Calabacillo Type', 'Improved White Landrace Clone Carmelo', 'Landrace Type', and 'Rabo Lagarto'. The collection activities were carried out during March and April 2022. The variety 'Improved White Landrace Clone

Carmelo' was selected due to its recognized organoleptic profile and its distinction granted by the Mexican government under the Plant Varieties Law through a breeder's title. The remaining four varieties were chosen based on information provided by local producers, taking into account the general fruit morphology and the experiential knowledge of producers from the municipalities of Cárdenas and Cunduacán, Tabasco, as illustrated in Figure 1. It is important to note that the collected samples were subjected to a varietal validation process to ensure the accuracy of their classification. The sampling followed methodologies proposed by Restrepo-Quiroz *et al.* (2018) and López-Hernández *et al.* (2021). Each sampling site was georeferenced using a Global Positioning System (GPS), and mature fruits were randomly harvested in each plantation to ensure representativeness and minimize selection bias. Subsequently, the samples were recorded and labeled using a passport data system, which included information regarding the locality of origin, geographic coordinates, collection date, producer, and relevant morphoagronomic characteristics such as fruit and seed descriptors.

Physical (Morphoagronomic) Characterization)

Each cocoa sample was assigned an identification code that included the sample number, variety, and place of origin. The samples were processed at the Food Technology Laboratory of the Multidisciplinary Academic Unit Reynosa-Aztlán, part of the Autonomous University of Tamaulipas. For the physical characterization, morphoagronomic descriptors were used, consisting of physical measurements obtained using a HER-411 digital Vernier caliper (STEREN brand), an analytical balance, and a colorimeter (model NR110). Table 1 presents the twenty morphoagronomic descriptors employed in this study.

Chemical characterization (phytochemicals)

Phytochemicals present in cocoa seeds and paste were extracted following the methodology proposed by Ordoñez *et al.* (2020). From the collected cocoa seed and paste samples, 2.5 g of dry sample was mixed with 25 mL of a methanol-water solution

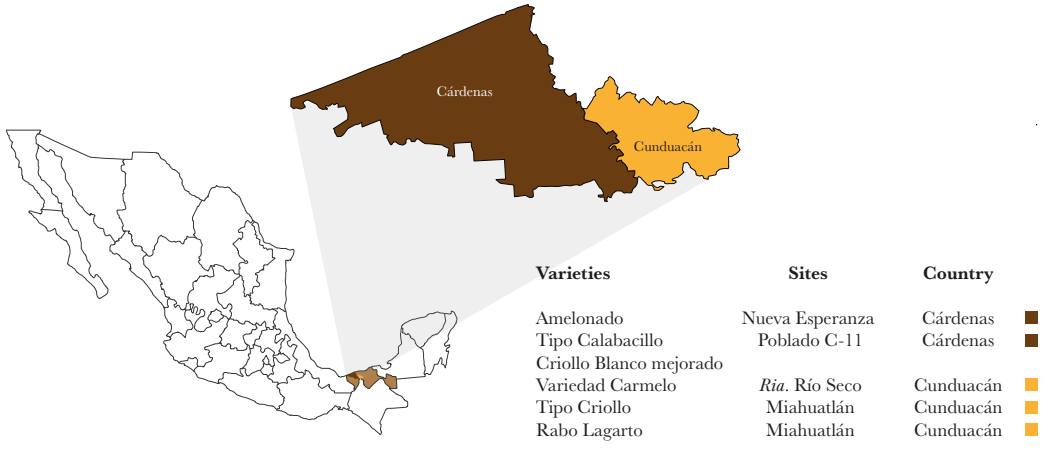

Figure 1. Geographical location of the collection sites for five cocoa varieties in the municipalities of Cárdenas and Cunduacán, Tabasco, Mexico. Source: Authors' own elaboration.

Table 1. Descriptors used for the morphoagronomic characterization of five cocoa varieties.

