Stereospecific Analysis of Fish Oil Triacyl-*sn*-glycerols

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Stereospecific analysis of fish oil triacyl-*sn*-glycerols was carried out by novel high-performance liquid chromatography on a chiral stationary phase. The positional distributions of fatty acids were determined without accumulation of errors in a particular position and preferential hydrolysis for a particular fatty acid. High-resolution gas-liquid chromatography on an open tubular column detailed the distribution of unsaturated fatty acid isomers having olefinic bonds in different positions. The distribution of fatty acids was not independent of other fatty acids. The distribution of long-chain highly unsaturated fatty acids 22:6(n-3), 22:5(n-3) and 20:5(n-3) was governed by total amounts of 20:1 and 22:1 in triacyl-*sn*-glycerols. Long-chain monounsaturated acids 20:1 and 22:1 were influenced by the position of the olefinic bond in 20:1 occurring in triacyl-*sn*-glycerols. Shorter-chain C14-C18 fatty acids seemed to be influenced by total fatty acid composition. These results introduce a concept of mutual interaction between fatty acids to the investigation of positional distribution of fatty acids.

KEY WORDS: Chiral stationary phase, fish oil, high-performance liquid chromatography, monoacylglycerols, stereospecific analysis, triacyl-*sn*-glycerols.

The positional distributions of fatty acids in depot fat of many aquatic animals were determined by Brockerhoff and his coworkers in 1968 (1). On the basis of their results, Litchfield (2,3) found that the exogenous long-chain fatty acids, 22:6, 22:5 and 22:1, follow regular distribution patterns in certain aquatic species. However, the enzymatic methods that have been used for the stereospecific analysis of triacyl-*sn*-glycerols have some drawbacks. The main drawback of Brockerhoff's method (4) is that the result for position 3 is subject to cumulative experimental errors because the fatty acid composition in this position is not determined directly. The other drawback is the specificity of enzymes to certain fatty acids or fatty acid combinations. Triacyl-*sn*-glycerols from natural sources, especially aquatic animal sources, contain many kinds of fatty acids. However, there is no evidence of nonpreferential hydrolysis for any fatty acids. In a recent study (5), a method different from that of Brockerhoff was used, but it was also enzymatic and it seemed to be burdened with similar disadvantages.

Recently, we (6) have reported a new chemical method suitable for stereospecific analysis of marine biogenic lipid triacyl-*sn*-glycerols. Triacyl-*sn*-glycerols were partially hydrolyzed with ethyl magnesium bromide, and 1-monoacylglycerol products were resolved into *sn*-1 and *sn*-3 monoacylglycerol fractions by high-performance liquid chromatography (HPLC) on a chiral column after derivatization. Fatty acid methyl esters prepared from the original triacyl-*sn*-glycerols, 1- and 2-monoacylglycerol fractions, and *sn*-1 and *sn*-3 monoacylglycerol fractions were analyzed by gas-liquid chromatography (GLC). The positional distributions were calculated on the basis of data obtained. This method has no disadvantages characteristic of enzymatic methods and gives stereospecific distributions of minor components when high-resolution capillary GLC is employed at the final stage.

In this study, a stereospecific analysis of fish oil triacyl-*sn*-glycerols was carried out by this new method. The results showed that the distribution pattern of fatty acids was not as simple as reported by Litchfield. This paper presents some new concepts governing the positional distribution of long-chain unsaturated fatty acids. We selected saury, herring, capelin, sardine, menhaden and dolly varden oils for examination of these concepts.

MATERIALS AND METHODS

Triacyl-sn-*glycerols.* Saury (*Cololabis saira*), herring (*Clupea pallasi*) and capelin (*Mallotus villosus*) were obtained at food markets in Hakodate. Dolly varden (*Salvelinus malma*), which is a fresh-water fish, was obtained at a local fish farm. Lipids were extracted from the flesh by the method of Bligh and Dyer (7). Menhaden (*Brevoortia tyrannus*) and sardine (*Sardinops melanostictus*) oils were obtained as industrial products. Triacyl-*sn*-glycerols were isolated from the lipids or oils by preparative thin-layer chromatography (TLC) on Kiesel Gel 60G plates (Merck, Darmstadt, Germany) with *n*-hexane/diethyl ether (80:20, v/v) for development.

Partial hydrolysis of triacyl-sn-*glycerols.* Stereospecific analysis was carried out by the method described earlier (6). The first step is partial hydrolysis with ethyl magnesium bromide by the modified Brockerhoff procedures (4). Triacyl-*sn*-glycerols (100 mg) were dissolved in 3 mL of dry diethyl ether, and ethyl magnesium bromide in dry diethyl ether (0.33 mL of 3 M solution) was added. The mixture was shaken for 1 min, and then 0.1 mL glacial acetic acid followed by water (3.3 mL) was added to stop the reaction. The products were extracted with diethyl ether. The ether extract was washed five times with 2% aqueous sodium bicarbonate, then washed with water, and dried over anhydrous sodium sulfate. After removal of the solvent at ambient temperature, 1- and 2-monoacylglycerols were isolated by preparative TLC on boric acid-impregnated silica gel plates (20 × 20 cm, 0.5 mm thickness, boric acid 10% to Kiesel Gel 60G) developed in chloroform/methanol (98:2, v/v) under nitrogen. The yields of 1- and 2-monoacylglycerols were generally 5–7 mg and 1–2 mg, respectively.

Enantiomer separation of 1-monoacylglycerols by HPLC. About a half of 1-monoacylglycerols obtained was dissolved in dry toluene (500 μL) and reacted overnight at ambient temperature with 3,5-dinitrophenyl isocyanate (10–20 mg) in the presence of dry pyridine (50 μL). The chloroform solution of the products was spotted on the Kiesel Gel 60G plate and developed by *n*-hexane/1,2-dichloroethane/ethanol (40:15:6, v/v/v) under nitrogen. HPLC separations of the 1-monoacylglycerol di-3,5-dinitrophenylurethane derivatives were carried out by using a Hitachi L-6000 isocratic pump (Hitachi Co., Tokyo, Japan) with a Jasco 875-UV ultraviolet spectrophotometric detector

