Lipid Composition of Wild Ecuadorian *Theobroma subincanum* Mart. Seeds and Comparison with Two Varieties of *Theobroma cacao* L.

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The present work analyzes the lipid fraction from seeds of wild Ecuadorian *Theobroma subincanum* and selected commercial varieties of *Theobroma cacao* from Mexico (var. Criollo) and Ecuador (var. Arriba). The lipid fraction was obtained from the seeds through supercritical fluid extraction and analysis performed by preparatory thin-layer chromatography followed by gas chromatography. The results revealed that in *T. subincanum* the triglycerides contain fatty acids with longer chains. The melting point and peroxide and saponifiable numbers were determined for each *Theobroma* sample. The results lead to the conclusion that *T. subincanum* would produce a poorer quality butter than *T. cacao*. Nevertheless, the results do point toward a significant commercial use of *T. subincanum* for low-profile products.

Keywords: *Theobroma subincanum; Theobroma cacao; cocoa butter; triglycerides; fatty acids; sterols; supercritical fluid extraction*

INTRODUCTION

Of the various species belonging to the genus *Theobroma* (Sterculiaceae), *T. cacao* has certainly been studied the most (Chatt, 1953; Sotelo and Alvarez, 1991; Liendo et al., 1997; Willson, 1999). Due to the extensive use of the oleaginous seeds as foods, drugs, and cosmetics, such research has been aimed at determining the quantity and quality of the lipid fraction (cocoa butter), alkaloid fraction (caffeine, theobromine), and all compounds of commercial interest (Morgan, 1994).

The commercial importance of *T. cacao* has resulted in an ongoing selection of cultivars to increase production. This objective was intensively developed in the 1950s and has seen the introduction of intensive farming—with disregard for the ecological impact on the original habitat—and species selection for the most highly productive varieties—without regard to their response to pathogens and parasites (Chatt, 1953; Hunter, 1990; Willson, 1999).

To obviate these problems, a great deal of interest is currently being focused on comparison with wild *Theobroma* species; indeed, these species have naturally developed a strong response versus etiological agents and, at the same time, could offer a qualitatively and quantitatively interesting product (Hunter, 1990).

The present work evaluates the lipid fraction of seeds from the wild species *Theobroma subincanum*—native to the eastern Ecuadorian Amazon—and compares them to seeds of two commercial cultivars of *T. cacao*: one from Mexico (var. Criollo) and another from Ecuador (var. Arriba). The purpose has been to identify the commercial potential and uses for *T. subincanum* in terms of quality and quantity of the seed lipid fraction. The studies of this species found in the literature are limited to the purinic alkaloid content (Marx and Maia, 1991; Hammerstone et al., 1994); the fatty acid and phytosterol compositions of the seeds have been studied for only one variety from Brazil (Carpenter et al., 1994). The supercritical fluid extraction (SFE) technique was employed to reduce the extraction time and still obtain results qualitatively representative of the lipid fraction composition. In fact, this technique has proved to be suitable for the extraction of various substances, including lipid-bearing materials (Bartle, 1990; Li and Hartland, 1996), even cacao (Rossi et al., 1989).

MATERIALS AND METHODS

Plant Material. In September 1997, on the outskirts of the Wasak'entsa reserve in eastern Ecuador (77° 15′ W/2° 35′ S), the *T. subincanum* samples (fruits) were collected from trees the natives call "*wakam*" by applying the wild species identification indications of Villacres (1995) and Martinez (1996). A sample of *T. subincanum* was deposited in the Herbarium Universitatis Ferrariensis of the Department of Biology, Section of Botany, University of Ferrara (code 002018 3G1). The *T. cacao* samples came from two commercial cultivars: one from Mexico, *T. cacao* var. Criollo, and the other from Ecuador, *T. cacao* var. Arriba.

For each sample the seeds were separated from the rest of the fruit and the skin was discarded. The seeds were then ground in a blade to a maximum diameter of 1 mm. These grains were then dried using an Edwards Modulyo freeze-dryer. The resulting grains were extracted with supercritical CO_2 using an Applied Separations Speed SFE extractor. The resulting extracts were stored in the dark, at −10 °C in an inert atmosphere.

Physical and Chemical Characteristics Determination. Melting point, saponification number, and peroxide number were determined according to AOAC methods (AOAC, 1984).

