Modification of Selected Edible Vegetable Oils to High Oleic Oils by Lipase-Catalyzed Ester Interchange†

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High amounts of polyunsaturated fatty acids (PUFA) present in edible oils such as peanut, sunflower, safflower, soybean, and linseed oils are susceptible to oxidative deterioration and consequently could contribute to carcinogenesis and atherogenesis. Long-chain (C_{20-24}) saturated fatty acids (LSFA) present in the *sn*-3 position of peanut oil play a role in atherogenesis. Monounsaturated fatty acid, oleic acid, does not exhibit these adverse effects. Hence, the replacement of LSFA and PUFA in peanut oil and of PUFA in sunflower, safflower, soybean and linseed oils with oleic acid was investigated by a batch-stirred interesterification reaction of the oil in hexane with methyl oleate using 1,3-specific lipase (*Mucor miehei*) for 4 h. The triacylglycerols (TAG) of interesterified peanut, sunflower, safflower, soybean, and linseed oils were enriched with oleic acid from 48, 42, 13, 24, and 23% to 66, 63, 47, 54, and 56%, respectively. The TAG of peanut oil were depleted of LSFA from 6.6 to 2.0%.

INTRODUCTION

The fatty acid composition of edible oils (Hilditch, 1964) plays an important role in shelf life, nutrition, and health. Long-chain saturated fatty acids (LSFA), namely arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0), predominantly present in the *sn*-3 position of peanut (*Arachis hypogea*) oil are reported to contribute to atherogenesis (Kritchevsky, 1971). Linoleic acid (18:2) is present in major amounts of 18–38, 20–72, 57–80, and 50–60%, respectively, in peanut, sunflower (*Helianthus annus*), safflower (*Carthamus tinctorius*), and soybean (*Soja max*) oils. In addition to linoleic acid (8–17%), linolenic acid (18:3) is present in high amount (45–67%) in linseed (*Linum usitatissimum*) oil. It is also present in significant quantity (4–10%) in soybean oil. Due to the presence of significant amounts of polyunsaturated fatty acids (PUFA), these oils are susceptible to oxidation and the products of oxidation could contribute to atherosclerosis and carcinogenesis (Kubow, 1990). Studies on experimental animals indicated that excessive amounts of linoleic acid promote carcinogenesis (Gammal et al., 1967), raise the risk of gall stones (Sturdevant et al., 1973), alter the composition of cell membranes (King and Spector, 1978), and reduce high-density lipoproteins (HDL) (Vega et al., 1982). Recent studies indicate that oleic acid can be as beneficial as PUFA in the reduction of LDL cholesterol without affecting the levels of HDL (Mattson and Grundy, 1985). Low linolenic soybean and high oleic safflower and sunflower varieties have been obtained by chemical mutagenesis and genetic selection (Wilson et al., 1976; Purdy, 1985; Yodice, 1990). The possibility of modifying the fatty acid composition of vegetable oils by lipase-catalyzed interesterification has also been explored (Macrae, 1983; Graille et al., 1987; Schuch and Mukherjee, 1987; Kaimal and Saroja, 1989). The feasibility of reducing PUFA in sunflower, safflower, soybean, and linseed oils and both PUFA and LSFA in peanut oil by replacing them with oleate through lipase-catalyzed ester interchange forms the subject of the present paper.

EXPERIMENTAL PROCEDURES

Materials. Refined peanut oil, sunflower oil, and safflower oil were purchased from the local market. Linseed oil was hexane-extracted from linseed in the laboratory. Refined soybean oil was purchased from A. K. Mathews & Co., Nagpur, India. Lauric (12:0), myristic (14:0), palmitic (16:0), and stearic (18:0) acids, 2′,7′-dichlorofluorescein, and Celite were purchased from Loba Chemie, Bombay. Oleic acid and silica gel G were purchased from Acme Synthetic Chemicals Limited, Bombay. Lipid standards and *Candida cylindracea* lipase were obtained from Sigma Chemical Co., St. Louis, MO. *C. cylindracea* lipase was immobilized on Celite according to the method of Tanaka (1981). *Mucor miehei* lipase, immobilized on macroparticulate ion-exchange resin (Lipozyme), was a gift from Novo Industri, A/S, Copenhagen. Distilled solvents were used throughout. Column packing for gas–liquid chromatography (GLC) was obtained from Supelco Inc., Bellefonte, PA.