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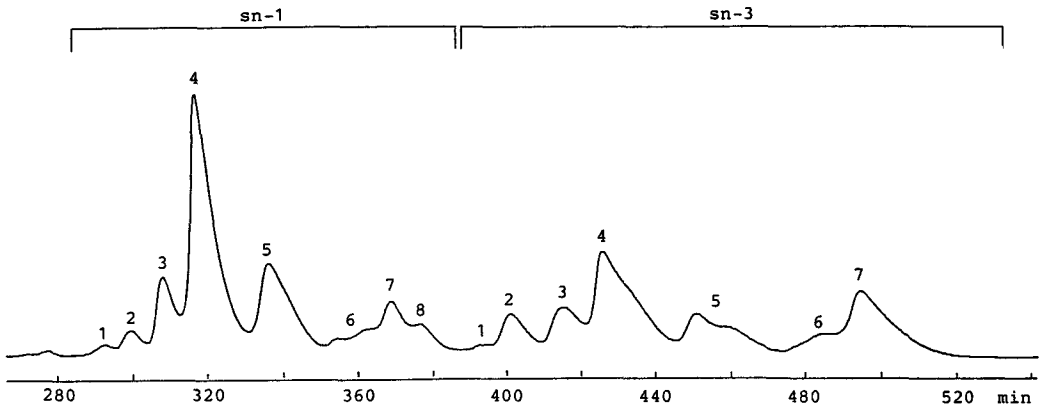

FIG. 1. HPLC of 1-monoacylglycerols, formed by partial hydrolysis of sardine oil triacyl-*sn*-glycerols with ethyl magnesium bromide, as their di-3,5-dinitrophenylurethane derivatives on a chiral column, OA-4100 (50 cm × 4 mm i.d.). Mobile phase, hexane/1,2-dichloroethane/ethanol (40:10:1, v/v/v). Flow rate, 0.5 mL/min. Temperature, ambient. Detection, 254 nm UV.

(Japan Spectroscopic Co., Tokyo, Japan) and a Shimadzu C-R3A integrator (Shimadzu Co., Kyoto, Japan). For optimum resolution, a chiral column (50 cm × 4 mm i.d.) packed with 5-μm particles of N-(*R*)-1-(α-naphthyl)ethylaminocarbonyl-(*S*)-valine chemically bonded to γ-aminopropyl silanized silica, Sumichiral OA-4100 (Sumitomo Chemical Co., Osaka, Japan) was used with a mixture of *n*-hexane/1,2-dichloroethane/ethanol (40:12:3, v/v/v) as a mobile phase at a constant flow rate of 0.5 mL/min. The samples were dissolved in the minimum volume of chloroform for injection. Detection was at 254 nm.

GLC analysis of methyl esters. Total triacyl-*sn*-glycerols, 1- and 2-monoacylglycerols (1–2 mg), and the urethane derivatives of *sn*-1 and *sn*-3 monoacylglycerols (0.1–0.3 mg) were placed into 10-mL centrifuge tubes. Two mL of diethyl ether and 1 M sodium methoxide-methanol solution (25 μL) were added. The content was gently shaken for 5 min before glacial acetic acid (6 μL) was added to stop the reaction. After removing the precipitate by centrifugation for 2 min at 2000 rpm, and evaporating the solvent, products were extracted with *n*-hexane. The extracts were injected into a Shimadzu GC-6AM instrument, which was equipped with a WCOT column (50 m × 0.25 mm i.d., 0.2-μm film thickness) coated with Silar 5CP, and a flame ionization (FID) detector. Peak area percentages and retention times were measured with a Shimadzu C-R6A integrator. Column temperature was 190°C, and injector and detector temperatures were 230°C. Hydrogen was the carrier gas.

RESULTS AND DISCUSSION

Stereospecific analysis. Figure 1 shows HPLC resolutions of 1-monoacylglycerols, prepared from sardine oil triacyl-*sn*-glycerols, in the form of their di-3,5-dinitrophenylurethane derivatives. The chromatogram was obtained from a 50-cm Sumichiral OA-4100 with *n*-hexane/1,2-dichloroethane/ethanol (40:10:1, v/v/v) as mobile phase at ambient temperature. Because the chiral stationary phase has a polar functional group, monoacylglycerol derivatives elute from the column in a descending order of the acyl chainlength and in an ascending order of the number of olefinic bonds in them (8,9). The chromatogram in Figure 1 shows many peak overlaps caused by the existence of a wide variety of fatty acids in fish oil. The numbered peaks were analyzed by GLC as methyl esters of component fatty acids after collection and were identified as monoacylglycerols mainly having the following fatty acid residues: peak 1, 24:1; peak 2, 22:1; peak 3, 18:0 and 20:1; peak 4, 16:0 and 18:1; peak 5, 14:0, 16:1 and 18:2; peak 6, 18:3 and 22:5; peak 7, 18:4, 20:5 and 22:6; and peak 8, 16:4. This chromatogram shows the separation of 1-monoacylglycerols into *sn*-1 and *sn*-3 monoacylglycerols. In micro-preparative HPLC, however, the first peak of the *sn*-3 group and the last peak of the *sn*-1 group overlapped under such conditions.

Figure 2 shows chromatograms obtained at −9°C with *n*-hexane/1,2-dichloroethane/ethanol (40:12:3, v/v/v) as mobile phase. In Figure 2A, 1-monoacylglycerols were clearly resolved into two groups, *i.e.*, *sn*-1 and *sn*-3 monoacylglycerol groups. Though the peak resolutions among the enantiomeric homologous components diminished considerably, a better resolution of *sn*-1 and *sn*-3 monoacylglycerol groups was favorable for collection of these fractions. Rechromatography of the collected separate fractions showed little cross-contamination during peak collection at low temperature (Figs. 2B and 2C).

On a stationary phase of reverse chirality (Sumichiral OA-4000), the elution order changed so that the *sn*-1 group eluted after the *sn*-3 group. Figure 3 shows the resolution of herring oil 1-monoacylglycerols on OA-4000. The elution profile of the latter part of the sardine oil *sn*-1 group (Fig. 2A) was similar to that of the herring oil *sn*-1 group shown in Figure 3. Since this part is free from *sn*-3 monoacylglycerol components (Fig. 3), the similarity supports the clear resolution of *sn*-1 and *sn*-3 groups in Figure 2A.

