Incorporation of Eicosapentaenoic and Docosahexaenoic Acids into Groundnut Oil by Lipase-Catalyzed Ester Interchange

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Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were incorporated into groundnut oil by interesterification with a 1,3-specific lipase from *Mucor miehei*. The resultant EPA and DHA concentrations of the groundnut oil were 9.5 and 8.0%, respectively.

KEY WORDS: Docosahexaenoic acid, eicosapentaenoic acid, ester interchange, n-3 fatty acid incorporation, groundnut oil, 1,3-specific *Mucor miehei* lipase.

Seafood diets rich in long-chain n-3 polyunsaturated fatty acids (PUFA), namely eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), are known to reduce both thrombotic tendency and hypertriglyceridemia (1). EPA and DHA also possess antitumor, anti-inflammatory, antiviral and other beneficial qualities (2). The production of EPA and DHA from linolenic acid (18:3n-3), an essential fatty acid, may be impaired due to deficiency in Δ6 desaturase activity (3,4), and this could lead to various clinical disorders. The extent of conversion of linolenic acid to EPA in humans is slow compared to the conversion in animals, particularly when the intake of linoleic acid (18:2n-6) or saturated fatty acids is high (5). The long-chain n-3 PUFA in fish oil from dietary sources provide much more EPA and DHA than would be formed from 18:3n-3 of plant origin (6). An increased supply of preformed long-chain n-3 PUFA in the diet may enhance the concentration of these in membrane lipids and can be advantageous in circumventing the metabolic control steps (7). Supplementation of diet with n-3 fatty acids during infancy is reported to result in doubling of the membrane DHA concentration, improving visual activity and lessening accumulation of body fat (8). The n-3 fatty acid requirement varies during the human lifespan; for example, it is 0.54% of energy for a six-year-old (9), and only half of that for an immobilized, elderly person (10). At present, n-3 PUFA ethyl esters are sold as physiologically functional supplements. However, recent reports suggest that the absorption of fatty acid ethyl esters is highly dependent on the amount of co-ingested fat (11) and that n-3 PUFA in the triacylglycerol (TAG) form are absorbed more easily than in the ethyl ester form by humans (12). However, fish oils contain significant amounts of cholesterol (13), and many consumers find them unpalatable, except in capsule form. Fish or fish oil are not acceptable to some sectors of the population on religious grounds. Therefore, commonly consumed edible oils would be more acceptable media for providing EPA and DHA in the diet. Groundnut oil (GNO) is a major edible oil in the world and it constitutes about 50% of the oil consumption in India. However, GNO contains little or no n-3 fatty acids. GNO does contain about 6% long-chain saturated fatty acids (LSFA), such as arachidic (20:0), behenic (22:0) and lignoceric (24:0) acids. These fatty acids are predominantly in the *sn*-3 position of its triacylglycerols, which may contribute to atherogenesis (14). Hence, incorporation of EPA and DHA into GNO should replace the LSFA and improve nutritional quality of the oil. This incorporation was investigated by ester interchange reaction with a 1,3-specific lipase.

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MATERIALS AND METHODS

Refined GNO (Postman™, Ahmad Mills, Bombay, India) was purchased from the local market. Fish oil capsules (MaxEPA®) were purchased from Universal Generics Ltd. (Bombay, India).

Long-chain n-3 PUFA concentrate was obtained from fatty acids of MaxEPA by the urea adduction method as described by Haagsma *et al.* (15) and converted to fatty acid methyl esters (FAME) with BF_3-methanol (16). A mixture of GNO (100–200 mg) with methyl esters of the long-chain n-3 PUFA concentrate (1:0.5, w/w) and Lipozyme™ (10% of the wt of the reactants) was magnetically stirred in hexane (3 mL) at 60°C under nitrogen atmosphere. Lipozyme (donated by Novo Industry, Copenhagen, Denmark) is a 1,3-specific lipase from *Mucor miehei* immobilized on a macroparticulate ion-exchange resin. The reaction was monitored at intervals of 2, 4 and 6 h. The TAG were isolated from the interesterified GNO by preparative thin-layer chromatography (TLC) on silica gel G (Acme Synthetic Chemicals, Bombay, India) with hexane/diethyl ether (80:20, vol/vol) as developer, converted to FAME with BF_3-methanol and analyzed for fatty acid composition by capillary gas-liquid chromatography (GLC). A Tracor 540 gas chromatograph (Tracor Instruments Inc., Austin, TX) fitted with a flame-ionization detector and a Nelson PC Integrator (Nelson Analytical, Inc., Cupertino, CA) was used. A fused-silica capillary column (0.24 mm × 30 m) coated with SP 2330 (film thickness 0.2 μm) was used. The column temperature was programmed (5°C/min) from 160°C (held for 2 min) to 220°C (held for 10 min). The temperatures of injector and detector were maintained at 250 and 300°C, respectively. Nitrogen was used as carrier gas and the pressure was maintained at 15 psig. Peaks were identified by comparison with standard FAME and quantitated by using methyl heptadecanoate as internal standard.