#	Descriptor	Code	#	Descriptor	Code
1	Color of ripe fruit.	CFM.	11	Total number of seeds per ear.	NSTM.
2	Shape of the apex.	FAF.	12	Number of rows.	NH.
3	Basal construction of the fruit.	CBF.	13	Wet seed mass.	MHS
4	Shape of the fruit.	FF.	14	Dry seed weight.	PSS
5	Roughness of the fruit.	RF.	15	Seed length.	LS.
6	Anthocyanin intensity in fruit spines.	IALF.	16	Seed diameter.	DS.
7	Separation between pairs of spines.	SPL.	17	Thickness of the seed.	GS.
8	Fruit length.	LF.	18	Longitudinal shape of the seed.	FLS.
9	Fruit width	AF.	19	Cross-sectional shape of the seed.	FSTS.
10	Thickness of the fruit.	GF.	20	Predominant color of the cotyledon.	CPC.

Source: Restrepo-Quiroz *et al.*, 2018.

(50:50 v/v). The suspension was subjected to constant agitation at 160 rpm for 24 hours at 20 °C using an Innova 4900 incubator. The mixture was then filtered using a Büchner funnel, filter paper, and a Buchi V-300 vacuum pump. The filtrate was centrifuged at 10,000 rpm for 10 minutes, and the resulting supernatant was transferred to porcelain capsules and dried under controlled conditions at 40 °C±2 °C. The dried extract was stored in amber glass bottles with screw caps at 3 °C±1 °C until phytochemical characterization.

Determination of phenolic compounds

Total phenols (TP) were quantified using the Folin-Ciocalteu (FC) method. A 125 μL aliquot of the extract was mixed with 500 μL of deionized water and 125 μL of Folin reagent (Sigma-Aldrich, St. Louis, MO). The mixture was left to stand for six minutes in the absence of light. Then, to neutralize the reaction, 1,250 μL of 7% sodium carbonate (Na_2CO_3) solution was added. The final volume was adjusted with 1,000 μL of deionized water. After 90 minutes, absorbance was measured at 750 nm using a spectrophotometer. Quantification was performed using a gallic acid standard curve (Sigma Chemical Co.) within the range of 1,000-10 μg/mL, with an R^2=0.9926. Results were expressed as mg gallic acid equivalents per gram of dry sample (mg GAE g^{-1}).

Determination of Flavonoids

Flavonoid content (FL) was determined using the method of Adom and Liu (2002). A 250 μL aliquot of cocoa seed or paste extract was mixed with 75 μL of 5% (w/v) sodium nitrite ($NaNO_2$), 150 μL of 10% (w/v) aluminum chloride ($AlCl_2$), 500 μL of 1 M sodium hydroxide (NaOH), and 2,500 μL of distilled water. After five minutes of rest, absorbance was measured at 517 nm. Quantification was performed using a quercetin standard curve ranging from 10-500 mg/mL, with an R^2=0.9962. Values were expressed as mg quercetin equivalents per gram of dry sample (mg QE g^{-1}).

Determination of procyanidins (tannins)

Condensed tannins (procyanidins) were quantified following the methodology of Broadhurst and Jones (1978). A 50 μL aliquot of the extract was mixed with 3,000 μL of 4% (w/v) methanol-vanillin solution and 600 μL of concentrated hydrochloric acid (HCl). The mixture was allowed to react for 15 minutes at room temperature, and absorbance was measured at 500 nm using methanol as a blank. Quantification was based on a quercetin standard curve within a range of 10-500 mg/mL, with an R^2=0.9707. Results were expressed as mg quercetin equivalents per gram of dry sample (mg QE g^{-1}).

Evaluation of antioxidant capacity

The antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH*) method, as described by Brand-Williams *et al.* (1995). A 100 μL aliquot of the cocoa seed and/or paste extract was mixed with 3,900 μL of DPPH* reagent. Absorbance was measured at 517 nm using a spectrophotometer, with readings taken every 3 minutes for 30 minutes. Antiradical activity was expressed in µmol equivalents. All samples were analyzed in duplicate.