Table 1 shows the positional distributions of fatty acids in fish oil triacyl-*sn*-glycerols examined. The fatty acid migration affecting the accuracy and precision was low for the procedures used in this method. Our previous study showed that each position of the triacyl-*sn*-glycerol molecule was contaminated with up to about 3% of acyl groups that had migrated from other positions (6). The

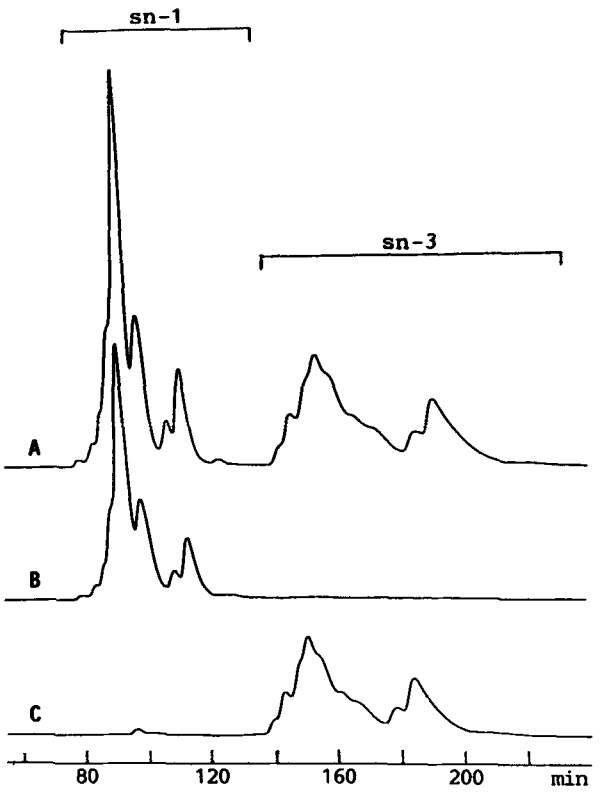

FIG. 2. HPLC of di-3,5-dinitrophenylurethane derivatives of original sardine oil 1-monoacylglycerols (A) and of the *sn*-1 (B) and *sn*-3 (C) monoacylglycerols fractions on a chiral column, OA-4100 (50 cm × 4 mm i.d.). Mobile phase, hexane/1,2-dichloroethane/ethanol (40:12:3, v/v/v). Flow rate, 0.5 mL/min. Temperature, −9°C. Detection, 254 nm UV.

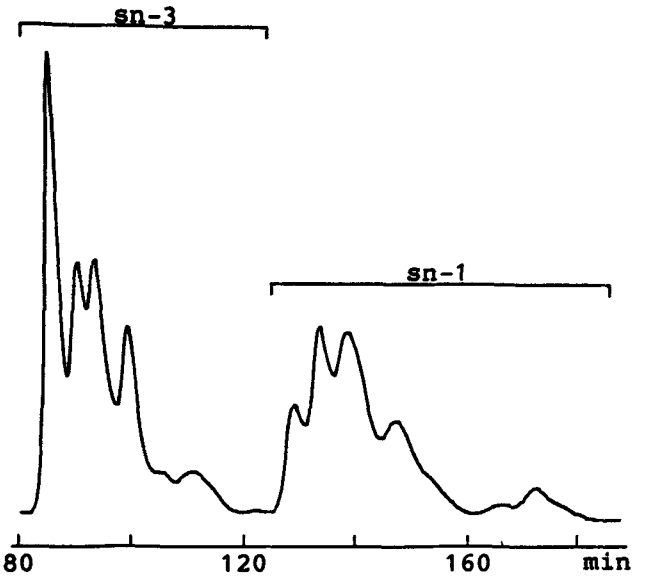

FIG. 3. HPLC of 1-monoacylglycerols, formed by partial hydrolysis of herring oil triacyl-*sn*-glycerols with ethyl magnesium bromide, as their di-3,5-dinitrophenylurethane derivatives on a chiral column, OA-4000 (50 cm × 4 mm i.d.). Mobile phase, hexane/1,2-dichloroethane/ethanol (40:12:3, v/v/v). Flow rate, 0.5 mL/min. Temperature, −7°C. Detection, 254 nm UV.

results shown in Table 1 also confirm the view that acyl migration was low. For example, saury oil contained 14:0 at a concentration of 19.43 mole % in position 1 and 6.74 mole % in position 3, but it was only 1.14 mole % in position 2. The same oil contained 22:6(n-3) at concentrations of 21.57 mole % in position 2 and 3.45 mole % in position 3, while it was only 1.15 mole % in position 1. Such low isomerization of monoacylglycerols in these procedures indicates that the results obtained in the stereospecific analysis are accurate and reliable.

In menhaden oil, 18:0, 18:1(n-9), and 18:1(n-7) were more abundant in the primary positions, whereas 22:6(n-3), 22:5(n-3), 16:4(n-1), and 16:3(n-4) were concentrated in the secondary position. Of the acids in the primary position, 16:0 and 18:0 showed preference for the *sn*-1 position, whereas 20:5, 22:5, and 22:6 showed preference for the *sn*-3 position. Myher *et al.* (5) have reported the distribution of the major fatty acids among three positions of menhaden oil triacyl-*sn*-glycerols. Although their analysis was enzymatic and gave the distribution of only about one-half of the fatty acids listed in Table 1, the present results were similar to those obtained by them. The similarity supports the fact that the results shown in Table 1 are accurate and reliable.

Factors governing the distribution of long-chain highly unsaturated acids. Brockerhoff and his coworkers (1) pointed out the general tendency of 22:6, 22:5 and 20:5 to be preferentially esterified at position 2 in fish and invertebrate triacyl-*sn*-glycerols. Litchfield (2,3) has discussed the relationship between the percent of 22:6 esterified at each position and the total percent of 22:6 in fish and invertebrate triacyl-*sn*-glycerols, and showed that the positional distribution of 22:6 can be predicted by the following simple proportionality equations:

$$\% \text{ 22:6 in position 1} = 0.28\ x \quad [1]$$

$$\% \text{ 22:6 in position 2} = 2.06\ x \quad [2]$$

$$\% \text{ 22:6 in position 3} = 0.66\ x \quad [3]$$

where x shows mole percent of 22:6 in the total triacyl-*sn*-glycerols and $0 < x < 30$. The existence of these proportionality equations suggests that the esterification of 22:6 at each position is independent of other fatty acids.

