アブラボウズのトリグリセリド組成 I

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Triglyceride Composition of Black Cod Lipid—I

Triglyceride Composition based on the Acyl Carbon Number and Degree of Unsaturation

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The triglyceride composition of lipids of black cod, *Erilepis zonifer*, was investigated by high-performance liquid chromatography (HPLC) and gas liquid chromatography (GLC). The fatty acid composition of the triglyceride was also analyzed by GLC.

The fatty acid composition of the triglyceride was characterized by a high ratio of monoenoic acid content (71.7%) to polyenoic acid content (2.3%).

In HPLC, the triglycerides were separated into seven fractions on the basis of their partition numbers. Each of these collected fractions gave three to eight peaks in the GLC chromatograms according to the carbon number of the triglyceride.

From these results, it was found that the major triglycerides of black cod lipid are those of C–52 with 2 double bonds, C–54 with 2 double bonds, C–54 with 3 double bonds, C–56 with 2 double bonds, C–56 with 3 double bonds, and C–58 with 3 double bonds, amounting to 54.2% in all.

From the various points of view, the triglycerides of fish lipids have been studied so far. LITCHFIELD *et al.*[1] and IKEKAWA *et al.*[2] showed the triglyceride compositions, based on the acyl carbon number, of some fish lipids using gas liquid chromatography (GLC). BROKERHOFF *et al.*[3,4], DOLEV and OLCOTT,[5] and BOTTINO[6] reported on the positional distribution of fatty acids in triglycerides of various aquatic animals, sable fish, and cod-liver and whale oils, respectively, by means of pancreatic lipase hydrolysis. However, no detailed information concerning fatty acid combinations in individual triglycerides of fish lipids has been available.

Recently, reverse phase high-performance liquid chromatography (HPLC) has been used for the investigation of triglycerides of natural fats and oils. In our previous studies[7,8], the triglycerides of soybean oil and beef lipids were investigated using HPLC in combination with GLC and the fatty acid combinations in individual triglycerides and their contents were demonstrated.

In the present study, the triglyceride composition of lipids of black cod, *Erilepis zonifer*, a deep-sea fish, was investigated by the same procedure as used in the previous studies[7,8]. Black cod lipid was chosen as one of fish lipid samples, since the lipids are known to be rich in monoenoic acids and contain a limited amount of polyenoic acids which complicate the triglyceride analysis.

Experimental

Triglyceride

The black cod was caught around Ten-noh Kaizan in the central sea of the Pacific Ocean in summer, 1977.

The lipids were extracted with acetone from minced muscle of the fish. After removal of the solvent, the triglyceride fraction was separated from the lipids by column chromatographies[9] on Bio-beads SX–2 and Sephadex LH–20.

HPLC

HPLC was carried out under the same conditions as described in the previous paper.[7] For the separation of triglycerides, a stainless steel tubing (1′×1/4″ i.d.) packed with μ-Bondapack C_{18} was used. Column temperature was maintained at 27°C. A differencial refractometer was used as a detector. The solvent system was a mixture of methanol–chloroform (9: 1) of which flow rate was 1.5 ml/min.

GLC

The glass tubing (0.5 m×3 mm i.d.) packed with 1% JXR on Gas Chrom Q (60–80 mesh) was used

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as a column for triglyceride analysis based on acyl carbon number. Column temperature was programed from 170 to 320°C at the rate of 3°C/min.

For fatty acid analysis, the glass tubing (3 m × 3 mm i.d.) packed with 15% DEGS on Chromsorb W (60–80 mesh) was used at 195°C. Interesterification of triglyceride was carried out using BF_3–MeOH.

Results and Discussion

The fatty acid composition of whole triglyceride is shown in Table 1. The monoenoic acids and the polyenoic acids amounted to 71.7% and 2.3% of the total, respectively. The content of monoenoic acids is considerably high compared with that reported[10] on the other marine fishes so far, while the content of polyenoic acids is low. In fatty acid composition of marine species, in general, polyenoic acid content has a tendency to decrease with increase in depth of habitat, while monoenoic acid content to increase[11–13]. This seems to be true for black cod lipid. These results coincide essentially with those in earlier study[14] of KANEDA who determined the fatty acid composition of black cod lipid by fractional distillation.

Table 1. Fatty acid composition of whole triglyceride of black cod lipid

Fatty acid	mol%
$C_{14:0}$	3.2
$C_{14:1}$	tr.
$C_{15:0}$	0.5
$C_{15:1}$	tr.
$C_{16:0}$	16.0
$C_{16:1}$	5.2
$C_{17:0}$	0.8
$C_{17:1}$	0.8
$C_{18:0}$	4.1
$C_{18:1}$	43.6
$C_{18:2}$	1.0
$C_{19:0}$	tr.
$C_{19:1}$	0.3
$C_{20:1}$	14.4
$C_{20:2}$	0.3
$C_{22:1}$	6.6
$C_{22:6}$	1.0
$C_{24:1}$	0.8
unknown	1.4
sat.	24.6
unsat. (mono.)	71.7
unsat. (poly.)	2.3

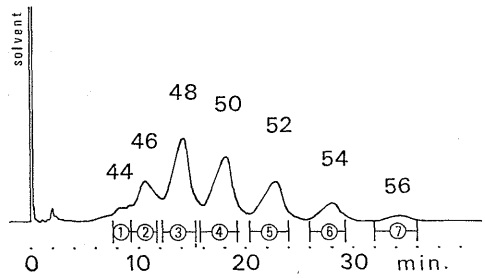

Fig. 1. Typical HPLC chromatogram of black cod lipid triglyceride.
The figures in circles and above the tops of peaks indicate fraction numbers and partition numbers, respectively.

