The Production of Fish Oils Enriched in Polyunsaturated Fatty Acid-Containing Triglycerides

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A simple concentration technique was developed and used for the production of fish oils highly enriched with respect to the polyunsaturated triglycerides. The method involves the rapid solidification of fish oil droplets in liquid nitrogen followed by extraction with acetone at −60°C. The combined percentage of *cis*-5,8,11,14,17-eicosapentaenoic acid (20:5) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (22:6) after enrichment of a crude South African Anchovy (*Engraulis capensis*) oil was 57.4. A maximum percentage of 66.0 was attained for n-3 fatty acids after enrichment of a crude Chilean fish oil. A maximum yield of 26.0% was achieved for a crude sardine (*Sardina pilchardus*) oil. Triglycerides containing only saturated fatty acids or a combination of saturated and monoenoic acids were totally removed by the process, as assessed by silver-ion high-performance liquid chromatography of the triglyceride oils. This process permits the production of significant quantities of highly unsaturated triglycerides, which may be used in physiological and oxidative studies or for structural analysis of these triglycerides, many of which are present at extremely low concentrations in the original oils.

KEY WORDS: Enrichment of EPA- and DHA-containing triglycerides, low-temperature solidification, low-temperature solvent extraction, n-3 polyunsaturated fatty acids, triglycerides.

The triglyceride composition of fish oils is diverse. This is, in part, a consequence of the wide-ranging fatty acid composition of these oils, which incorporate short-chain saturated acids such as tetradecanoic acid and the long-chain polyunsaturated fatty acids (PUFAs) *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA) (1). The composition of virtually all fish oils can be described by reference to eight major fatty acids (2). These are 14:0, 16:0, 16:1, 18:1, 20:1, 22:1, 20:5(n-3) and 22:6(n-3). An additional thirteen acids are often present above a level of 0.3% and include 15:0, 16:2, 16:3, 16:4, 18:0, 18:2, 18:3(n-3), 18:4(n-3), 20:0, 20:4(n-6 and n-3), 21:5 and 22:5(n-3) (3). Furthermore, trace quantities of the odd-numbered fatty acids 17:0 and 17:1 are often detected (4) while methyl-branch fatty acids are also present as minor components (5).

The triglyceride distribution of fish oils has been studied by silver-ion high-performance liquid chromatography (Ag^+-HPLC) (3,6) and reverse-phase chromatography (7). These studies have shown that fish oils contain triglycerides with only saturated fatty acids present in the molecule and those where each fatty acid contains at least five double bonds. Between these two extremes, there is a wide range of triglycerides of varying composition.

The increased interest in both the fatty acid composition and triglyceride distribution of fish oils is a direct result of the association of high fish-containing diets and the consumption of fish oils with the prevention and treatment of a variety of human diseases. The influence of the n-3 acids EPA and DHA, which are regarded as being the important components of the fish oils, has been observed in relation to cardiovascular disease (8–10), inflammatory diseases (11,12) and cancer (13,14). A wide range of fish oil supplements containing approximately 18% EPA and 12% DHA is readily available at retail outlets (15). Supplements containing higher percentages of EPA and DHA are generally derived from ethyl or methyl esters. In this report we present a method for the production of triglyceride fish oils enriched with respect to the polyunsaturated fatty acids.

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MATERIALS AND METHODS

Chemicals. Hexane, methanol and acetonitrile were from Rathburn Chemicals Ltd. (Walkerburn, Scotland). Hipersolv 1,2-dichloroethane, used for the HPLC, and Aristar acetic acid were from BDH Chemicals Ltd. (Poole, England). Acetone used for the enrichment was a general-purpose reagent from the same company. Diethyl ether, chloroform and benzene were Pronalys AR from May and Baker (Dagenham, England).

Sodium sulfate, copper sulfate, orthophosphoric acid and sodium hydroxide were AnalaR-grade chemicals from BDH Chemicals Ltd. Lipid standards were purchased from Sigma Chemical Co. Ltd. (Poole, England).