Statistical analysis

Statistical analysis was initially conducted using STATGRAPHICS Data Analysis Solution. A multivariate analysis was performed to identify descriptors with the highest degree of correlation ($p>0.70$). For the physicochemical characterization, ANOVA ($p<0.05$) was employed to verify model assumptions. Data normality was assessed using the Anderson-Darling test prior to ANOVA application. When necessary, homogeneity of variances was verified using Levene's test. Data were transformed and standardized (mean=0, standard deviation=1) to avoid scale-related bias. To perform the multivariate analysis Principal Component Analysis (PCA) Python 3.11 was used, utilizing the 'Scikit-learn' and 'Scipy' libraries for computation and 'matplotlib' for visualization. Each variable was measured in five independent samples with three biological replicates (n=15 observations per variable), applied to the 20 descriptors, ensuring the robustness of the experimental design.

RESULTS AND DISCUSSION

Physical analysis, morphological descriptors

The qualitative and quantitative morphological descriptors allowed for the differentiation and characterization of cocoa fruit and seed traits. This analysis was conducted using 20 morphological descriptors, through which the cocoa accessions were distinguished based on both qualitative and quantitative features of fruits and seeds. Tables 2 and 3 present the results of the morphoagronomic analysis applied to the studied varieties.

Table 4 presents a summary of the statistical analysis of the morphological descriptors evaluated in the cocoa samples, which allow for a statistical assessment of the intrinsic characteristics of the studied variety. Although the analysis framework encompasses the morphology of the cacao tree including stems, flowers, leaves, fruits, and sedes this study

Table 2. Qualitative descriptors of seeds and fruits from five cocoa (*Theobroma cacao* L.) varieties.

Variety	Location	CFM.[A]	FAF.[B]	CBF.[C]	FF.[D]	RF.[E]	IALFM.[F]	SEPL.[G]	FLS.[H]	FSTS.[I]	CPC.[J]
Amelonado	Nueva Esperanza Cárdenas	3	5	1	2	0	1	0	3	1	2
Type Calabacillo	Poblado C-11, Cárdenas	2	6	0	5	0	0	1	3	1	3
Landrace Var. Carmelo	Ria. Río Seco. Cunduacán	3	4	2	2	2	1	3	4	1	1
Type Landrace	Miahuatlán, Cunduacán	3	4	1	1	2	1	3	2	3	1
Rabo Lagarto	Miahuatlán, Cunduacán	2	2	1	4	3	0	4	4	1	3

Where: CFM, color of the ripe fruit; FAF, shape of the fruit apex; CBF, basal construction of the fruit; FF, fruit shape; RF, fruit roughness; IALFM, intensity of anthocyanins on the fruit ridges; SEPL, separation between pairs of ridges; FLS, longitudinal shape of the seed; FSTS, cross-sectional shape of the seed; CPC, predominant color of the cotyledon.
A) 1 intense yellow; 2 intermediate yellow; 3 light yellow; 4 orange-yellow; 5 light orange-yellow; 6 intense red; 7 intermediate red; 8 reddish-orange. B) 1 attenuated; 2 serrated; 3 sharp; 4 nipple-shaped; 5 obtuse; 6 rounded. C) 0 absent; 1 slight; 2 intermediate; 3 strong. D) 1 elliptical; 2 oblong; 3 obovate; 4 ovate; 5 orbicular; 6 oblate. E) 0 absent; 1 slight; 2 intermediate; 3 strong. F) 0 absent; 1 slight; 2 intermediate; 3 intense. G) 1 fused; 2 slight; 3 intermediate; 4 wide or equidistant. H) 1 oblong; 2 elliptical; 3 ovate; 4 irregular. I) 1 flattened; 2 intermediate; 3 rounded. J) 1 white; 2 violet; 3 purple.

Table 3. Quantitative descriptors of seeds and fruits from five cocoa (*Theobroma cacao* L.) varieties.