SFE. Three supercritical CO_2 extractions for each *Theobroma* sample were performed using an Applied Separations

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Speed SFE extractor under the following operating conditions: CO_2 flow rate, 2 L/min; oven temperature, 40 °C; pressure, 400 bar; restrictor temperature, 70 °C. During tuning of the operating conditions it was found that the extract yield was directly linked to an increase in temperature and pressure, in agreement with the findings of Rossi et al. (1989). The first phase involved 3 min of static extraction at 400 bar. This was followed by 13 min of dynamic extraction at the same pressure. The sample was directly collected in a freezer container.

Gas Chromatography (GC) of the Fatty Acid Methyl Esters (FAME). The FAME were prepared by transmethylation using sodium methoxide in the presence of methyl acetate following the method laid out by Christie (1982). Analysis was performed under the following conditions: a Carlo Erba HRCG gas chromatograph equipped with a Carlo Erba EL490 amplifier and an FID detector; injection temperature, 300 °C; detector temperature, 350 °C; split ratio, 1:50; carrier gas, helium, at a flow rate of 2 mL/min; Restec Corp. (Bellefonte, PA) RTX-2330 cyanopropyl column (cyanopropylphenyl 10%, biscyanopropylphenylpolysiloxane 90%), i.d. = 0.25 mm and length = 20 m, film thickness = 0.20 μm, programmed for a 5 °C/min temperature increase from 150 to 200 °C and maintaining the temperature at 200 °C for 6 min. The fatty acid standards were obtained from Alltech (Deerfield, IL).

GC of the Triglycerides. The raw silanized fat (pyridine/hexamethyldisilazane/trimethylchlorosilane 5:2:1) was analyzed by GC using a MEGALAP column (MEGA, Legnano, Italy; i.d. = 0.32 mm and length = 25 m; film thickness = 0.1 μm) programmed for a 3 °C/min temperature increase from 250 to 350 °C and isotherm maintenance at 350 °C for 40 min, installed on a Carlo Erba Auto HRGC MFC 500 gas chromatograph equipped with a Fisons EL980 processor, a Carlo Erba Mega series integrator, an FID detector, and helium as carrier gas.

Phytosterol Derivatives. The unsaponifiable fraction was obtained by treating 2 g of raw fat with methanolic KOH and then extracting with ethyl ether. This was then characterized by planar thin-layer chromatography (TLC) and GC (Lercker and Caboni, 1985). The TLC silica gel plates (Merck 1.05715 Kieselgel 60_{F254}) were eluted with 60:40 hexane/ethyl ether. The unsaponifiable components were separated by preparatory TLC on silica gel plates (Merck 5717 Kieselgel 60_{F254} 20 × 20, 2 mm thick). Plate detection was performed with fluorescein (Fluka, Buchs, Switzerland). The phytosterol bands were extracted with ethyl ether and silanized (pyridine/hexamethyldisilazane/trimethylchlorosilane 5:2:1) before being injected into the column. GC analysis was performed on a MEGA SE52 column, i.d. = 0.32 mm and length = 25 m, film thickness = 0.15 μm, installed on a Fisons 9130 VIC 900 9000 series GC equipped with a Fisons EL980 processor and programmed temperature increase of 5 °C/min from 100 to 300 °C, with the temperature held at 300 °C for 6 min. Injector temperature was 300 °C, FID temperature was 350 °C, helium flow rate was 2 mL/min, and split ratio was 1:40. The campesterol, stigmasterol, and β-sitosterol standards were obtained from Fluka.

RESULTS AND DISCUSSION

SFE was used to obtain the total lipid fraction from seeds of wild *T. subincanum* and from seeds of two commercial varieties of *T. cacao*, one from Mexico (var. Criollo) and the other from Ecuador (var. Arriba). The results obtained show that the method used provides a high extract yield for *T. subincanum*. In fact, under the same extraction conditions, the total lipid yield was greater for *T. subincanum* than for either of the varieties of *T. cacao* (Table 1). Similar research using SFE performed on *T. cacao* but over an extended period of time (5 h) provided similar results (Rossi et al., 1989).

A preliminary TLC screening was then performed on the lipid matrix, revealing that, in both species, the triglycerides significantly exceeded the mono- and diglyceride component as well as unsaponifiable compounds.