Fish oils. Sardine (*Sardina pilchardus*) oil and a refined stabilized fish body oil were provided by Isaac Spencer and Co. Fleetwood (1920) Ltd. (Fleetwood, England). Menhaden (*Brevoortia* spp) oil was from Zapata Haynie Corporation (Reedville, Virginia). A white fish oil was provided by United Fish Products Ltd. (Aberdeen, Scotland) while a commercial-grade fish oil was supplied by HBP Ltd. (Fraserburgh, Scotland). Sun 'n' Sea cod liver oil (Cupar Ltd., Blackburn, England) was purchased from a local retailer. A Chilean fish oil, South African anchovy (*Engraulis capensis*) oil and a Danish fish oil were taken from samples held at Torry Research Station (Aberdeen, Scotland).

Analysis of the original, enriched and residual fish oils. The fatty acid distributions of the original, enriched and residual fish oils were determined as described by Moffat *et al.* (16).

The triglyceride distribution of the original, enriched and residual fish oils was determined by Ag^+-HPLC with a 1,2-dichloroethane, acetone, acetone/acetonitrile (75:25, vol/vol) ternary gradient system (3). The gradient was altered to give a shorter analysis time and improved resolution of the PUFA-containing triglycerides. This is summarized in Table 1.

The original and enriched oils were further analyzed by high-performance thin-layer chromatography (HPTLC), to assess the effect of the enrichment process on free fatty acids, using LHP-K linear HPTLC plates (20 × 10 cm, Whatman International Ltd. Maidstone, England), as described previously (3).

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TABLE 1

Ternary Gradient Conditions for Elution of Triglycerides from the Original and Enriched Fish Oils[a]

Time (min)	% $(H_2CCl)_2$	% $COMe_2$	% $COMe_2$/MeCN (75:25, vol/vol)
0.0	100	0	0
12.0	60	40	0
13.0	10	83	7
25.0	10	83	7
35.0	0	75	25
45.0	0	70	30
50.0	0	53	47
55.0	0	0	100
60.0	0	0	100
65.0	100	0	0
80.0	100	0	0

[a]The period from 60.0 to 80.0 min was required to recondition the column. $(H_2CCl)_2$, 1,2-dichloroethane; $COMe_2$, acetone; MeCN, acetonitrile.

Enrichment of the fish oils. Fish oil (200 g) was run down a finely drawn-out Pasteur pipette. The small droplets thus produced fell into a conical flask containing, and surrounded by, liquid nitrogen. The oil droplets solidified instantly, thus producing very small beads of fish oil with a high surface area to volume ratio. The beads sank to the bottom of the flask. Once all the oil was converted into small beads, the liquid nitrogen was allowed to boil off, and the beads were poured into acetone (1800 mL) at −60°C. The mixture was stored at −60°C for 42 h, with intermittent mixing, after which the solid was separated from the supernatant by filtration at −60°C through a glass sinter funnel. The acetone was removed by rotary evaporation (water bath 40°C) with final traces of solvent being removed with a high-vacuum pump. The antioxidant 2,6-di-*tert*-butyl-4-methylphenol was added to the oil to give a final concentration of 0.01%.

RESULTS

The yields of enriched fish oil varied from 26.0% for a sardine oil to 2.8% for the Danish fish oil (Table 2). Fish oils that gave the higher yields (greater than 15%) were characterized by a low concentration of the monoenoic acids eicosenoic acid (20:1) and docosenoic acid (22:1) (Table 3). These were the sardine, South African anchovy, Chilean and menhaden fish oils.

TABLE 2

Percentage Yield and Degree of Enrichment for the Enriched Fish Oils[a]

Fish oil	Yield (%)	(% EPA + % DHA) enriched / (% EPA + % DHA) original
Sardine	26.0	1.54
South African anchovy	18.1	1.67
Chilean	15.6	1.72
Menhaden	15.2	1.62
HBP fish oil	14.1	1.81
White fish oil	9.2	2.14
Cod liver oil (Sun 'n' Sea)	8.4	2.20
Refined stabilized fish body oil	6.3	2.52
Danish	2.8	2.12

