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Profile of cacao cultivated in Colombia: a study based on standardized methods, indicators of quality and variety

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ABSTRACT

Several modifications have been reported for methods used to recognize varieties and the quality of cocoa during post-harvest. This situation has limited comparable and competitive profiles. For this reason, the aim of this study was to standardize the methodologies to evaluate the bromatological, and physicochemical profile of raw, fermented and dried cocoa of four clones from Colombia, in order to identify its quality during post-harvest and between varieties. Fat content: Six solvents were evaluated using Soxhlet and an alternative method assisted by Ultrasound. Total acidity: It was optimized with respect to time by using centrifugation. Antioxidant capacity: Two solvent systems were evaluated to obtain the higher recovery of cocoa extract in the determination of polyphenols and antioxidant capacity. Fermentation index: The difference among the varieties as well as raw and well-fermented cocoa was calculated by using the anthocyanins absorbance ratio. Finally, the experiments were conducted in a completely randomized design. One-way analysis of variance. Hexane was the most efficient solvent for the extraction of fat content. The use of centrifugation instead of filtration during the determination of total acidity reduced the time of analysis in 25 min. acetone:water: acetic acid (70:29.5:0.5) mixture was the best system for the extraction in the determination of the antioxidant activity. The ratio of anthocyanins <1 was an indicator of well-fermented beans and raw cocoa varieties. Proteins, fiber, anthocyanins, and phenols showed significant differences between varieties, which may be convenient to classify cocoa beans.

Keywords: Theobroma cacao L., ethereal extracted, total acidity, anthocyanins, ORAC, total phenols content.

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INTRODUCTION

According to international standards, the implementation of productive models for cocoa (*Theobroma cacao* L.) crops by intervening post-harvest stages through efficient processes and quality control methods, is one of the main challenges of the countries producing this plant material. International Cocoa Organization (ICCO) has estimated that the production of this material has been about 4700 tons during 2016-2017 (1).

Araujo & Fernandes (2014) consolidated a series of tests to define the quality index which refers to the most representative properties according to different sectors involved in the whole production chain and the numerous scientific reports that must be subject to traceability during the processes of benefit and industrialization. The most important tests are the ethereal extract content for its commercial value, the percentage of acidity and pH as a parameter of good fermentation, antioxidant activity as an indicator of its functional properties, as well as heavy metals, among others (2)

An example of this diversity is the time and temperature of extraction and variety of solvents used for the determination of the fat content (3–6), as well as the prolonged time used for the sample preparation used in the analysis of total acidity and pH (7–11).

On the other hand, the implementation of methods to define the profile of the chemical composition of cocoa beans has an additional interest in the quality monitoring using robust tests for the differentiation of cocoa varieties as the content of alkaloids such as theobromine-caffeine ratio (Carrillo, Londoño-Londoño, & Gil, 2013). In this sense, the phenolic compounds content, support in a quantitative way the characteristics of the three large groups that comprise the varieties known as Criollo, Forastero, and Trinitario in Colombia. This classification influences their sensorial attributes and their positioning in the market as fine or common cocoa, which have a different commercial value (12),(13). However, it is required that the implementation of standardized methods be selected in a way that allows to take quick decisions and generate reliable technical data, using statistical tools (14–16). Thus, the aim of this study was to standardize the methodologies to evaluate the bromatological, and physicochemical profile of raw, fermented and dried cocoa of four clones from Colombia in order to identify their quality during post-harvest and among varieties.

MATERIALS AND METHODS

Materials

Cobs of cocoa were obtained from the cocoa farm “Cannes” of the harvest in May of 2016 with an average production of 1800 kg/hectare. The farm is in the Betulia village of Maceo (Antioquia-Colombia) at 6° 33´ 12´´ N - 74° 47´ 14´´ W, altitude average 950 m.a.s.l.

The plant material consisted on four hybrids identified by the phenotypic characteristics as a *Modern Creole* (MC) and *San Vicente* 155 (FSV-155) and two Forasteros *Iquitos Marañón Collection* 67 (IMC-67) and *Caucasia* 51 (CAU-51).

Hexane, diethyl ether, petroleum ether, cyclohexane, methanol, chloroform, sulfuric acid, hydrochloric acid, sodium hydroxide, copper sulfate, n-octanol, acetone anhydride, phthalate potassium acid, phenolphthalein and Tashiro indicator, were obtained from Merck (Darmstadt, Germany) and were analytical grade. 6-hydroxy-2, 5,7-teramethylchroman-2-carboxylic acid (Trolox), fluorescein, and 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) were obtained from Sigma Chemical Co. (Missouri; USA).

