Thin-Layer Silver Ion Chromatography and Supercritical Fluid Chromatography of Baltic Herring (*Clupea harengus membras*) Triacylglycerols

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The Baltic herring flesh triacylglycerols (TAGs) were separated in eight fractions by Ag^+ thin-layer chromatography (TLC), and the four most unsaturated fractions were analyzed by capillary supercritical fluid chromatography according to their acyl carbon numbers (ACN). In the most unsaturated fraction the major ACNs were 58, 60, and 62, whereas in the three subsequent fractions ACN values of 52, 54, and 56 typically dominated. Fifty-four fatty acids (FAs) were identified from the TAGs by gas chromatography–mass spectrometry as their methyl esters, and the content of 37 major acids was analyzed in each TAG fraction. The most abundant FAs were *cis*-9-octadecenoic (21.0%), *n*-hexadecanoic (18.6%), *all-cis*-4,7,10,13,16,19-docosahexaenoic (12.5%), *cis*-9-hexadecenoic (11.1%), and *all-cis*-5,8,11,14,17-eicosapentaenoic acid (6.4%). In the three most unsaturated TAG fractions 28% was docosahexaenoic acid and 11% eicosapentaenoic acid.

1. INTRODUCTION

In fish lipid studies the main emphasis has typically been on the total lipid content, lipid classes, and fatty acid composition. It is well-known that the depot fat of marine and fresh water fatty fish is composed mainly of triacylglycerols (Linko, 1967; Exler et al., 1975; Ratnayake and Ackman, 1979; Linko et al., 1985). Moreover, the fatty acid compositions of lipids of most food fishes are generally known to be very complex and characterized by their long-chain polyunsaturated fatty acids (Ackmann, 1976; Kinsella et al., 1977; Linko et al., 1985).

The Baltic herring fat content shows variations year by year as well as season by season during the year (Linko, 1967; Pesonen et al., 1957; Aneer, 1975). The fatty acids of Baltic herring flesh lipids have been reported by Linko (1967), Linko and Karinkanta (1970), Kaitaranta et al. (1977), and Linko et al. (1985).

The aim of the present work was to verify an analytical method to study the long-chain polyunsaturated fatty acids in triacylglycerol species of Baltic herring flesh lipids with various molecular weights and degrees of unsaturation.

2. MATERIALS AND METHODS

2.1. Isolation and Purification of Triacylglycerols. The Baltic herring (*Clupea harengus membras*) was caught by trawling in the archipelago of Turku at the end of November 1988. The fresh fish, ca. 15 cm in length, were transported chilled and filleted within 12 h. The lipids were extracted from 65 g of pooled samples according to the method of Bligh and Dyer (1959), which included homogenization in $CHCl_3$/MeOH followed by treatment with $CHCl_3/H_2O$, and, after filtering, retreatment with the former solvent pair. The combined filtrate solution was sealed under nitrogen and stored in the dark overnight. The separated $CHCl_3$ phase was dried with anhydrous Na_2SO_4 and evaporated to dryness in a vacuum. The lipid residue was weighed and dissolved in $CHCl_3$. The headspace gas of the solution was replaced by nitrogen, and the sample was stored at −18 °C.

The "neutral lipids" were isolated with activated silicic acid column chromatography (Rouser et al., 1967). Lipids, 150–200 mg in 5 mL of $CHCl_3$, were applied to a column of silicic acid (15 g, 100–200 mesh; i.d. 18 mm) and eluted with 175 mL of the same solvent at a rate of 2 mL/min. The neutral lipid fraction contained sterols, sterol esters, acylglycerols, free fatty acids, and hydrocarbons.