[a]EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Fatty acid composition of the original and enriched fish oils. Similar trends were observed for the changes in the fatty acid composition of the fish oils after enrichment. The saturated fatty acids comprised less than 7.2% of the total, corresponding to less than one-fifth that of the original oils. Palmitic acid (16:0) was significantly reduced while octadecanoic acid (18:0) was absent from all the enriched fish oils (Table 3). The concentration of palmitoleic acid (16:1) increased by a factor of 1.1 to 1.3, while the percentage octadecenoic acid (18:1) decreased slightly except for the Danish fish oil. The concentration of 20:1 and 22:1 decreased except for the sardine oil where 22:1 showed a small increase. All fatty acids containing two or more double bonds were present at a higher concentration in the enriched oils. Significant increases were observed in the concentration of EPA and DHA. The minimum combined percentage of these two fatty acids (41.7%) was observed for the menhaden fish oil, while a maximum of 57.4% was noted for the enriched South African anchovy oil. The degree of enrichment was more significant in the oils that gave the lowest yields (Table 2). The refined stabilized fish body oil was enriched, with respect to EPA and DHA, by a factor of 2.52 but the yield was 6.3%. The highest concentration of the six n-3 acids (66.0%), which included 18:3, 18:4, 20:4, 20:5, 22:5 and 22:6, was observed for the enriched Chilean fish oil, but the enriched South-African anchovy oil contained the highest concentration (76.2%) of fatty acids with two or more double bonds.

Ag^+-HPLC of the original and enriched sardine oil. The original sardine oil gave a typical Ag^+-HPLC profile (Fig. 1A), which was conveniently divided into three regions. The first region (0 to 17 min) was comprised of triglycerides containing only saturated fatty acids (3.0 min) together with those containing one (5.0 min), two (8.0 min) or three (11.5 min) monoenoic acids. A small proportion of dienoic acids was present in peaks eluting after 11.5 min. These triglycerides were readily identified by reference to standard triglycerides and previous Ag^+-HPLC fractionation of fish oils (3). Triglycerides eluting between 17 and 30 min (region 2) contained increasing proportions of PUFAs with the progressive introduction of more highly unsaturated fatty acids, including EPA and DHA. The third region (30 to 60 min) contained highly unsaturated triglycerides with equivalent retention times to trilinolenin (TL) and triarachidonin (TA; Fig. 1A). The most highly unsaturated triglycerides, those with more than 13 double bonds per triglyceride, were present at very low concentrations and were eluted after 44 min.

The enrichment process radically altered the Ag^+-HPLC profile for the sardine oil (Fig. 1B). Almost all the triglycerides from the first region, those containing saturated, monoenoic and a small proportion of dienoic acids, were removed by the treatment. In contrast, the concentration of the most highly unsaturated triglycerides, those eluted after 44 min, was greatly increased, with a series of discrete peaks evident on the HPLC chromatogram. In addition to the PUFA-containing triglycerides, the process was shown, by HPTLC, to concentrate the free fatty acids.

TABLE 3

Fatty Acid Composition (% of nominated acids) and Percentage Fatty Acids with Specific Number of Double Bonds for the Original (Orig.) and Enriched (Enr.) Fish Oils[a]