Variety	Location	n	PF[A]	LF[B]	AF[C]	GF[D]	NSTM	NH	MHS[E]	PSS[F]	DS[G]	LS[H]	GS[I]
Amelonado	Nueva Esperanza Cárdenas	3	428	135.33	83.33	11.40	48	6	2	1.3	13	22.7	4.5
Type Calabacillo	Poblado C-11, Cárdenas	3	554	136	81.33	9.93	40	5	3	1.2	12	20.07	6.5
Landrace Var. Carmelo	Ria. Río Seco. Cunduacán	3	572	169.67	85.33	12.97	44	5	3.54	1.1	12.5	20.47	6.2
Type Landrace	Miahuatlán, Cunduacán	3	650	186.33	86.67	14.83	45	5	3.20	1.6	11.5	19.97	7.2
Rabo Lagarto	Miahuatlán, Cunduacán	3	406	162	71.67	8.97	35	5	3.0	1.2	12.2	20.73	7.3

Where: n, number of fruits; PF, fruit weight; LF, fruit length; AF, fruit width; GF, fruit thickness; NSTM, total number of seeds per pod; NH, number of rows; MHS, fresh seed mass; PSS, dry seed weight; DS, seed diameter; LS, seed length; GS, seed thickness. A) fruit weight in grams (g); B) fruit length in millimeters (mm); C) fruit width (g); D) fruit thickness (mm); E) fresh seed mass (g); F) dry seed weight (g); G) seed diameter (mm); H) seed length (mm); I) seed thickness (mm).

focused on values obtained from fruits and seeds. These data enabled differentiation among cocoa types based on fruit length and width (Restrepo-Quiroz *et al*., 2018).

Table 5 shows that the variance associated with each principal component differs and decreases in order. The Kaiser criterion (eigenvalue ≥1) was used to select significant eigenvalues, as it is appropriate for the model employed. The first component accounted for 38.41% of the total variance. The distribution of coefficients in the first vector and correlation values (≥0.63) indicated that fruit length (FL) contributed most positively to this component. In contrast, the variables fruit apex shape (FAS), fruit shape (FS), number of rows (NR), seed length (SL), seed diameter (SD), and seed longitudinal shape (SLS) contributed negatively. The second principal component explained 28.74% of the variance, with fruit width (FWi) showing a negative correlation (≥0.63) and being the main contributing variable.

Table 4. Characteristic vectors of 20 morphological descriptors measured in cocoa (*Theobroma cacao* L.).

Descriptor	PC_1	PC_2	PC_3
CFM.	0.009	−0.057	−0.073
FAF.	−0.037	−0.123	0.027
CBF.	0.017	−0.016	−0.110
FF.	−0.045	0.0138	0.065
RF.	0.035	0.070	−0.214
IALF.	0.210	−0.030	0.036
SPL.	0.460	0.098	−0.104
LF.	0.989*	0.064	−0.014
AF.	0.064	−0.710*	0.051
GF.	0.072	−0.0217	0.031
NSTM.	0.120	−0.0607	−0.054
NH.	−0.011	−0.027	−0.109
MHS	0.290	0.021	0.113
PSS	0.105	0.012	0.012
LS.	−0.028	−0.041	−0.036
DS.	−0.022	−0.004	0.026
GS.	0.033	0.088	0.065
FLS.	−0.007	0.060	−0.051
FSTS.	0.029	−0.037	0.028
CPC.	0.030	0.088	0.038
$\sum^2$=Eigenvalue	2.865	1.6422	1.257

Source: own elaboration

The third principal component accounted for 14.73% of the variance; however, some variables in this component did not meet the Kaiser criterion. The total accumulated variance was 81.96%. The eigenvalues of each component revealed two highly explanatory variables, primarily found in the first and second principal components, which presented the highest coefficients. Table 6 summarizes the variance values for the three principal components.

In this context, several studies have demonstrated the impact of morphoagronomic characterization on cocoa seeds. For example, Montaleza-Armijos *et al.* (2020) evaluated

Table 5. Variance values corresponding to the three principal components obtained.

Principal Component (PC)	Explained Variance (%)	Cumulative Variance (%)
1	38.49	38.49
2	28.74	67.23
3	14.73	81.96

Source: own elaboration.

Table 6. Quantification of polyphenols in five varieties of cocoa beans.

