Triglyceride Composition of *Madhuca butyraceae* Seed Fat

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ABSTRACT

Fatty acid and triglyceride compositions of phulwara butter (*Madhuca butyraceae* seed fat) have been determined by combination of the techniques of systematic crystallization at low temperatures, pancreatic lipase hydrolysis, and gas liquid chromatography of methyl esters. The percentages of individual fatty acids were found to be palmitic, 55.6; stearic, 5.2; oleic, 35.9; and linoleic, 3.3. The special characteristic of the phulwara butter is its content of POP, 52.5%; PLP, 4.9%; POSt, 8.6%; POO, 14.4% and PPP, 7.7% (P, palmitic; St. stearic; O, oleic; and L, linoleic). 2-Monoglycerides obtained by lipolysis of this fat and its least soluble fraction contained 13.0% and 29.3% saturated acids, respectively. Phulwara butter may be a potential source of palmitic acid for the pharmaceutical industry.

INTRODUCTION

Seed fat of the species *Madhuca butyraceae* (Syn. *Diploknema butyraceae;* family, Sapotaceae) is known in the trade as phulwara butter. It is white in color, harder in consistency, and has a higher titre test than most vegetable fats. Because of its pleasant taste, odor, and good keeping quality, phulwara butter is commonly used as a substitute for cocoa butter or as an adulterant of ghee.

The species *M. butyraceae,* a large deciduous tree, is commonly found in the sub-Himalayan tract and outer Himalayas of India up to an altitude of 15,000 ft. The species is also found in the Andaman Islands. The plant flowers in the winter season, and the fruits ripen in June-July. Fruits are elipsoid (0.8-1.8 in.), and each encloses one to three black seeds (0.8 g in wt). White almond-shaped kernels (70% of the weight of the seeds) contain 60-70% oil (1).

Fats of the Sapotaceae family generally have low iodine values and are composed mainly of oleic and saturated acids. The saturated acids in most cases are stearic and palmitic acids (2). Seed fats of the genus *Madhuca* of this family, however, have been the subject of greater interest than others because of their more variable compositions and characteristics (3-8). Total content of stearic and palmitic acids in the seed fats of *Madhuca latifolia, Madhuca longifolia,* and *Madhuca mottleyana* generally lies within the range of 28-62%. The proportion of stearic acid in these fats is considerably high (14-45%). Stearic acid, however, constitutes only a minor component (3%) in phulwara butter. Its major components are oleic and palmitic acids, the latter being present in unusually large proportion (56%). Low content of linoleic acid (3.8%) is also another significant feature (8).

Bushell and Hilditch (8) determined the triglyceride composition of phulwara butter by the technique of low temperature segregation. According to these authors, the major glycerides are oleoyldipalmitoylglycerol (62%), palmitoyldioleoylglycerol (23%), tripalmitoylglycerol (8%), and oleoylpalmitoylstearoylglycerol (7%). The content of trisaturated glycerides was more abundant than is usual in the seed fats with the observed proportions of saturated and unsaturated acids (9). Determination of triglyceride composition based on a low temperature crystallization technique has been reported to be satisfactory so long as a fat does not contain more than 50% linoleic or total polyethenoid acids (9), but the method does not give a detailed picture of the triglyceride composition. Investigations of triglyceride composition based upon techniques such as selective enzymatic hydrolysis and thin layer chromatography give more specific information. The triglyceride composition of *M. butyraceae* seed fat based upon the recently developed techniques has not yet been reported, even though this fat appears to be of a special type among the Sapotaceae seed fats. Moreover, the fatty acid composition of *M. butyraceae* seed fat indicates potential as a commercial source of palmitic acid which is in great demand in the pharmaceutical industry. India's requirement of palmitic acid for this purpose in 1983-84 has been estimated as 50 tons per annum.