RESULTS AND DISCUSSION

A long-chain n-3 PUFA concentrate was obtained by urea adduction of fatty acids of a fish oil (MaxEPA). The fatty acid compositions of MaxEPA and the n-3 PUFA concentrate are given in Table 1. EPA was enriched from 14.0 to 34.9% and DHA from 13.8 to 30.4%. Table 2 gives the fatty acid composition of TAG of GNO interesterified with the methyl esters of the concentrate. Ester interchange for 4 h resulted in the incorporation of 9.2% of EPA and 7.4% of DHA. Increasing the reaction time to 6 h resulted only in marginal increments of EPA and DHA. Since a 1,3-specific lipase was used for acyl interchange, the saturated fatty acids predominantly present in the 1,3-positions and the LSFA in the 3-position of TAG of GNO were presumed to be replaced by EPA and DHA. This can be seen by reduction in saturated fatty acids

TABLE 1

Composition (wt%) of Methyl Esters of MaxEPA Oil and of Its Long-Chain n-3 Polyunsaturated Fatty Acid (PUFA) Concentrate Obtained by Urea Adduction

Fatty acid	MaxEPA oil	PUFA concentrate
14:0	5.0	—
14:1	0.4	0.4
16:0	14.3	0.2
16:1	7.2	3.9
16:2	0.2	2.2
18:0	4.0	1.7
18:1	14.8	4.9
18:2	6.8	2.2
18:3	7.2	1.2
18:4	4.2	7.5
20:0	0.4	0.2
20:1	0.2	0.5
20:3	3.6	0.3
20:4	0.8	1.8
20:5	14.0	34.9
22:1	1.0	2.1
22:4	0.1	1.5
22:5	2.0	4.1
22:6	13.8	30.4

TABLE 2

Fatty Acid Composition (wt%) of Triacylglycerols of Groundnut Oil Interesterified with Methyl Esters of Long-Chain n-3 Polyunsaturated Fatty Acids Concentrate of MaxEPA Oil

	Duration of interesterification (h)			
Fatty acid	0	2	4	6
16:0	10.7	8.0	8.0	7.8
16:1	—	0.8	1.0	1.0
16:2	—	0.5	0.6	0.6
18:0	3.5	3.0	2.8	2.6
18:1	50.8	46.6	37.7	36.8
18:2	27.8	27.1	24.0	24.3
18:3	—	0.3	0.4	0.4
18:4	—	0.2	1.8	2.2
20:0	1.5	1.5	1.0	1.0
20:1	1.3	1.3	1.0	1.0
20:4	—	0.2	0.5	0.4
20:5	—	4.3	9.2	9.5
22:0	3.1	3.1	1.9	1.7
22:1	—	0.1	0.4	0.2
22:4	—	—	0.4	0.6
22:5	—	0.8	1.1	1.2
22:6	—	0.9	7.4	8.0
24:0	1.3	1.3	0.8	0.7

from 20.1% to 13.8%, and particularly in LSFA from 5.9% to 3.4%, as well as a concurrent increase in EPA and DHA from 0 to 17.5%.

The results demonstrate the feasibility of appreciable incorporation of EPA and DHA into GNO by the lipase-catalyzed ester interchange reaction. The GNO, thus enriched, can be considered a specialty edible oil for specific nutritional and clinical needs. Further studies on the rate of interchange of specific PUFA, optimum yield of interesterified product, and on physical, stability and nutritional properties should be of interest.

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