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Characterization of cocoa butter extracted from hybrid cultivars of *Theobroma cacao L*

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SUMMARY. Cocoa butter is the most important fat used in the confectionery and chocolate industries. The main objective of the present study was to determine the physical and chemical characteristics of cocoa butter extracted from hybrid cultivars belonging to the germplasm bank of the Fondo Nacional de Investigaciones Agropecuarias (National Foundation for Agricultural Research). AOAC methods were used for the assesment ot the proximal composition of the beans, physical and chemical characteristics as well as for the fatty acid profile of the fat. It was found that there were statistical differences in the proximate composition of the cocoa beans among the cultivars studied as well as the iodine and saponification indices of the butter. Saturated fatty acids were present in higher proportions than unsaturated fatty acids, with palmitic and stearic acid as the main fractions. Oleic acid content was higher than linoleic acid. The fatty acid profile found is the main factor that influences the hard texture of the cocoa butter from Venezuelan cocoa hybrids cultivars.
Key words: *Theobroma cacao* L., hybrids, cocoa butter, fatty acid profile, proximate analysis

RESUMEN. Caracterización de la manteca de cacao extraída de cultivares híbridos de *Theobroma cacao L.* La manteca de cacao es la grasa más importante usada en la industria de la confitería y del chocolate. El objetivo principal del presente estudio fue determinar las caracaterísticas físicas y químicas de la manteca de cacao extraída de cultivares híbridos pertenecientes al banco de germoplasma del Fondo Nacional de Investigaciones Agropecuaria (FONAIAP). Se determinó la composición proximal de las almendras, las características físicas y químicas y el perfil de ácidos grasos de acuerdo a los métodos del AOAC. Se encontró que entre los cultivares estudiados existian diferencias estadísticamente significativas en la composición proximal de las almendras de cacao asi como para el índice de yodo y saponificación de la manteca. La cantidad de ácidos grasos saturados presentes fue mayor que la de insaturados, encontrándose como principales fracciones el palmítico y el esteárico. El contenido de ácido oleico fue mayor que el de linoleico. El perfil de los ácidos grasos presentes es el principal factor que influye en la dureza de la manteca de cacao de los cultivares venezolanos estudiados.
Palabras clave: *Theobroma cacao L.*, híbridos, manteca de cacao, perfil de ácidos grasos, análisis proximal.

INTRODUCTION

Historically cocoa butter has been the fat in greatest demand at the international level, and one of the most expensive edible fats. It is qualified as a standard of reference that many have tried to imitate for years, and is a product of great commercial value due to its physical and chemical characteristics. It is the most important fat used in confectionery, not only because it is the natural ingredient of chocolate, but also because the melting characteristics are unique, in having a high solid fat content at room temperature and melting at a narrow temperature range just below body temperature (1-3). Cocoa butter is unique because it presents a strong polymorphism which is extremely important for chocolate making. In general, the amount and position of the fatty acids on the glycerol molecule affect the physical and chemical characteristics of fats and oils, such as the melting point, iodine, and saponification indices. In cocoa butter these characteristics will provide important evidence about its functional properties, as well as its convenience when used in certain products in the pharmaceutical, cosmetic and chocolate industries. Martin Jr. (4), and Charman (5) report that the combination and position of fatty acids on the glycerol molecule of cocoa butter produce a complex mixture of melting ranges.

The plasticity range of cocoa butter is very narrow compared with other edible fats and its melting properties are intimately associated with consumers perception of the quality of good chocolate.

Venezuelan cocoa has a unique taste and aroma which are largely appreciated in the international market (6). In spite of this, in Venezuela there is very little information about the chemical and physical characteristics of the fat extracted from the different cultivars of cocoa (*Theobroma cacao* L.) that are grown. On the other hand, we also evidence the lack of specific data on the chemical composition of the beans obtained from these cultivars and only those studies related to the agronomic aspects, with the intent to improve the selection of new genetic material, have received the necessary attention.

The purpose of this study is to determine the proximate composition of cocoa beans and the chemical and physical

characteristics of fat extracted from different hybrid cultivars (Amazon x Forastero and Amazon x Criollo) of cocoa (*Theobroma cacao* L.,) that are most representative of the collection of the Germplasm Bank of the Fondo Nacional de Investigaciones Agropecuarias (FONAIAP) at Ocumare de la Costa, Aragua State, Venezuela.