In our search for less known indigenous seed oils specially suitable for utilization in the pharmaceutical industry, phulwara butter was examined. Its triglyceride composition was determined by combination of the techniques of selective enzymatic hydrolysis, thin layer chromatography (TLC), and gas liquid chromatography (GLC). Studies were also extended to the preparation of a concentrate of palmitic acid by low temperature segregation from the mixed fatty acids of *M. butyraceae* seed fat.

EXPERIMENTAL PROCEDURE

Fatty acid composition of the *M. butyraceae* seed fat was determined by GLC of the methyl esters in an F and M analytical gas chromatograph (model 700-R-12) with flame ionization detector. The column (6 ft x 1/4 in.) packed with 10% polyester of diethylene glycol adipate on 60-80 mesh Gas Chrom Z was operated at 166 C with carrier gas flow of 40 ml/min. Peak areas were determined as the product of peak height and the width at half height.

Triglyceride composition of the oil was determined by combination of the techniques of segregation of the oil and selective enzymatic hydrolysis of each of the segregated fractions. Segregation of the oil into fractions with varying degrees of unsaturation was effected by the low temperature crystallization technique of Hilditch and Williams (9). Selective enzymatic hydrolysis by pancreatic lipase was by the method suggested by Coleman (6). Hydrolysis was carried out at pH 8.5 and 37.5 C with addition of calcium ions and bile salts. The partial glycerides thus formed were separated on a thin layer (0.3-0.4 mm) of silica gel developed with a solvent system of *n*-hexane-diethyl ether-acetic acid (80:20:0.25). The 2-monoglyceride fraction detected with 2′,7′-dichlorofluorescein was extracted with hot ethanol. 2-Monogalycerides and the original triglycerides were converted into methyl esters by the semi-micro-method of Luddy et al. (10). Methyl esters were analyzed by GLC for their fatty acid compositions.

RESULTS

M. butyraceae seeds were procured from the Indian Botanic Garden, Howrah. On extraction with petroleum ether (bp 40-60 C), the seed kernels yielded 44.4% of a light colored fat with 5.2% free fatty acids. Since alkali refining of the oil as a 50% solution in *n*-hexane (11) proved troublesome, free acids were removed by extraction with ethanol. After refining, the oil showed the following characteristics: free acid, 0.8% as oleic; unsaponifiable, 3.5% by wt; saponification equivalent, 286.4; iodine value,

TABLE I

Fatty Acid Composition (mole %) of Different Triglyceride Fractions of *Madhuca butyraceae* Seed Oil and of the Corresponding 2-Monoglycerides

Fatty acids	Native seed oil		Fractions[a] A (27.3%)		B (46.2%)		C (12.5%)		D (8.8%)		E (5.2%)		Computed values (A-E)	
	TG	MG	TG	MG	TG	MG	TG	MG	TG	MG	TG	MG	TG	MG
$C_{16:0}$	58.0	11.7	65.5	27.2	60.6	5.6	50.2	5.1	39.6	4.7	34.1	4.2	57.5	11.3
$C_{18:0}$	4.9	1.3	5.1	2.1	2.6	---	3.4	---	2.5	---	3.0	---	3.3	0.6
$C_{18:1}$	34.0	79.5	27.5	68.0	34.3	88.2	35.4	83.5	47.8	75.1	45.6	69.6	34.3	80.0
$C_{18:2}$	3.1	7.5	1.9	2.7	2.5	6.2	11.0	11.4	10.1	20.2	17.3	26.2	4.9	8.1

[a]TG = triglyceride; MG = 2-monoglyceride.

TABLE II

Triglyceride Composision[a] (mole %) of *M. butyraceae* Seed Fat and Its Fractions

Samples	Percent of total	Glycerides SSS	SSU	SUS	USU	SUU	UUU
Seed fat	---	10.1	2.8	67.2	0.1	18.5	1.3
Fraction A	27.3	24.3	4.8	59.0	0.2	11.1	0.6
Fraction B	46.2	4.8	0.8	80.0	---	13.9	0.5
Fraction C	12.5	3.1	1.7	57.5	0.3	32.8	4.6
Fraction D	8.8	1.8	2.2	35.3	0.7	45.3	14.7
Fraction E	5.2	1.2	2.0	27.3	1.0	47.8	20.7
Computed Values (A-E)		9.4	2.2	64.9	0.1	20.1	3.3

[a]S represents saturated fatty acids; U represents unsaturated. The sequence represents that on the glycerine moiety.