The triacylglycerols (TAGs) were purified on a silicic acid column prepared with 12 g of absorbent in hexane (Carroll and Serdarevitch, 1967). The neutral lipids, 120–180 mg, were separated by increasing the polarity of the eluent fraction by fraction. The hydrocarbons were removed with 45 mL of hexane, followed by elution of sterol esters with 95 mL of hexane/diethyl ether (99:1 v/v). The TAG fraction was eluted with 60 mL of the mixture of hexane/diethyl ether (95:5 v/v), and the solvent was evaporated in a vacuum. The oil, which was shown not to contain any free fatty acids, was weighed, dissolved in hexane, and stored under nitrogen at −18 °C.

2.2. Silver Ion Chromatography of Triacylglycerols. The TAGs were fractionated according to the degree of unsaturation on 0.5 mm silica gel 60 G plates containing 8% $AgNO_3$ (Dudley and Anderson, 1975). The activated plates (110 °C, 30 min) were stored in a dark desiccator and cleaned with a polar solvent system according to the method of Culp et al. (1965) before use. TAGs, 120–180 mg, dissolved in hexane, were applied as a streak on the starting line and developed twice with a mixture of $CHCl_3$ and methanol (94:6 v/v) in the dark. The developed plates were dried under a nitrogen stream, and a part of each was sprayed with 2′,7′-dichlorofluorescein. The unsprayed parts of the plates were divided into eight fractions, which were scraped off and extracted with diethyl ether/methanol (90:10 v/v) containing 0.1 g/L *tert*-butylhydroxytoluene. The extraction was carried out three times with 5-, 4-, and 4-mL portions of the solvent by shaking vigorously and centrifuging in between. The supernatants were combined, and the solvent was evaporated with a nitrogen stream. The average degree of unsaturation of each fraction was calculated after the analysis of the fatty acids in each fraction.

2.3. Supercritical Fluid Chromatography of Triacylglycerols. The TAGs of the TLC fractions were analyzed by capillary supercritical fluid chromatography (SFC) at 150 °C by using a flame ionization detector, and their ACN values were determined by co-injecting the samples with known TAG mixtures (Huopalahti et al., 1988; Kallio et al., 1989). The sample was introduced into the column with a 200-nL Rheodyne 7520 (Cotati, CA) injector and separated with either DB-1 or DB-5 fused silica capillary columns (2 m, i.d. 50 μm, d_f 0.2 μm; J&W Scientific, Folsom, CA). After 2 min at 13.8 MPa, the pressure was raised over 26 min to 27.6 MPa and then over 17 min to 34.5 MPa.

2.4. Analysis of the TAG Fatty Acids. The methyl esters were prepared of the purified TAG samples as well as of the separated TAG fractions according to the method of Metcalfe et al. (1966), further developed by van Wijngaarden (1967). The saponification was carried out by incubating a TAG sample of

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25–50 mg for 5 min at 85 °C with 1 mL of 0.5 N NaOH under nitrogen; 1 mL of 10% BF_3–CH_3OH was added, and the incubation was continued another 5 min. After cooling, 1 mL of hexane and 0.5 mL of saturated NaCl/water solution were added. The tubes were shaken, and the hexane layer was decanted. The extraction was repeated with 1 mL of hexane. The organic layers were combined, dried with anhydrous Na_2SO_4, concentrated with a nitrogen stream, and stored at −18 °C.

Analysis of the fatty acid methyl esters was carried out by using a Varian 3300 (Limerick, Ireland) gas chromatograph equipped with a Schimadzu C-R3A (Kyoto, Japan) integrator. The column was an NB-351 fused silica capillary column (25 m, i.d. 0.32 mm, d_f 0.20 μm; Nordion, Helsinki, Finland), which is equivalent to, e.g., OV-351 [nitroterephthalate-modified poly(ethylene glycol)]. The split injector kept at 225 °C was opened 15 s after injection. After a 2-min isothermal hold at 70 °C, the oven temperature was programmed at a rate of 3 °C/min to 230 °C and kept at this temperature for 20 min. Temperature of the flame ionization detector was 240 °C. Retention indices (I_r) of the fatty acid methyl esters were determined by co-injecting *n*-alkanes from nonadecane to tetratriacontane with the sample. Nonadecane, docosane, pentacosane, and tetratriacontane, which were shown not to elute with any of the fatty acid methyl esters to be analyzed, were used as internal standards. Retention patterns, commercial reference compounds, mass spectra recorded, and library mass spectra were used to verify the structures of the methyl esters.