Sample Treatment

The mucilage was removed from the raw and unfermented seeds and packed separately in airtight bags at -20°C.

The unfermented seeds were dried in a convection oven (Memmert UFB model 500)

according to the method reported by Gil, M *et al* (2016) at 60 ºC with an air velocity of 0.16 m/s, the seeds were turned each 2 h until reaching a moisture content of 7 % (17),(18).

Raw seeds with their respective mucilage were fermented in wooden boxes. The fermentation process had an anaerobic period for 24 h followed by an aerobic one with periodic agitation, the process took 4 days for MC and FSV-155 and 6 days for ICS-67 and CAU-51. The cocoa beans were then, dried over a marquee six during days until the grain showed the characteristics of dryness identified by the farmer (< 7 %).

Samples of fermented and unfermented dried cotyledon obtained were grounded in a mill (Cuisinart, USA) without husk and passed through a sieve with a particle size of 0.45 µm. The final product was packed in screw-top polyethylene bottles and stored in a plastic container with silica gel disposed at room temperature (17 °C) with controlled relative humidity (65%).

Bromatological characterization

The standardized assay of the bromatological characterization in the samples was fat content known as ethereal extract (EE). The complementary proximal analysis included ashes, total nitrogen and protein, moisture and total crude fiber (19).

Extraction of Ethereal Extract (EE) by Soxhlet

3 g (P1) of the sample was previously treated into a cellulose thimble. Subsequently, 90 mL of chloroform-methanol, dichloromethane, cyclohexane, diethyl ether or n-hexane were added to a pre-weighed crucible (P0). The assembly of Soxhlet was carried out at 130 °C in four batches that consisted of 2 hours of extraction by immersion, 1 h of washing and 30 min of solvent recovery. Crucible was transferred to a desiccator and weighed (P2) after each batch. % EE was calculated by using the following equation (equation 1).

$$\%\mathrm{EE} = \frac{\mathrm{P_2 - P_0}}{\mathrm{P_1}} \times 100 \qquad (1)$$

Extraction of EE assisted by Ultrasound

The quantification of % EE at low temperature was carried out according to the method reported by Carrillo et al. (2014) with slight modifications and the results were compared with those obtained by Soxhlet. 100 mg of sample was weighed into a plastic reaction tube with 1.5 mL of hexane and taken to an ultrasonic bath for 10 min at 25 Hz, a frequency of 99% and 25 °C, after centrifuging at 14000 rpm, 15 min and 10 °C. The supernatant was removed, and the procedure was repeated four times by combining the supernatants until the EE content coincided with the percent by Soxhlet (20).

Total Acidity (TA) and pH

TA and pH of cocoa beans during fermentation and at the end of drying process were determined by a potentiometric method according to international standard A.O.A.C 925.34 with some modifications suggested by Nazzaduri (2006) and Guehi (2010)(8,21,22).

The standardization of the method consisted, first, in selecting the way to separate the solid and the supernatant by centrifugation instead of filtration. Second, the way to prepare the cocoa sample (mucilage and cotyledon) in the different stage (raw, fermented and dried).

Mucilage

Mucilage and husk were removed from 10 seeds (raw and during the fermentation period) with a scalpel to separate from the cotyledon. 4 g of mucilage and husk were placed in Falcon tubes with 36 mL of distilled water and mixed at medium velocity during 1 min. The mix was centrifuged at 5000 rpm x 15 min, 15 °C. Finally, 5 mL of the supernatant was used to register the pH using a potentiometer (InoLab® pH 7110 New) at 20 ±5 ° C and the same among tested was titrated with NaOH (0.01 N) until reaching a pH between 8.27to 8.33.

Cotyledon (raw and dried)

1 g from 10 cotyledons (raw, obtained during fermentation or dried) without mucilage and husk were placed in Falcon tubes with 9 mL distilled water at 90 °C and placed into an

incubation bath at 85 °C during one hour with agitation at medium velocity. Then, the mix was filtered under vacuum using a Whatman N° 4 during 24h or centrifuged for 25 min at 15 °C and 5000 rpm, pH was measured in 5 mL of the supernatant. Another aliquot of 5 mL of the supernatant was titrated with NaOH (0.01 N) until reaching a pH range of 8.27 - 8.33, to calculate the total acidity (TA) as meq of NaOH per 10 g of dry cotyledon or mucilage

Fermentation index (FI)

The FI of the cocoa used to evaluate the evolution of the fermentation process was calculated by anthocyanin absorbance ratio. 0.1 g of ground cocoa beans were subjected to an extraction with 10 mL of a mixture methanol: HCl (97: 3). The homogenate was left at 8 °C for 17 hours. Then, the supernatant was filtered with vacuum and 1 mL was transferred to a quartz cell in order to measure the absorbance ratio at 460 nm and 530 nm by a fluorometer Synergy HT (Biotek Instruments Inc, USA) (23).