Fatty acid	Sardine oil		South African anchovy oil		Chilean fish oil		Menhaden fish oil		HBP fish oil		White fish oil		Sun 'n' Sea cod liver oil		Refined stabilized fish body oil		Danish fish oil	
	Orig.	Enr.	Orig.	Enr.	Orig.	Enr.	Orig.	Enr.	Orig.	Enr.	Orig.	Enr.	Orig.	Enr.	Orig.	Enr.	Orig.	Enr.
14:0	6.7	2.6	10.6	3.0	6.4	2.2	8.6	3.9	6.6	1.9	6.1	1.4	6.2	2.9	6.8	1.9	8.3	2.6
15:0	trace	—	0.5	—	0.8	trace	0.6	trace	0.5	—	trace	—	trace	trace	0.5	—	0.6	trace
16:0	19.0	1.8	16.1	1.0	20.3	1.5	21.2	3.2	16.7	1.7	13.6	1.2	10.5	2.3	15.4	1.7	15.4	2.4
16:1	8.8	10.8	11.4	12.1	5.9	7.1	10.6	13.1	5.9	6.5	5.3	5.3	7.4	8.7	4.9	6.3	4.9	6.5
16:2	0.7	1.1	1.1	1.5	0.5	0.7	1.4	2.1	0.6	0.9	trace	trace	0.6	0.9	trace	trace	trace	0.5
16:3	1.0	1.5	1.1	1.8	0.8	0.5	1.5	2.4	0.5	1.0	trace	trace	0.8	1.3	trace	trace	trace	trace
16:4	2.1	3.7	2.5	4.6	0.7	1.4	0.9	1.8	1.0	2.1	trace	0.7	1.2	2.7	trace	0.8	0.5	1.1
18:0	3.4	—	2.8	—	4.3	—	3.3	—	2.0	—	2.3	—	1.6	—	1.7	—	1.3	—
18:1	17.1	16.7	10.2	7.8	17.8	15.6	13.2	13.2	13.1	12.0	16.6	16.2	14.3	12.8	16.2	16.9	11.1	12.0
18:2	1.1	1.4	1.0	1.1	1.4	1.8	1.4	1.7	2.0	2.4	1.8	1.9	0.9	1.1	2.0	2.6	2.3	3.1
18:3[b]	trace	0.9	0.4	0.7	0.8	1.2	1.3	1.8	1.4	2.1	1.1	1.3	0.5	0.6	1.5	1.7	1.6	2.2
18:4	2.6	4.4	1.5	2.5	2.2	3.8	4.1	7.1	4.1	8.6	3.6	6.6	2.3	4.5	2.9	6.5	4.2	9.0
20:0	trace	—	0.4	—	trace	—	0.4	—	trace	—	—	—	—	—	—	—	trace	—
20:1	2.5	1.8	0.5	trace	1.3	1.0	1.2	0.8	8.7	5.3	7.8	3.2	18.6	9.2	8.6	5.5	8.7	6.6
20:4	1.6	1.3	1.7	2.6	1.8	2.8	1.9	3.6	1.3	2.4	1.6	2.9	0.4	0.7	1.2	2.7	0.7	1.9
20:5	19.0	29.0	24.6	42.2	13.5	25.2	13.4	23.5	11.6	22.9	9.4	19.4	12.8	28.1	7.4	18.2	8.8	19.4
22:1	1.0	1.2	1.0	trace	trace	trace	trace	—	8.9	4.8	14.3	3.4	12.4	3.5	19.8	6.0	19.3	7.1
21:5	0.6	1.0	0.8	1.2	trace	0.8	0.6	0.9	0.5	1.0	trace	0.7	0.5	1.0	trace	0.6	trace	0.5
22:5	1.9	2.7	1.9	2.8	3.1	4.8	2.0	2.8	1.2	1.9	1.8	3.3	1.2	2.2	1.3	2.8	0.9	1.5
22:6	11.0	17.2	9.8	15.2	18.5	29.7	12.4	18.2	13.4	22.4	14.9	32.6	8.0	17.6	10.0	25.6	11.5	23.7
Sat.	29.1	4.4	30.4	4.0	31.8	3.7	34.1	7.1	25.8	3.6	22.0	2.6	18.3	5.2	24.4	3.6	25.6	5.0
Mono.	29.4	30.5	23.1	19.9	25.0	23.7	25.0	27.1	36.6	28.6	44.0	28.1	52.7	34.2	49.5	34.7	44.0	32.2
Di.	1.8	2.5	2.1	2.6	1.9	2.5	2.8	3.8	2.6	3.3	1.8	1.9	1.5	2.0	2.0	2.6	2.3	3.6
Tri.	1.0	2.4	1.5	2.5	1.6	1.7	2.8	4.2	1.9	3.1	1.1	1.3	1.3	1.9	1.5	1.7	1.6	2.2
Tet.	6.3	10.4	5.7	9.7	4.7	8.0	6.9	12.5	6.4	13.1	5.2	10.2	3.9	7.9	4.1	10.0	5.4	12.0
Pent.	21.5	32.7	27.3	46.2	16.6	30.8	16.0	27.2	13.3	25.8	11.2	23.4	14.5	31.3	8.7	21.6	9.7	21.4
Hex.	11.0	17.2	9.8	15.2	18.5	29.7	12.4	18.2	13.4	22.4	14.9	32.6	8.0	17.6	10.0	25.6	11.5	23.7
EPA + DHA	30.0	46.2	34.4	57.4	32.0	54.9	25.8	41.7	25.0	45.3	24.3	52.0	20.8	45.7	17.4	43.8	20.3	43.1
n-3[c]	35.3	55.4	36.8	64.2	38.9	66.0	34.5	56.1	32.5	64.1	31.7	64.6	25.2	53.7	23.9	56.2	27.7	57.1