2.5. Mass Spectrometry. The measurements were carried out by using a VG 7070E mass spectrometer with a VG 11-250 data system. The fatty acid methyl esters were analyzed with the same column and program as described earlier. The temperature of the ion source (EI mode) was 280 °C and the ionization energy 70 eV.

The TAG samples were introduced through the solid-state inlet and ionized by electron impact at an energy of 70 eV. The ion source was kept constantly at 180 °C, and the temperature of the platinum wire probe was programmed at the rate of 2 °C/min.

3. RESULTS AND DISCUSSION

3.1. Preparation of the Samples. The composition of Baltic herring oil varies greatly according to the time of the year, and the total lipid content is known to reach its maximum in late fall (Linko, 1967). The material studied was typical late November–early December Baltic herring, trawled with short sweeps under the ice. The relative long rigor mortis and the short, cold transport guaranteed the proper quality of the fish flesh to be analyzed.

The total lipid content of the flesh varied from 4.0 to 5.8% (w/w), the average being 5.2%, which is in agreement with previous studies (Pesonen et al., 1957; Linko, 1967). Two pooled flesh samples were processed in duplicates through the whole procedure. The Bligh and Dyer method was chosen from among several possibilities because it is especially suitable for large fish samples. According to Christie (1982), the yield with this method is typically higher than 95%.

The proportion of neutral lipids varied between 65.1 and 77.3%, being on average 69.1%, which was equivalent to 3.6% on a fresh weight basis; 61.5% of this neutral fraction was triacylglycerols, the lowest and highest values being 57.1 and 67.0%. Linko (1967) and Linko et al. (1985) reported higher values of TAGs, 85.1 and 86.4% of the corresponding fraction, respectively. The TAG fraction was shown not to contain any free fatty acids, which might occur when silicic acid is used as the sorbent material (Litchfield, 1972).

Before the TAGs were fractionated by Ag^+-TLC, the effects of $AgNO_3$ content and MeOH content were thoroughly tested. By use of 8% $AgNO_3$ in the silica gel based adsorbent and 5% MeOH in the $CHCl_3$ eluent, the resolution of the most unsaturated TAG fractions was most effective. Eight fractions were discerned mainly on the basis of the degree of unsaturation. Even though the fractions were nicely distinguished, the fractionation was, as is known, not complete (Laakso et al., 1990).

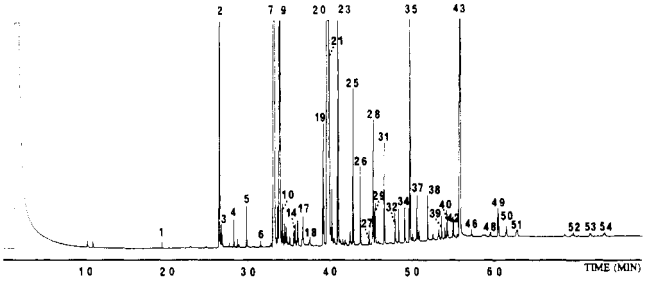

Figure 1. Gas chromatogram of the methyl esters of Baltic herring TAG fatty acids. Numbering is as in Table II.

Table I. Fractionation of the Baltic Herring TAGs on Ag-TLC According to the Degree of Unsaturation

fraction no.	R_f value	av no. of double bonds[a]	% of the total TAGs[a]
1	0.05	10.0	14
2	0.11	8.7	24
3	0.25	6.8	13
4	0.35	5.6	10
5	0.54	4.2	4.5
6	0.76	3.2	4
7	0.85	2.8	6
8	0.93	2.1	25

[a] Calculated according to the analysis of fatty acids.