Table 1. Operating Conditions and Yield of the Extraction Experiments[a]

pressure (bar)	temp (°C)	yield (%)
	T. subincanum	
200	40	8.3
400	40	41.5
400	80	42.7
	T. cacao Var. Criollo	
400	40	18.2
	T. cacao Var. Arriba	
400	40	17.5

[a] The results are the average of three determinations.

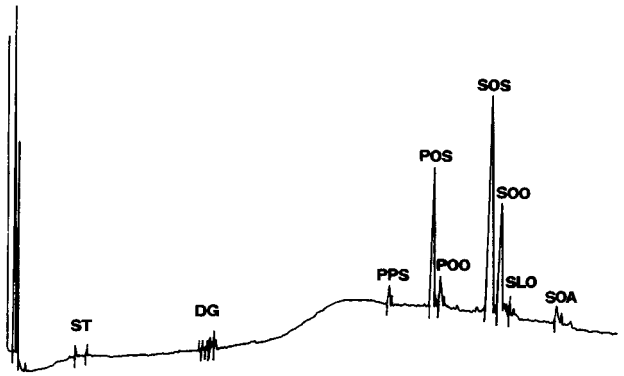

Figure 1. Total fat GC spectrum of *T. subincanum*: ST, sterols; DG, diglycerides; PPS, dipalmitostearin; POS, palmitoleostearin; POO, palmitodiolein; SOS, oleodistearin; SOO, stearodiolein; SLO, stearolinolein; SOA, stearoleoarachidin.

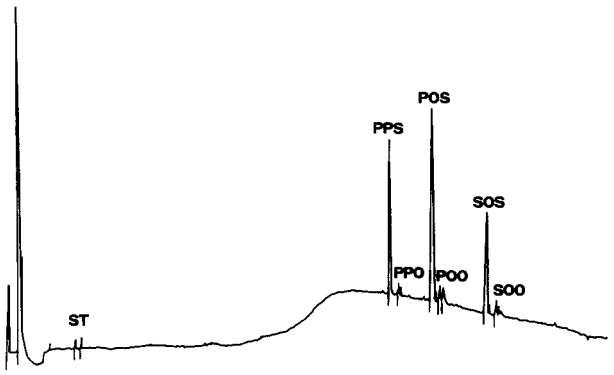

Figure 2. Total fat GC spectrum of *T. cacao* var. Criollo: ST, sterols; PPS, dipalmitostearin; PPO, dipalmitolein; POS, palmitoleostearin; POO, palmitodiolein; SOS, oleodistearin; SOO, stearodiolein.

The triglycerides were then characterized through GC. The GC spectra for the two varieties of *T. cacao* were practically identical, whereas they differed significantly from the *T. subincanum* spectrum, in terms of both composition and amount of compounds separated (Figures 1−3).

The qualitative and quantitative differences in the individual compounds found are worthy of note (Table 2). The presence of stearolinolein (SLO) and stearoleoarachidin (SOA) in the fatty fraction from *T. subincanum* leads one to assume the presence of linoleic acid (C18:2) and arachic acid (C20:0). The greater presence of dipalmitostearin (PPS) in *T. cacao*, on the other hand, leads to the conclusion that the palmitic acid (C16:0) fraction is markedly lower in *T. subincanum*. It is also

Table 2. Total Fat Composition of *T. subincanum* and *T. cacao* (Criollo and Arriba Varieties)[a]

	total fat composition[b] (%)									
sample	sterols	DG	PPS	PPO	POS	POO	SOS	SOO	SLO	SOA
T. subincanum	0.8	1.1	1.3	N	16.3	2.5	53.3	19.6	2.6	2.5
T. cacao var. Criollo	1.1	ND	20.3	1.6	44.9	2.9	25.9	3.3	ND	ND
T. cacao var. Arriba	0.4	1.3	14.2	1.0	61.7	2.0	16.5	2.0	0.6	0.3

[a] The results are the average of three determinations. [b] DG, diglycerides; PPS, dipalmitostearin; PPO, dipalmitolein; POS, palmitoleostearin; POO, palmitodiolein; SOS, oleodistearin; SOO, stearodiolein; SLO, stearolinolein; SOA, stearoleoarachidin; ND, not detected.