In all fish oil samples, 22:6(n-3) was preferentially esterified at position 2. Smaller amounts of 22:6(n-3) were found at positions 1 and 3, but it showed a preference for position 3. Table 2 shows the positional distribution of 22:6(n-3) as the ratio of the 22:6(n-3) content in each position to that of the total triacyl-*sn*-glycerols. The total averages of the ratios for positions 1, 2 and 3 were 0.28, 2.13 and 0.56, respectively. These values do not differ much from the proportionality constants presented by Litchfield. However, it is noteworthy that the ratios at position 2 were higher than the predicted value (2.06) in all high-20:1 and 22:1 fish oils (herring, saury and capelin oils), whereas they were lower than 2.06 in all low-20:1 and 22:1 fish oils (menhaden, dolly varden and sardine oils). The average of the ratios for position 2 in menhaden, dolly varden and sardine oils was 1.89, while that in herring,

TABLE 1

Positional Distribution of Fatty Acids in Fish Oil Triacyl-*sn*-glycerols (mole %)

Fatty acids	Saury				Herring				Capelin			
	Total	*sn*-1	*sn*-2	*sn*-3	Total	*sn*-1	*sn*-2	*sn*-3	Total	*sn*-1	*sn*-2	*sn*-3
13:0	—	—	—	—	—	—	—	—	1.04	0.25	2.29	0.71
14:0	9.45	19.43	1.14	6.74	9.67	11.89	6.74	9.73	9.79	8.01	18.03	4.10
4,8,12-TMTD	0.12	0.18	—	0.18	0.33	0.12	0.37	0.49	0.29	0.09	0.62	0.19
14:1(n-11)	0.14	0.07	—	0.36	0.14	—	0.12	0.31	—	—	—	—
14:1(n-5)	0.08	0.09	0.12	0.03	0.09	0.06	0.14	0.07	—	—	—	—
iso-15:0	0.48	0.83	0.13	0.44	0.42	0.46	0.35	0.43	0.38	0.28	0.69	0.21
anteiso-15:0	0.12	0.12	0.15	0.10	0.13	0.08	0.18	0.12	—	—	—	—
15:0	0.77	1.05	0.44	0.80	0.59	0.43	0.85	0.50	0.31	0.13	0.66	0.18
iso-16:0	0.10	0.15	0.04	0.10	0.08	0.05	0.14	0.04	—	—	—	—
Pristanic	—	—	—	—	—	—	—	—	0.41	0.12	0.36	0.75
16:0	13.51	20.87	4.24	14.79	15.70	15.15	17.87	12.49	9.98	6.34	19.53	5.00
16:1(n-13)	0.17	0.17	0.18	0.16	0.17	0.05	0.24	0.21	—	—	—	—
16:1(n-11)	0.55	0.81	0.16	0.66	0.30	0.53	0.31	—	0.11	0.05	0.21	0.08
16:1(n-9)	0.26	0.17	0.41	0.21	0.23	0.08	0.46	0.12	0.12	0.03	0.29	0.05
16:1(n-7)	2.84	4.23	0.85	3.33	4.98	6.37	3.82	4.30	12.13	11.28	19.41	6.36
iso-17:0	0.85	1.30	0.15	1.07	0.48	0.63	0.37	0.40	0.28	0.25	0.43	0.18
16:1(n-5)	0.12	0.18	—	0.18	0.11	0.03	0.12	0.18	—	—	—	—
anteiso-17:0	0.06	0.05	0.05	0.08	0.09	0.05	0.14	0.07	—	—	—	—
16:2(n-7)	0.06	0.10	—	0.08	0.09	0.02	0.17	0.07	0.17	0.09	0.38	0.06
17:0	1.13	1.31	0.47	1.61	0.61	1.44	0.27	—	0.93	0.60	0.91	1.28
16:2(n-4)	0.25	0.31	0.12	0.33	0.42	0.46	0.59	0.14	—	—	—	—
16:3(n-4)	0.33	0.32	0.49	0.17	0.29	0.06	0.57	0.20	0.53	0.86	0.12	0.56
iso-18:0	0.15	0.29	—	0.15	0.09	0.06	0.09	0.12	—	—	—	—
17:1(n-4)	0.18	0.22	0.06	0.26	0.16	0.15	0.18	0.14	—	—	—	—
17:2(n-9)	0.11	0.28	0.02	—	0.09	—	0.12	0.15	—	—	—	—
16:4(n-1)	0.20	0.23	—	0.38	0.44	0.88	0.35	—	0.66	0.32	1.42	0.32
18:0	1.59	2.22	0.58	1.93	0.93	0.93	0.94	0.84	0.77	0.80	0.97	0.56
18:1(n-13)	0.07	0.09	—	0.12	0.08	0.01	0.12	0.12	—	—	—	—
18:1(n-11)	—	—	—	—	—	—	—	—	0.63	0.76	0.68	0.45
18:1(n-9)	4.38	4.01	4.15	5.04	9.93	11.28	8.68	8.99	6.39	7.85	6.59	4.71
18:1(n-7)	1.04	1.32	0.47	1.31	1.49	2.36	0.82	1.14	2.52	3.47	1.81	2.19
18:1(n-5)	0.87	1.03	0.28	1.30	0.46	0.61	0.45	0.26	0.51	0.59	0.61	0.33
anteiso-19:0	0.07	0.05	0.09	0.07	0.05	0.04	0.07	0.05	—	—	—	—
18:2(n-9)	0.06	0.08	—	0.10	0.05	0.03	0.03	0.09	—	—	—	—
18:2(n-6)	1.60	1.74	0.32	2.87	1.21	1.16	1.37	0.98	0.69	0.56	1.16	0.40
19:0	0.22	0.29	—	0.37	0.21	0.45	0.13	—	0.13	0.07	0.29	0.05
18:3(n-6)	0.12	0.05	0.26	0.06	0.08	0.15	0.07	—	—	—	—	—
18:3(n-3)	1.63	1.41	1.34	2.19	1.37	1.22	1.59	1.16	0.14	0.12	0.23	0.08
18:4(n-3)	6.50	5.62	5.45	8.61	3.76	4.58	3.87	2.37	0.42	0.33	0.71	0.25
18:4(n-1)	0.09	—	0.27	—	0.10	0.19	0.09	—	—	—	—	—
20:0	0.17	0.20	0.10	0.20	0.13	0.12	0.11	0.16	0.13	0.14	0.10	0.15
20:1(n-11)	11.83	6.15	15.21	14.77	1.18	1.41	0.55	1.55	1.04	1.26	0.71	1.12
20:1(n-9)	2.89	2.27	1.74	4.80	11.75	16.26	4.19	14.37	21.07	29.15	8.68	24.08
20:1(n-7)	0.20	0.18	0.14	0.28	0.18	0.45	0.05	—	1.18	1.50	0.67	1.31
20:2(5,11)	—	—	—	—	—	—	—	—	0.09	0.12	0.05	0.10
20:2(n-6)	0.26	0.16	0.26	0.37	0.18	0.07	0.12	0.37	0.15	0.15	0.11	0.19
20:3(n-9)	—	—	—	—	0.05	—	0.14	—	—	—	—	—
20:4(n-6)	0.31	—	0.47	0.50	0.16	0.03	0.22	0.23	—	—	—	—
20:3(n-3)	0.16	0.05	0.20	0.24	0.09	—	0.06	0.23	—	—	—	—
20:4(n-3)	1.46	0.73	2.25	1.47	0.52	0.97	0.48	—	0.11	—	0.13	0.21
20:5(n-3)	5.96	5.97	10.60	1.08	5.87	2.97	10.49	3.33	2.28	1.07	4.20	1.78
22:1(n-11),(n-13)	15.12	10.83	20.02	14.88	16.02	12.32	5.24	31.63	20.75	20.24	4.93	35.67
22:1(n-9)	0.72	0.46	1.03	0.69	0.75	0.19	1.51	0.45	2.37	2.11	0.68	4.17
22:1(n-7)	—	—	—	—	—	—	—	—	0.41	0.34	—	0.86
21:5(n-3)	0.32	—	0.65	0.34	0.29	0.30	0.51	—	—	—	—	—
22:5(n-6)	0.15	—	0.46	—	0.07	—	0.19	—	—	—	—	—
22:5(n-3)	0.99	0.23	2.52	0.24	0.49	0.16	1.20	—	0.16	0.04	0.39	0.08
22:6(n-3)	8.58	1.15	21.57	3.45	6.14	2.19	14.87	—	0.36	—	0.79	0.33
24:1(n-9)	0.63	0.92	0.32	0.62	0.72	0.52	0.28	1.41	0.59	0.66	0.20	0.87

