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Title: Bioactive compounds in Mexican genotypes of cocoa cotyledon and husk.

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ABSTRACT

A characterization of the phenolic profile of 25 cocoa genotypes established in a Mexican gene bank was carried out. From five different extraction methods commonly used for phenols, extraction with acidified methanol-water was chosen as the best to quantify the concentrations of theobromine and individual phenols in cocoa beans. High concentrations of individual and total phenols were found for genotypes originally from Mexico (like RIM105, MO31, and MO33) or from Peru and Ecuador (INI10), but not the commercial mix (CAF), and were directly associated with their antioxidant activities. Despite the loss of some theobromine and phenols during fermentation, epicatechin remained in the fermented cotyledon in high concentrations. This study could help promote the commercialization of Mexican genotypes of cocoa and reports the possibility of upcycling fermented cocoa husks, which are rich in bioactive compounds and fiber, as novel functional extracts for use in food formulation or for nutraceutical purposes.

Keywords: cocoa genotypes, theobromine, flavonoids, antioxidant, phenolic extract, upcycling.

1. INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a perennial tree widely cultivated in America, Asia, and Africa, whose beans are extracted in order to produce the main ingredient of cocoa powder and chocolate (Cuatrecasas, 1964). Phenolic compounds constitute about 10% of cocoa beans and are responsible for their antioxidant, cardiovascular protective, antitumor, anti-inflammatory (Jolic, Redovnikovic, Markovic, Sipusic, & Delonga, 2011; Steinberg, Bearden, & Keen, 2003; Corti, Flammer, Hollenberg, & Luscher, 2009; Keen, Holt, Oteiza, Fraga, & Schimitsz, 2005; Selmi, Cocchi, Lanfredini, Keen, & Gershwin, 2008; Rusconi & Conti, 2010; Aprotosoiaie, Luca, & Miron, 2016), antineurodegeneratives (Nehlig, 2013), antibacterial, and anticariogenic properties (Ferrazamo, Amato, Ingenito, De Natale, & Pollio, 2009). Phenolic compounds are secondary metabolites that are stored in the pigmented cells of the cotyledon and which perform various functions at the plant, such as the regulation of its development, among others. Flavonoids are the largest and most diverse group of phenolic compounds found in the cocoa bean, which, depending on the structural composition of its central heterocyclic ring, may be divided into six different subclasses including flavones, isoflavones, flavanones, flavon-3-ols, flavanols, and anthocyanins (Oracz, Nebesny, & Żyżelewicz, 2015a).

The most common compounds in cocoa beans are the flavanols epicatechin, catechin, and procyanidins. According to various studies, epicatechin is the compound found in the greatest amounts in cocoa beans, representing about 35% of the total phenolic content, but other polyphenolic compounds have also been identified, including gallocatechin, epigallocatechin, epicatechin-3-O-gallate, quercetin, quercetin 3-

glucoside, quercetin-3-arabinoside, clovamide, and deoxyclovamide (Sanbongi, Osakabe, Natsume, Takizawa, Gomi & Osawa, 1998; Oracz et al., 2015a).

Theobromine and caffeine are two compounds from the group of methylxanthines that are also present in cocoa (Timbie, Schrist, & Kenney, 1978; Belscak, Komes, Horzic, Kovacevic, & Karlovic, 2009) whose physiological effects on the central nervous, cardiovascular, gastrointestinal, respiratory, and renal systems are well known (Nehlig, Dava, & Debry, 1992; Spiller, 1998; Nehlig, 2013).

The phenolic profile in foods, including cocoa, may be affected by various factors: environmental (origin of the sample, variety, maturity, and climate) and processing (tempered, alkalization, and storage) (Oracz, Żyżelewicz, & Nebesny, 2015b; Rusconi, & Conti, 2010; Jolic et al., 2011). Flavonoids are water soluble and highly susceptible to enzymatic and non-enzymatic oxidation (Oracz et al., 2015a). It is estimated that the phenolic content can be reduced from 100 to 10% during the manufacturing process of cocoa (fermentation, drying, and roasting) (Rusconi & Conti, 2010; Oracz et al., 2015b; Afoakwa, Ofose-Ansah, Budo, Mensah-Brown, & Takrama 2015). This can be caused by the presence of the enzyme polyphenoloxidase, epicatechin epimerization caused by pH changes during the fermentation process, and the polymerization of quinones during drying, among other reasons (Oracz et al., 2015b; Afoakwa et al., 2015).