46.9; and slip point, 39 C. The mixed fatty acids prepared by the usual procedures had the characteristics: saponification equivalent, 267.8; and iodine value, 41.2.

Refined oil (40.7 g) was next segregated according to Hilditch and Williams (9) into five fractions (A, 10.5 g; B, 17.8 g; C, 4.8 g; D, 3.4 g; and E, 2.0 g) with varying degrees of unsaturation (iodine values: 26.8, 33.6, 50.8, 59.2, and 70.9, respectively) by stepwise crystallization at low temperatures. The oil was crystallized from 10 volumes of acetone at -40 C to +20 C increasing the temperature by 10 C in each successive stage. In all seven, crystallizations were carried out and smaller fractions with closer iodine values were combined together.

In this process of segregation, the solid that separated at -40 C did not dissolve completely in 10 volumes of acetone at room temperature (25 C). On analysis, this insoluble fraction (2.2 g) was found to be highly unsaturated (iodine value, 196.0). This fraction was soluble in chloroform but did not give any test for phospholipids. Since this fraction was not triglyceride, it was not examined further.

Aliquot portions from each of the segregated fractions and the oil were subjected to pancreatic lipase hydrlysis. Fatty acid composition of the triglycerides and the corresponding 2-monoglycerides of all the fractions were determined by GLC analysis. Weight percentages were converted into mole percentages, and the compositions are given in Table I.

Triglyceride compositions of the *M. butyraceae* seed fat and its five fractions were calculated from the fatty acid composition (mole %) of the original triglycerides and the corresponding 2-monoglycerides using the assumptions of Vander Wal (12) and Coleman (6). Results are given in Table II.

Mixed fatty acids (42.7 g), free from unsaponifiables, were next segregated by low temperature crystallization into four fractions (A_1, 19.3 g; B_1, 3.7 g; C_1, 7.4 g; and D_1, 12.3 g) with iodine values, 2.7, 29.1, 54.3, and 86.0, respectively. Crystallization was effected three times, each at -20 C, the first one from 10 volumes acetone and the remaining two from 10 volumes ether. Fatty acid composition of the most saturated fraction, A_1, was determined by GLC as $C_{16:0}$, 90.1%; $C_{18:0}$, 5.7% and $C_{18:1}$, 4.2%.

DISCUSSION

Results of the present investigation along with previous findings on the fatty acid compositions of different seed fats of the genus *Madhuca* are shown in Table III. Present findings are in good agreement with those of Bushell and Hilditch (8). The seed fat of *M. butyracea,* containing a very high percentage of palmitic acid, is an exceptional one among the seed fats of the genus *Madhuca.* Stearic and linoleic acids together constitute only 8.5% of this fat. Its oleic acid content is also much lower than the amount present in other fats of the same genus.

Bushell and Hilditch (8) also determined the triglyceride composition of *M. butyraceae* seed fat utilizing the techniques of low temperature segregation and methyl ester fractionation. For a more comprehensive view, previous findings on the triglyceride compositions of different seed fats of the genus *Madhuca,* as determined by modern techniques, are grouped together in Table IV along with the present one. Present findings show a general agreement with the composition reported by Bushell and Hilditch (8). The chief glycerides, as determined by those authors (8), were oleoyldipalmitoylglycerol (OPP and POP), 62.0%; oleoylpalmitoylstearoylglycerol (OPSt and POSt), 7.0%; palmitoyldioleoylglycerol (OPO and POO), 23.0%; and tripalmitoylglycerol (PPP), 8.0%. According to the present findings, prominent triglycerides in this seed fat are: POP, 52.5%; PLP, 4.9%; POSt, 8.6%; POO, 14.4%; and PPP, 7.7%. The difference between these two sets of observations becomes significant in the case of oleoyldipalmitoylglycerol (62.0%

