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Thermal Characteristics and Composition of Fats from *Theobroma* Species[1]

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ABSTRACT

Thermal behaviors and compositions of fats from *T. angustifolia*, *T. bicolor* (from Costa Rica and Brazil), *T. gileri*, *T. grandiflora* and *T. mammosa* were evaluated in comparison to cocoa butter. *Theobroma* fats, except for that from Costa Rican *T. bicolor*, exhibited lower solid fat contents (SFC) than that of cocoa butter at room temperature (20-25°C). This is the result of the high oleic and linoleic acid contents which made up more than 50% of their total fatty acid composition. Cocoa butter (*T. cacao*) and Costa Rican *T. bicolor* contained 37.8% and 47.7% of both oleic and linoleic acid, respectively. The melting behavior of the Costa Rican *T. bicolor* fat was similar to that of cocoa butter, but its high SOO (S = stearic acid, O = oleic acid) content (22%) would be incompatible with cocoa butter. However, both Costa Rican and Brazilian *T. bicolor* fats contained high SOS. An SOS fraction could be used to improve the inferior quality cocoa butter.

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COMPENDIO

La evaluación del comportamiento térmico y la composición fueron utilizados para comparar las grasas de *T. angustifolia*, *T. bicolor* (de Costa Rica y Brasil), *T. gileri*, *T. grandiflora* and *T. mammosa* con la manteca de cacao (*T. cacao*). Al ser comparadas a temperatura ambiente (20-25°C), las grasas de las especies *Theobroma*, con excepción de la grasa de la especie *T. bicolor* de Costa Rica, presentaron contenidos más bajos de grasas sólidas (SFC) que la manteca de cacao. Esto fue debido al alto contenido de ácidos oléico y linoléico, los cuales representaron más del 50% del total de ácidos grasos. El contenido de los ácidos oléico y linoléico fue 37.8% en la manteca de cacao (*T. cacao*) y 47.7% en las grasas de la especie *T. bicolor* de Costa Rica. La forma en que la grasa de la especie costarricense *T. Theobroma* se derrite, fue, similar al comportamiento térmico de la manteca de cacao, pero la alta concentración (22%) de triglicéridos de la forma SSO (S = ácido esteárico, O = ácido oléico) es diferente a la concentración observada en la manteca de cacao. Sin embargo, ambas especies de *T. bicolor*, costarricense y brasileña, tuvieron un alto contenido de SOS. Una fracción de SOS podría ser utilizada para mejorar la manteca de cacao de baja calidad.

INTRODUCTION

Cocoa butter is a major ingredient in chocolate and comprises 30-40% of its weight. The melting characteristics of cocoa butter are unique, in having a high solid fat content at room temperature and melting at a narrow temperature range just below body temperature. The high price of cocoa butter encourages the confectionery industry to search for economical fats that have similar thermal characteristics. Theoretically, alternative fats have to contain the same types and proportions of triacylglycerols as cocoa butter in order to be fully compatible with cocoa butter. Over 70% of cocoa butter triacylglycerols are POP, POS, and SOS (P = palmitate, O = oleate, and S = stearate). In other words, cocoa butter alternatives should contain high concentrations of C16-18 fatty acids and monounsaturated triacyglycerols that have oleic acid at the sn-2 position. Up to now, most of the cocoa butter alternatives are obtained from the modified vegetable oils, e.g. palm oil, soybean oil, cotton seed oil, and coconut oil (8). Modification of these oils, which usually involve hydrogenation and fractionation, increases the price of cocoa butter alternatives. Some alternatives, such as lauric cocoa butter substitutes (CBS), are inexpensive but are not compatible with cocoa butter. Natural fats, of similar composition from plants of the same genus as the cacao tree, could be potential cocoa butter alternatives.

There are 22 other species of the genus *Theobroma* in Central and South America, but they are not widely cultivated as cacao trees (10). In addition to *T. cacao*, the commonly used species in this genus are *T. grandiflora* and *T. bicolor*. Fatty acid compositions and thermal characteristics of fats from these last two species have been studied (1, 2). Fats from *T. bicolor* and *T. grandiflora* contain high oleic and stearic acid. *T. bicolor* fat contains 43.2% oleic acid and 40.3-41.0% stearic acid, whereas *T. grandiflora* fat contains 41.4% and 31.8-34.0% oleic and stearic acid, respectively. Both fats are softer than cocoa butter, but have similar melting points. *T. grandiflora* fat had the same melting point as cocoa butter, whereas *T. bicolor* fat had a higher melting point, 32°C and 35°C, respectively (1). Fats from other species of *Theobroma*, however, have not been extensively investigated.