Each genotype of cocoa has its own distinguishing characteristics including bean size, chemical composition, organoleptic properties, and degree of fermentation. In Mexico one of the agricultural products with the greatest potential for cultivation in tropical regions is cocoa. In fact, *Theobroma cacao* is now grown in an area of about 60,000 hectares, mainly distributed in the states of Tabasco and Chiapas, and yields a

production volume of 27,000 tons of cocoa beans (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, 2015). Although several studies have focused on the study of the phenolic compounds of the cocoa beans commercially produced in Mexico (Ramírez, Cely Niño, & Ramírez, 2013; Romero-Cortes et al., 2013; Quiroz, Aguilar, Ramírez, & Ronquillo, 2013), no study has yet to carry out a characterization by genotype. Several studies on different genotypes of cocoa in other countries report varied results for the content of phenols and methylxanthines after processing, which give rise to big differences in terms of sensory characteristics and the final quality of the beans (Cruz, Paula Bacelar, Sergio Eduardo, & Eliete da Silva, 2015). Therefore, the characterization of the phenolic profile of Mexican cocoa genotypes established in a national gene bank could have utility in clonal selection, and the phenolic profile and antioxidant capacity of the genotypes could be used as a marker of nutraceutical quality. To this end, this study contributes to the characterization of the phenolic profile of 25 genotypes and one commercial mix of fermented cocoa harvested in the Gene Bank of the Mexican National Institute of Forestry, Agriculture and Livestock. A comparison of different methods of extraction of phenolic compounds in a homogeneous sample of cocoa beans was first performed; then the most effective extraction methods were chosen for the subsequent characterization of phenols and the phenolic profile of the 25 different Mexican genotypes of cocoa selected and a commercial mix used in Mexico.

2. MATERIALS AND METHODS

2.1. Samples

Twenty-five different genotypes of dry and fermented cacao were used (Table 1) in comparison with a commercial mix sample fermented (CAF) or non fermented (CAL).

All of them were harvested in the 2012–2013 season and belong to the Germplasm *Bank of* the National Institute of Forestry, Agriculture and Livestock Research (INIFAP), Huimangillo, Mexico. The samples were fermented and dried following the industrial method, namely the fermentation of the cocoa beans in boxes (100 kg of fresh samples per box) and subsequent drying in platforms with up to 7% humidity.

2.2. Chemicals

Trifluoroacetic acid (TFA), anthrone, and Folin–Ciocalteu phenol were purchased from Sigma-Aldrich (Madrid, Spain). Na_2CO_3 and methanol were from Panreac Química S.A. (Barcelona, Spain). The standard of gallic acid (GA) was purchased from Sigma-Aldrich. Acetonitrile was of HPLC grade purity (Romyl, Teknokroma, Barcelona, Spain) and acetone was obtained from Sharlau (Barcelo, Spain).

2.3. Extraction methods

The samples of cocoa seed and cocoa husk were manually separated, milled, and homogenized to a particle size of 0.5 μm for the extraction using five different methods. The cocoa seed samples were defatted by extraction with n-hexane for six hours using a Soxhlet apparatus.

Method A. Methanol-water extraction. 1 gram of cocoa seed or cocoa husk sample was extracted twice with 3 mL of methanol-water 80:20 (v/v) at 70 °C for one hour with stirring. The two filtered liquids were mixed to form the final liquid extract.

Method B. Ethanol-acidified water extraction. 1 gram of cocoa seed or cocoa husk sample was extracted with 5 mL of ethanol:water 30:70 (v/v) acidified up to pH 3 with concentrated HCl. After stirring for two hours at room temperature, the mix was filtered

and the solid was extracted again with acetone:water 70:30 (v/v). The liquids obtained from both extractions were mixed.

Method C. Water extraction. 1 gram of cocoa seed or cocoa husk sample was extracted twice with 6 mL of distilled water with stirring at 70 °C for one hour. The liquids obtained from each extraction were mixed.

Method D. Methanol-acidified water extraction. 1 gram of cocoa seed or cocoa husk sample was extracted using method A but with the methanol-water mix acidified to pH 3 with HCl.