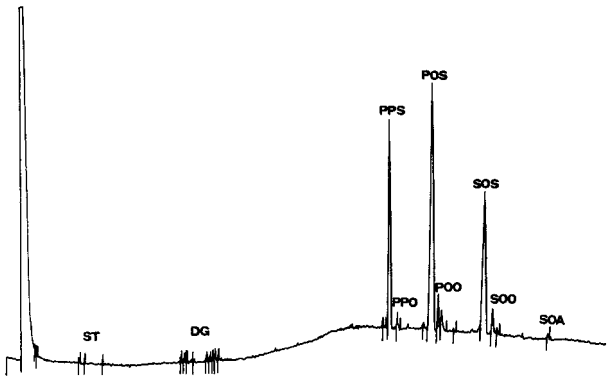

Figure 3. Total fat GC spectrum of *T. cacao* var. Arriba: ST, sterols; DG, diglycerides; PPS, dipalmitostearin; PPO, dipalmitolein; POS, palmitoleostearin; POO, palmitodiolein; SOS, oleodistearin; SOO, stearodiolein; SOA, stearoleoarachidin.

Table 3. Physical and Chemical Characteristics of the Fat of *Theobroma* Seeds[a]

sample	mp (°C)	peroxide no., mequiv of active O_2/kg of sample	saponification no., mg of KOH/g of sample
T. subincanum	39.1	2.6	180.2
T. cacao var. Criollo	33.3	1.3	189.4
T. cacao var Arriba	32.6	1.5	191.7
cocoa bean fat	29−35	<2	189−200

[a] The results are the average of three determinations.

worth noting the different quantitative breakdown in the triglycerides oleopalmitosterin (POS), oleodistearin (SOS), and stearodiolein (SOO) for two species. The presence of triglycerides containing longer chain fatty acids in *T. subincanum* suggests the rheology of the resulting butter would be different from that of *T. cacao*. Moreover, the physical and chemical characteristics of the fat (Table 3) showed that for *T. subincanum* all of the values are outside the commercial parameters. In particular, the higher melting point of the butter means poorer quality and lower market value (Chatt, 1953; Schmidt-Ebbel, 1966; van Ejik, 1992; Willson, 1999). These considerations have been confirmed by GC analysis of the transmethylated fatty acids. In fact, the GC analyses showed that the fatty acid contents in the seeds of the two varieties of *T. cacao* were substantially similar, whereas there were significant differences with that of *T. subincanum*. In fact, in *T. subincanum* there is little palmitic acid (C16:0) and more stearic (C18:0) and oleic (C18:1) acid than in *T. cacao*. In addition, arachic acid (C20:0) was present in *T. subincanum* but not in *T. cacao* (Table 4). The fatty acid content in *T. cacao* proved to be analogous to the data reported in the literature (Sotelo and Alvarez, 1991). On the other hand, some differences were seen for *T. subincanum*. In particular, stearic acid (C18:0) was more abundant, whereas arachic acid was less available. This could reflect the different geographic origin of this sample.

Table 4. *T. subincanum* and *T. cacao* (Criollo and Arriba Varieties) Fatty Acid Compositions[a]

	fatty acid composition (%)				
sample	C16:0	C18:0	C18:1	C18:2	C20:0
T. subincanum	7.2	41.9	40.6	3.0	3.2
T. cacao var. Criollo	26.1	34.3	34.8	1.0	ND[b]
T. cacao var. Arriba	29.8	33.0	32.5	0.8	ND

[a] The results are the average of three determinations. [b] ND, not detected.

Table 5. *Theobroma* Seed Sterolic Composition and Unsaponifiable Yield[a]

	sterolic composition (%)			
sample	campesterol	stigmasterol	β-stigmasterol	yield
T. subincanum	9.4	24.1	53.0	2.5
T. cacao var. Criollo	10.0	23.2	55.6	2.0
T. cacao var. Arriba	9.8	23.6	55.2	1.9

[a] The results are the average of three determinations.

The unsaponifiable fraction (phytosterols) was analyzed using planar chromatography and GC (Table 5). In particular, this revealed that campesterol, stigmasterol, and β-sitosterol, in order of increasing abundance, were the most common such compounds present in all three varieties studied. Comparison of the quantitative data did not reveal any substantial differences between the commercial varieties and *T. subincanum*. This result is qualitatively similar to what was found for sterols in both *T. subincanum* and *T. cacao* (Carpenter et al., 1994). The total absence of diglycerides in *T. cacao* var. Criollo may be due to the higher quality of the original cultivar (Pires et al., 1998). The analogous sterol fraction composition—mainly unsaponifiable compounds—indicates that the quality of the butter would be basically equivalent for this parameter.