The objective of this study was to determine the thermal behavior and composition and of fats from five *Theobroma* species in comparison to cocoa butter.

MATERIALS AND METHODS

Sample preparation. Dry unfermented seeds of *T. angustifolia*, *T. bicolor* (from Brazil and Costa Rica), *T. cacao*, *T. gileri*, *T. grandiflora*, and *T. mammosa* were obtained as follows: Mature pods were collected, opened in the field laboratory and the seeds were washed with sawdust and water. Seeds were sun-dried for 20 min and oven-dried at 60°C for 48 h with mixing. Following collection, the seeds were stored in a dry atmosphere until being shipped to the Pennsilvania State University Laboratory. Upon receipt of the seeds, they were shelled by hand and ground with dry ice to prevent the fat from melting.

Table 1. Fat content of *Theobroma* seeds and melting points of their fats.

Sample	Fat content (% dry basis)	Melting point (°C)[1]
T. cacao	52.0	29.9
T. bicolor (Costa Rica)	27.0	29.7
T. bicolor (Brazil)	34.1	28.3
T. angustifolia	58.6	27.0
T. grandiflora	36.7	28.2
T. mammosa	49.6	28.3
T. gileri	1.2	–[2]

1 Based on onset temperature by DSC.
2 Liquid at room temperature.

Fat content. Fats were solvent-extracted from 2 g of the dry ground samples using a Tecator Soxtech system 1043. Samples were boiled in petroleum ether for 45 min and refluxed with the same solvent for 1 h. Solvent was removed by heating at 60°C. Percentages of fat content were calculated on a dry weight basis (db).

Melting point and solid fat content. Fat samples were completely melted at 100°C. Three microliters of the samples were transferred into differential scanning calorimeter (DSC) aluminum pans. Samples were tempered at 4°C for 24 h to initiate crystallization and, subsequently, at 27°C for three weeks to produce the stable polymorph V crystals. Tempered samples were analyzed for their melting points and solid fat content (SFC) profiles by using a Perkin Elmer DSC-4. Samples were heated from 0°C to 50°C at the rate of 20°C/min. Melting points were determined by measuring the onset temperatures of the endotherms (5). SFC profiles were obtained using a partial area program on the same DSC thermograms.

Fatty acid composition. Fatty acid compositions of the total lipid fractions were determined as fatty acid methyl esters using a Hewlett-Packard model 5730A gas chromatograph (GC) equipped with a flame ionization detector. Analyses were performed isothermally at 180°C on a 1.82 m x 2 mm column packed with GP 10% DEGS-PS on 80/100 Supelcoport (Supelco, Inc.). The carrier gas (helium) was at the flow rate of 20 ml/min.

Triacyglycerol compositions Triacyglycerol compositions were evaluated (6) with a Perkin Elmer HPLC pump and a water differential refractometer detector model 401. Mobile phase acetonitrilechloroform 6:4(v/v) was pumped at 0.7 ml/min through an adsorbosphere C-18 reverse phase column (Alltech Assoc.). Ten microliters of 10% (w/v) samples in chloroform were injected.

RESULTS

Seeds of *Theobroma*, except for those of *T. gileri*, contained high fat ranging from 27% to 58.6% (db) (Table 1). Triacylglycerol compositions of *Theobroma* fats were evaluated and the results are shown in Fig. 1 and Table 3. Peaks were identified by comparing their retention times with those of cocoa butter triacylglycerols from previous work (9) and quantification was based on peak area percentages. Cocoa butter contained mainly monounsaturated triacylglycerols; namely POP, POS, and SOS, which comprise more than 80% of the total triacylglycerols. Other species of *Theobroma* had high concentrations of di- and triunsaturated triacylglycerols, namely OOO, AOO and SOO (A = arachidate). In addition, fats from *T. angustifolia*, *T. grandiflora*, and *T. mammosa* had higher concentrations of high molecular weight triacylglycerols, namely SOA and OAA compared to cocoa butter and *T. bicolor* fats.