Method E. Acidified water extraction. 1 gram of cocoa seed or cocoa husk sample was extracted using method C but with the distilled water mix acidified to pH 3 with HCl.

2.4. Determination of total phenols

Total phenolic content was determined by the Folin-Ciocalteu spectrophotometric method and was expressed as grams of gallic acid equivalents (Singleton & Rossi, 1965).

2.5. Analysis of phenols

HPLC-DAD: Phenols were quantified using a Varian Prostar liquid chromatography system with a C-18 column (*Kinetex*[®] *Biphenyl* 100 Å, 250 mm x 4.6 mm, i.d. 5 µm) and diode array detector (DAD; the wavelengths used for quantification were 254, 280, and 340 nm) with automatic Rheodyne injection valves (20 µL loop). The mobile phases were 0.01 % trichloroacetic acid in water and acetonitrile, utilizing the following gradient over a total run time of 55 min: 95 % A initially, 75 % A in 30 min, 50 % A in 45 min, 0 % A in 47 min, 75 % A in 95 min, and 95 % A in 52 min until completion of

the run. Quantification was carried out by integration of the peaks at different wavelengths in function of the compounds, with reference to calibrations made using external standards. All determinations were carried out in triplicate and the samples were filtered (0.45 μm) before the analysis. For the theobromine and epicatechin quantification, the initial extract was diluted 1:4; for the other quantifications, no dilution was needed.

HPLC-MS: The phenolic compounds present in the different extracts were identified by electron impact mass data collected on a quadrupole mass analyzer (ZMD4, Micromass, Waters Inc.; Manchester, U.K.). Electron spray ionization (ESI) mass spectra were obtained at ionization energies of 50 and 100 eV in negative mode and 50 eV in positive mode; the capillary voltage was 3 kV, the desolvation temperature was 200 °C, the source temperature was 100 °C, and the extractor voltage was 12 V. The flow was maintained at 1 mL min⁻¹ in split mode (UV detector MS) for each analysis. The *Kinetex*[®] Biphenyl 100 Å C-18 column (250 mm x 4.6 mm i.d. 5 μm) was used. The mobile phases were 0.01% trichloroacetic acid in water and acetonitrile utilizing the same gradient used in HPLC-DAD.

2.6. Determination of antiradical activity

The antioxidant activity of each phenolic extract was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method as described in a previous study (Mrabet, García-Borrego, Jiménez-Araujo, Fernández-Bolaños, Sindic, & Rodríguez-Gutiérrez, 2017). This method is based on the measurement of the free-radical scavenging capacity of the antioxidant against the stable radical DPPH. A microplate reader (Bio-Rad 550 model, Hercules, CA, US) was used for the absorbance measurements. The result of the activity

of each extract was expressed as an EC₅₀ (effective concentration, mg/mL). The amount of antioxidant necessary to decrease the initial absorbance by 50 %, EC₅₀, was calculated from a calibration curve by linear regression for each antioxidant solution.

2.7. Statistical analysis

Results were expressed as mean values $\pm$ standard deviations. STATGRAPHICS® plus software was used for statistical analyses. Comparisons amongst samples were made using one-way analysis of variance (ANOVA) and the LSD method. A p-value of 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1. Extraction method comparison

A mix of the 25 different cocoa cotyledons and the commercial one was used to compare the five methods in terms of phenol and theobromine extraction. After the extractions, the total phenolic content and the HPLC-UV profile were determined and compared for each method (Figure 1). Despite the higher values of total phenols obtained by method B, the best phenolic profile was achieved with method D, in which the peaks were significantly higher than those obtained with the other methods. Thus, method D was chosen for the phenolic quantification of the 25 cocoa genotypes and the commercial mix. The quantification of each phenolic compound obtained using the five different methods was also carried out with quantification using external standards (Figure 1). The major compound identified in the cocoa cotyledon samples was theobromine, followed by the flavonoids epicatechin and catechin and their derivatives.

The proportion of these main components was similar in all the extractions, but method D extracted the highest concentration of all the quantified compounds. Acidification of the extraction solvent increased the concentration of phenolic compounds recovered in the case of methanol (compare results for methods A and D), allowing the quantification of caffeic acid and the catechin derivatives, but not in the case of distilled water (compare results for methods C and E), in which case only the concentration of catechin was increased.