Cacao seeds sample	Phenols[1]	Flavonoides[2]	Procianidinas[2]
Amelonado	6.55±0.10	28.90±0.10	3.37±0.64
Calabacillo	8.66±0.05	31.23±0.09	3.72±0.66
Criollo	6.27±0.10	29.40±0.07	2.68±0.31
Clon Carmelo	7.08±0.08	16.52±0.005	1.62±0.59
Rabo Lagarto	8.95±0.009	38.62±0.05	3.96±0.36

[1] Milligrams of gallic acid equivalents per gram of dry sample.
[2] Milligrams of quercetin equivalents per gram of dry sample.
Source: Authors' own elaboration.

37 accessions of nacional cocoa in southern Ecuador using 22 morphoagronomic descriptors derived from leaves, flowers, fruits, and seeds. They identified eight principal components explaining 77.62% of the total variance and found that components 3 and 4 were correlated with the descriptors corresponding to fruit length (FL) and fruit width (FWi), which are consistent with the findings of the present study. Similarly, Ballesteros-Possu (2011) characterized 102 cocoa genotypes and explained the observed variation using five principal components. The descriptors contributing to these components included fruit length (FL), fruit width (FWi), shell thickness (STh), fruit width-to-length ratio (FWLR), and seed weight (SW), yielding a cumulative variance of 70.17%. The first principal component was found to contain the variables with the highest positive (FL) and negative (FWi) loadings, results that align with those of the current research. Additionally, López-Hernández *et al.* (2021) performed morphoagronomic characterization of ten cocoa accessions and identified nine key descriptors explaining the variability within their collections: fruit weight (FW), fruit length and diameter (FL, SD), seed index using dry seed weight (SID), seed weight with mucilage (SWWM), seed length and diameter (SLSD), ratio of seed weight without mucilage to seed weight with mucilage (SWWM/SWM), shell weight (ShW), percentage of seed with mucilage (SWM), and seed index (SI). This analysis shares only one descriptor with those found in the present study.

Chemical analysis: polyphenol characterization in cocoa seeds and paste

The polyphenolic content of cocoa seeds and paste was evaluated in this study. For the quantification of polyphenols, calibration curves were prepared for total polyphenols and flavonoids. Standard solutions were prepared using gallic acid and quercetin for the quantification of total phenols, flavonoids, and procyanidins, respectively. Each concentration corresponded to an absorbance value according to Heimler *et al.* (2005) for total phenols, Ghafar *et al.* (2017) for flavonoids, and Oña and Novillo (2017) for procyanidins. The quantification of polyphenol content was based on the linearity of the method; for this purpose, standard solutions of gallic acid and quercetin were prepared, and calibration curves were generated at concentrations ranging from 10 to 1000 μL for total phenols, flavonoids, and procyanidins.

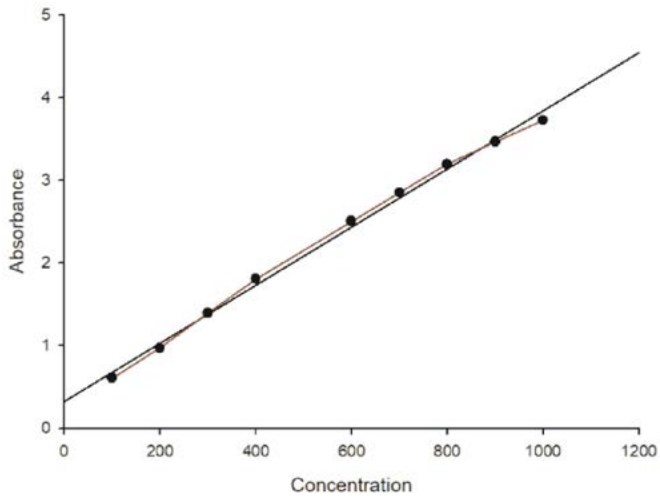

Figure 2. Gallic acid curve.