Fatty acid compositions of the fats support the patterns of triacylglycerol content. *T. angustifolia*, *T. grandiflora*, and *T. mammosa* fats contained high arachidic acid; 12.7, 12.1, and 13.0%, respectively (Table 3). These fats were also high in oleic and linoleic acid, which together composed more than 50% of the total fatty acid content. Fatty acid composition of *T. bicolor* from both Costa Rica and Brazil were most similar to cocoa butter, i.e., high in stearic and low in arachidic acid content. However, *T. bicolor* fats were approximately 20% lower in palmitic acid and about 15% higher in oleic acid content than cocoa butter. *T. gileri* was remarkably different from the other species studied. It contained only 1% fat and that fat was characterized by being low in stearic and high in linoleic acids. The triacylglycerol profile was atypical (Fig. 1). The *T. gileri* came from a very restricted area in Ecuador. The striking differences from the other *Theobroma* spp. may be due to: 1) it was separated genetically from the other species for a long time and/or 2) it belongs to another genera. It is apparent from the data that further studies are needed to clarify the nature of *T. gileri*.

The effect of differences in composition among fats from *Theobroma* spp. on physical behavior can be observed through DSC thermograms. After tempering at 27°C for three weeks, cocoa butter was solidified into the stable polymorph V, whereas *T. gileri* fat remained liquid at this temperature. Melting points of solid phases were similar among different species, ranging from 27.0 to 29.9°C (Table 1).

Fig. 2 shows the melting profiles of the fats as represented by SFC at various temperatures. Most of

Table 2. Triacylglycerol composition of *Theobroma* species (%).

Triacylglycerol	*T. cacao*	*T. bicolor* (Costa Rica)	*T. bicolor* (Brazil)	*T. angustifolia*	*T. grandiflora*	*T. mammosa*
OLiO	–	0.6	1.3	1.9	1.0	2.1
PLiO	0.8	1.0	1.4	1.4	1.9	2.1
PLiP	2.6	0.5	0.5	0.6	1.1	0.5
OOO	0.6	5.6	9.5	7.5	6.0	6.0
SliO	–	0.3	4.2	4.4	3.2	5.7
POO	4.5	3.5	5.8	3.2	6.2	3.1
PliS	4.8	2.1	2.4	–	2.9	1.9
POP	18.5	1.7	2.2	1.4	5.7	1.3
SOO	6.0	22.0	28.0	17.0	14.9	22.8
SLiS	–	4.6	3.2	–	–	–
POS	37.4	11.9	10.7	4.7	8.3	5.3
OOA	0.7	2.3	3.5	15.8	16.0	14.6
SOS	22.0	38.6	24.0	16.1	14.0	14.4
PSS	0.6	0.7	0.7	5.5	3.9	4.2
SOA	1.0	4.0	0.4	0.8	1.0	0.9
SSS	0.4	1.0	0.4	0.8	1.0	0.9
OAA	–	–	–	5.5	0.5	0.3
AAA	–	0.7	–	0.8	2.7	3.8

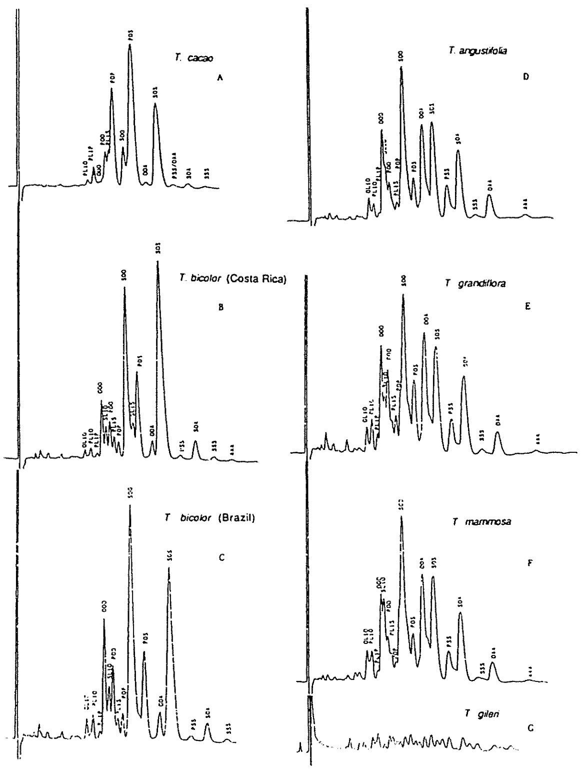

Fig. 1. Triacylglycerol chromatograms of *T. cacao* (A), Costa Rican *T. bicolor* (B), Brazilian *T. bicolor* (C), *T. angustifolia* (D), *T. grandiflora* (E), *T. mammosa* (F), and *T. gileri* (G). (P = palmitate, S = stearate, O = oleate, Li = linoleate, A = arachidate).

