魚類のトリグリセライドおよびコレステロールエステルの組成

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The Composition of Triglycerides and Cholesteryl Esters in Some Fish Oils of Salt, Brackish and Fresh Water Orygins

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The triglycerides and cholesteryl esters of six fish oils were analysed by gas chromatography after preliminary separation by thin layer chromatography. Generally, fish oils contained C-even triglycerides from C–30 to C–60 with small amounts of C-odd triglycerides. The oils of fresh-water fish contained mainly C-even triglycerides from C–40 to C–60, while oils from salt-water fish contained in addition those from C–30 to C–40. A characteristic of salt-water fish oils was the predominance of C–odd triglycerides over C-even below C–45, in contrast to the normal predominance of C-even triglycerides above this length.

Cholesteryl esters in fish oils consisted mainly of C–16 and C–18 esters in fresh-water fish and C–14 to C–26 esters in salt-water fish, with emphasis on the longer chain length in the latter.

Recent advances in gas chromatography have enabled workers to directly analyse triglycerides without derivatisation. The quantitative aspects of direct analysis by gas chromatography and other factors affecting the analysis have been reported by KUKSIS,[1] LITCHFIELD *et al.*,[2] WATTS and DILS[3] and the present authors.[4,5]

Although animal lipids have been studied, extentively on the triglyceride composition, only two reports are available on those of fish oils.[6,7] These show the presence of triglycerides possessing an odd number of carbon atoms (C-odd triglycerides) in addition to the dominant C-even triglycerides in the fish oils. We present here some comparative results of direct gas chromatographic analysis of triglycerides and cholesteryl esters from the oils of several fish species from different aquatic environments in which short-chain C-odd triglycerides occur in an unexpected and unusual way.

Materials and Methods

Materials The species studied were basking shark *Acipenser mikadoi* and sardine *Sardinops melanosticta*, representing salt water fish, carp *Cyprinus carpio*, "yamame", a land-locked form of trout *Oncorhynchus masou* and rainbow trout *Salmo irideus*, re-

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presenting fresh water fish, and eel *Anguilla japonica* representing brackish water fish. Carp and rawinbow trout were cultivated fish reared on experimental diets as described in a previous paper.[10] The others were fished from normal habitats.

Standard samples of triglycerides and cholesteryl esters were obtained from Applied Science Laboratories, Inc.

Extraction Extraction of fats from the fish was carried out using a chloroform-methanol (2: 1) mixture as described by FOLCH *et al.*[8] In the cases of carp, trout and eel, the oils were extracted from whole bodies, and in the basking shark and sardine from muscles only. The triglycerides and cholesteryl esters were separated prior to gas chromatography by thin-layer chromatography on 0.3 mm plates coated with silica gel (Wako Gel B–5) which had been activated for 1 hr at 120°C. The plates were run using petroleum ether: diethyl ether: acetic acid (74: 15: 1)[9] and developed by charring with