saury and capelin oils was 2.37. Such deviations from the predicted value show that the positional distribution of 22:6(n-3) is not independent of other fatty acids but is governed by the amounts of 20:1 and 22:1 in fish oil triacyl-*sn*-glycerols.

The same equations were also applied to 22:5, which is present in smaller amounts (2,3). The positional distribution of 22:5(n-3) is also governed by the amounts of 20:1 and 22:1 in total triacyl-*sn*-glycerols (Table 3). The total averages of the ratios for positions 1, 2 and 3 (0.39, 2.08

TABLE 1 (continued)

Positional Distribution of Fatty Acids in Fish Oil Triacyl-*sn*-glycerols (mole %)

Fatty acids	Sardine				Menhaden				Dolly varden			
	Total	*sn*-1	*sn*-2	*sn*-3	Total	*sn*-1	*sn*-2	*sn*-3	Total	*sn*-1	*sn*-2	*sn*-3
12:0	0.15	0.10	0.18	0.14	—	—	—	—	—	—	—	—
13:0	—	—	—	—	—	—	—	—	0.64	0.28	1.31	0.35
14:0	8.87	9.61	8.60	8.41	11.14	12.40	13.00	8.21	2.64	3.08	1.49	3.34
4,8,12-TMTD	—	—	—	—	—	—	—	—	0.08	0.05	0.12	0.07
iso-15:0	0.37	0.40	0.41	0.32	0.23	0.21	0.30	0.21	0.19	0.23	0.12	0.22
anteiso-15:0	0.12	0.08	0.20	0.10	—	—	—	—	—	—	—	—
15:0	0.70	0.56	0.88	0.67	0.56	0.53	0.77	0.38	0.21	0.15	0.26	0.22
iso-16:0	0.12	0.08	0.15	0.10	—	—	—	—	—	—	—	—
Pristanic	—	—	—	—	—	—	—	—	0.03	0.02	0.05	0.02
16:0	20.54	25.45	16.83	19.36	12.98	16.37	13.93	9.07	13.61	18.52	5.54	16.67
16:1(n-13)	0.20	0.14	0.35	0.10	—	—	—	—	—	—	—	—
16:1(n-11)	0.53	0.96	0.59	—	—	—	—	—	0.12	0.19	0.02	0.14
16:1(n-9)	0.31	0.10	0.67	0.14	0.18	0.13	0.33	0.11	0.41	0.24	0.56	0.44
16:1(n-7)	5.64	7.24	4.80	4.87	14.86	17.24	14.67	12.55	7.28	6.83	8.44	6.62
iso-17:0	0.77	0.92	0.50	0.63	0.49	0.57	0.39	0.49	0.19	0.25	0.06	0.26
16:1(n-5)	0.19	0.20	0.18	0.18	—	—	—	—	0.17	0.19	0.09	0.23
anteiso-17:0	0.14	0.16	0.15	0.12	—	—	—	—	—	—	—	—
16:2(n-7)	0.11	0.12	0.03	0.18	0.33	0.25	0.48	0.23	—	—	—	—
16:2(n-4)	0.63	0.58	0.59	0.71	2.89	3.33	2.55	2.87	0.52	0.39	0.64	0.53
16:3(n-4)	0.64	0.18	1.14	0.61	1.61	1.44	2.11	1.29	0.35	0.09	0.71	0.25
iso-18:0	0.25	0.26	0.18	0.28	—	—	—	—	—	—	—	—
17:1(n-4)	0.10	0.16	0.06	0.08	—	—	—	—	—	—	—	—
17:2(n-9)	0.11	—	0.12	—	—	—	—	—	—	—	—	—
16:4(n-1)	0.94	0.92	1.11	0.79	2.15	1.76	2.97	1.74	0.14	—	0.28	0.14
18:0	2.52	3.98	1.11	2.51	2.26	4.33	0.80	1.84	3.19	4.77	0.96	3.81
18:1(n-11)	—	—	—	—	—	—	—	—	0.41	0.58	0.33	0.33
18:1(n-9)	9.36	11.12	6.35	10.67	4.96	6.47	2.61	5.87	29.32	27.65	32.76	27.71
18:1(n-7)	2.89	4.34	1.46	2.87	3.22	4.95	1.43	3.41	4.30	5.90	1.70	5.28
iso-19:0	0.08	0.10	0.06	0.08	—	—	—	—	—	—	—	—
18:1(n-5)	0.51	0.74	0.29	0.49	—	—	—	—	0.30	0.52	0.07	0.31
18:2(n-9)	—	—	—	—	—	—	—	—	0.09	0.01	0.07	0.19
18:2(n-6)	1.60	2.03	1.17	1.62	1.43	1.53	1.10	1.67	9.40	7.91	11.87	8.50
19:0	0.32	0.36	0.20	0.36	0.42	0.62	0.21	0.47	0.18	0.11	0.22	0.21
18:3(n-6)	0.05	—	0.09	—	0.52	0.45	0.56	0.51	0.15	0.17	0.12	0.15
19:1(n-8)	0.05	0.02	0.09	0.04	0.34	0.32	0.27	0.40	0.13	0.04	0.30	0.05
18:3(n-3)	1.16	1.43	0.73	1.29	0.88	0.85	0.71	1.06	0.82	0.61	1.16	0.70
18:4(n-3)	3.83	4.12	3.78	3.58	2.92	2.82	2.97	2.95	0.69	0.30	1.47	0.33
18:4(n-1)	0.17	0.24	0.09	0.18	0.54	0.72	0.21	0.72	0.12	—	0.37	—
20:0	0.17	0.16	0.18	0.16	0.16	0.17	0.12	0.19	0.09	0.10	0.05	0.12
20:1(n-11)	2.83	2.47	2.28	3.74	0.09	0.04	0.15	0.08	2.28	2.60	1.06	3.17
20:1(n-9)	1.66	1.83	1.35	1.82	0.59	0.16	0.24	0.89	2.86	3.55	1.30	3.71
20:1(n-7)	0.17	0.54	—	0.49	0.18	0.21	0.06	0.26	0.26	0.38	0.13	0.27
20:2(n-9)	—	—	—	—	—	—	—	—	0.17	0.23	0.06	0.22
20:2(n-6)	0.20	0.22	0.18	0.20	0.18	0.30	0.06	0.19	0.65	0.80	0.38	0.77
20:3(n-6)	0.13	0.08	0.20	0.10	0.26	0.19	0.27	0.28	0.33	0.30	0.26	0.43
20:4(n-6)	0.70	0.42	0.88	0.81	0.70	0.40	0.62	1.00	0.55	0.40	0.71	0.54
20:3(n-3)	0.12	0.34	—	0.38	—	—	—	—	—	—	—	—
20:4(n-3)	0.94	1.07	0.67	1.11	1.30	1.38	0.53	1.97	0.49	0.54	0.41	0.52
20:5(n-3)	11.66	9.01	12.20	13.72	21.20	14.43	17.40	30.87	3.35	2.69	3.98	3.39
22:1(n-11),(n-13)	2.98	1.95	2.81	4.20	0.09	0.08	—	0.21	1.66	2.05	1.21	1.73
22:1(n-9)	0.37	0.26	0.35	0.53	0.13	0.13	—	0.28	0.42	0.48	0.30	0.48
22:1(n-7)	—	—	—	—	0.04	—	—	0.11	—	—	—	—
21:5(n-3)	0.41	0.30	0.47	0.49	0.87	0.45	0.77	1.31	0.24	—	0.73	—
22:4(n-6)	—	—	—	—	—	—	—	—	0.09	—	0.27	—
22:5(n-6)	—	—	—	—	0.31	—	0.42	0.51	0.22	0.40	0.26	—
22:5(n-3)	1.55	0.82	2.69	1.13	2.36	0.76	4.16	2.06	1.93	1.24	2.91	1.65
22:6(n-3)	11.53	3.08	22.07	9.09	6.44	2.67	12.86	3.60	8.39	4.47	14.89	5.95
24:1(n-9)	0.62	0.80	0.56	0.55	0.21	0.42	—	0.25	0.23	0.69	—	—