Table I summarizes the R_f values, the calculated average number of double bonds (according to the fatty acid analysis reported later), and the relative proportions of the fractions.

3.2. Composition of Fatty Acids. The analysis of the fatty acid methyl esters with the NB-351 column indicated the presence of at least 70 FAs in the Baltic herring TAG pool, 54 of which were identified according to retention indices, reference compounds, and mass spectra (Table II). As a result, the quantities of the 37 most abundant compounds, which comprised about 96 mol % of the fatty acids, were analyzed in each silver ion chromatographic fraction. Figure 1 shows a chromatogram of the methyl esters of the total fatty acids of the TAGs. No detector response corrections were carried out.

The most abundant saturated fatty acids were, as is known (Linko, 1967; Linko et al., 1985), *n*-hexadecanoic acid (16:0, 18.6 mol %), *n*-tetradecanoic acid (14:0, 4.1 mol %), and *n*-octadecanoic acid (18:0, 1.2 mol %). Very small amounts of saturated *n*-15:0, *n*-17:0, and *n*-24:0 were also observed. The total proportion of the eight detected branched acids was small, the most abundant being the methyl-substituted hexadecanoic acids.

Among the monoenoic acids, *cis*-9-octadecenoic acid (18:1ω9, 21.0 mol %) and *cis*-9-hexadecenoic acid (16:1ω7, 11.1 mol %) were the most abundant compounds. The 15 identified monoenoic acids corresponded to about 39 mol % of the total fatty acids. The longest among this group were *cis*-17-tetracosenoic (24:1ω7) and *cis*-15-tetracosenoic (24:1ω9) acids. The last mentioned acid together with *cis*-17-hexacosenoic (26:1ω9) acid has been isolated earlier from the flesh lipids of Baltic herring by Linko and Karinkanta (1970). All the ω7 and ω9 series acids were monoenoic. One special feature was the very low content of 20:1 and 22:1 acids. Baltic herring oil may thus be categorized as a "basic fish oil" according to the definition of Ackman et al. (1988), and special attention is paid to its further fractionation.

Table II. Fatty Acid Composition of Baltic Herring Triacylglycerols, Basis of the Identification (M, Mass Spectrum; R, Reference Compound; I, I_r), Retention Indices (I_r), and Mole Fractions of the Methyl Esters of Fatty Acids