Chemical characterization

This characterization was focused on the total phenols content and antioxidant capacity.

Extract of antioxidant compounds and alkaloids

100 mg of defatted cocoa (item 2.3.2) with 1 mL of acetone/water MilliQ/acetic acid solution (70/29.5/0.5, v/v/v) was mixed in a Vortex for1 min and subjected to an ultrasonic bath for 3 min, 25 Hz frequency, 25 °C and 99% power, then centrifuged at 14000 rpm, for 10 min at 10 °C. The supernatant was collected and this procedure was repeated twice, both extracts were combined and used for the determination of antioxidant capacity and alkaloids (24).

Total Phenols Content (TPC)

TPC was determined using the Folin-Ciocalteu reagent. (Singleton & Rossi, 1965). Briefly, 30 µL of gallic acid dilution (10, 20, 40, 60, 80, 100 µg/mL) and 30 µL of the sample previously diluted with distilled water in a ratio 1/60 was added to a 96-well plate polystyrene microplate (Costar, United States). After 6 minutes, 30 µL NaCO2 (10 %w/v) was added, followed by 15 µL of Folin-Ciocalteu reagent except for the Blank (water), then the mixture was incubated at 25 °C for 60 minutes and record the absorbance at 760 nm in a plate reader (Synergy HT, United States). The TPC was expressed as the equivalent of one milligram of gallic acid per gram of dried sample material (mg GAE/g dried sample). All measurements were performed in triplicate.

Determination of Antioxidant Capacity by ORAC

The procedure was based on previous reports of Ou and others with some modifications. Trolox was used as a standard for the calibration curve in concentrations of 5.10, 25, 50, 100, 150, 200 µM, in a 10 Mm phosphate buffer at pH 7.4. The sample previously extracted were added in a strict order fluorescein, phosphate buffer was diluted in a phosphate buffer in a 1/200 ratio. Then, samples, standards and blank were incubated for 30 min at 37 °C. After this time the AAPH solution was added to each sample and the fluorescence intensity was measured in triplicate every 2 min during 2 hours with an excitation and emission wavelength of 485 and 520 nm, respectively, excitation intensity (I) was read at 493 nm and the slit of excitation was 5; the emission intensity (I) at 515 nm and the slit of emission was 13, with an attenuator of 1% and with no attenuator plate (25).

Antioxidant protection was measured from the fluorescence area under the curve (AUC) calculated by the following expression:

$$\mathrm{AUC} = \left(0{,}5 + \left(\sum_{i=1}^{i=31} \frac{f_i}{f_1}\right)\right) \mathrm{x}\ \mathrm{CT} \qquad (2)$$

I: number of cycles, F: fluorescence units, CT: time of each cycle in minutes (CT = 2)

The protective effect of the antioxidant was calculated by the differences in areas under the decay curve of fluorescein between the blank and the sample. It was then compared to the Trolox curve and was expressed in micromoles of Trolox per gram of sample (mmol Tx/g sample), according to Equation (3):

$$ORAC = ABC\_AH / ABC\_Trolox \times [Trolox] / [AH] \quad (3)$$

Experimental design

The experiments were conducted in a completely randomized design (DBA). One-way analysis of variance (ANOVA) was performed using the statistical software R Studio (Version 0.98.1103 GNU Affero General of Public Licenses) and, significant differences between the methods and varieties were determined by Tukey's test at 95% confidence level ($p < 0.05$) (26).).

The evaluation of the effects between treatments during drying, was followed: using equation 4:

$$X\_ij = \mu + \tau\_i + 〖\beta\_j + Ɛ〗\_ij, i = 1,2, ..., k; j = 1,2, ..., r \quad (4)$$

Where:

X_ij: the j-th repetition of the i-th treatment,

μ: general population mean

τ_i: effect of the i-th treatment ($\mu\_i-\mu$)

β_j: effect of j-th block

ε_ij: deviation of the j-th repetition of the i-th treatment with respect to the mean, μ_i, of the i-th treatment

RESULTS AND DISCUSSION

Ethereal Extract (EE)

The EE content is a determinant parameter because it represents one of the main compounds of cocoa (higher than 50% in most materials), which implies a representative economic value for the industry to obtain a product of wide application as the cocoa butter. The EE content has also been used as an indicator to select novel plant materials (16,27). Finally, EE can be considered as an interfering agent to quantify compounds present in low concentration, for example, polyphenols, acrylamide, asparagine, pesticides, among others (28),(29).