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Table I. Comparison of 1,3-Specific and Nonspecific Lipases for the Removal of Long-Chain Saturated Fatty Acids in Triacylglycerols of Peanut Oil by Ester Interchange[a]

	fatty acid, mol %						
enzyme	16:0	18:0	18:1	18:2	20:0	22:0	24:0
	12.0	4.1	48.2	29.1	1.5	3.6	1.5
M. meihei (1,3-specific)	10.0	2.9	53.7	28.7	1.4	2.4	0.9
C. cylindracea (nonspecific)	11.0	3.7	52.9	25.8	1.5	3.4	1.5

[a] Oil:ester, 1:1 mole ratio.

Ester-Interchange Reactions. Fatty acid methyl esters (FAME) for ester-interchange reaction were prepared by esterifying the fatty acids with 2% sulfuric acid in methanol. Ester interchange of oils with FAME was carried out by magnetically stirring the mixture of oil, FAME (100–200 mg), and immobilized enzyme (10% of total weight of the reactants) in hexane (2–3 mL) at 38 or 60 °C for 4 h. The enzyme was filtered off and the product purified.

Purification of Triacylglycerols (TAG). The TAG were isolated by preparative thin-layer chromatography using hexane–diethyl ether (80:20 v/v) as developer and detected with 2′,7′-dichlorofluorescein.

Fatty Acid Analysis. FAME were obtained from TAG by using a 1% methanolic solution of sodium methoxide (Luddy et al., 1960) and analyzed by GLC for fatty acid composition by using a Hewlett-Packard 5840A unit fitted with a hydrogen flame ionization detector and a data processor (Hewlett-Packard Co., Palo Alto, CA). A glass column [1.8 m × 4.8 mm (i.d.)] packed with 10% Silar 10C on Chromosorb W HP (60–80 mesh) was used. The detector and injection port were maintained at 300 and 250 °C, respectively. The column temperature was main-

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Table II. Fatty Acid Composition of Triacylglycerols of Vegetable Oils Interesterified with Methyl Oleate Using Lipozyme

oil	ester/oil (mole ratio)	fatty acid, mol % 16:0	18:0	18:1	18:2	18:3	20:0	22:0	24:0	P/M[a]
peanut		12.0	4.1	48.2	29.1		1.5	3.6	1.5	0.6
	1	8.6	2.4	59.1	27.0		0.8	1.5	0.5	0.5
	2	6.3	1.8	64.5	25.1		0.8	1.1	0.4	0.4
	3	5.6	1.7	66.5	24.1		0.6	1.0	0.4	0.4
sunflower		6.5	3.9	41.9	46.8		0.9			1.1
	1	4.1	2.4	55.9	37.5		0.1			0.7
	2	3.1	2.0	58.0	36.8					0.6
	3	3.0	1.7	63.2	32.1					0.5
safflower		6.9	2.3	13.4	76.8	0.1	0.3	0.2		5.7
	1	4.5	1.6	35.6	57.8	0.1	0.3	0.2		1.6
	2	3.4	0.9	42.3	53.2			0.2		1.3
	3	2.6	0.9	46.9	49.6					1.1
soybean		11.0	3.6	24.5	53.6	6.9	0.4			2.5
	1	7.3	2.3	41.2	44.2	4.7	0.2			1.2
	2	5.6	1.6	46.6	41.6	4.6				1.0
	3	4.4	1.3	54.3	36.2	3.8				0.7
linseed		5.7	6.1	23.1	11.9	52.8	0.2	0.2		2.8
	1	3.1	3.1	37.4	9.2	46.9	0.1	0.2		1.5
	2	2.9	3.0	48.0	8.5	37.3	0.1	0.2		1.0
	3	2.5	3.0	55.7	8.1	30.4	0.1	0.2		0.7

[a] P = 18:2 + 18:3; M = 18:1.