and 0.53) were close to the predicted values presented by Litchfield (0.28, 2.06 and 0.66). However, the ratios for position 2 in low-20:1 and 22:1 fish oils, 1.51–1.76, were considerably lower than the predicted value, 2.06. In high-20:1 and 22:1 fish oils, the ratios, 2.44–2.55, were much higher than the predicted value, 2.06. These results show that the positional distribution of 22:5(n-3) is also influenced by the total 20:1 and 22:1 content in fish oil triacyl-*sn*-glycerols.

Menhaden, dolly varden, saury and herring oils contained the 22:5(n-6) isomer as a minor component with major 22:5(n-3) isomer (Table 1). In saury and herring oils,

TABLE 2

Influence of Total 20:1 and 22:1 Content on Positional Distribution of 22:6(n-3)

Samples	20:1 and 22:1 (mole %)	Ratio[a] *sn*-1	*sn*-2	*sn*-3
Menhaden	1.12	0.41	2.00	0.56
Dolly varden	7.48	0.53	1.77	0.71
Sardine	8.01	0.27	1.91	0.79
Average		0.40	1.89	0.69
Herring	29.88	0.36	2.42	—
Saury	30.76	0.13	2.51	0.40
Capelin	46.82	—	2.19	0.92
Average		0.16	2.37	0.44
Total average		0.28	2.13	0.56
Prediction[b]		0.28	2.06	0.66

[a]Ratio = mole % 22:6(n-3) in each position/mole % 22:6(n-3) in total triacyl-*sn*-glycerols.

[b]From empirical formulas presented by Litchfield (2,3).

TABLE 3

Influence of Total 20:1 and 22:1 Content on Positional Distribution of 22:5(n-3)

Samples	20:1 and 22:1 (mole %)	Ratio[a] *sn*-1	*sn*-2	*sn*-3
Menhaden	1.12	0.32	1.76	0.87
Dolly varden	7.48	0.64	1.51	0.85
Sardine	8.01	0.53	1.74	0.73
Average		0.50	1.67	0.82
Herring	29.88	0.33	2.45	—
Saury	30.76	0.23	2.55	0.24
Capelin	46.82	0.25	2.44	0.50
Average	—	0.27	2.48	0.25
Total average		0.39	2.08	0.53
Prediction[b]		0.28	2.06	0.66

[a]Ratio = mole % 22:5(n-3) in each position/mole % 22:5(n-3) in total triacyl-*sn*-glycerols.

[b]From empirical formulas presented by Litchfield (2,3).

22:5(n-6) was esterified exclusively at position 2 of the triacyl-*sn*-glycerols. However, the 22:5(n-6) isomer in menhaden and dolly varden oils was enriched in positions 3 and 1, respectively. According to these limited results, the 22:5(n-6) isomer does not have the same positional distribution as the major 22:5(n-3) isomer and 22:6(n-3).