Several methods to quantify EE in the cocoa matrix have been reported (Table 1) with some similarities in the operating conditions, but the differences may conduct to a lack of reproducibility and confidence in data result to take decisions or compare with competitors. Notice that the main variation in the methods consists of the type of solvent used and the duration. For this reason, the solvents identified in the previous reports were evaluated, which led to six treatments (A: Chloroform: methanol, B: Cyclohexane, C: Diethyl ether, D: Petroleum ether, E: Methanol, F: Hexane) and the variable response defined for the selection of the solvent was the efficiency of the first cycle (first 4 h), which is the batch that presented the highest capacity of extraction.

Table 2 showed that there were significant differences ($p<0.05$) between treatments. Chloroform-methanol mixture presented the highest yield but did not differ significantly from that obtained with hexane, but the burned appearance and stale odor of the oil obtained for (A), this was considered as a defect in the oil to be used for later applications. This change in color could be due to the reaction of chloroform, not only with the fat content of the seed but also with the proteins (30).

Likewise, there were no significant differences between extraction with hexane and diethyl ether, but the volatile nature of the solvents (boiling temperature C: 36.4 °C; F: 68 °C) avoid enough recovery of diethyl ether (less than 5 %) upon reaching this stage at the end of the treatment, opposite to hexane (>45 %). In addition, a rapid loss was observed during the immersion and washing stages, which involves controlling that the solvent stays in contact with the sample by regulating the recirculation of the solvent.

The other solvent did not have the best performance during the extraction, including petroleum ether, recommended by AOAC for cocoa products. Cyclohexane was the most expensive, opposite to the extraction with methanol that has the lowest cost, but the appearance of the oil obtained with methanol was similar to chloroform-methanol, furthermore, it is a restricted reagent by

antinarcotic law in Colombia, a reason to discard it.

According to the results described above the hexane was the selected solvent due to its recovery capability and the clear appearance of the oil.

With respect to the variation in the duration of the extraction, the method was carried out in four cycles to avoid the exhaustion or saturation of the solvent. The whole cycles took 18 h until the ethereal extract in the sample was exhausted, thus there was no more than 1% the difference of recovery with the last extraction cycle. The results are reported in Table 3.

Table 1. Methods reported since 2010 to 2016 for extraction of ethereal extract from cocoa and by-products

Food matrix	Method	Solvent	Reference
Raw and grounded cocoa beans Powder cocoa (85%, 72% , 70% of cocoa) Hershey's, dark chocolate, milk chocolate (60 %, 60 % and 40 % of cocoa, respectively)	Extraction by agitation and recovery of the supernatant with centrifugation, no temperature or mechanical force was used on the sample	Hexano	(Ramirez-Sanchez, Maya, Ceballos, & Villarreal, 2010)
Well-fermented cocoa beans were obtained from Ivory Coast Jacobs-Suchard Research and Development	Extraction by Soxhlet at 70 C for 16 hours, performing two repetitions each of 8 hours	Petroleum ether	(Voigt et al., 1994)
fermented beans from Ecuador, Madagascar, Camerún, Ghana, Indonesia, Dominic Republic and Trinidad and Tobago	Only a described parameter: particle size which was 0.5 mm	Chloroform: Ethanol	(Hue et al., 2016)
Cocoa beans Forastero from Karnataka, India	Extraction by Soxhlet at 60 °C during 8 hours	Petroleum ether	(Sandhya et al., 2016)
Fermented cocoa without drying from Minchen, Alemania	Samples were immersed in liquid nitrogen until freezing, milling was performed and the particle size was then determined which was 1mm	Hexane	(Esatbeyoglu, Wray, & Winterhalter, 2015)
Cocoa beans from Ivory Cost	Extraction by Soxhlet at 90 °C during 2 hours	Ciclohexano	(Diomande et al., 2015)
Fermented and dried cocoa beans from Brasil (Forastero) and Ecuador (Nacional) Papua Nueva Guinea (Trinitario), Venezuela (Trinitario), Ghana (Alto Amazon) Amazon Forastero hybrid, UAF), Camerún (Trinitario × Alto Amazons) Forastero hybrid, T × UAF) and Indonesy (Trinitario × Alto Amazons) Forastero hybrid, T × UAF)	Rotational vortex agitation at 300 rpm and centrifuged at 6000 rpm x 10 min	Petroleum ether	(Oracz & Nebesny, 2014)
Fermented and dried cocoa beans: Creole from Rome and Italy	The extraction was performed 3 times, after which the remaining solvent was evaporated under a stream of nitrogen.	Hexane	(F. Ioannone et al, 2015)
Fermented and dried cocoa beans: Forastero from Seongnam, Korea	Extraction by Soxhlet for 17 hours	Ether diethyl	(Hu, Kim, & Baik, 2016)
Raw cocoa beans unfermented from different regions of Huila, Colombia	Freeze with liquid nitrogen, grind, freeze-dry and store at -20 ° C, to degrease the sample with sonic hexane 15 min and centrifuge at 1565 g (13000 rpm) x 10 min repeat 3 times	Hexane	(Carrillo, Londoño-Londoño, & Gil, 2014)