The concentration of total phenols does not correspond to the sum of the individual phenols and, curiously, the highest value of total phenols was obtained with method B, for which the concentration of individual phenols was lower in comparison to methods A and D. The colorimetric method for total phenol determination not only quantifies polymerized phenols that are not present in the UV-profile but also gives an error when the phenols are not isolated but rather present in a complex matrix with proteins and sugars (Sánchez-Rangel, Benavides, Heredia, Cisneros-Zevallos, & Jacobo-Velázquez, 2013).

3.2. Identification and quantification of theobromine and phenols

A study of one cotyledon sample following extraction with method D with (CAL) and without (CAF) fermentation was used to identify all peaks by HPLC-MS (Figure 2). The complete profile of the non-fermented sample allowed the identification of theobromine and eight different phenolic compounds as shown in Figure 2. The HPLC-MS spectrum of the de-protonated molecule $[M-H]^-$ with ions (m/z 289) and (m/z 245) confirmed the presence of epicatechin and catechin as well as four of their derivatives with a retention time in the range of 21 to 26 minutes and a λ_{max} of 280 nm.

Theobromine and caffeic acid showed a de-protonated molecule $[M-H]^-$ of the same ion (m/z 179) but at different λ_{max} , of 280 and 324 nm, respectively.

Fermentation of the cotyledon is a natural process used to obtain the final cocoa seed for its commercialization. There are clear differences in the chromatograms of the fermented and non-fermented cotyledon samples (Figure 2), with the non-fermented sample having a richer profile. The concentrations of theobromine and phenolic compounds were also determined for the fermented and non-fermented cotyledon samples of husk and cotyledon for the same genotype (Table 2). All the values except for catechin diminished following the fermentation of cotyledon samples; this is in contrast to the husk sample, for which the concentration of phenolic compounds tended to increase after fermentation, particularly in the case of catechin. The reduction of these compounds in cotyledon following fermentation has been reported previously (Forsyth & Quesnel, 1957; Kim & Keeney, 1984; Elwers, Zambrano, Rohsius, & Lieberei, 2009) and directly depends on the reaction time (Brunetto *et al.*, 2007). The sum of the values for the total sample of cotyledon and husk are similar before and after the fermentation treatment for the major components theobromine, catechin, and epicatechin, whereas the concentration of the derivatives and epigallocatechin are significantly different (Table 4). Nazaruddin and colleagues (2006) determined that the decrease of theobromine and caffeine in fermented cocoa cotyledon is due to their diffusion during the fermentation process. The considerable increase in the concentration of bioactive compounds in the husk after fermentation means that husk can be considered as a natural source of theobromine and phenols such as epicatechin. Previous studies have shown that cocoa husk extracts have antibacterial activity and can help to diminish the concentration of residual glucose for the prevention of tooth decay (Kim, Lee, Kim, Park, Kwon, & Lee, 2004; Matsumoto *et al.*, 2004). Currently, cocoa

husk is used for animal feed or is disposed of directly; thus, the presence of bioactive compounds in this by-product of cocoa bean production could be of interest for the upcycling of the final fiber-rich solid for food formulation or dietetic purposes.

The concentration of theobromine and the main phenols was determined for the fermented cotyledon of each of the 25 genotypes and the commercial mix using method D (Table 3). Theobromine was the major bioactive compound for most genotypes, with a range of 24.28 ± 0.01 and 9.79 ± 0.03 mg/g of dry and defatted sample. This *methylxanthine* is one of the most attractive molecules in cocoa because it is considered to be a diuretic, smooth muscle relaxant, myocardial stimulant, and vasodilator (Dorland, 2007).

The second most abundant compound was the flavonoid epicatechin, ranging widely across the different genotypes from 6.13 ± 0.08 to 51.57 ± 0.02 mg/g of dry and defatted sample, followed by catechin and their derivatives. The epicatechin concentrations were higher than the values found for fermented cotyledon genotypes from other countries (Elwers et al., 2009). This fact makes these Mexican genotypes particularly interesting because the concentration of the main phenol, epicatechin, is higher in the fermented cotyledon, and thus in the final cocoa product to be commercialized. Besides the well-known beneficial effects of these compounds on the vascular system and cerebral blood flow, flavonoids have an effect on the inhibition of neuronal death by apoptosis induced by neurotoxins and oxygen radicals, and promote neuronal survival and synaptic plasticity (Nehlig, 2013).