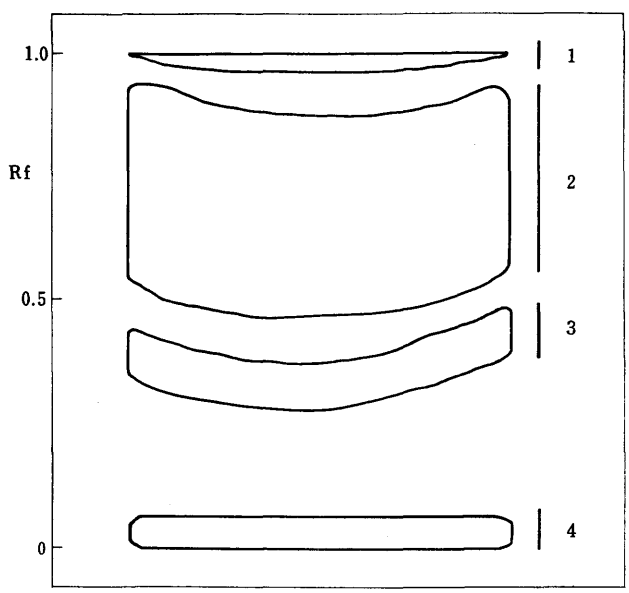

Fig. 1. Separation of triglycerides and steryl esters (plus hydrocarbons) from fish lipids by preparative thin-layer chromatography on silicic acid.
Adsorbent layer scored after development to facilitate band recovery. Operating conditions: Thin-layer chromatograph plate coated with 0.3 mm layer of Wako Gel B–5; development with 74/15/1 petroleum ether/diethyl ether/acetic acid; bands visualized with sulfuric acid.
1. Hydrocarbons and steryl esters. 2. Triglycerides. 3. Sterols. 4. Phospholipids.

sulfuric acid (Fig. 1). To recover the compounds, the triglyceride and cholesteryl ester bands were scraped off the plates. This silica gel adsorbent was extracted with chloroform-methanol (2: 1), and the extract analysed by gas chromatography.

Gas Chromatography A Shimadzu Seisakusho Model GC–4APF gas chromatograph

equipped with a hydrogen flame ionization detector was used. A glass column, 50 cm in length×3 mm i.d., was packed with 2% OV-17 (phenyl methyl silicone) supported on 80–100 mesh silanized Shimalite W. The flow rate of nitrogen carrier gas was 80 ml/min. For triglyceride analyses, the column temperature was programmed from 100° to 320°C at 4°/min. In both cases, detector temperature and injection port temperature were 350°C.

Results and Discussion

To separate triglycerides by gas chromatography, one should use short column, from 35 to 75 cm in length, and operate it at a higher temperature, 280–340°C. Triglycerides having the same number of carbon atoms show the same retention time in such a short column irrespective of the degree of unsaturation of the fatty acids. If a column of longer length is used for analysis, irreversible adsorption of comparatively high molecular weight

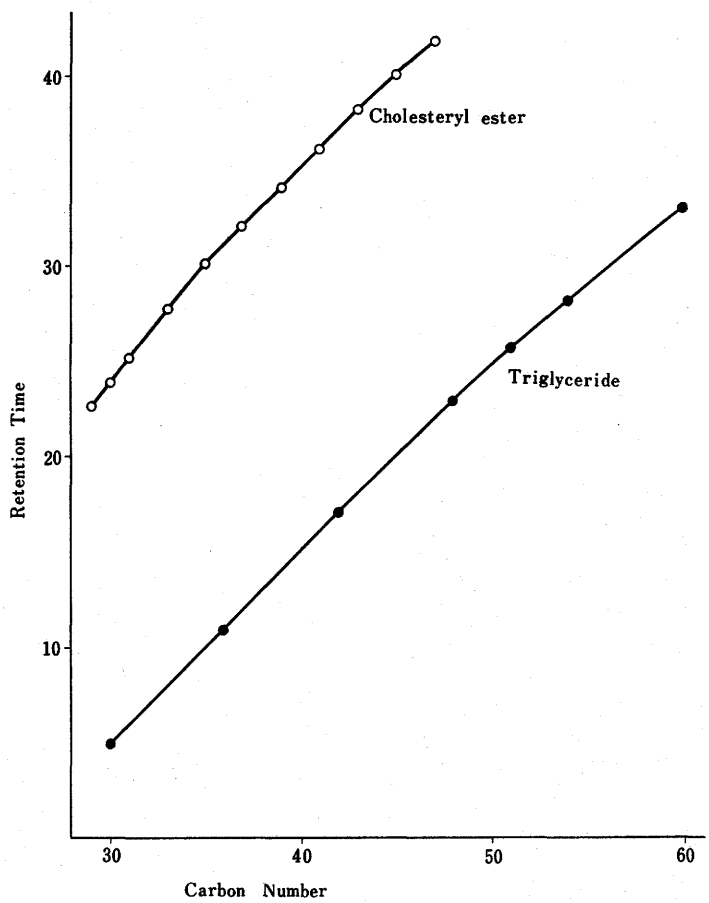

Fig. 2. Correlation curves between retention time and carbon number for triglycerides and cholesteryl esters.

components will occur. A 35 cm column is ordinarily used for the analysis of plant oils because they contain mainly C-even triblycerides which can be separated easily. A 50 cm column was used for analysis of fish oils because they also contain C-odd triglycerides. In the case of plant oils, the triglycerides can be analysed by direct injection of the oils, but fish oil contains various kinds of lipids other than triglycerides in considerable amounts, so that a preliminary separation by means of preparative thin layer chromatography is necessary prior to gas chromatographic analysis.