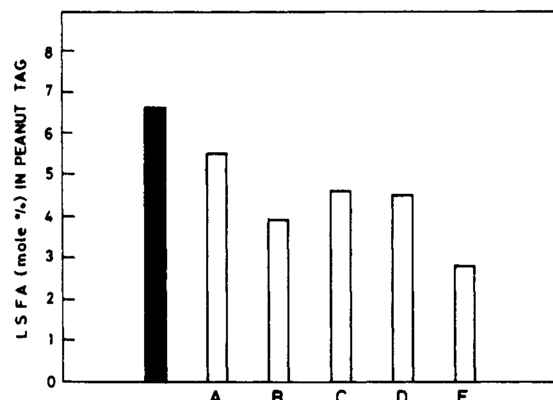

Figure 1. Comparison of removal of long-chain saturated fatty acids (LSFA) from peanut triacylglycerols (TAG) by ester interchange with various fatty acid methyl esters. (■) LSFA in peanut oil; (□) LSFA in TAG of peanut oil interesterified with methyl ester, 12:0 (A), 14:0 (B), 16:0 (C), 18:0 (D) and 18:1 (E).

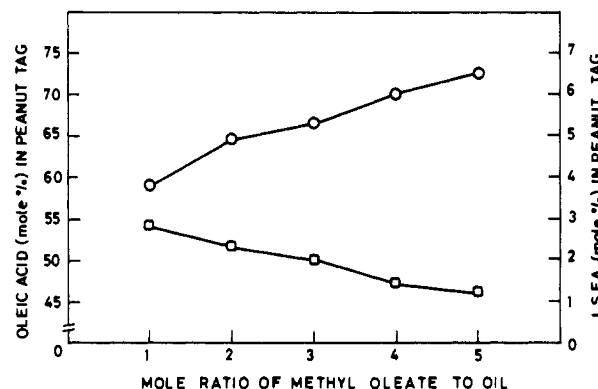

Figure 2. Removal of LSFA and incorporation of oleate in peanut TAG interesterified with varying mole proportions of methyl oleate. (□) LSFA content; (○) oleate content. Peanut oil initially contained 48.2% oleic acid and 6.6% LSFA.

tained at 190 °C for isothermal operations or held at 190 °C for 4 min and programmed to 230 °C at 10 °C/min. Nitrogen was used as carrier gas (35 mL/min). Peaks were identified by using standard FAME and quantitated by using methyl heptadecanoate (17:0) as internal standard.

RESULTS AND DISCUSSION

Ester-interchange reactions catalyzed by *sn*-1,3-specific *M. miehei* (Lipozyme) and nonspecific *C. cylindracea* lipases were carried out with equimolar mixtures of peanut oil and methyl oleate in hexane at 38 °C for 4 h. The fatty acid composition of interesterified products given in Table I indicates that the incorporation of methyl oleate was similar in both reactions but the reduction of LSFA was negligible with *C. cylindracea*.

To choose suitable fatty acid for maximum removal of LSFA, methyl esters of saturated fatty acids varying in chain lengths (12:0, 14:0, 16:0, 18:0) and oleic acid (18:1) were interesterified with peanut oil at 60 °C for 4 h by using Lipozyme as the catalyst. Incorporation of oleate by ester interchange resulted in the maximum reduction of LSFA followed by myristate, stearate, palmitate, and laurate (Figure 1).

The incorporation of oleate and reduction of LSFA in peanut oil by Lipozyme-catalyzed ester interchange at 60 °C is indicated in Figure 2. Increase in the mole ratio of methyl oleate to the oil from 1 to 5 increased oleate content from 48.2 to 72.7% and reduced the LSFA content from 6.6 to 1.2%. The LSFA are predominantly present in the *sn*-3 position of peanut TAG (Myher et al., 1977). Employing *sn*-1,3-specific Lipozyme as the catalyst in the ester-interchange reaction reduced the LSFA significantly from 6.6 to 2.8% even at a low mole ratio (1:1) of peanut oil to methyl oleate.

