Technical

♣Composition of the Fat Extracted from the Seeds of Theobroma Bicolor

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ABSTRACT

The fat from *Theobroma bicolor* was analyzed for glyceride content by thin layer chromatography (TLC), and for fatty acid composition and triglyceride (carbon number) composition by gas liquid chromatography (GLC). The fat was then separated into glycerides of different degrees of unsaturation by means of silver nitrate TLC. Then, the bands were examined by GLC before and after conversion to methyl esters. From the results obtained, the distribution of the fatty acids on the individual glycerides was calculated. The fat consisted of 96.5% triglyceride with only 2.5% diglyceride and 1.7% free fatty acid. The major fatty acids present were 42.3% C18:0, 45.2% C18:1, and 6.0% C16:1. Most of the triglycerides were of carbon number 52 (18.0%) and 54 (77.6%). The major triglycerides were 38.6% 1-stearyl-2,3 diolein (SOO), 25.4% 2-oleyl-1,3 distearin (SOS) and 13.8% 1-palmito-2-oleyl-stearin (POS). Only 44.3% of the fat consisted of monounsaturated triglycerides.

INTRODUCTION

Theobroma bicolor is a noncultivated plant related to *Theobroma cacao*. The latter is used for the manufacture of chocolate and cocoa. The seeds of *T. bicolor* have been claimed to have been commercially mixed with those of *T. cacao* and that *T. bicolor* contains a considerable quantity of good-quality cocoa butter (1).

At the time of this work, no reports had been published on the chemical composition of the fat, although, being of the same family as *T. cacao*, it was thought likely to be similar to cocoa butter. If *T. bicolor* is not similar, then commercial mixing of the seeds with *T. cacao* might yield a product with undesirable physical properties, causing problems in the tempering of chocolate manufactured from such mixtures. Therefore, the composition of the fat from these seeds was investigated.

Since this work was carried out, the fatty acid composition and some physical properties of one sample of *T. bicolor* have been published with those of *Theobroma grandiflora* (2).

EXPERIMENTAL

Materials

A small sample of air-dried seeds was supplied by Instituto Nacional de Investigaciones Agropecuarias, Quito, Equador. Precoated silver nitrate/silica gel TLC plates were supplied by Supelco Inc., Bellforte, PA. Chemicals were analytical grade from British Drug Houses, Ltd., Poole, England, as were standard Merck 5721 silica gel G plates.

Extraction of Oil

Two seeds (ca. 6) were ground whole in a coffee grinder, since removing the outer shell proved impossible. The fat was extracted with petroleum ether (40-60 C) in a Soxhlet for 12 hours, and then most of the solvent gently evaporated in a water bath, the last traces being removed under vacuum at 40 C. This procedure was repeated with two further samples of seeds so that analysis could be carried out in triplicate.

Analysis of Glyceride Classes

Glyceride composition was determined after TLC separation of ca. 50 mg on a silica gel G plate, developing once with hexane/diethylether/formic acid (60:40:1) and spraying with 0.1% ethanolic 2,7-dichlorofluorescein solution. The bands corresponding to the free fatty acids, mono-, di- and triglyceride, were scraped off and extracted in a Soxhlet for 2 hours with diethylether. After the addition of 1 mL of 0.1% methyl heneicosanoate, the mixture was transesterified using boron trifluoride/methanol (3), and the methyl ester composition was determined using a Hewlett Packard 5880 gas chromatograph.

TABLE I

Analysis of Lipid of *T. bicolor* v. Typical *T. cacao*

	T. bicolor[a]	*T. cacao*
% Monoglyceride	ND	ND
% Diglyceride	2.5 ± 0.02	1.7
% Triglyceride	96.5 ± 0.4	98.0
% Free fatty acid	1.7 ± 0.1	0.3
trans Fatty acid	ND	ND
Unsymmetrical monounsaturated triglyceride	ND	ND
Unsaponifiable matter	0.58 ± 0.02	0.49 ± 0.01

ND = None detectable.

[a]Three analyses of fat from *T. bicolor* were carried out on 3 samples of fat, each extracted separately from 2 seeds of *T. bicolor*. The values were found to be similar, therefore the average values, together with the variation observed, are given here.