HPLC chromatogram of the black cod lipid triglyceride is shown in Fig. 1. The triglycerides were separated into seven peaks, of which partition numbers (PN* = TC** − 2 × DB***) were estimated to be 44, 46, 48, 50, 52, 54, and 56 on the basis of a linear relationship between the partition numbers and the logarithms of retention times as described elsewhere.[8] The percentages of each fraction were calculated from their peak areas in the chromatogram and are shown in Table 2. In this calculation, it was assumed that in HPLC the area percentage is equivalent to the weight percentage. The total peak areas of these seven fractions amounted to 88.5% in all. Remainder, 11.5%, will be reported in the near future.

The triglyceride composition of whole triglyceride and the seven fractions collected based on total acyl carbon number are shown in Table 3.

The major constituent triglycerides of whole triglyceride are C–54, C–52, C–56, C–58, and C–50, amounting to 92.6% of the total on the basis of their peak areas.

Fraction 1 having the partition number of 44,

Table 2. Percentages of each triglyceride fraction separated by HPLC

Fraction No.	PN	%
1	44	4.1
2	46	12.5
3	48	27.2
4	50	22.5
5	52	14.2
6	54	6.1
7	56	1.9
Others		11.5

* PN: Partition number, ** TC: Total acyl carbon number, *** DB: Number of double bond.

Table 3. Triglyceride composition based on the carbon number and double bond in acyl chains

TC		44	46	48	50	52	54	56	58	60	62
Whole TG		tr.*	tr.	3.1	10.8	23.4	25.9	21.3	11.2	4.3	tr.
	DB	0	1	2	3	4	5	6	7		
Fraction No. (PN)		↑	↑	↑	↑	↑	↑	↑	↑		
1	(44)	tr.* (tr.)**	3.6 (0.15)	14.6 (0.60)	21.7 (0.89)	18.3 (0.75)	20.0 (0.82)	16.5 (0.68)	5.3 (0.22)	—	—
2	(46)	—	tr. (tr.)	11.5 (1.44)	38.8 (4.85)	33.7 (4.21)	10.7 (1.34)	5.3 (0.66)	—	—	—
3	(48)	—	—	—	11.0 (2.99)	47.4 (12.9)	38.6 (10.5)	3.0 (0.82)	—	—	—
4	(50)	—	—	—	tr. (tr.)	10.9 (2.45)	41.3 (9.29)	40.2 (9.05)	6.1 (1.37)	1.5 (0.34)	—
5	(52)	—	—	—	—	tr. (tr.)	8.8 (1.25)	43.8 (6.22)	44.1 (6.26)	3.3 (0.47)	—
6	(54)	—	—	—	—	—	—	7.4 (0.45)	43.3 (2.64)	49.3 (3.01)	—
7	(56)	—	—	—	—	—	—	tr. (tr.)	9.3 (0.18)	47.9 (0.91)	42.8 (0.81)

* mol%, ** Figures in parentheses indicate the percentages in the whole triglyceride of black cod lipid.

a minor component, revealed eight peaks corresponding to those of C–44, C–46, C–48, C–50, C–52, C–54, C–56, and C–58, which were easily calculated to contain 0, 1, 2, 3, 4, 5, 6, and 7 double bonds, respectively. Similarly, the percentages and the number of double bond of constituent triglycerides in fractions 2, 3, 4, 5, 6, and 7 were calculated and are shown Table 3.

The percentages in parentheses in Table 3 indicate the triglyceride composition of whole triglyceride, based on the acyl carbon number and degree of unsaturation, calculated from the data in Tables 2 and 3. Major triglycerides are those of C–52 with 2 double bonds, C–54 with 3 double bonds, C–54 with 2 double bonds, C–56 with 3 double bonds, C–56 with 2 double bonds, and C–58 with 3 double bonds, amounting to 54.2% in all. The triglycerides having double bonds more than 5 seem to be minor components.

In the previous studies[7,8] on soybean oil and beef lipids, the fatty acid combinations in individual triglycerides and their contents were estimated from the data of the triglyceride composition based on the acyl carbon number, the degree of unsaturation, and the fatty acid composition of each fraction. In the case of the black cod lipid, however, it was difficult to estimate the fatty acid combinations in individual triglycerides, because the triglycerides in each fraction were composed of a wide variety of fatty acids. In order to determine the possible fatty acid combinations in the triglycerides, the triglycerides must be separated into the fractions in which triglycerides contain a limited kind of constituent fatty acids. For this purpose, silver ion adsorption thin-layer chromatography might be a useful technique, because it is capable of separating the triglycerides depending on their degree of unsaturation. Further study will be needed to estimate the detailed triglycerides composition of black cod lipids.

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