Relationships were observed between the determined compounds, such as between catechin and epicatechin as well as between theobromine and epicatechin, but not between the individual and the total phenols (Figure 3). Samples of the RIM105, MO31,

and MO33 genotypes were the most concentrated in theobromine, epicatechin, and catechin. These three genotypes are from Mexico and are currently cultivated in Chiapas. RIM105 and MO33 are very similar in their morphology and color and different to the genotype MO31 (Table 1). The INI10 genotype had some of the lowest concentrations of individual phenols, yet its total phenol concentration and antioxidant activity were the highest of all the genotypes studied (81.99 ± 5.03 mg/g of dry and defatted sample and (EC_{50} 4.83 ± 0.67 , respectively). This genotype comes from Peru and Ecuador and has a particularly low weight of cotyledon and high percentage of husk.

The cocoa sample with some of the lowest concentrations of bioactive compounds was CAF, although its concentration in total phenols was mid-range. Technically, CAF is not a genotype but rather a mix of commercial genotypes from Mexico and is commonly used in the Tabasco cacao industry. The sample of CAF shows a medium concentration of total phenols, but a low concentration of theobromine, epicatechin, and catechin.

3.3. Antioxidant activity

Beside the phenolic concentration, another important aspect of cocoa is the antioxidant capacity of the cotyledon. The concentration of epicatechin has been related with the antioxidant activity (Othman, Jalil, Weng, Ismail, Ghani, & Adenan, 2010). All the genotypes have a high concentration of epicatechin except for the commercial mix (CAF), in which the major part of this compound is transferred to the husk during fermentation. Free radical *scavenging activity* was determined according to the elimination of *DPPH* radicals and used as a measure of the antioxidant capacity of each sample (Table 3). The results show that the genotypes previously identified as having

the highest concentrations of theobromine, epicatechin, and catechin (M031, M033, and INI10) have some of the lowest EC_{50} values and consequently the highest antioxidant activities, significantly higher than the commercial CAF. However, RIM105 is the exception, having a low antioxidant activity (EC_{50} 8.03 ± 0.05) despite its high epicatechin concentration. The commercial mix of genotypes (CAF) had a low antioxidant activity but not the lowest among the genotypes studied (EC_{50} 8.02 ± 0.57). The direct relationship between the total phenolic concentration and antioxidant activity is shown in Figure 3. The *in vitro* determination of the antioxidant capacity in aqueous solution gives an idea about the biological properties of each sample of fermented cotyledon. However, further assays would be required to determine each the real bioactivity of each genotype *in vitro* and *in vivo*.

4. CONCLUSION

Five different methods for phenolic extraction from cocoa beans were compared, and extraction with acidified methanol-water was shown to be the best method for quantifying the composition of the bioactive compounds theobromine and individual phenols of each genotype of cocoa cotyledon. This characterization identified theobromine as the major component of cocoa beans, followed by epicatechin and catechin phenolic compounds, with important differences established between the genotypes studied. Curiously, the commercial mix of genotypes, CAF, contained some of the lower concentrations of theobromine, epicatechin, and catechin. Samples of the genotypes RIM105, M031, and M033 were the most concentrated in theobromine, epicatechin, and catechin, and are Mexican in origin. The INI10 genotype had the highest concentration of total phenol and antioxidant activity. This study offers the opportunity to select new genotypes originating from Mexico based on their biological

activity and favorable concentrations of bioactive and phenolic components, which are even higher following the fermentation process. Moreover, this study identified for the first time that cocoa husk has important concentrations of bioactive compounds after its fermentation, which, together with its high fiber content and antibacterial activity, could make it an interesting novel ingredient for the food industry to consider. Further studies should be carried out to determine the organoleptic properties of these genotypes and establish their commercial potential.

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Figure Captions.

Figure 1. Phenolic profile of the cocoa cotyledon mix following HPLC-UV of the five different extraction methods. Concentration (mg/g of dry and defatted sample) of the identified components from cotyledon samples according to the five extraction methods (A to E). Derivatives I, II, and III are the catechin and epicatechin derivatives.

Figure 2. Phenolic profile at 280 nm of cotyledon, A) without fermentation and B) with fermentation. Theobromine and phenolic identification by HPLC-MS of the extract obtained from the cotyledon samples.