Table 2. Differences between the mean values of the percentage of ethereal extract according to the Tukey's test

Comparision	Difference	Low Confidence Level	Upper Confidence Level	p value	Significance
A-B	10.4	2.98	17.82	0.0052	**
A-C	7.7	0.31	15.15	0.0394	*
A-D	10.9	3.51	18.35	0.0035	**
A-E	15.5	8.05	22.89	0.0002	***
A-F	3.6	-3.79	11.05	0.5879	
B-C	-2.7	-10.09	4.75	0.8256	
B-D	0.5	-6.89	7.95	0.9999	
B-E	5.1	-2.35	12.49	0.268	
B-F	-6.8	-14.19	0.65	0.0818	.
C-D	3.2	-4.22	10.62	0.7	
C-E	7.7	0.31	15.15	0.0394	*
C-F	-4.1	-11.52	3.32	0.4698	
D-E	4.5	-2.89	11.95	0.3706	
D-F	-7.3	-14.72	0.12	0.0548	.
E-F	-11.8	-19.25	-4.41	0.0018	**

A: Chloroform: methanol; B: Cyclohexane; C: Diethyl ether; D: Petroleum ether; E: Methanol; F: Hexane Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3. Percent of protein, ethereal extract, total fiber, ash and moisture of dried unfermented and fermented cotyledons (Modern Creole and FSV-155) from Maceo, Antioquia

Clones	Protein		Ethereal Extract, EE		Fiber		Ash		Moisture	
	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented
Modern Creole	13.0 ± 0.418	12.8 ± 0.0152	12.0 ± 1.14	7.4 ± 1.19	12.0 ± 1.14	7.4 ± 1.19	6.0 ± 0.006	7.0 ± 0.008	5 ± 0.001	5 ± 0.001
FSV-155	12.1 ± 0.63	11.5 ± 0.46	6.6 ± 0.49	5.1 ± 1.02	6.6 ± 0.49	5.1 ± 1.02	5.0 ± 0.005	4.0 ± 0.017	6.8 ± 0.0001	7.0 ± 0.0001
CAU-39	12.1 ± 0.22	14.1 ± 0.587	11.4 ± 2.5	8.4 ± 0.85	11.4 ± 2.5	8.4 ± 0.85	7.0 ± 0.003	6.0 ± 0.003	6.5 ± 0.001	5.0 ± 0.0015
IMC-67	13 ± 0.243	12.4 ± 0.32	9.5 ± 0.68	6.9 ± 0.28	9.5 ± 0.68	6.9 ± 0.28	5.0 ± 0.001	5.0 ± 0.0009	6.8 ± 0.0001	5.0 ± 0.0006

From the standardized method parameters, the EE content in the samples was evaluated, as described in Table 3. The fat content of MC and FSV-155 were similar to those reported by Villamir (2013) and Martínez (2015) who reported values of % EE from 56.7 % to 59.8 %. According to National Federation of Cocoa from Colombia, the value found of EE for CAU-39 and IMC-67 were between the range expected (51.3 % – 59.7 %) and (55% – 58.8 %), respectively (31,32). The difference may be due to origins, plant materials and treatments in the processes of post-harvest[3,26,](34).

An indirect use of the obtaining of EE is to get the defatted cocoa for other analysis, but the

high temperatures required during the Soxhlet method can propitiate thermal decomposition of some compounds (Wang & Weller, 2006). In order to avoid the loss of the thermosensitive compounds of cocoa, the extraction assisted by ultrasound technique at room temperature is one of the best alternative (35). The four studied clones were summited to this method, and in order to reach the same percent of the fat extracted by Soxhlet, it was necessary four cycles of extraction under the method described above (2.3.2). The difference with the Soxhlet method was maximum ± 1.0 % and the time was reduced to 14 h.