MATERIALS AND METHODS

The Germplasm Bank of FONAIAP, in Ocumare de la Costa, Aragua State provided the pods of hybrid cultivars (*Theobroma cacao* L.). The cultivars (IMC11 x OC60, IMC11 x OC61, IMC11 x OC73, IMC11 x OC77, and IMC67 x SC10) were the result of crosses between Amazon cocoa cultivars brought from Peru, the Iquito Mixed Calabacillo (IMC) and the Santa Cruz (SC) a cocoa Forastero cultivar from Barlovento (Venezuela) or Criollo cocoa cultivars (OC) from Ocumare de la Costa, Venezuela.

The pods were harvested individually from 7 trees of each cultivar, chosen at random between February and April 1995. The degree of ripeness was established according to the physical characteristics of the pods.

The pods were individually opened to remove the beans, from which the mucilage was removed with sawdust. The beans were dried on aluminum trays in a Uniterm Drier mechanical desiccator with forced ventilation at 60°C for a period of 24 hours. After the pods were dry, the testa was removed manually. All the beans from the same cultivar were mixed and ground in a Bel Art Products Micro-Mill, and then sifted to obtain a fine powder on which the proximal analysis was carried out. The moisture content of fresh-shelled cotyledons free of germ was determined according to the AOAC (7) and the results were expressed as g /100g.

The cocoa butter was extracted with light petroleum ether (40-60°C) in a Soxhlet apparatus for a period of 24 hours. After the solvent was evaporated, the fat residue was stored at 5°C until it was needed for analysis.

Proximate composition

Moisture, ash and crude fibre analyses of the cocoa beans were carried out according with the official methods of the AOAC (7). The proteins and the fat were determined according with the Office International du Cacao et du Chocolat (8) methods. Carbohydrates were calculated by difference.

Physical and chemical characteristics

The iodine (Wijs), saponification, and refractive indices, and the melting point of the cocoa butter were determined according with the AOAC (7) methods. All the analyses were run in triplicate, using analytical grade reagents.

Fatty acid profile

The fatty acid profile of cocoa butter was determined following the AOAC (7) methods 9698.33 and 963.22, with a Hewlett Packard (HP) model 5730A Gas Chromatograph fitted with a flame ionization detector set at 300°C, and an HP model 3380S integrator (Hewlett Packard de Venezuela, Caracas). Separation was carried out in a stainless steel Alltech column (4 mm i.d. x 3 m length) at 190°C, packed with Hi-EEF® - IBF Diethylene glycol succinate 15% on Chromosorb W/AW 8420 80/100 (Albis Venezolana CA., Caracas). The injector temperature was 250°C. Nitrogen was the carrier gas (60 ml/min). Fatty acid identification was done by comparison with standards: L205 NIFH, C12-C18 and K128 (Alltech).

Statistical analysis

Results were expressed as mean values ± standard deviation of three separate determinations. An analysis of variance (ANOVA) showed the variation of the data according to cultivar. Means were compared ($P \leq 0.05$) by Duncan's multiple range test (9).

RESULTS AND DISCUSSION

Proximate composition

Table 1 present the results of the moisture of the fresh beans and Table 2 the proximate analyses of the ground dried beans for the different cultivars considered in this study. Table 1 reveals that there are statistical differences ($P \leq 0.05$) among the moisture content of some cultivars. The values fall in the range of 29.12±0.23 to 34.21±0.49 g/100g and are lower than the ones reported by Rohan (10) , for cocoa Forastero (35g/100g). These results are similar to the ones obtained by Liendo *et al.* (11) for Criollo cultivars, which fall in the range of 31.64 to 38.42 g/100g.

TABLE 1
Moisture content (g/100g) of fresh cotyledons of hybrid cultivars of *Theobroma cacao* L[1]

Hybrids	Moisture
IMC 11 x OC 61	29.12±0.23a
IMC 11 x OC 63	34.21±0.49d
IMC 11 x OC 73	33.13±0.29bc
IMC 11 x OC 77	32.26±0.86b
IMC 67 x SC 10	33.32±0.36c

1 Mean of n = 3 ± standard deviation. Different letters are significantly different ($P \leq 0,05$).

The moisture content of the dried powder was higher than the results reported by Offem (12), whose highest value was 4.08 for F_3 Amazon cultivar. This might be due to differences in treatment. Nevertheless, our results are lower than the ones reported by Alvarado *et al.* (13) for Arriba and EET-19 cultivars grown in Ecuador, and similar to results for Criollo cultivars studied by Liendo *et al.* (11), which were in the range

of 4.35 to 7.06.