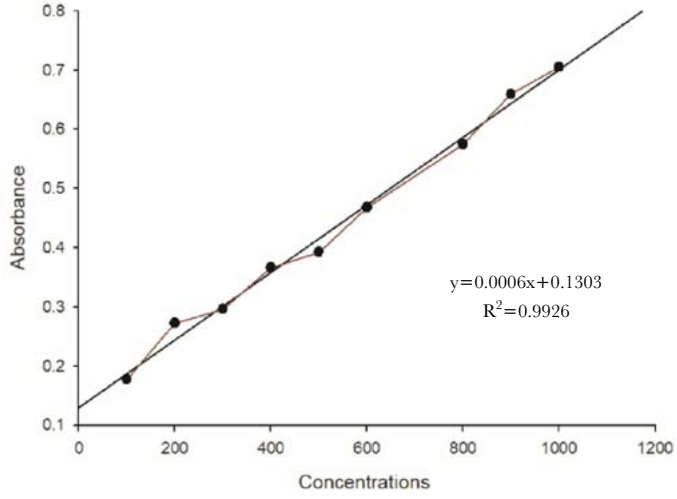

Figure 3. Quercetin curves.

In a study conducted by Urbanska and Kowalska (2019), it was reported that the polyphenolic content present in cocoa beans depends on the regions where the crop is grown, and that the concentration of these compounds is primarily influenced by temperature. Polyphenols are the most abundant phytochemicals in plants. They are secondary metabolites that play a protective role in plants and influence flavor due to their astringent properties (Wollgast & Anklam, 2000). The presence of polyphenols in cocoa has been documented in numerous studies. Urbanska and Kowalska (2019) noted that although polyphenols naturally occur in cocoa beans, their concentrations are influenced by external factors such as geographic region and climate specifically temperatura as a determinant of plant response and compound accumulation in the beans. Additionally, Benítez-Correa *et al.* (2023) highlighted the importance of the solvent type on the kinetics of polyphenol extraction from cocoa seeds, a factor

that must be considered in such analyses. Polyphenols in cocoa are mainly stored in cotyledon cells, whose coloration ranges from white to deep purple (Wollgast & Anklam, 2000). In the present study, the flavonoid content in cocoa seeds of the 'Rabo Lagarto' variety was 38.62±0.05 mg EQ/g, compared to 16.52±0.005 mg EQ/g for the 'Clon Carmelo' variety, as shown in Figure 4. For total phenols, the values were 8.95±0.009 and 6.27±0.10, with the highest concentration observed in 'Rabo Lagarto' and the lowest in the 'Amelonado' variety (Figure 5). In the case of procyanidins, the quantified ranges were 3.96±0.36 and 1.62±0.59, corresponding to the 'Rabo Lagarto' and 'Clon Carmelo' varieties, respectively (Figure 6). Table 6 presents a summary of the analyses performed. The variation in compound concentrations is attributed to environmental

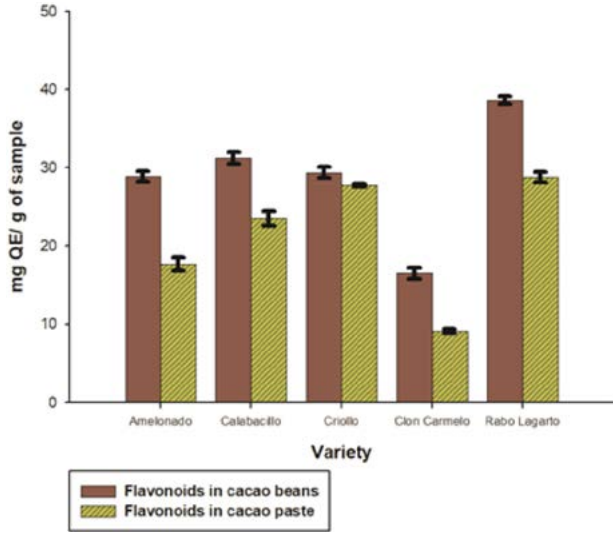

Figure 4. Average total flavonoid content by variety in cocoa seed and paste.