Bromatological characterization

Total fiber, proteins, ashes, and moisture were evaluated following AOAC methods, in Table 3 are shown the results obtained for dried unfermented and fermented cotyledon of the four clones.

The fiber content on the evaluated samples was lower than the reported by Villamir (2013) but the protein content (12.5%) was similar. This difference can be due to the type of the soil and the system of fertilization. The moisture content for all clones was in the range recommended by International Cocoa Organization ICCO < 7 %)(36).

Total Acidity (TA) and pH

TA and pH are considered important tests to determine the quality of the cocoa beans after and during the fermentation process due to the changes of the organic acids. The control of the values of both analyses has an influence on the overall quality and sensorial profile of the cocoa after drying (7,17). Therefore, the concentration of the organic acids can affect its price in the market.

Figure 1. Acidity (A) and pH (B) of the Modern Creole, FSV-155, CAU-39 and IMC-67 dried unfermented and fermented cotyledons

The filtration is the main operation to separate the dissolved acids from the solid cocoa matrix before measuring both parameters, but this procedure can take between 8 h and 24 h. In the present study, the centrifugation was considered as an option to replace the filtration, which led to a reduction of time in two hours. However, there were not significant differences between both methods to determinate TA and pH ($p<0.05$). Figure 1 shows the pH and acidity expressed as acetic acid mile-equivalent per 10 g of a dried sample for dried unfermented and fermented cotyledons.

Figure 1 shows that the pH values of dried fermented cotyledons were between 5.2 and 5.4, a range that according to Portillo et al (2007) corresponds to a cocoa with aromatic potential and it is classified as a well fermented dry cocoa (pH range between 4.8 and 5.5) and the acidity results fit the recommended range between 0.1 and 0.2 meq NaOH/10 g^{-1} (37,38).

Fermentation Index

The group of anthocyanins is mainly responsible for raw cocoa oriented to a strong purple color when the concentration is at maximum level and as the fermentation progresses the anthocyanin content is affected because the glycosidic bond of the two main anthocyanin fractions present in the cocoa, that is, cyanidin-3-O-galactoside and cyanidin-3-O-arabinoside, are hydrolyzed to anthocyanidins by the action of the enzyme glucosidase that has optimal conditions of 45 ° C and pH between 3.8 - 4.5, which can be observed by a change of color to a pale pink at the end of the fermentation processes (39).

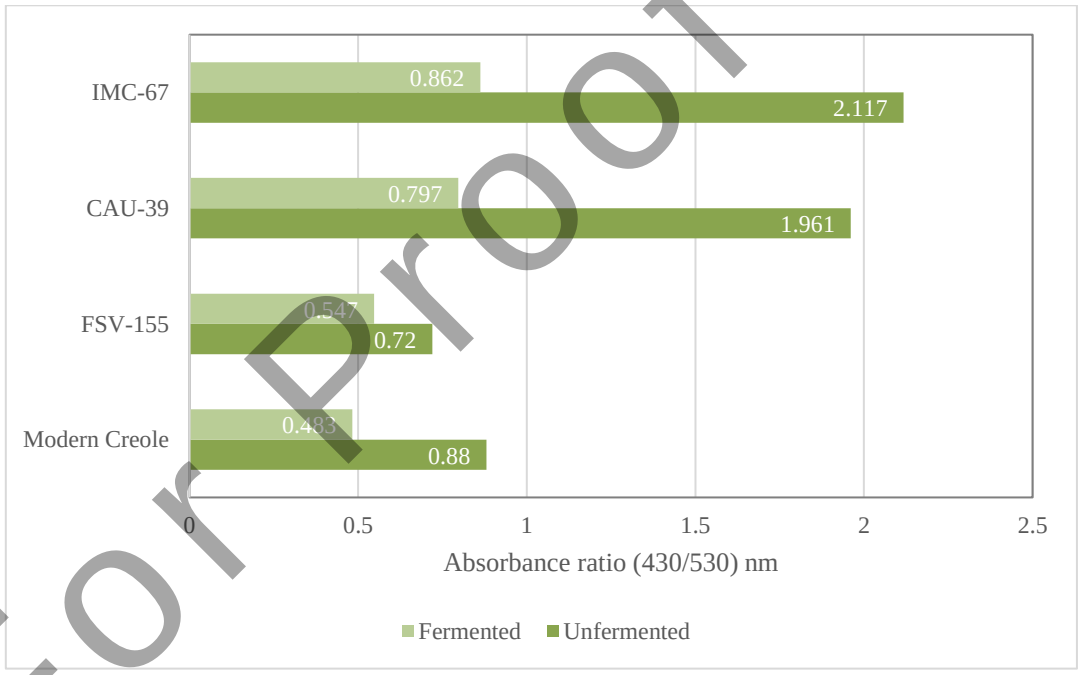

Figure 2. Content of total anthocyanins for raw and dried fermented cocoa beans of the varieties Modern Creole, FSV-155, CAU-39 and IMC-67