The protein analysis did not show significant differences ($p \leq 0.05$) according to the Duncans tests among some of the cultivars. The average protein content was of 15.58±0.58 g/100g. Alvarado et al. (13) did not find highly significant differences in the protein content ($p \leq 0.01$) of the different varieties studied. The average protein values found by these investigators for the cocoas cultivated in Ecuador, were 13.98 and 14.22 g/100g for the Arriba and EET-19 cultivars respectively. The latter value is closer to the average of 15.58 g/100g obtained with the varieties studied here, but lower than the average of 17.25 g/100g for Criollo cultivars found by Liendo et al. (11). However, Offem (12) found higher protein values in the range of 23.82 to 26.26 g/100g, for younger cocoa samples and trees interspersed with palm trees; apparently, these accumulated more crude protein. due to certain environmental and ecological factors such as the nitrogen content of the soil (14).

TABLE 2

Proximate composition (g/100g) *Theobroma cacao L.* cotyledons from different hybrid cultivars[1]

Cultivar	Moisture	Protein (N x 6,25)	Fat	Ash	Crude fibre	Carbohydrates
IMC11 x OC61	5.86± 0.05	16.00 ±0.02 b	52.49±0.26c	3.37±0.10a	7.85±0.07b	20.29
IMC11 x OC63	7.17± 0.03	15.88±0.06a	50.16± 0.21b	3.56±0.06b	8.09±0.51b	21.31
IMC11 x OC73	5.94±0.03	16.21±0.08c	54.21±0.30e	3.54±0.14b	5.69±0.14a	20.35
IMC11 x OC77	5.85±0.09	14.98± 0.12a	47.27±0.14a	3.79 ±0.06c	8.67±0.47b	25.29
IMC67 x SC10	5.98±0.09	14.82±0.12a	53.54±0.30d	3.86±0.05c	8.79±0.49b	20.99

[1] Mean of n= 3 ± standard deviation. Different letters in the same column are significantly different ($P \leq 0.05$). Data is indicated on dry weight basis

The ash content values ranged from 3.37±0.10 to 3.86±0.05 g/100g. These results are higher than those obtained by Wood 15 for African and Forastero cocoa, with an average value of 2.36 g/100g, whereas they are very similar to those of Alvarado *et al*, (13) for Arriba and EET-19 cultivars (4.12 and 3.69 g/100g). Only the IMC11 x OC73 presented any significant difference for crude fiber ($P \leq 0,05$). The variation interval was between 5.69±0.14 to 8.79±0.49 g/100g. These results are much higher than the 3.2 g/100g average reported by Wood (15), but lower than the average of 4.26 reported by Liendo *et al.* (11). The carbohydrate content for these hybrids presented an average of 21.65 g/100g.

The fat content values ranged from 54.21±0.30 g/100g for IMC11 x OC73 to 47.27±0.14 g/100g for IMC11 x OC77 with an average of 51.51 g/100g, and there were significant differences among all cultivars ($p \leq 0.05$) These results show that hybridization between IMC11 and some criollo cultivars of the Ocumare group produced, a decrease in the fat and ash content. Hybridization in general produces a decrease in the fat content. The IMC11 cultivar is an Amazon variety which apparently has a lower fat content than the Criollo cultivars, this seems to be true, since cultivars from Nigeria of the Amazon variety presented a fat content around 46.34% (12), and Vello *et al.* (16), reported that the Amazon clones slightly decreased the fat content of the hybrids studied by them. The exception is the hybrid IMC11 x OC73 which has a higher fat content (54.21 g/100g) when compare to the fat level of 46.08 g/100g detected for the OC73 cultivar (11).

Dominguez (17) found that in the Venezuelan cocoas analyzed, a high fat content was associated with cultivars obtained from hybridization between Criollo and Forastero (45.87 g/100g) while the cross between Forastero and Forastero gave a lower fat content (41.42 g/100g) Nevertheless, the fat content by acid hydrolysis, found for the hybrid cultivars studied here, was higher than the ones reported by Dominguez (17), probably due to differences in the extraction method.