The positional distribution of 20:5(n-3) was also influenced by the amounts of 20:1 and 22:1 found in the total triacyl-*sn*-glycerols. Table 4 shows that the ratio, 20:5(n-3) mole % in position 2/20:5(n-3)mole % in total triacyl-*sn*-glycerols, is much higher in the high-20:1 and 22:1 fish oil samples than in the low-20:1 and 22:1 fish oil samples. In addition, the amount of 20:5(n-3) itself in total triacyl-*sn*-glycerols also seems to influence the distribution of 20:5(n-3), mainly in low-20:1 and 22:1 fish oils. Table 5 shows that 20:5(n-3) in sardine oil and especially in menhaden oil was preferentially located in position 3. In

TABLE 4

Comparison of Distribution of 20:5(n-3) in Low 20:1 and 22:1 Fish Oils and High 20:1 and 22:1 Fish Oils

Position	Low 20:1 and 22:1 fish oils[a]		High 20:1 and 22:1 fish oils[b]
20:1, 22:1 contents (mole %)	1.12–8.01		29.88–46.82
sn-1	0.68–0.77[c,d]	=	0.47–1.00
sn-2	0.82–1.19	<	1.78–1.84
sn-3	1.01–1.46	>	0.18–0.78

[a]Menhaden, dolly varden and sardine oils.

[b]Herring, saury and capelin oils.

[c]The ratios of mole % 20:5(n-3) in each position to mole % 20:5(n-3) in total triacyl-*sn*-glycerols.

[d]Minimum found value — maximum found value.

TABLE 5

Influence of Total 20:5(n-3) Content on Distribution of 20:5(n-3) in Low-20:1 and 22:1 Fish Oil Triacyl-*sn*-glycerols

Position	Menhaden		Sardine		Dolly varden
Total content (mole %)	21.20		11.66		3.35
sn-1	0.68[a]	<	0.77	<	0.80
sn-2	0.82	<	1.05	<	1.19
sn-3	1.46	>	1.18	>	1.01

[a]Ratio of mole % 20:5(n-3) in each position to mole % 20:5(n-3) in total triacyl-*sn*-glycerols.

dolly varden oil, the 20:5(n-3) content in positions 2 and 3 was approximately equal, with a slight preference for position 2. The amount of 20:5(n-3) in sardine and menhaden oils is much higher than that in other fish oils. Table 5 shows that 20:5(n-3) preferred to esterify at position 3 in accordance with the increase of the total 20:5(n-3) content in triacyl-*sn*-glycerols. However, the total 20:5(n-3) content does not seem to be the superior factor governing the positional distribution of 20:5(n-3), compared with the total 20:1 and 22:1 content. The total content of 20:5(n-3) was approximately equal in capelin oil (2.28 mole %) and dolly varden oil (3.35 mole %), but the ratio, mole % 20:5(n-3) in position 2 to mole % 20:5(n-3) in total triacyl-*sn*-glycerols, for dolly varden oil (1.19) was much lower than for capelin oil (1.84) and was close to that for sardine oil (1.05), which contains much more 20:5(n-3) (11.66 mole %).

A factor governing the distribution of long-chain monounsaturated acids. Litchfield (3) found a regular distribution pattern of 22:1 at position 3 in triacyl-*sn*-glycerols of aquatic animals examined:

$$\% \text{ 22:1 in position 3} = 0.901\ x + 0.0525\ x^2 \quad [4]$$

where x = mole % 22:1 in the total triacyl-*sn*-glycerols and

TABLE 6

Content of 22:1 in Position 3 in Fish Oil Triacyl-*sn*-glycerols

Fish oil	22:1 content (mole %)		
	Total	Found	Calcd[a]
Saury	15.84	15.57	27.44
Herring	16.77	32.08	29.87
Menhaden	0.26	0.60	0.24
Dolly varden	2.08	2.21	2.10
Sardine	3.35	4.73	3.61
Capelin	23.53	40.07	50.27

[a]Calculated by the formula presented by Litchfield (3).

$0 < x < 25$. Curiously, there was no pronounced correlation of the amount of 22:1 found at positions 1 and 2 with the 22:1 content in triacyl-*sn*-glycerols. Table 6 shows a comparison of the values obtained in this study with the calculated ones. A large difference between the calculated and experimental values for 22:1 was observed for saury oil compared with the other oils. The 22:1 content in saury and herring oils was approximately equal, and thus the calculated values for them were also approximately equal. An agreement between the calculated and experimental values was good for herring oil, while the value found for saury oil was about half as much as the calculated one. Litchfield's correlation formula seems not to be the best method for estimating the distribution of 22:1 in position 3 for all fish oil triacyl-*sn*-glycerols.

Brockerhoff *et al.* (1) presented the positional distribution of eleven fatty acids listed in Table 7. For herring oil, the reported data do not differ much from the data obtained in this study. On the other hand, the data found for saury oil did not agree with those for herring oil, although the fatty acid composition in saury oil was close to that in herring oil. The main difference between herring and saury oils was that the major isomer of 20:1 was 20:1(n-9) in herring and 20:1(n-11) in saury.

The positional distributions of 20:1(n-11) and 20:1(n-9) were influenced by the proportion of 20:1(n-11) and 20:1(n-9) occurring in fish oil triacyl-*sn*-glycerols. The influence is shown in Table 8 by using the ratios of mole % 20:1(n-9) to mole % 20:1(n-11) [20:1(n-9)/20:1(n-11)]. In the fish oils having higher values of the ratio (herring and capelin), both 20:1(n-11) and 20:1(n-9) preferred to esterify at position 1. On the other hand, the fish oils having lower values of the ratio (saury and sardine) contained the lower proportions of 20:1(n-11) and 20:1(n-9) in position 1. The 20:1(n-11) and 20:1(n-9) content was approximately equal in dolly varden oil (ratio = 1.25) and thus the level of these fatty acids at position 1 in this oil was lower than that in herring and capelin oils and higher than that in saury and sardine oils.

The potential distribution of 22:1 in position 2 was also influenced by the ratio of 20:1(n-9)/20:1(n-11) in the same manner as 20:1(n-9) and 20:1(n-11) (Table 8). In herring and capelin oils, which have high values of the ratio, the level of 22:1 at position 2 was about one-third of the percentage found in the total triacyl-*sn*-glycerols, and large amounts of it were concentrated at position 3, followed by position 1. In saury oil with extremely low values of the ratio, this fatty acid was preferentially esterified in position 2, followed by position 3.