Therefore, it is possible to conclude that the butter obtained from seeds of this wild variety of *T. subincanum* has less commercial value than that of *T. cacao* because of the presence of diglycerides and longer chain fatty acids in its lipid fraction and because of its higher melting point. Nevertheless, the differences found suggest that *T. subincanum* could find significant commercial use in low profile products.

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LITERATURE CITED

AOAC. *Official Methods of Analysis*, 14th ed.; Williams, S., Ed.; Association of Official Analytical Chemists: Arlington, VA, 1984; p 1141.

Bartle, K. D. *Supercritical Fluid Chromatography*; Smith, R. M., Ed.; Royal Society of Chemistry: Cambridge, U.K., 1990; p 204.

Carpenter, D. R.; Hammerstone, J. F., Jr.; Romanczyk, L. J., Jr.; Aikten, W. M. Lipid composition of *Herrania* and *Theobroma* seeds. *J. Am. Oil Chem. Soc.* **1994**, *71*, 845−851.

Chatt, E. M. *Cocoa—Cultivation, Processing, Analysis*; Interscience Publishers: New York, 1953; p 302.

Christie, W. W. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. *J. Lipid Res.* **1982**, *23*, 1072−1075.

Hammerstone, J. F., Jr.; Romanczyk., L. J., Jr.; Aitken, W. M. Purine alkaloid distribution within *Herrania* and *Theobroma*. *Phytochemistry* **1994**, *35*, 1237−1240.

Hunter, J. R. The status of Cacao (*Theobroma cacao*, Sterculiaceae) in the Western Hemisphere. *Econ. Bot.* **1990**, *44*, 425−439.

Lercker, G.; Caboni, M. F. Analisi degli insaponificabili mediante GLC. *Riv. Ital. Sostanze Grasse* **1985**, *62*, 193−198.

Li, S. F.; Hartland, S. A new industrial process for extracting cocoa butter and xanthines with supercritical carbon dioxide. *J. Am. Oil Chem. Soc.* **1996**, *73*, 423−429.

Liendo, R.; Padilla, F. C.; Quintanta, A. Characterization of cocoa butter extracted from criollo cultivars of *Theobroma cacao*. *Food Res. Int.* **1997**, *30*, 727−731.

Martinez, C. C. *Etno Botanica del Ecuador*; Abya Yala: Quito, Ecuador, 1996; p 197.

Marx, F.; Maia, J. G. S. Purine alkaloids in seeds of *Theobroma* species from the Amazon. *Z. Lebensm. Unters. Forsch.* **1991**, *193*, 460−461.

Morgan, J. Chocolate: a flavour unlike any other. *Am. J. Clin. Nutr.* **1994**, *60*, 1065−1067.

Pires, J. L.; Cascardo, J. C. M.; Lambert, S. V.; Figueira, A. Increasing cocoa butter yield through genetic-improvement of *Theobroma cacao* L. seed fat content variability, inheritance and association with seed yeld. *Euphytica* **1998**, *103*, 115−121.

Rossi, M.; Arnoldi, C.; Salvioni, G.; Schiraldi, A. Characterization of Cocoa extracts obtained with supercritical carbon dioxide. *Ital. J. Food Sci.* **1989**, *3*, 41−50.

Schmidt-Hebbel, H. *Quìmica y Tecnologìa de los Alimentos*; Editorial Salesiana: Santiago, Chile, 1966; p 313.

Sotelo, A.; Alvarez, R. G. Chemical composition of wild *Theobroma* species and their comparison to the Cacao bean. *J. Agric. Food Chem.* **1991**, *39*, 1940−1943.

van Ejik, T. The flavor of Cocoa and chocolate and the flavoring of compound coatings. *Dragoco Rep.* **1992**, *1*, 3−24.

Villacres, O. V. *Bioactividad de Plantas Amazonicas*; Abya Yala: Quito, Ecuador, 1995; p 378.

Willson, K. C. *Coffee, Cocoa and Tea*; CABI Publishing: New York, 1999; p 300.

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