[a] The percentage of the saturated (Sat.), monoenoic (Mono.), dienoic (Di.), trienoic (Tri.), tetraenoic (Tet.), pentaenoic (Pent.) and hexaenoic (Hex.) acids were summed to assess the overall double bond distribution in the triglycerides. Trace (<0.3%). The percentages include the various isomers where appropriate (20:4 n-6 and n-3).

[b] n-3 isomer, *cis*-9,12,15-octadecenoic acid (*α*-linolenic acid).

[c] Includes n-3 isomers of 18:3, 18:4, 20:4, 20:5, 22:5 and 22:6.

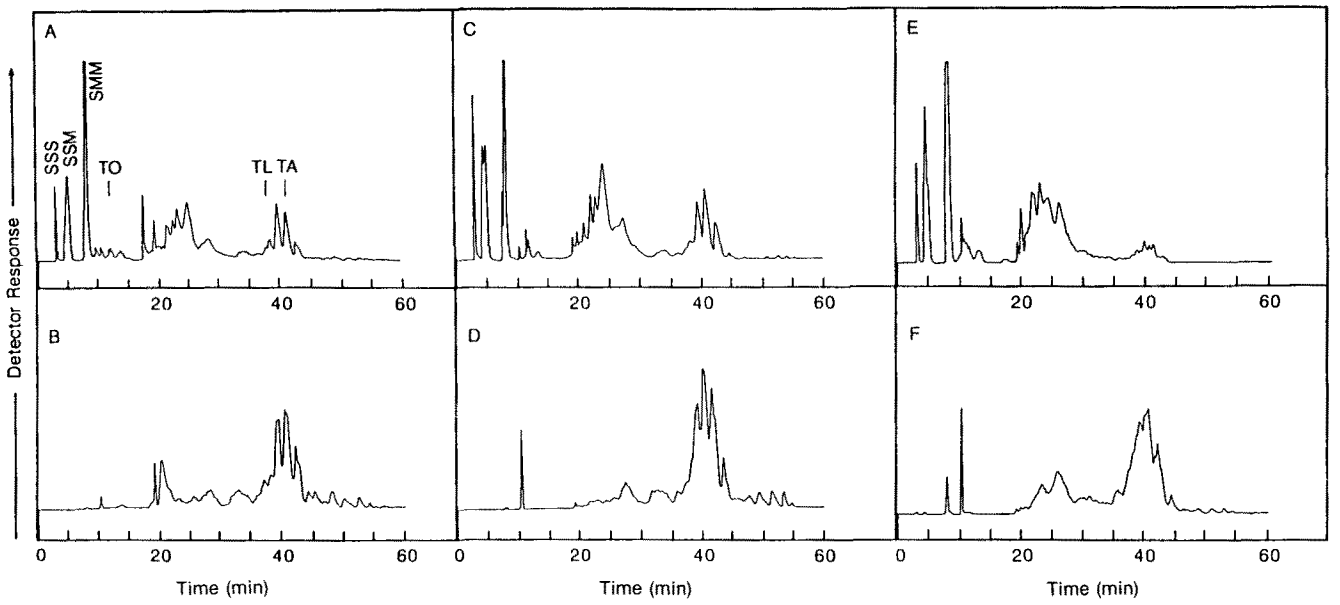

FIG. 1. Silver-ion high-performance liquid chromatography of the original and enriched fish oil triglycerides. A, original sardine oil; B, enriched sardine oil; C, original Chilean fish oil; D, enriched Chilean fish oil; E, original Danish fish oil; F, enriched Danish fish oil. S = saturated, M = monounsaturated, TO = triolein, TL = trilinolenin, TA = triarachidonin.