Identifications of the triglyceride and cholesteryl ester peaks on the chromatograms were effected through correlation curves between the numbers of carbon atoms and retention times. Those obtained by measurement of retention time for authentic standards of triglycerides and cholesteryl esters are shown in Fig. 2. Triglycerides containing branched-chain fatty acid and hydroxy fatty acids were not identifiable because of their low levels and the lack of suitable standards.

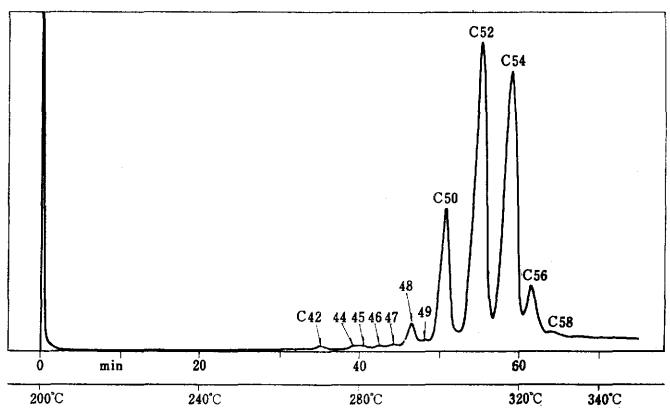

Fig. 3. Gas chromatogram of triglycerides in carp oil.
Operating conditions: Shimadzu Seisakusho Model GC-4APF gas chromatograph with a hydrogen flame ionization detector; 50 cm×3 mm i.d. glass column packed with 2% OV-17 (phenyl methyl silicone) on 80/100 mesh silanized Shimalite W; column programmed 200–340°C at 2°C/min. with 80 ml/min. Nitrogen carrier gas; detector and injection port temperatures at 350°C.

The chromatogram of carp triglycerides is shown in Fig. 3. Carp oil contained C-even triglycerides from C-42 to C–58 and C–odd triglycerides in small amounts. The dominant compoeents are the C–50 to C–56 triglycerides. The distribution pattern of triglyceride peaks on the chromatogram of "yamame" oil was similar to that of carp with slight emphasis on longer chain length within the same overall range of C–43 to 3–60 triglycerides as shown in Fig. 4. The triglyceride pattern of rainbow trout was almost the same as that of "yamame". It is of interest that there is a difference in the dominant components for carp and rainbow trout in spite of the fish being fed on the same diet which contained soybean oil and halibut liver oil. The carp oil chromatogram resembles

slighly that of soybean oil, while that of trout oil is closer to a halibut liver oil chromatogram. The characteristic distribution of triglycerides in fish fat is therefore due to both the diet of the fish and the specificity of the mechanisms of incorporating these compounds into the body fat.

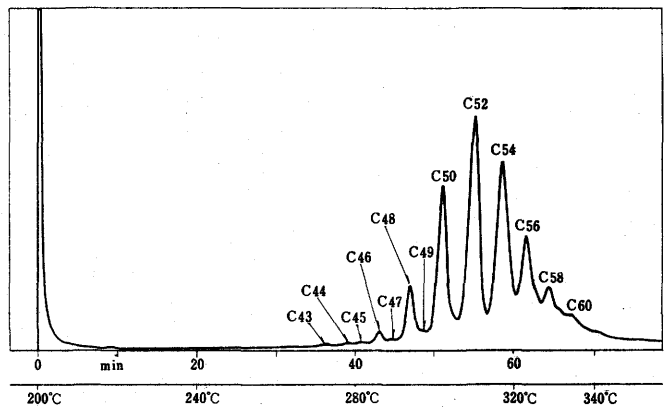

Fig. 4. Gas chromatogram of triglycerides in "yamame" oil. Gas chromatography conditions as for Fig.3.