TABLE II

Fatty Acid Composition of Triglyceride of *T. bicolor* Compared with Fat from Typical *T. cacao*

Fatty acid	% *T. bicolor*[a]	% *T. cacao*
C14:0	ND	0.2
C16:0	6.6 ± 0.2	25.8
C16:1	ND	0.2
C17:0	0.2	0.2
C18:0	42.9 ± 1.1	35.9
C18:1	45.1 ± 0.3	33.3
C18:2	3.0 ± 0.2	3.2
C20:0	2.0 ± 0.3	1.0
C20:1	ND	0.1

ND = None detected.

[a]Three analyses of fat from *T. bicolor* were carried out on 3 samples of fat, each extracted separately from 2 seeds of *T. bicolor*. The values were found to be similar, therefore the average values, together with the variations observed, are given here.

TABLE III

Triglyceride (Carbon Number) Composition of *T. bicolor* (3 Samples) Compared with Typical *T. cacao*

Carbon number	% *T. bicolor*[a]	% *T. cacao*
48	0.1 ± 0.1	1.8
50	1.5 ± 0.1	18.9
52	18.1 ± 0.2	44.6
54	77.7 ± 0.2	33.9
56	2.5 ± 0.2	0.8
58	0.2 ± 0.1	ND

ND = None detectable.

[a]Three analyses of fat from *T. bicolor* were carried out on 3 samples of fat, each extracted separately from 2 seeds of *T. bicolor*. These values were found to be similar, therefore the average values, together with the variation observed, are given here.

Analysis of Triglycerides by Carbon Number

The analysis was carried out on a Hewlett Packard 5880 using a 60 cm × 3 mm ID column packed with conditioned 3%OV-1 on Gaschrom Q (4). The proportion of triglycerides of carbon number C54 up was corrected for column losses by means of a curve prepared using standard solutions containing known quantities of tristearin, tripalmitin, tribehenin, trierucin and triarachidin.

Analysis of Fat for Unsymmetrical Monounsaturated Triglycerides

This analysis was carried out by TLC of the sample on silica gel plates coated with silver nitrate (5).

Determination of trans Acid Content

Using infrared analysis, *trans* acids were determined by measuring the absorption at 10.36 μ compared with a cocoa butter standard (6).

Determination of Unsaponifiable Matter

This determination was carried out in duplicate on pooled samples of fat by the AOAC method (7).

Analysis of Component Triglycerides of Fat

This analysis was performed on only one (sample 1) of the three samples extracted. Because the levels of the mono- and diglycerides were found to be low, nontriglyceride material was not removed before TLC. The removal would have been necessary if high levels of diglyceride, for example, had been present.

A 50 mg portion of fat was dissolved in 1 mL of hexane and spread in a narrow band about 1 cm from the lower edge of a silver nitrate-coated silica gel TLC plate. This was repeated for a second plate. The plates were then lowered into a TLC tank containing sufficient redistilled chloroform to immerse about 1 cm in the solvent. After the plates had been developed once, they were removed from the tanks and the chloroform evaporated under a stream of nitrogen. The plates were then developed for a second time in the same solvent. After evaporating the solvent as before, the plates were liberally sprayed with 0.1% ethanolic dichlorofluorescein solution to show up the individual lipid bands under UV light. The bands were scraped into an extraction thimble, corresponding bands from the two plates being bulked together. The contents of each thimble were extracted in a Soxhlet using diethylether, containing 0.005% BHT, as solvent. The solvent was then evaporated to dryness.

The triglyceride extracts were analyzed by GLC for triglyceride composition by carbon number. Each band was then quantitatively transferred, using small portions of diethylether, into a 50 mL flask and 1 mL of a 0.3% solution of methyl heneicosanoate was added as the internal standard. The methyl esters were then prepared and analyzed as before (3). From the percentage of internal standard present in each band, the relative proportions of each band can be calculated (8,9).

RESULTS AND DISCUSSION

The seeds of *T. bicolor* yielded 38% of a soft yellow fat, containing 0.6% unsaponifiable matter. As can be seen from Table I, the partial glyceride and free fatty acid levels were quite low although slightly higher than normal cocoa butter. No *trans* fatty acids or unsymmetrical monounsaturated triglycerides were detected. The fatty acid and triglyceride compositions (Tables II and III) were very different from cocoa butter. Much higher levels of stearic and oleic acids, and lower levels of palmitic acid, resulted in very high C54 levels. The figures for fatty acid composition are similar to those reported by Berbert (2).