		ID				proportion, mol % in the Ag^+-TLC fractions								
peak no.	fatty acid	M	R	I	I_r	1	2	3	4	5	6	7	8	total
1	12:0i[c]	x		x	1800	tr[a]	tr	tr	tr	tr	tr	tr	tr	
2	14:0	x	x	x	2008	1.7	3.1	3.7	4.3	5.2	3.3	4.2	6.3	4.1
3	14:1ω9		x	x	2017							tr	tr	
4	15:0i[c]	x	x	x	2061	tr	tr	0.2	tr	0.3	tr	tr	tr	tr
5	15:0	x	x	x	2110	tr	0.4	0.3	0.5	0.5	tr	0.3	0.4	0.3
6	16:0i[c]	x	x	x	2164		tr	tr	tr	tr	tr			
7	16:0	x	x	x	2217	10.4	16.2	18.4	22.6	16.2	13.0	17.0	25.6	18.6
8	16:1ω9	x		x	2233	0.4	0.3	0.5	0.4	0.4	0.7	0.6	0.5	0.5
9	16:1ω7	x		x	2241	5.5	6.8	8.7	7.1	7.9	10.5	23.3	18.1	11.1
10	16:1[a]	x			2253	0.4	0.3	0.5	0.3	0.4	1.1	0.6	0.6	0.5
11	17:0[b]	x			2261						tr	tr		
12	17:0i[c]	x	x	x	2269		0.2	0.2	0.2	tr	tr	0.3	0.3	0.2
13	16:2ω6	x		x	2284							tr	tr	
14	17:0[b]	x	x	x	2306		0.3	0.3	0.2	0.6	1.4	0.4	0.4	0.3
15	17:0	x		x	2318		0.2	0.3	0.3		tr	tr	tr	0.1
16	17:1ω7	x		x	2335					tr				
17	18:0[b]	x			2340	tr	0.2	tr	0.5	2.5	tr	tr	0.4	0.3
18	18:0i[c]	x	x	x	2369				tr					
19	18:0	x	x	x	2423	0.8	1.4	1.2	1.5	1.6	0.9	0.8	1.1	1.2
20	18:1ω9	x	x	x	2445	12.4	14.7	16.7	15.8	17.9	36.9	28.1	30.6	21.0
21	18:1ω7	x		x	2451	2.9	3.4	3.6	2.9	3.1	5.6	4.3	4.4	3.7
22	18:1[a]	x			2464	0.3	0.5	0.5	0.5	0.7	tr	0.7	2.3	0.5
23	18:2ω6	x	x	x	2491	4.2	3.4	2.2	1.6	10.5	19.3	12.8	4.4	5.0
24	18:2[a]	x			2512						tr			
25	18:3ω3	x	x	x	2560	2.7	0.5	1.5	3.7	19.2				1.9
26	18:4ω3	x		x	2592	1.3	0.5	1.4	9.6					1.5
27	20:0	x	x	x	2631						tr	tr	tr	
28	20:1ω11	x		x	2652	0.8	0.9	0.8	1.2	1.1	tr	1.1	1.4	1.1
29	20:1ω9	x	x	x	2661	0.3	0.4	0.4	0.5	0.6	tr	0.4	0.5	0.4
30	20:1ω7	x		x	2674						tr	tr	tr	
31	20:2ω6	x	x	x	2702	1.4	1.2	0.7	0.6	1.7	3.9	2.7	0.9	1.2
32	20:4ω6	x	x	x	2755	0.9	tr	0.2	1.9					0.4
33	20:3ω3	x	x	x	2772	1.4	tr	0.7	1.1	4.9				0.6
34	20:4ω3	x		x	2800	1.6	tr	1.6	3.6					0.8
35	20:5ω3	x		x	2826	7.9	4.9	24.2	9.8					6.4
36	22:1ω11	x		x	2860	0.3	0.4	0.4	0.5	0.5	tr	0.3	0.5	0.4
37	22:1ω9	x	x	x	2869					tr	tr			
38	22:2ω6	x		x	2912	0.7	0.7	0.7	0.4	tr	2.0	1.4	0.4	0.7
39	22:3ω3	x		x	2983	1.1	tr	0.4	0.5	3.1				0.4
40	22:4ω3	x		x	3010	1.7	0.4	1.5	3.2					0.9
41	22:5ω6			x	3027	tr								
42	22:5ω3	x		x	3038	0.9	0.7	2.2						0.6
43	22:6ω3	x	x	x	3076	29.5	35.4	2.7	1.2					12.5
44	24:0	x	x	x	3076					0.8	1.3	0.6	0.8	0.4
45	24:1ω9	x	x	x	3093	0.6	0.2							0.1
46	24:1ω7	x		x	3110	tr								
47	24:2ω6	x		x	3122				tr	tr	tr			
48	24:3ω3			x	3194	0.4	tr							0.1
49	24:4ω3	x		x	3219	3.3	0.4	1.7	3.5					1.1
50	24:5ω3			x	3243	1.4	0.6	1.6	tr					0.6
51	24:6ω3			x	3273	2.6	1.2	tr	tr					0.4
52	26:4ω3			x	3417	tr								
53	26:6ω3			x	3467	tr								
54	28:0			x	3507	tr	tr	tr	tr					

[a] Unknown isomer. [b] Branched. [c] Iso fatty acid, methyl branched at position ω2.