In figure 2, can be observed that the content of anthocyanins decreases in the Criollo cocoa (MC and FSV-155), which agrees with a study by Elwers (2009), where they found a very low, almost null content of anthocyanins for this variety, opposite to Trinitario and Forastero (IMC-67 and CAU-39) (40). According to the results, in order to differentiate varieties, unfermented Forasteros values are above 1 and Criollos below 1.

Regarding the results of the fermented clones, independent of the variety, the ratio of absorbance of anthocyanins is below 1, indicating that it could no as an indicator of the

end of fermentation. In order to do the verification of this approximation, 30 samples (100 grains each one) of a mixture of Forastero cocoas denominated by producers as well-fermented were evaluated and 93 % confirmed the results, see Figure 3.

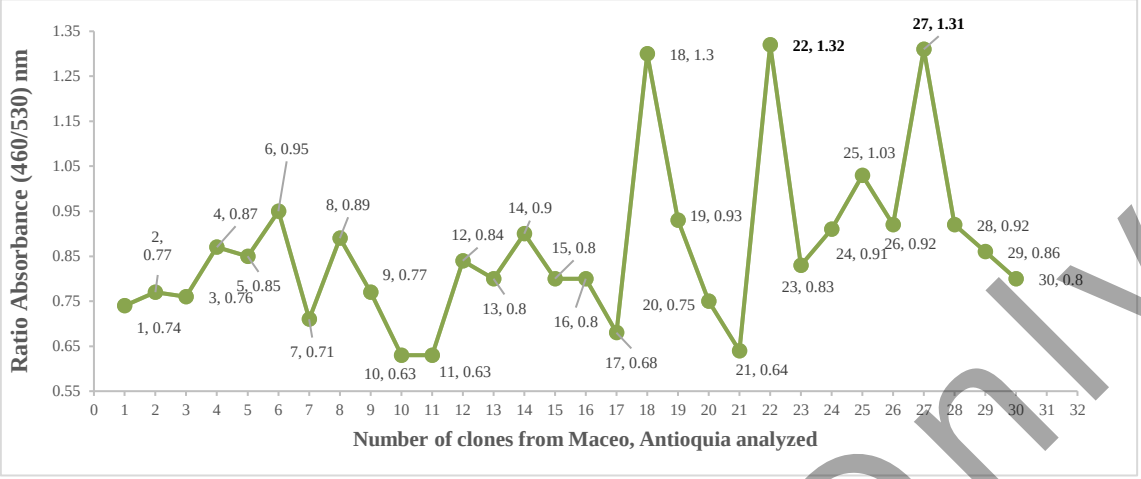

Figure 3. Ratio Absorbance (460/530) nm of clones from Maceo, Antioquia classified as well-fermented by producers

Total Phenol Content and Antioxidant Capacity

Two systems of solvent-extraction were evaluated according to previous studies to find the highest recovery of phenolic compounds (20,41). The first system consisted in a ratio ethanol: water (70:30) and the second was acetone: water Milli-Q: acetic acid (70:29.5:0.5), the last one was the best with a recovery of 75.04 % (data not shown) (42)·(24). This mixture of acetone: water Milli-Q: acetic acid was chosen to evaluate TPC and ORAC.

The content of phenolic compounds corresponding to the fermented and dried cocoa of the four clones studied is detailed in Figure 4. These values are similar to those reported by previous studies Aranzazu (2015), who reported 47.63 -55.65 mg/g (IMC-67), 54 mg AG/g (FSV-155), 54 mg AG/g (CAU- 39)(43).