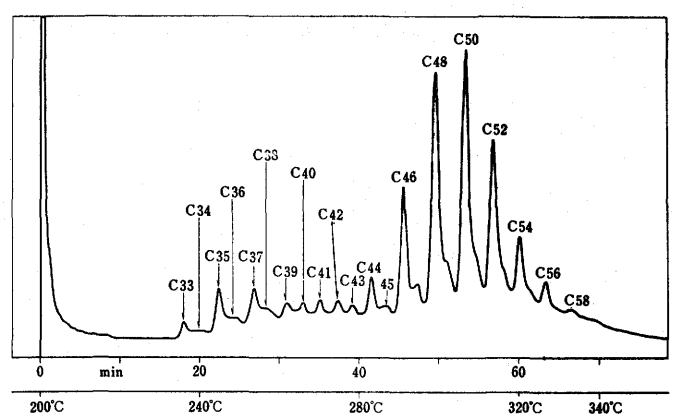

Fig. 5. Gas chromatogram of triglycerices in sardine muscle oil. Gas chromatography conditions as for Fig. 3.

In salt water fish, the triglycerides are distributed differently, as demonstrated by gas chromatography. In the case of the above fresh-water fish triglycerides below C–40 could not be detected. Although the triglyceride patterns of salt-water fish were reported by LITCHFIELD *et al.*[7] to contain principally C–42 to C–68 triglycerides, in the cases of our two samples of salt water fish, fairly large amounts of triglycerides below C–40 were also found. Generally, below C–45, the C–odd triglycerides were present in higher levels than the C–even. Above C–45 the C–even triglycerides were dominant. As an example, the chromatogram of sardine oil containing triglycerides ranging from C–33 to C–58 is

shown in Fig. 5. Characteristically, the C–odd triglycerides predominate below C–42, while the C–even predominate above C–42. The fatty acid analysis of the sardine oil triglyceride after hydrolysis proved that it contained small amounts of C–9 to C–13 fatty acid (2%) as well as the usual large amounts of C–14 to C–22 fatty acids (98%). A similar triglyceride pattern was obtained for the oil of basking shark which has C–50 to C–60 triglycerides as dominant components.

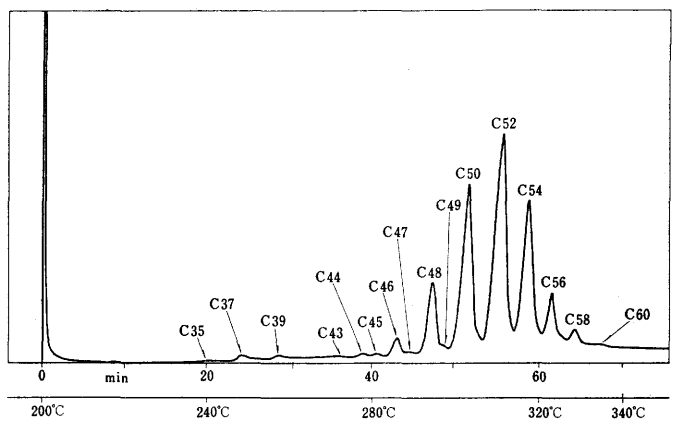

Fig. 6. Gas chromatogram of triglycerides in eel oil. Gas chromatography conditions as for Fig. 3.

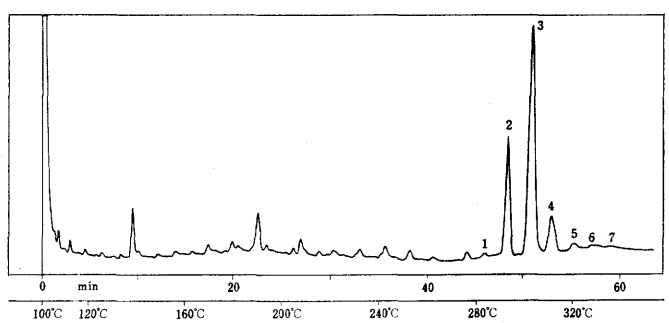

Fig. 7. Gas chromatogram of cholesteryl esters in carp oil.
Gas chromatography conditions as for Fig. 3. except column temperature. Column programed 100–320°C at 4°C/min.