Theobroma fats were softer than cocoa butter at room temperature (20-25°C) which is indicated by lower SFC values. *T. bicolor* from Costa Rica had melting characteristics similar to cocoa butter at room temperature but had higher SFC at temperatures above 30°C.

DISCUSSION

Theobroma seeds, except for *T. gileri*, were high in fat, which could have some commercial significance. Among fats from *Theobroma* seeds, the fat from Costa Rican *T. bicolor* was most similar to cocoa butter in both composition and thermal characteristics. This fat exhibited a high SFC value at room temperature and had a sharp melting range at temperatures above 30°C. One disadvantage is that this fat had a high SFC value at temperatures over 35°C (Fig. 2), which may cause an undesirable waxy mouthfeel. This could be a result of a high SOS content. Although the Costa Rican *T. bicolor* fat has a melting behavior similar to cocoa butter, the use of this fat as a cocoa butter substitute could be complicated. The difficulty is due to its 43.4% oleic acid content, which is about 10% higher than contained in cocoa butter. Oleic acid exists primarily in the SOO forms in *T. bicolor* and is known to promote softness. The extra double bond in the sn-3 position of the SOO glycerol backbone may disturb the molecular packing of the major components, monounsaturated triacylglycerols, in cocoa butter (3). However, *T. bicolor* fat contained high SOS, a major triacylglycerol in cocoa butter. Previous work indicates that,

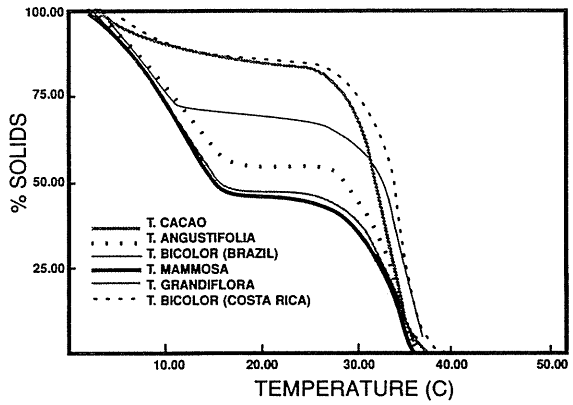

Fig. 2. Solid fat content profiles of fats from various *Theobroma* species.

Table 3. Fatty acid composition of *Theobroma* fats (%).

Sample	Fatty acid						
	16:0[1]	16:1	18.0	18:1	18:2	18:3	20:0
T. cacao	28.9	0.2	32.0	34.0	3.8	0.2	1.0
T. bicolor (Costa Rica)	6.8	–	43.5	43.4	4.3	–	1.9
T. bicolor (Brazil)	7.8	–	34.0	51.8	5.0	–	1.9
T. angustifolia	4.8	–	25.9	48.8	7.5	–	12.7
T. grandiflora	10.0	0.2	21.7	47.4	8.6	–	12.1
T. mammosa	5.6	0.2	25.9	44.1	10.7	0.5	13.0
T. gileri	11.8	0.8	12.2	34.2	27.5	2.4	11.2

1 Carbon chain length: number of double bonds.

in most cases, adding 3-5% of SOS to cocoa butter can improve the crystallization behavior of an inferior cocoa butter (7). It may not be commercially feasible to use the Costa Rican *T. bicolor* directly as a cocoa butter alternative; however, fractionation of SOS could be a viable alternative for improving the quality of an inferior cocoa butter. The Brazilian *T. bicolor* fat can be used in a similar way. The difference between the *T. bicolor* fats from the two sources is that the Brazilian one had higher unsaturated components, namely, SOO and OOO. The nature of the composition of the Brazilian *T. bicolor* fat results in a softer fat than the Costa Rican fat. The higher degree of unsaturation in the Brazilian *T. bicolor* fat could be a result of differences in variety or the temperature in the growing areas. Plants grown at low temperature generally contain high unsaturated fatty acids (4).

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