Positional distribution of shorter-chain fatty acids. In general, there was no consistency in the distribution pattern of shorter-chain fatty acids (Table 1). Positional distribution of these fatty acids seems to be governed considerably by the content of other fatty acids and their positional distribution. Saury and capelin had specific fatty acid compositions. The capelin oil sample had 23.29 mole % 20:1 and 23.53 mole % 22:1, which were preferentially esterified to positions 1 and 3, but had no more than 3 mole % of highly unsaturated fatty acids, which are preferentially esterified to position 2. In this oil, most of up to C18 fatty acids were esterified to position 2 instead of 22:6(n-3) and 20:5(n-3). Saury oil had also large amounts of 20:1(n-11) (11.83 mole %) and 22:1(n-11) (n-13) (15.12 mole %), but they were preferentially located in position 2 in the same manner as 22:6(n-3) and 20:5(n-3). In this oil, the shorter-chain fatty acids were esterified to the remaining positions 1 and 3. The only exception was 18:1(n-7), which had a simple distribution pattern. This fatty acid was preferentially esterified to positions 1 and 3 in all fish oil samples, while the 18:1(n-9) isomer was randomly distributed among the three positions (Table 1).

Origin of fatty acids. Long-chain monounsaturated fat-

TABLE 7

Positional Distribution of Fatty Acids in Saury and Herring Oil Triacyl-*sn*-glycerols (mmole %)

Fatty acids	Saury				Herring							
	Found				Found				Reported[a]			
	Total	*sn*-1	*sn*-2	*sn*-3	Total	*sn*-1	*sn*-2	*sn*-3	Total[b]	*sn*-1	*sn*-2	*sn*-3
14:0	9.45	19.43	1.14	6.74	9.67	11.89	6.74	9.43	7	6	10	4
16:0	13.51	20.87	4.24	14.79	15.70	15.15	17.87	12.49	12	12	17	7
16:1	3.94	5.56	1.60	4.54	5.79	7.06	4.95	4.81	9	13	10	5
18:0	1.59	2.22	0.58	1.93	0.93	0.93	0.94	0.84	1	1	1	1
18:1	6.36	6.45	4.90	7.77	11.96	14.26	10.07	10.51	11	16	10	8
18:2	1.66	1.82	0.32	2.97	1.26	1.19	1.40	1.07	2	3	3	1
20:1	14.92	8.60	17.09	19.85	13.11	18.12	4.79	15.92	17	25	6	20
20:5	5.96	5.97	10.60	1.08	5.87	2.97	10.49	3.33	8	3	18	4
22:1	15.84	11.29	21.05	15.57	16.77	12.51	6.75	32.08	23	14	5	50
22:5	1.14	0.23	2.98	0.24	0.56	0.16	1.39	—	2	1	3	1
22:6	8.58	1.15	21.57	3.45	6.14	2.19	14.87	—	5	1	13	1

[a]Data from Brockerhoff *et al.* (1).

[b]Total = (*sn*-1 + *sn*-2 + *sn*-3)/3.

TABLE 8

Influence of the Ratio 20:1(n-9)/20:1(n-11) on Distribution of 20:1 and 22:1

Fish oils	20:1(n-9)/ 20:1(n-11)	20:1[a] (n-9) *sn*-1	20:1[a] (n-11) *sn*-1	22:1[b] *sn*-2
Saury	0.24	0.79	0.52	1.33
Sardine	0.58	1.10	0.87	0.94
Dolly varden	1.25	1.24	1.14	0.73
Herring	9.96	1.38	1.19	0.40
Capelin	20.26	1.38	1.21	0.24

[a,b]Distribution was shown as the ratio of mole % in position 1[a] and 2[b] to mole % in total triacyl-*sn*-glycerols.

ty acids in fish depot fat originate mainly from fatty alcohols with similar chainlength in the wax esters of components of the food chain (10). Long-chain polyunsaturated fatty acids are also mainly of dietary origin (10). In this study, the distributions of 22:6(n-3), 22:5(n-3) and 20:5(n-3) were influenced by the amount of 20:1 and 22:1 in total triacyl-*sn*-glycerols. This 20:1 and 22:1 content factor shows the interrelation between long-chain monounsaturated acids and long-chain highly unsaturated acids originated from the diet.

On the other hand, fish can biosynthesize monounsaturated fatty acids from raw food material and are capable of elongating monounsaturated fatty acids from, for example, C18 to C22 (10). In this study, the ratio 20:1(n-9)/20:1(n-11) was a factor governing the positional distribution of 22:1. A chain extension from 20:1(n-11) to 22:1(n-11) in fish with low values of the ratio may take place in a manner different from that in fish having a high value. In the former fish, 22:1(n-11) may be a mixture of fatty acids originated from the diet and from chain elongation. The existence of this factor suggests that the positional distribution of fatty acids is related to their origin, and this may be useful to investigate the positional distribution pattern.

Saturated fatty acids and C16 and C18 monounsaturated acids can be biosynthesized *de novo* by fish (10). The positional distribution of such fatty acids was governed considerably by the content of other fatty acids and their positional distribution. Brockerhoff *et al.* (1) suggested that the 11-olefinic monounsaturated fatty acid isomer, which was more likely to be exogenous than the 9-olefinic isomer, might follow a simple and rigid pattern, though this has not been proven experimentally. Our results obtained for 18:1(n-7) and 18:1(n-9) indicate that their suggestion is true for the distributions of 18:1.

Closer stereospecific analysis. Brockerhoff and his coworkers (1) summarized their results obtained for aquatic animals, and reported the general tendencies in positional distributions of fatty acids. In fish oil triacyl-*sn*-glycerols, position 1 attracts saturated and monounsaturated fatty acids; position 2 polyunsaturated and short fatty acids; position 3 long fatty acids. Position 2 is also high in 16:0. However, the contents of the 11 components reported (1) were about 80% of those of the acyl groups obtained in this study. The stereospecific analysis in this study could confirm that the distribution pattern of fatty acids is not so simple as that reported, and give some new factors for investigation of the positional distributions of fatty acids in fish oil triacyl-*sn*-glycerols. However, the stereospecific analysis of individual triacyl-*sn*-glycerol molecular species might be more important than that of their sum, since it would give us information regarding a preferential molecular association of different fatty acids in individual triacyl-*sn*-glycerol molecules. Hølmer (11) stressed in the review on natural aquatic triacylglycerols that only a detailed analysis would give the right answers. The method used in this study was useful for a close stereospecific analysis, and will be usable for the analysis of triacyl-*sn*-glycerol molecular species after some modifications.

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