The ω3 series polyunsaturates (24 mol %) are known to be well represented in the Baltic herring TAGs (Linko, 1967; Linko et al., 1985). Among these, *all-cis*-4,7,10,13,16,19-docosahexaenoic acid (22:6ω3, 12.5 mol %) and *all-cis*-5,8,11,14,17-eicosapentaenoic acid (20:5ω3, 6.4 mol %) were the most prominent. The ω6 series was less abundant. Six of them were, however, identified and *cis,cis*-9,12-octadecadienoic acid (18:2ω6) comprised 5 mol % of the total fatty acids. In a recent paper Rezanka (1990) analyzed, from unspecified herring oil, polyunsaturated fatty acids up to 32:6ω6 with a combination of silver ion HPLC and positive ion chemical ionization GC–MS. The possible existence of these extremely long chain acids was not confirmed in Baltic herring in our work. If they were present, their percentiles were less than 0.05%.

3.3. Composition and Distribution of Triacylglycerols. The four most unsaturated Ag^+-TLC fractions of the TAGs were analyzed by capillary SFC. Table III summarizes the ACN distribution of the Ag^+-TLC fractions 1–4, which were the most interesting ones, because the 22:6ω3 and 20:5ω3 acids were solely concentrated in these samples (Table II). In the most unsaturated TAG fraction (no. 1) the major acyl carbon numbers were 58, 60, and 62, whereas in the three subsequent fractions (no. 2–4) ACNs 52, 54, and 56 typically dominated. The nonpolar liquid phase (DB-1) separated the compounds according to their acyl carbon numbers (ACN) only.

When the slightly more polar DB-5 phase with 5% phenyl groups was applied instead of the pure methylsilicone phase (DB-1), the peaks became broader and shoulders

Table III. Molecular Weight Distribution of the Triacylglycerols in the Four Most Unsaturated Silver Ion Thin-Layer Chromatography Fractions of Baltic Herring Fillets Measured by Capillary Supercritical Fluid Chromatography by Flame Ionization Detector

	proportion, %								
fraction no.	ACN48	ACN50	ACN52	ACN54	ACN56	ACN58	ACN60	ACN62	ACN64
1	0	2	4	9	17	21	18	22	6
2	1	4	18	24	23	13	9	5	2
3	1	7	20	27	19	16	9	1	0
4	5	5	17	29	26	14	7	0	0

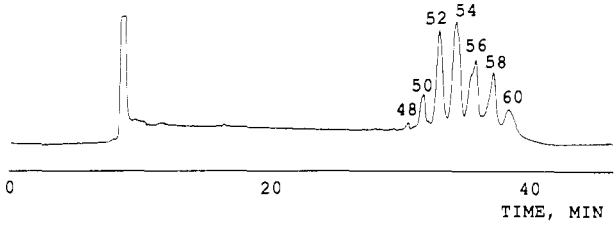

Figure 2. Supercritical fluid chromatogram of TLC fraction 3 of Baltic herring TAGs analyzed by DB-5 column. Numbering (ACN) is as in Table III.

appeared. However, no useful information concerning the double-bond species or fatty acid combinations was available for interpretation. The greater the separation between the individual TAG species, the poorer the resolution became between the peaks appearing on the chromatogram. An example of this situation is shown in Figure 2, which is a chromatogram of TAG fraction 3 analyzed by the DB-5 column.

Demirbüker and Blomberg (1990) introduced a sophisticated method to separate TAGs according to their degree of unsaturation by using microcolumn argentation SFC. The various double-bond species of rapeseed oil were well separated according to the level of unsaturation, but the resolution was not sufficient to be successfully applied for the more complex fish oil sample. It is clear that the resolution efficiency of these two SFC methods at their present status is not even close to what is needed for detailed fish oil analysis (Kallio and Laakso, 1990).