Figure 3. Pearson's correlation coefficients (R) between different components, total phenols, and antioxidant activity.

TABLES

Table 1. Origin, crosses, crop, and morphology of each genotype of cocoa studied.

	Origin	Crosses	Color	Weight (g/cotyledon)	Husk %	Shape	Crop locus
CAF	FOREIGN	N.A.	VIOLET	1.08	14.07	ELLIPTICAL	TABASCO AND NORTHERN CHIAPAS
CONA 1	MEXICO	N.A.	IVORY	1.10	28.36	ELLIPTICAL	TABASCO
CONA 2	MEXICO	N.A.	DEEP VIOLET	1.39	15.30	OVOID	TABASCO
CRC	MEXICO	N.A.	IVORY AND SLIGHTLY VIOLET	1.51	15.72	ELLIPTICAL	TABASCO
CRP	MEXICO	N.A.	IVORY AND SLIGHTLY VIOLET	1.14	17.13	ELLIPTICAL	TABASCO
INI1	MEXICO AND ECUADOR	RIM 76A (MEXICO) x EET400 (ECUADOR)	DEEP VIOLET	1.14	19.23	ELLIPTICAL	TABASCO
INI4	MEXICO AND PERÚ	RIM 76 (MEXICO) x POUND 7 (PERU)	SLIGHTLY VIOLET	1.34	17.25	OVOID	TABASCO
INI9	MEXICO AND COLOMBIA	RIM 75 (MEXICO)x SPA 9 (COLOMBIA)	IVORY AND SLIGHTLY VIOLET	2.20	14.62	ELLIPTICAL	TABASCO
INI10	PERU AND ECUADOR	POUND 7 (PERU) x EET48 (ECUADOR)	DEEP VIOLET	0.98	17.71	OVOID	TABASCO
INI8	MEXICO Y ECUADOR	RIM 76A (MEXICO) x EET48 (ECUADOR)	SLIGHTLY VIOLET	1.63	14.86	OVOID	TABASCO
M001	MEXICO	N.A.	IVORY AND SLIGHTLY VIOLET	0.92	11.28	OVOID	CHIAPAS
M002	MEXICO	N.A.	VIOLET	1.11	13.00		CHIAPAS
M003	MEXICO	N.A.	IVORY AND VIOLET	1.06	14.93	ELLIPTICAL	CHIAPAS
M008	MEXICO	N.A.	SLIGHTLY VIOLET AND VIOLET	1.31	13.72	OVOID	CHIAPAS
M022	MEXICO	N.A.	VIOLET	1.10	16.30	ELLIPTICAL	CHIAPAS
M024	MEXICO	N.A.	IVORY AND SLIGHTLY VIOLET	1.00	17.33	ELLIPTICAL	CHIAPAS
M025	MEXICO	N.A.	SLIGHTLY VIOLET AND VIOLET	0.82	11.41	ELLIPTICAL	CHIAPAS
M026	MEXICO	N.A.	SLIGHTLY VIOLET	1.08	17.34	OVOID	CHIAPAS
M028	MEXICO	N.A.	IVORY	1.16	15.74	OVOID	CHIAPAS
M029	MEXICO	N.A.	SLIGHTLY VIOLET	1.20	43.91	ELLIPTICAL	CHIAPAS
M031	MEXICO	N.A.	VIOLET	1.00	19.48	ELLIPTICAL	CHIAPAS
M032	MEXICO	N.A.	IVORY AND SLIGHTLY VIOLET	1.02	13.09	ELLIPTICAL	CHIAPAS
M033	MEXICO	N.A.	IVORY AND SLIGHTLY VIOLET	1.17	14.02	ELLIPTICAL	CHIAPAS
RIM 44	MEXICO	N.A.	IVORY	0.99	10.71	ELLIPTICAL	CHIAPAS
RIM 88	MEXICO	N.A.	IVORY AND SLIGHTLY VIOLET	1.42	12.85	ELLIPTICAL	CHIAPAS
RIM 105	MEXICO	N.A.	IVORY AND SLIGHTLY VIOLET	1.16	15.20	ELLIPTICAL	CHIAPAS

Table 2. Concentrations (mg/g of dry and defatted sample) of theobromine and phenols present in husk and cotyledon samples for one genotype of cocoa.