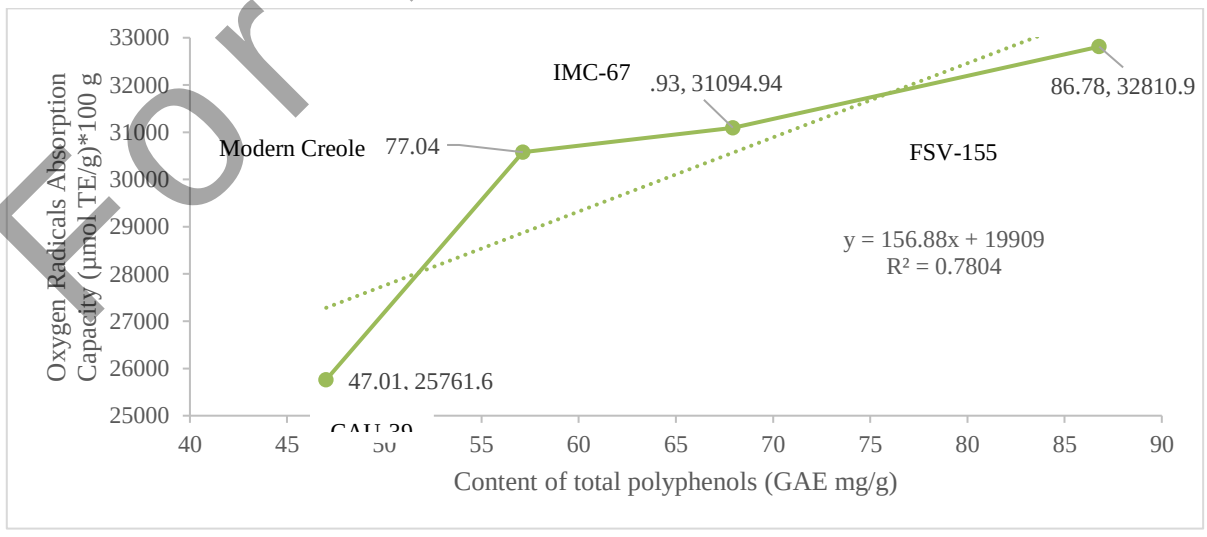

Figure 4. Content of total polyphenols (GAE mg/g) and Oxygen Radicals Absorption Capacity (µmol TE/g)*100 g) of dried and fermented cotyledons of Modern creole, FSV-155, FSV-155, CAU-39 and IMC-67

The correlation between ORAC and TPC was 78.04 % then can be improved between both Criollo clones and IMC-67. The differences of the clones can be attributed to the agronomic, environmental and postharvest conditions (44).

The physical, chemical and bromatological profile described is relevant for decision making in the different stages of the cocoa production chain, as discussed by Araujo (2014) and Gil (2018), when defining the evidence that can be associated with commercial quality, functional, sensory or the distinction between a fermented cocoa of a raw cocoa, among others (2,45). The differentiation between the varieties of cocoa is another characteristic that can be identified by one of the chemical tests such as the ratio of theobromine/caffeine reported by Carrillo (2015), but according to the results obtained in this study, other tests that can help this differentiation is summarized in the content of total polyphenols ($p = 0.0024$), anthocyanins ($p = 0.00206$) and total protein ($p = 0.000132$) since those were the tests that presented significant differences between the Trinitarian clones (IMC-67 and CAU-39).) and Criollos (FSV-155 and Modern Criollo).

The results obtained can be evidenced by the values associated with the content of anthocyanins to each variety of cocoa shown in Figure 2 and these compounds, in turn, are part of the polyphenols. On the other hand, the difference between the protein content that can be directly related to the nutritional requirements of the plant will have a significant impact after fermentation, generating a greater number of free amino acids and low molecular weight peptides (46).

Conclusions

The quality management systems of the cocoa industry are demanding effective analyzes in a way that provides reliable, reproducible, and comparable information that allows quick results to take decisions.

With this study, it was possible to standardize methodologies from previous reports using new technologies, in order to respond to the exposed need. Thus, for the ethereal extract content, the solvent with the highest performance was found, preserving the desired characteristics in the oil. As an alternative method, the conditions for keeping thermosensitive compounds were found, and for both the conditions were guaranteed to obtain the maximum content of ethereal extract. For the determination of total acidity, the time was reduced using centrifugation instead of solid separation, without affecting the results and with the minimum sample amount.

A reference value was found to verify the quality of the fermentation and distinguish between varieties. This quantitative method might replace subjective decisions in the farm and does not require an expensive equipment.

Regarding the antioxidant capacity and polyphenols content, a mixture was determined to obtain a reliable extraction and to be able to relate these two functional properties on cocoa.

Finally, the physicochemical profile of four cocoa materials including a promissory Modern Creole cultivated in Antioquia, Colombia was determined. Protein, anthocyanins, and total contain polyphenols were identified as the tests to identify between varieties or the quality of the cocoa beans during postharvest.

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