TABLE III

Fatty Acid Composition of the Seed Fats of the genus *Madhuca*

Species[a]	Habitat	Component fatty acids (% wt) $C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
M. latifolia (3)[b]	—	—	23.7	24.1	37.6	14.4
M. latifolia (4)	India	0.2	23.5	21.6	39.3	15.4
M. longifolia (5)	Ceylon	—	28.2	14.1	48.8	8.9
M. longifolia (6)	—	—	17.0	45.6	37.4	37.4
M. mottleyana (7)	Borneo	—	10.0	18.5	69.0	2.5
M. butyraceae (8)	India	—	56.6	3.6	36.0	3.8
M. butyraceae (Present work)	Bengal	—	55.6	5.2	35.9	3.3

[a]Numbers in parentheses indicate reference number.

[b]Also contains 0.2% $C_{16:1}$.

TABLE IV

Triglyceride Compositions (mole %) of the Seed Fats of the Genus *Madhuca*

Sample[a]	Method	Saturated acids	Triglycerides[b] SSS	SSU	SUS	USU	SUU	UUU
M. latifolia (3)	Lipolysis	43.0	4.0	46.0	46.0	41.0	41.0	9.0
M. longifolia (6) (Illip'e butter)	Lipolysis	62.6	3.5	0.7	80.4		14.6	0.7
M. butyraceae (8)	Crystallization	62.0	8.0	69.0	69.0	23.0	23.0	0.0
M. butyraceae (13)	Calculated[c]	62.0	8.0	72.0	72.0	18.0	18.0	2.0
M. butyraceae (Present work)	Crystallization and lipolysis	62.9	10.1	2.8	67.2	0.1	18.5	1.3

[a]Numbers in parentheses indicate reference number.

[b]S represents saturated fatty acids; U represents unsaturated; The sequence represents that on the glycerine moiety.

[c]Calculated according to Restricted Random Theory (14) on the basis of experimental data of Bushell and Hilditch (8).

and 54.7%) and palmitoyldioleoylglycerol (23.0% and 14.5%). Content of tripalmitoylglycerol was found to be same in both the cases (8.0% and 7.7%). More significant are the deviations observed between the triglyceride composition of the seed fat of *M. longifolia* (6) and that of *M. butyraceae* as determined in the present instance, although both the samples were constituted of 62.0% saturated acids. Methodology used in both cases was very similar. In these cases, differences are very prominent in the contents of SSS (3.5% and 10.1%) and SUS (80.4% and 67.2%). Phulwara butter thus appears to be a remarkable one among the seed fats of the *Madhuca* genus or of the Sapotaceae family in general The content of tripalmitoylglycerol is more abundant in this seed fat than is usual with the observed proportions of saturated acids. Similar deviations have also been observed earlier in the case of palm oil (saturated acids, 50.1%) which was constituted of SSS, 5.8%; SSU, 7.0%; and USU, 2.2% (6,16). Present findings on the triglyceride composition of *M. butyraceae* seed fats are also agreeable to the composition calculated (13) according to the restricted random distribution theory of Kartha (14).

M. butyraceae seed fat is similar to cocoa butter in the content of saturated acids. Both the samples contain 60% saturated acids. But in the case of *M. butyraceae,* the saturated acid is mostly palmitic acid while in the case of cocoa butter, the saturated are a mixture of palmitic and stearic acids (25.2% and 35.5%, respectively). The contents of oleic and linoleic acids in the two samples are nearly the same. *M. butyraceae* seed fat is commonly used as a substitute for cocoa butter since these samples have slip points close to each other (39 C and 35 C), but it will be evident from the Table V that a close agreement does not exist between the triglyceride compositions of these fats. Cocoa butter contains 72.9% 1,3-disaturated-2-oleoylglycerol and 8.6%, 1-saturated 2,3-dioleoylglycerol; but in the case of phulwara butter, the contents of these two types of glycerides amount to 61.5% and 15.6%, respectively. Moreover in phulwara butter 1,3-disaturated-2-oleoylglycerol is