Samples	Theobromine	Caffeic acid	Catechin	Epicatechin	Deriv. I	Deriv. II	Deriv. III	Deriv. IV	Epigallocatechin
<i>Cotyledon sample</i>									
CAL (raw sample)	18.07 ± 0.01 ^a	traces ^b	1.27 ± 0.00	22.05 ± 0.39	2.99 ± 0.00	2.81 ± 0.04	0.88 ± 0.00	0.97 ± 0.01	1.72 ± 0.00
CAF (fermented sample)	9.79 ± 0.03	traces	1.48 ± 0.09	6.13 ± 0.08	0.49 ± 0.00	0.09 ± 0.00	traces	traces	traces
<i>Husk sample</i>									
CAL (raw sample)	3.90 ± 0.15	traces	nd ^c	4.20 ± 0.10	1.1±0.02	0.12±0.02	traces	traces	nd
CAF (fermented sample)	12.00 ± 0.10	traces	1.2±0.1	17.70 ± 0.10	0.2±0.02	0.12±0.02	traces	traces	0.20 ± 0.01
<i>Total sample</i>									
CAL (raw sample)	21.97 ± 0.16	traces	1.27 ± 0.00	26.25 ± 0.49	4.09 ± 0.02	3.01 ± 0.06	0.88 ± 0.00	0.97 ± 0.01	1.72 ± 0.00
CAF (fermented sample)	21.79 ± 0.13	traces	1.48 ± 0.09	23.83 ± 0.18	0.69 ± 0.02	0.21 ± 0.02	traces	traces	0.20 ± 0.01

^aStandard deviations (n=3); ^btraces: values detected ≤0.01 mg/g of dry and defatted sample; ^c nd: not detected.

Table 3. Concentrations (mg/g of dry and defatted sample) of theobromine, total phenols, and phenols for 26 genotypes of fermented cotyledon of cocoa. Derivative (Deriv.) IV and Epigallocatechin were found in traces in all samples. DPPH: Antioxidant activity (using the DPPH method) expressed as EC₅₀ in mg of dry extract/mL.