The fatty acid compositions of peanut, sunflower, safflower, soybean, and linseed oils interesterified with methyl oleate are given in Table II. The reaction catalyzed by Lipozyme resulted in considerable reduction in the linoleic and linolenic acid contents of these oils. The palmitic, stearic, and linolenic acid contents in the soybean oil were reduced by more than 50% while the oleic acid content increased by 2-fold. Linoleic acid also decreased substantially by 32%. According to a panel of scientists from Iowa State University and other industrial consultants (Smith, 1990), raising oleic acid at the expense of linolenic and linoleic acids would greatly increase the oxidative stability of soybean oil. The panel predicted

that a soybean oil containing 40–60% oleic, 30–40% linoleic, and 2–3% linolenic acid and the rest saturated fatty acids would have enough oxidative stability to eliminate the need for partial hydrogenation and it would be an attractive salad and cooking oil as well as frying and liquid shortening oil. The fatty acid composition of interesterified soybean oil was well within these specifications. When used in deep fat frying, soybean oil gives off fishy and painty odors due to its significant linolenic acid content between 6 and 10% (Prévôt et al., 1990). After ester interchange, the linolenic acid content in soybean oil was reduced from 6.9 to 3.8%.

The ester interchange reduced the linoleic acid content of safflower oil by 35% while increasing the oleic acid content by more than 3-fold. The interesterified safflower oil had almost equal amounts of oleic and linoleic acids in addition to reduced amount of saturates. Together the oleic and linoleic acids contributed 96.5% of the total fatty acids. At this stage, the interesterified safflower oil resembled the original sunflower oil in its fatty acid composition. High oleic sunflower and safflower seed progenies derived from normal seed by chemical mutagenesis exhibit excellent resistance to oxidative deterioration (Purdy and Campbell, 1967). Genetically modified hybrid high oleic sunflower oils are also reported to impart excellent nutritional properties, especially with respect to cholesterol metabolism (Yodice, 1990). In the present investigation, the ester interchange of sunflower oil aided in increasing the oleate content from 41.9 to 63.2% while reducing the linoleate content from 46.8 to 32.1%.

Linseed oil is primarily considered an industrial oil, although it is consumed as edible oil in some parts of the world. Attempts are being made to isolate linseed mutants having reduced linolenic acid content (Green and Marshall, 1984). Ester interchange of linseed oil with methyl oleate catalyzed by Lipozyme resulted in an increase of the oleic acid content from 23.1 to 55.7% and a reduction of the linolenic acid content from 52.8 to 30.4% (Table II).

High levels of polyunsaturates decrease not only cholesterol and LDL levels but also the beneficial HDL level. When the level of monounsaturates is at least 50% or more of the total unsaturates and the level of saturates is low, a decrease in cholesterol and LDL levels is observed while a desirable level of HDL is maintained (Yodice, 1990). Such beneficial ratios of polyunsaturates to monounsaturates were found in the interesterified peanut, sunflower, safflower, soybean, and linseed oils.

The results reported in the present investigation demonstrate the potential of ester-interchange reaction catalyzed with 1,3-specific lipase from *M. miehei* for the preparation of high oleic and low PUFA oils from peanut, sunflower, safflower, soybean, and linseed oils. The atherogenic LSFA could also be reduced in peanut oil by this technique. The interesterified soybean oil with reduced linolenic acid and high oleic acid contents may find increased application for cooking, frying, and liquid shortening purposes. The interesterified sunflower and safflower oils with high oleate and low saturates may find use for decreasing the cholesterol and LDL levels and maintaining the beneficial level of HDL.

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Registry No. 18:1, 112-80-1; 18:1 methyl ester, 112-62-9; 12:0 methyl ester, 111-82-0; 14:0 methyl ester, 124-10-7; 16:0 methyl ester, 112-39-0; 18:0 methyl ester, 112-61-8; lipozyme, 9001-62-1.