The fatty acid composition of the bands of differing degrees of unsaturation are given in Table IV. A number of earlier workers (8-10) have combined figures for the fatty acid composition of the bands of differing degrees of unsaturation of a fat with figures obtained by lipase hydrolysis. Assuming that the fatty acids in the triglycerides are distributed in, for example, the 1,3-random-2-random distribution pattern, they have calculated the total triacylglycerol structure of the oil in question. If the figures for carbon number triglyceride composition are available, then, providing that isomers such as 1-stearo-2,3-diolein and the corresponding 2-stearo isomer are not differentiated,

TABLE IV

Fatty Acid Composition of Bands with Differing Degrees of Unsaturation of Fat from *T. bicolor*

Fatty acid	Tri-saturated	Mono-unsaturated	Di-mono-unsaturated	Mono-di-unsaturated	Tri-unsaturated
16:0	13.8	12.5	1.4	5.8	–
17:0	0.2	0.1	0.1	0.1	–
18:0	85.6	49.8	29.9	58.8	–
18:1	–	34.3	66.6	–	98.9
18:2	–	–	–	34.5	1.1
20:0	0.4	3.3	0.6	1.2	–
Band as % of total fat	1.5	44.3	40.8	9.8	3.8

TABLE V

Component Triglycerides of *T. bicolor*[b]

Carbon number	Tri-saturated band	Mono-unsaturated band	Di-mono-unsaturated band	Mono-diunsaturated band	Tri-unsaturated band
48	PPP[a] 0.1	–	–	–	–
50	PSP 0.1	POP 1.4	–	–	–
52	PSS 0.2	POS 13.8	POO 2.0	PLiS 1.6	–
54	SSS 1.1	AOP 0.9	SOO 38.6	AliP 0.1	OOO 3.8
		SOS 25.4		SliS 7.6	
56	–	AOS 2.5	AOO 0.2	AliS 0.3	–
58	–	AOA 0.3	–	–	–
Total for each band	1.5	44.3	40.8	9.6	3.8

[a]P = palmitic; S = stearic; O = oleic; Li = linoleic; A = arachidic.

[b]Glycerides present at less than 0.1% are ignored.

then the triglyceride structure may be calculated without the lipase hydrolysis data (11). In the case of the fat from *T. bicolor*, if the trace quantity of heptadecanoic acid is ignored, the triacylglycerol structure, ignoring positional isomers may be calculated without making any assumptions as to the distribution of the fatty acid residues. The results obtained are given in Table V. These figures correlate reasonably well with the analysis figures for the whole fat. As silver nitrate TLC had earlier shown the absence of any unsymmetrical monounsaturated triglycerides, all the triglycerides of this type have the unsaturated acid in the 2 position.

Unlike cocoa butter, which normally contains ca. 80% symmetrical monounsaturated triglycerides (11,12), this fat contained only 44% symmetrical monounsaturated triglycerides. The high level of diunsaturated triglyceride explains the softness and low melting point observed. Berbert attributes the softness to the level of unsaponifiable matter but this is probably a relatively unimportant factor as the unsaponifiable matter found (0.6%) was not significantly higher than the value for a typical cocoa butter (0.5%). If cocoa butter was adulterated with the fat of *T. bicolor* to any extent, then the product would probably be undesirably soft.

REFERENCES

1. Cuatrecasas, Cocoa and Its Allies, A Taxonomic Revision of the genus *Theobroma*; Contributions from the US National Herbarium, 1964, 35, Pt. 6, Smithsonian Institute, p. 463.
2. Berbert, P.R.F., Rev. Theobroma 11:91 (1981).
3. Association of Official Analytical Chemists, Official Methods of Analysis, 13th edn., 1980, method 28:053.
4. Padley, F.B., and Timms, R.E., Lebensm-Wiss. U. Technol. 11:319 (1978).
5. Dallas, M.S.J., and Padley, F.B., Ibid. 10:328 (1977).
6. Association of Official Analytical Chemists, Official Methods of Analysis, 13th edn., 1980, method 28:075.
7. Ibid., method 28:081.
8. Gunstone, F.D., and Padley, F.B., JAOCS 42:957 (1965).
9. Blank, M.L., Verdeno, B., and Privett, O.S., JAOCS 42:87 (1965).
10. Sempore, G., and Bezard, J., JAOCS 59:124 (1982).
11. Sidaway, A.F., personal communication.
12. Padley, F.B., et al., Int. Choc. Rev. 27:226 (1972).

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