Glendinning and Martinson (18) found fat content in the range from 61.7 g/100g for an hybrid of a forastero Amazon from Perú and 55.9% for a Trinitario hybrid. They also found that the hybrids related to the Scavina (SCA) cultivars presented lower fat content. However, our cultivar IMC67 x SC10, an hybrid between Amazon and Forastero presented a fat content of 53.54 g/100g which is a value close to the highest found in this study but lower than the ones reported by the above mentioned investigators.

Wessels and Toxopeus (19) observed significant differences in the fat content of the different hybrids tested and concluded that these variations were caused by genetic factors. These results suggest that the fat content depends on the type of hybridization, and might explain the differences found in our

study. Nevertheless, Baez and Enriquez (20) pointed out that the environment in which the cocoa tree grows has a significant effect on the fat content in cocoa clones and hybrids.

Physical and chemical characteristics of the cocoa butter

These results are presented in Table 3. The melting temperature of cocoa butter is an important factor for the chocolate industry. The spectrum of melting point ranges of cocoa butter is wide: incipient melting takes place between 31.2 and 32.7°C and complete melting occurs between 32 and 34°C. We cannot speak of one specific melting point, only of melting point ranges (4).

Lehrian *et al.*, (21) point out that, generally, the melting characteristics of cocoa butter are relatively constant, and variations of this behavior are caused by the changes in the type of triacylglycerols of cocoa butter.

TABLE 3

Physical and chemical indices of *Theobroma cacao* L. cocoa butter from different hybrid cultivars [1]

Cultivar	Refractive Index ND^{40}	Melting point °C	Iodine index $gI_2/100g$	Saponification m g KOH g^{-1}
IMC 11 x OC 61	1.4560	36	32.53 ± 0.03^a	193.41 ± 0.10^a
IMC 11 x OC 63	1.4556	35	34.11 ± 0.46^b	194.85 ± 0.77^b
IMC 11 x OC 73	1.4571	36	32.72 ± 0.48^a	194.90 ± 0.58^b
IMC 11 x OC 77	1.4561	35	33.69 ± 0.38^b	193.96 ± 0.66^{ab}
IMC 67 x SC 10	1.4546	35.5	33.04 ± 0.20^a	194.58 ± 0.08^{bb}

[1] Mean of n= 3 ±standard deviation. Different letters in the same column are significantly different (P≤0.05).

The average refractive index found was 1.4559 and all the cultivars' refractive indices were in the range from 1.4546 to 1.4571. The refractive indices for the hybrids are very similar to the ones found for the OC criollo cultivars (11) and lower than the ones reported by Chaiseri and Dimick (3) for cocoa butters from different geographic regions, which varied from 1.4572 to 1.4580

The determination of the iodine index can prove very useful in estimating the hardness of cocoa butter, since high iodine values indicate high content of unsaturated fatty acid components which contribute to the softness in cocoa butter. The iodine index in the hybrid cultivars presented a global average of 33.20g I_2 /100g of fat. The values for the IMC11 x OC77 and IMC11 x OC63 were significantly higher (P≤0,05) than those of the other cultivars, this suggest a higher content of unsaturated fatty acids and as a consequence they should have a slightly softer texture. When comparing these results for IMC x OC hybrids with the OC iodine index values (11) it can be seen that the hybrids in general showed lower iodine indices; the same tendency was observed in the melting point values.

Chaiseri and Dimick (3) point out that in the different countries of Latin America, Africa and Oceania the triacylglycerol composition had a certain influence over the cocoa butters texture and found that the cocoa butter from Venezuela was considerably harder than the cocoa butter from most other countries of the region with the exception of Mexico, because of the higher proportions of palmitic-oleic-palmitic (POP), palmitic-oleic-stearic (POS) and stearic-oleic-stearic (SOS) triacylglycerols, namely 20.4%, 40.4% and 28.8% respectively and lower than 5.2% ratios of palmitic-oleic-oleic (POO) + stearic-oleic-oleic (SOO).

Berbert and Alvim (22) suggested that in zones with an equatorial climate with very little seasonal change and high temperatures we should expect a low iodine index throughout the year. According with McHenry and Fritz (23) lower temperatures are more favorable for unsaturated fatty acid incorporation into the triacylglicerol.