1. Cholesteryl C–14 ester.
2. Cholesteryl C–16 ester.
3. Cholesteryl C–18 ester.
4. Cholesteryl C–20 ester.
5. Cholesteryl C–22 ester.
6. Cholesteryl C–24 ester.
7. Cholesteryl C–26 ester.

The eel, a brackish water fish, shows a triglyceride distributuon resembling both fresh and salt water fish (Fig. 6). The triglyceride pattern contains the same dominant components as fresh-water fish, but the components below C–40 are also present as in salt-water fish.

The distributions of cholesteryl esters in fresh water fish and salt water fish were also found to differ. In carp the C–16, and especially the C–18, cholesteryl esters, dominate (Fig. 7). The cholesteryl esters of salt-water fish generally have higher levels of the C–20 to C–26 esters. The chromatogram of basking shark cholesteryl esters shows that the

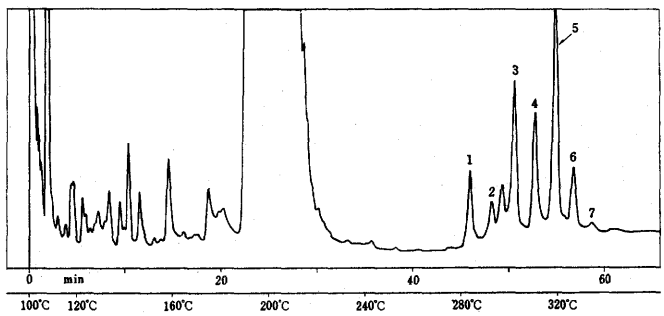

Fig. 8. Gas chromatogram of cholesteryl esters in basking shark muscle oil. Gas chromatography conditions as for Fig. 7.

1. Cholesteryl C–14 ester.
2. Cholesteryl C–16 ester.
3. Cholesteryl C–18 ester.
4. Cholesteryl C–20 ester.
5. Cholesteryl C–22 ester.
6. Cholesteryl C–24 ester.
7. Cholesteryl C–26 ester.

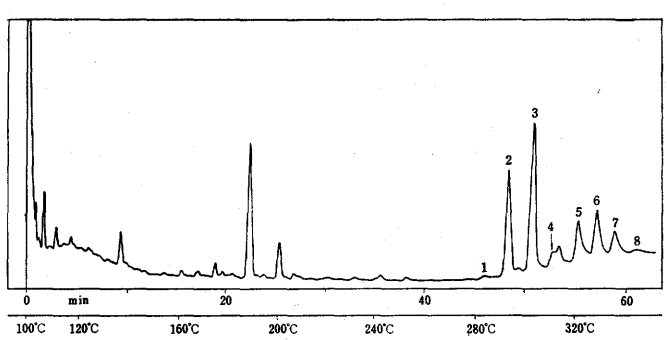

Fig. 9. Gas chromatogram of cholesteryl esters in eel oil. Gas chromatography conditions as for Fig. 7.

1. Cholesteryl C–14 ester.
2. Cholesteryl C–16 ester.
3. Cholesteryl C–18 ester.
4. Cholesteryl C–20 ester.
5. Cholesteryl C–22 ester.
6. Cholesteryl C–24 ester.
7. Cholesteryl C–26 ester.
8. Cholesteryl C–28 ester.

most important component is the cholesteryl C–22 ester (Fig. 8). The large off-scale peak in the neighbourhood of 220°C may be due to the squalene characteristic of the liver oil of this species. The cholesteryl esters of eel (Fig. 9) contained the C–20 to C–26 esters in moderate amounts and high levels of the C–16 and C–18 esters which are characteristic of fresh water fish. The brackish water fish is thus also intermediate between salt water and fresh water fish in its cholesteryl ester distribution.

Acknowledgement

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