TABLE V

Fatty Acid and Triglyceride Compositions (mole %) of Cocoa Butter[a] and Phulwara Butter

Major fatty acids	Cocoa butter (16)	Phulwara butter (Present work)
$C_{16:0}$	25.2	58.0
$C_{18:0}$	35.5	4.9
$C_{18:1}$	35.2	34.0
$C_{18:2}$	3.2	3.1
Major glycerides[b]		
PPP	0.1	7.7
PPSt	0.5	1.2
PPO	0.6	2.2
StStO	1.1	—
POP	12.0	52.5
POSt	34.8	8.6
StOSt	25.2	0.4
PLP	1.2	4.9
StLP	3.1	0.8
StLSt	2.0	—
POO	3.7	14.4
StOO	4.9	1.2
POL	0.4	1.2
PLO	0.3	1.3
OOO	0.5	1.0

[a]Number in parentheses indicates reference number.

[b]P, $C_{16:0}$; St, $C_{18:0}$; O, $C_{18:1}$: L, $C_{18:2}$.

mostly 1,3-dipalmitoyl-2-oleoylglycerol.

Mixed fatty acids of *M. butyraceae* seed fat, on segregation by low temperature crystallization, yielded a fraction amounting to 45% of the total composed of 90.1% palmitic, 5.7% stearic and 4.2% oleic acids. It is apparent that this fraction can be further purified by the urea adductation technique. So *M. butyraceae* seed fat shows potential as a source of palmitic acid, which is in demand in the pharmaceutical industry in this country.

REFERENCES

1. "The Wealth of India – Raw Materials," Publication and Information Directorate, Vol. III, CSIR, New Delhi, India, 1952, p. 88.
2. Eckey, E.W., "Vegetable Fats and Oils," Reinhold Publishing Corp., New York, 1954, p. 709.
3. Gunstone, F.D., and M. Ilyas Qureshi, JAOCS 42:961 (1965).
4. Chakrabarty, M.M., and K. Talapatra, Ibid. 45:172 (1968).
5. Child, R., and T.P. Hilditch, in "Chemical Constitution of Natural Fats," 4th Edition, Edited by T.P. Hilditch and P.N. Williams, Chapman and Hall, London, 1964, p. 327.
6. Coleman, M.H., JAOCS 38:685 (1961).
7. Zimmermann, J., Chem. Weekbl. 30:657 (1933).
8. Bushell, W.J., and T.P. Hilditch, J. Soc. Chem. Ind. 57:48 (1938).
9. Hilditch, T.P., and P.N. Williams, "The Chemical Constitution of Natural Fats," 4th Edition, Chapman and Hall, London, 1964, pp. 373, 441.
10. Luddy, F.E., R.A. Barford, S.F. Herb, and P. Magidman, JAOCS 45:549 (1968).
11. Chakrabarty, M.M., D. Bhattacharyya, and A. Basu, Fette Seifen Anstrichm. 69:403 (1967).
12. Vander Wal, R.J., JAOCS 37:18 (1960).
13. Mattil, K.F., F.A. Morris, A.J. Stirton, and D. Swern, "Bailey's Industrial Oil and Fat Products," 3rd Edition, Interscience Publisher, New York, 1964, p. 11.
14. Kartha, A.R.S., JAOCS 30:326 (1953).
15. Gunstone, F.D., Chem. Ind. 1214 (1962).
16. Jurriens, G., "Analysis and Characterisation of Oils, Fats and Fat Products," Vol. 2, Edited by H.A. Bockenoogen, Interscience Publishers, London, 1968, pp. 277, 291.

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