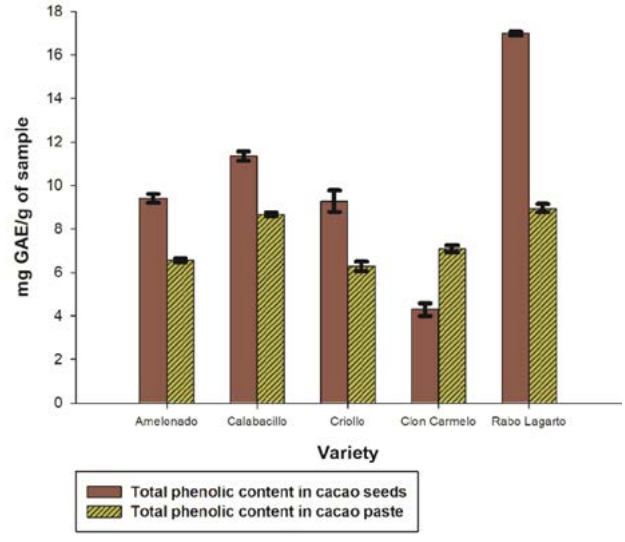

Figure 5. Average content of total phenols by variety in cocoa seed and paste.

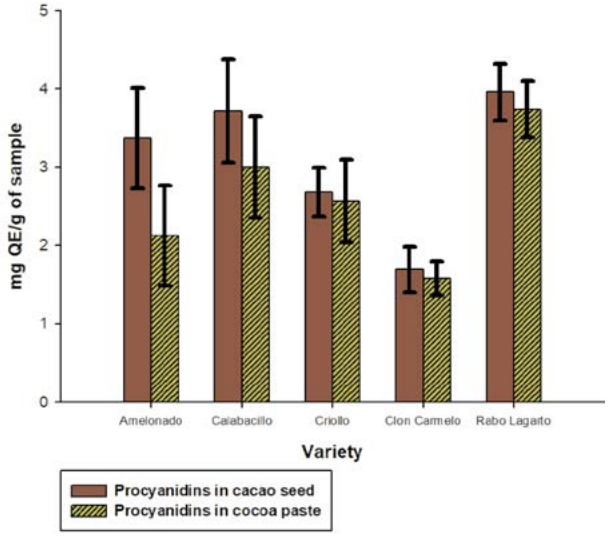

Figure 6. Average total flavonoid content by variety in cocoa seed and paste.

and physicochemical factors, as well as the type of solvent used (Benítez-Correa *et al.*, 2023; Niemenak *et al.*, 2006).

As is well known, epicatechins are a type of polyphenol with antioxidant properties. In this regard, Serafini *et al.* (2003) reported that these compounds can promote cardiovascular health. Similarly, Oracz and Zyzelewicz (2020) noted that cocoa-derived compounds are not only important in food applications but are also of significant interest to the cosmetic and pharmaceutical industries. Multiple studies have documented the positive effects of cocoa consumption on human health. The results of the present study confirm that flavonoids are the most abundant polyphenols in cocoa beans, specifically those of the procyanidin type, which consist of oligomers and polymers of (+)-catechin and (−)-epicatechin (Gu *et al.*, 2006). The polyphenols epicatechin and catechin present in cocoa beans undergo fermentation processes, during which they are oxidized into quinone structures. These oxidation products promote protein condensation, which affects the polyphenol profile and reduces the astringency and bitterness of the beans (Niemenak *et al.*, 2006). In this study, Figure 7 shows the degree of association between variables represented by vectors derived from the phytochemical characterization (polyphenol quantification) of cocoa seeds and paste, grouped into total phenols (TP), flavonoids (FL), and procyanidins (Pr). The distance between components, indicated by red lines, reflects differences among analytical groups, corresponding to the quantity of polyphenolic compounds present in each studied variety highlighting their relative importance. The biplot graphically illustrates the analysis of the five cocoa seed and paste varieties ('Amelonado', 'Calabacillo' type, 'Clon Carmelo', 'Landrace' type, and 'Rabo Lagarto'), each showing distinct associative behavior. The axes represent the principal components (PC1, PC2, and PC3), which capture the data variability in relation to polyphenolic content (see Figure 7).