The low iodine index determined in this study indicates the hardness of the fat obtained from the cultivars studied. We have also observed that the cultivars presented a high melting point (between 35 and 36°C) Table 3. This conforms with the low iodine index found in the cultivars, because the cocoa butters melting point is intimately related to the unsaturated degree of its fatty acids.

The saponification index indicates the average molecular weight of the fatty acids in fat, which are related to its organoleptic properties (firmness, taste and flavor). Comparison of saponification indices means by Duncans test showed some signifficant differences (p≤0.05) among the cultivars. The saponification index global average 194.32 mg KOH g^{-1} is similar to the value reported by Chaiseri and Dimick, (3) for Latin-American, African and Malaysian cocoa butters studied, and the average value (193.74) for the Criollo cultivars studied by Liendo *et al* (11).

Ekpa *et al.* (24) found that F3 Amazon, Amelonado and Trinitario cocoa butters had a variable iodine index from 37.05 to 39.16 gI_2 /100g and a saponification index from 191.21 to 198.68 mg KOH g^{-1} depending on the geographic location where the cocoa beans were harvested. In our study there is not

a high variation of the saponification values, since the samples were harvested at the same location.

The Venezuelan regulations (25) stipulate that cocoa butter must fulfill the following physical and chemical requirements: Refractive index from 1.456 to 1.458, melting point from 28 to 35°C; iodine index (Wijs) 33 to 40 gI2 /100g and saponification index from 188 to 200 mg KOH g^{-1}. The cocoa butters from these cultivars comply with all requirements except the melting point and iodine index, where some of the cultivars have a higher melting point and a lower iodine index than required, which means a harder texture.

Fatty acids profile

The hybrids revealed a variable fatty acid composition between 22.9 and 26.1% for palmitic, 34.7 to 37.3% for stearic, 36.2 to 39.9% for oleic and 1.5 to 1.6% for linoleic acids. The oleic acid showed the highest concentrations (Table 4).

TABLE 4
Fatty acid profile of cocoa butter extracted from different *Theobroma cacao L.* hybrid cultivars

Cultivar	Fatty Acids (%)							
	C16:0	C18:0	C18:1	C18:2	C18:3	Sat.	Unsat.	Sat/Unsat
IMC11xOC61	25.4	36.8	36.2	1.6	ND	62.2	37.8	1.64
IMC11xOC63	23.2	35.6	39.6	1.5	ND	58.8	41.1	1.43
IMC11xOC73	22.9	37.3	39.7	ND	ND	60.2	39.7	1.58
IMC11xOC77	23.9	34.7	39.9	1.5	ND	58.6	41.7	1.40
IMC67XSC10	26.1	35.9	38.0	ND	ND	62.0	38.0	1.63

ND = Not detectable

These results differ from those reported for commercial cocoa by Charman (5) and Martin Jr. (4). According to Alvarado *et al.* (13), the main fatty acids of cocoa butter are palmitic, stearic and oleic, and a small proportion of linoleic. The average content of stearic acid reported for the Arriba (Forastero) (28.07) and the EET- 19 (31.36) cultivars from Ecuador was less than the average (36.06) found in this study.

Table 4 also presents the distribution of saturated and unsaturated fatty acids in the different cultivars; we can observe the larger proportion of saturated fatty acids which would partially explain the melting behavior and low iodine index of the cultivars analyzed. When the average saturated and unsaturated fatty acid content of the IMC x OC cultivars (59.95 and 40.07 respectively) results are compared with the average value of saturated (61.65) and unsaturated (38.32) fatty acids found for the OC Criollo cultivars (11), there is an evident a decrease in saturation and increase in unsaturation; nevertheless the higher content of saturated fatty acids implies that there was higher availability of intermediate saturated fatty acids in the synthesis of monounsaturated triacylglycerides POP, POS and SOS, which are associated with a firmer cocoa butter from these cultivars. There is also a low content of di and triunsaturated fatty acids, which favor the constitution of a less stable polymorphic crystalline form. According to Lehrian *et al.* (21) this is a consequence of the temperature at which the cocoa pods developed, high temperatures mean less unsaturated fatty acids; as a result there is less diunsaturated triacylglycerols to interrupt the molecular packing of the monounsaturated types. The fatty acid profile found provides evidence for the melting point and the iodine index values found.

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