Genotype	Total phenols	Theobromine	Caffeic acid	Catechin	Epicatechin	Deriv. I	Deriv. II	Deriv. III	DPPH (EC ₅₀)
CAF	48.04 ± 2.63	9.79 ± 0.03 ^a	traces ^b	1.48 ± 0.09	6.13 ± 0.08	0.49 ± 0.00	0.09 ± 0.00	traces	8.02±0.57
CONA 1	30.61 ± 3.73	18.67 ± 0.02	0.42 ± 0.00	2.36 ± 0.03	19.98 ± 0.02	0.58 ± 0.01	0.35 ± 0.00	0.23 ± 0.00	9.69±0.76*
CONA 2	49.00 ± 1.68	20.29 ± 0.02	0.42 ± 0.00	1.63 ± 0.01	13.42 ± 0.02	0.90 ± 0.01	0.87 ± 0.01	0.38 ± 0.00	7.77±0.10
CRC	30.49 ± 6.73	16.51 ± 0.05	0.18 ± 0.00	1.49 ± 0.01	14.10 ± 0.00	0.34 ± 0.00	0.10 ± 0.00	traces	9.34±0.25*
CRP	51.94 ± 7.43	20.78 ± 0.01	0.78 ± 0.00	2.04 ± 0.01	25.12 ± 0.01	0.61 ± 0.01	0.32 ± 0.01	traces	8.17±0.31
INI1	39.08 ± 3.17	20.37 ± 0.01	traces	0.77 ± 0.00	14.89 ± 0.01	0.55 ± 0.01	0.40 ± 0.00	traces	7.74±0.25
INI4	40.35 ± 1.82	17.78 ± 0.04	0.12 ± 0.00	1.37 ± 0.00	23.04 ± 0.08	0.56 ± 0.00	0.35 ± 0.00	traces	9.36±0.73*
INI9	39.99 ± 1.93	14.83 ± 0.02	0.36 ± 0.00	2.35 ± 0.03	16.68 ± 0.04	2.15 ± 0.00	1.13 ± 0.00	traces	7.09±0.12
INI10	81.99 ± 5.03	21.07 ± 0.00	0.24 ± 0.00	1.67 ± 0.01	13.72 ± 0.03	1.78 ± 0.01	1.06 ± 0.00	traces	4.83±0.67**
INI813	58.39 ± 7.00	16.87 ± 0.22	0.54 ± 0.00	0.42 ± 0.00	11.38 ± 0.01	1.15 ± 0.01	traces	traces	6.87±0.42*
M001	45.64 ± 1.21	19.07 ± 0.04	0.24 ± 0.00	4.10 ± 0.03	11.11 ± 0.01	1.19 ± 0.01	0.09 ± 0.00	1.79 ± 0.00	7.75±0.74
M002	56.46 ± 4.05	20.84 ± 0.03	0.30 ± 0.00	2.42 ± 0.01	26.71 ± 0.02	1.43 ± 0.00	2.16 ± 0.03	1.40 ± 0.01	7.63±0.69
M003	33.08 ± 3.13	20.13 ± 0.02	traces	1.40 ± 0.02	15.28 ± 0.01	0.71 ± 0.00	0.79 ± 0.01	traces	9.10±0.45
M008	41.28 ± 4.45	21.85 ± 0.02	0.30 ± 0.00	2.78 ± 0.00	15.52 ± 0.08	1.21 ± 0.00	1.19 ± 0.01	traces	7.75±0.18
M022	47.28 ± 7.29	21.31 ± 0.03	0.54 ± 0.00	2.90 ± 0.02	16.02 ± 0.02	1.05 ± 0.00	0.91 ± 0.00	traces	6.80±0.61
M024	51.47 ± 2.58	19.70 ± 0.14	0.66 ± 0.00	3.38 ± 0.01	13.12 ± 0.86	0.91 ± 0.00	0.56 ± 0.01	traces	7.13±0.52
M025	43.59 ± 4.35	15.79 ± 0.21	traces	1.42 ± 0.01	7.06 ± 0.02	0.88 ± 0.01	0.91 ± 0.00	traces	9.07±0.11
M026	49.91 ± 2.17	22.21 ± 0.02	1.14 ± 0.00	3.56 ± 0.01	31.33 ± 0.10	0.85 ± 0.01	0.91 ± 0.00	traces	7.00±0.35
M028	39.29 ± 2.30	22.78 ± 0.04	traces	6.02 ± 0.00	24.87 ± 0.03	2.78 ± 0.01	2.09 ± 0.00	2.00 ± 0.00	7.61±0.04
M029	55.69 ± 2.47	17.90 ± 0.12	1.02 ± 0.00	2.77 ± 0.00	23.00 ± 0.07	2.13 ± 0.01	1.79 ± 0.00	1.12 ± 0.00	7.22±0.35
M031	84.95 ± 4.82	22.58 ± 0.04	0.90 ± 0.00	4.13 ± 0.00	37.46 ± 0.13	2.00 ± 0.01	2.07 ± 0.01	1.70 ± 0.01	5.25±0.04**
M032	71.66 ± 4.16	22.45 ± 0.11	traces	2.84 ± 0.00	31.25 ± 0.13	4.83 ± 0.00	3.41 ± 0.01	1.25 ± 0.00	6.61±0.18*
M033	58.65 ± 7.20	23.68 ± 0.01	traces	4.39 ± 0.04	39.77 ± 0.15	2.22 ± 0.01	1.88 ± 0.01	1.80 ± 0.00	6.10±0.21*
RIM 44	70.72 ± 9.97	23.59 ± 0.01	0.12 ± 0.01	1.66 ± 0.02	25.02 ± 0.02	1.03 ± 0.01	1.57 ± 0.00	traces	6.04±0.21*
RIM 88	46.40 ± 9.55	23.12 ± 0.08	0.54 ± 0.00	2.41 ± 0.01	30.17 ± 0.02	0.86 ± 0.01	0.81 ± 0.00	traces	7.85±0.46
RIM 105	43.35 ± 3.49	24.28 ± 0.01	1.02 ± 0.00	3.67 ± 0.00	51.57 ± 0.02	3.56 ± 0.01	2.03 ± 0.01	traces	8.03±0.05

^aStandard deviations (n=3); ^btraces: values detected ≤0.01 mg/g of dry and defatted sample;

*significant difference ($p < 0.05$) and **($p < 0.005$) respect to CAF (commercial mix genotypes) sample.

FIGURES

*Value ≤ 0.01 mg/g of dry and defatted sample.

Figure 1.

Figure 2.

Figure 3.