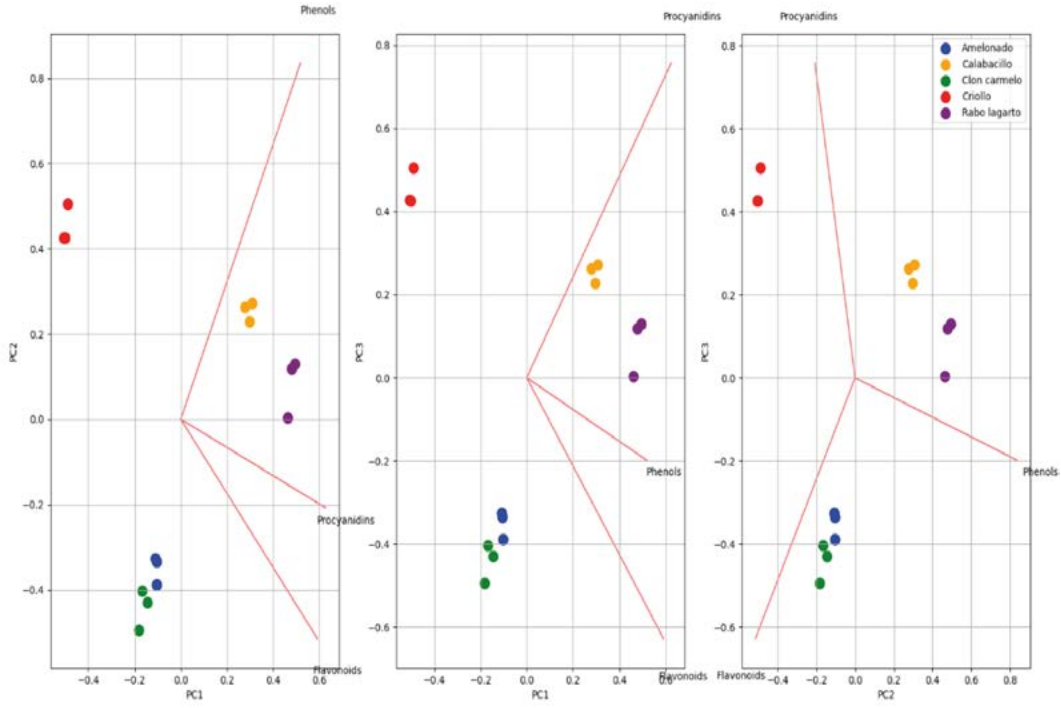

Figure 7. Distribution of accession variables on the first, second, and third principal components in the phytochemical characterization of cocoa seeds and cocoa paste.

CONCLUSIONS

The morphological and phytochemical analyses conducted on five cocoa varieties allowed for clear differentiation of both fruits and seeds based on qualitative and quantitative descriptors. Variables such as fruit length (FL) and fruit width (FWi) exhibited the greatest explanatory weight in the principal components, indicating that these parameters are key in characterizing the evaluated materials. The cumulative variance (81.96%) demonstrates that these variables effectively distinguish the diversity present among the accessions. Regarding the phytochemical analysis, significant variability was observed in the content of polyphenolic compounds (total phenols, flavonoids, and procyanidins). The phytochemical analysis enabled statistical differentiation ($p<0.05$) between cocoa seeds and paste. The 'Rabo Lagarto' variety exhibited the highest concentrations of these metabolites, positioning it as a material with high potential due to its antioxidant properties, whereas 'Clon Carmelo' showed the lowest values. Meanwhile, the 'Amelonado' and 'Calabacillo' varieties displayed similar profiles, while 'Landrace' and 'Clon Carmelo' shared some morphological characteristics but differed notably in their chemical profiles. These findings may inform preferences among both producers and consumers when health-promoting properties are of interest. The results suggest that both morphological traits and phytochemical profiles are complementary tools for differentiating and classifying cocoa germplasm. Furthermore, the variability observed is primarily associated with genetic factors, as 'Rabo Lagarto' corresponds to a Forastero-type cocoa, while 'Clon Carmelo' represents an improved Landrace variety. These findings not only contribute to

the advancement of morphoagronomic characterization of cocoa in the region, but also highlight the importance of conserving and valuing genetic diversity for its impact on bean quality and potential applications.

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