The MW distribution was also analyzed by direct inlet EI-MS-SIM analysis by monitoring the M^+ ions using the method described before (Kallio et al., 1989). The major MW species from ACN 50 to 64 were verified, but the quantitative results were poor because of the lack of proper reference compounds for the calibration. The corrections according to the varying intensities of the M^+ ions have to be calculated carefully, and also the varying delay in the evaporation of various TAG species from the direct inlet probe has to be taken into account.

The distribution of the most abundant fatty acids in the eight Ag^+-TLC fractions is shown in Table II. Nineteen of the 37 quantified fatty acids existed in each of the eight fractions. The quantitative results were based on four analyses, each carried out by using four internal standards.

Our TLC fractions were more complex than those obtained by Laakso et al. (1990) by silver ion HPLC. It is obvious that the differences were due not only to the less effective resolution on the TLC plates when compared to HPLC but also to different complexation patterns in the two systems. Affinity of the silver ions to the fatty acid moieties depends not only on the degree of unsaturation but also on the localization of the double bonds in the TAG molecule (Litchfield, 1972; Christie, 1988). The shifts in retention caused by the various positions of the double bonds might be different in a sulfonic acid ion-exchange column and on the silica gel plates.

All the tetra, penta, and hexa unsaturated fatty acids were located in fractions 1–4. Docosahexaenoic acid was the most prominent one in the two first TAG fractions and eicosapentaenoic acid in fraction 3. The total content of 22:6ω3 and 20:5ω3 acids exceeded one-third in the two most unsaturated TLC fractions, which verified the presence of TAGs containing at least two of these highly unsaturated acids, but the composition of the glycerides is unknown. The fatty acid analyses also show that the TAG species in ACN fractions 62–66 contain typically at least two highly unsaturated (four to six double bonds) C_{20}–C_{24} acids. This is in good agreement with the results of Laakso et al. (1990).

The mole proportion of the polyenoic fatty acids decreased gradually from 63.0 mol % in TAG fraction 1 to 5.7 mol % in fraction 8. At the same time, the average number of the double bonds of TAGs decreased. The triunsaturates had a clear maximum in fraction 5, where they evidently replaced the eicosapentaenoic and docosahexaenoic acids. The mole percentages of the monoenoic acids increased stepwise from 23.9 in the first fraction to 58.9 in fraction 8. No remarkable changes occurred in the ratios of fatty acid groups $C_{16}/C_{18}/C_{20}/C_{22}/C_{24}$.

The highest mole percent values of the saturated acids were found in TAG fractions 8 (35.3 mol %) and 4 (30.1 mol %). In every fraction the most abundant saturated fatty acids were the straight-chain 16:0 (58.5–80.6 mol % of the saturated FAs) and 14:0 (13.2–18.8 mol %). The odd-number fatty acids were 15:0 and 17:0; the latter existed typically also in branched chain forms.

The saturated 16:0 acid dominated in fraction 4, 18:3ω3 in fraction 5, and 18:1ω9 in the three most saturated fractions (no. 6–8). The acids 14:0, 16:0, 18:1ω7, 18:1ω9, and 18:2ω6 existed at least at the level of 1 mol % in all eight fractions. All the ω7 and ω9 series acids were monoenoic.

Table I clearly shows that even an extremely complex TAG mixture can be divided in fractions, which do not much resemble their original composition and which have new physical and evidently also physiological properties when compared to the crude fish oil. For example, the composition of Ag^+-TLC fractions 5–7, rich in 16:0, 18:1ω9, 18:2ω6, and 18:3ω3, are closer to some seed oils than to any unfractionated fish oil sample.

In addition to the knowledge about the fatty acid and MW distributions, the position isomers of the triacylglycerols should also be known. A method that might be common in the near future could be silver ion chromatographic separation of the TAG classes followed by MS and MS–MS analysis to verify the fatty acid composition and to discriminate between positions 1 and 2 or 3.

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