This was qualified by the presence of a broad peak at 20.3 min in the Ag^+-HPLC chromatogram (Fig. 1B). The relative concentration of triglycerides in the second region (17 to 30 min) was considerably reduced, although the triglycerides eluted between 26 and 30 min were of approximately the same peak intensity. The most intense peaks were those with retention times approximating to the standard triarachidonin, which contains 12 double bonds per triglyceride. The apparent percentage of triglycerides eluted after 30 min was 24.9 and 79.6% for the original and enriched oils, respectively. Due to the variation in detector response associated with longer chainlength and degree of unsaturation (3), these figures do not directly reflect a degree of enrichment.

Ag^+-HPLC of the original and enriched Chilean fish oil. The Chilean fish oil gave a similar triglyceride pattern (Fig. 1C) to the sardine oil (Fig. 1A) when analyzed by Ag^+-HPLC. The split peak with maxima at 4.5 and 5.0 min corresponds to α or β substitution, respectively, of the glycerol moiety by the monoenoic acid. There was no equivalent peak to the one observed at 17.4 min in the sardine oil, but thereafter the Ag^+-HPLC profile of the Chilean oil varied only with respect to peak intensities.

The enrichment process again removed all the triglycerides that eluted in the first region of the Ag^+-HPLC profile (Fig. 1D), although there was a peak at 10.4 min that was more intense relative to a peak with an equivalent retention time present in the original oil. This sharp gaussian peak was typical of all the enriched fish oils. The changes in the relative intensities of the peaks present after 17 min showed similar trends to the sardine oil. The most significant area of variation was between 43 and 49 min. The Chilean fish oil gave a significant peak at 43.5 min, while the sardine oil gave a peak of low amplitude in this region, which was coincident with a shoulder on the Chilean fish oil peak. Thereafter, the peak shapes and distribution were quite distinct until after 49 min when both oils gave four similarly shaped peaks.

The Chilean fish oil gave a significantly lower yield (Table 2), yet there were no stark differences in either the triglyceride profile of the original oils or the two enriched products.

Ag^+-HPLC of the original and enriched Danish fish oil. The Danish fish oil gave the lowest yield of all the fish oils on enrichment. The Ag^+-HPLC profile (Fig. 1E) was distinct from the other two oils with respect to the triglyceride pattern after 17 min. Prior to this time the profile was typical with trisaturated and monoenoic-substituted triglycerides. The peak intensities of the early eluting triglycerides in the second region (17 to 30 min) of the Ag^+-HPLC profile were greater for the Danish fish oil than for the sardine or Chilean fish oils. Furthermore, the peak at 26.0 min was more significant than for the other two oils. Thus, the triglyceride composition of the Danish oil was quite different. The third region (30 to 60 min) contained less than 10% of the detected triglycerides; there were no highly unsaturated triglycerides (greater than 44 min) detected. A low yield was, therefore, expected from this oil. The enriched Danish fish oil gave an Ag^+-HPLC profile similar to those obtained for the sardine and Chilean fish oils with the exception of the presence of a peak at 8.0 min. This arises from triglycerides that contain one saturated and two monoenoic acids per glycerol moiety (3). The relative concentration of triglycerides in the second region (17 to 30 min) was reduced, and there was only a slight change in peak intensities for triglycerides eluted between 30 and 35 min. The relative increase in the concentration of triglycerides with retention times equivalent to TL and TA was far greater for the Danish fish oil than for the other two oils. Although the peaks were not as discrete, it was apparent that the enrichment process extracted quite specific

triglycerides, those that were highly unsaturated. Such observations were confirmed by the Ag^+-HPLC profiles obtained for the other enriched oils. The presence of highly unsaturated triglycerides (eluted after 44 min, Fig. 1F) again typified the results for all the oils. Equivalent triglycerides were not identified in the Ag^+-HPLC profile of the original Danish fish oil (Fig. 1E), but it was apparent that such triglycerides were present in all the original fish oils, even though their concentration was extremely low.

Composition of the residual oil. The menhaden and HBP fish oils both gave mid-range yields of enriched fish oil (Table 2). The residual triglycerides still contained significant amounts of EPA and DHA (Table 4). Removal of the highly polyunsaturated triglycerides resulted in a 12.8% and 19.2% decrease in the combined percentage for EPA and DHA from the menhaden and HBP oils, respectively. A feature of the enriched oils was the absence of 18:0. This fatty acid showed an increase of 24.2% for the residual menhaden oil and a 25.0% increase in the residual HBP oil.

The changes observed in the Ag^+-HPLC profiles agreed closely with the increase in concentration of the saturated and monoenoic fatty acid-containing triglycerides and the corresponding concentration of polyunsaturated triglycerides.

TABLE 4

Fatty Acid Composition (% of nominated acids) and Percentage Fatty Acids with Specific Number of Double Bonds for the Residual Triglycerides from Menhaden and HBP Fish Oils[a]

Fatty acid	Residual triglycerides from menhaden fish oil	Residual triglycerides from HBP fish oil
14:0	9.3	7.0
15:0	0.7	0.6
16:0	24.3	18.4
16:1	10.0	5.6
16:2	1.3	0.5
16:3	1.4	0.4
16:4	0.7	0.8
18:0	4.1	2.5
18:1	13.3	12.4
18:2	1.3	1.9
18:3	1.2	1.3
18:4	3.6	3.5
20:0	0.4	—
20:1	1.4	8.2
20:4	1.8	1.2
20:5	11.6	9.3
22:1	—	14.1
21:5	0.6	0.5
22:5	1.9	1.1
22:6	10.9	10.9
Sat.	38.8	28.5
Mono.	24.7	40.3
Di.	2.6	2.4
Tri.	2.6	1.7
Tet.	6.1	5.5
Pent.	14.1	10.9
Hex.	10.9	10.9
EPA + DHA	22.5	20.2
n-3	29.7	26.5

[a]Abbreviations as detailed for Tables 2 and 3.

DISCUSSION

The process utilized for producing the enriched fish oils was simple and avoided chemical modification of the triglycerides. Large quantities of crude fish oil, with no prior refinement, can be enriched relatively quickly to give a product free of all particulate material. Furthermore, the by-products of the process contained a reduced proportion of PUFAs and as such may be of direct use to other processing industries where oils with a reduced PUFA content are required. The enriched oils gave a relatively consistent Ag^+-HPLC profile. Thus, specific triglycerides were being extracted from the solidified lipid by the acetone at −60°C. The rapid solidification is likely to produce an amorphous solid, rather than a crystalline material, which is at liquid nitrogen temperature when mixed with the cooled acetone. The structure of the solid probbably resembles that of triglycerides in the liquid state (17). During storage at −60°C, the triglyceride beads remain discrete hard spheres. The exact process that the biphasic system is undergoing is unclear but selective dissolution of the PUFA-containing triglycerides by the acetone is most likely. The solubility of fatty acids, containing up to three double bonds, in various solvents at a variety of temperatures has been studied in detail (18). Only linoleic and linolenic acids show significant solubility at −60°C in acetone. The solubility of the saturated triglycerides tricaprin, trilaurin and trimyristin in acetone decreases markedly with increasing chainlength, but the addition of one double bond per fatty acid, as in triolein, greatly enhances solubility in acetone. A further increase in the degree of unsaturation, as observed for trilinolenin, results in an improved solubility in acetone (19). On this basis, more highly unsaturated triglycerides should be readily soluble in acetone at −60°C.

Free fatty acids were concentrated by the process, but this was of little consequence when the original oil contained only low quantities of these molecules. They would, however, have to be removed from oils where their concentration was higher. The production of significant quantities of a high-quality enriched oil will provide a material that can be used for structural or oxidative studies and, in addition, could be used to further investigate the physiological effects of the consumption of such highly unsaturated n-3 acids.

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[Received June 22, 1992; accepted November 12, 1992]