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II. EEL (*Anguilla japonica*) LIVER AND *Channa argus* (CANTOR) LIVER

By

Masahiro HATA and Mitsuo HATA

Department of Fisheries, Faculty of Agriculture, Tohoku University, Sendai, Japan

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Many esters exist in fish liver. They are glycerides, sterol esters, vitamin A esters, wax esters, carotenoid esters etc. Since these esters have different molecular structures, metabolic pathways and physiological functions, it is considered that fatty acid composition of esters may be different from each other.

In general, the fatty acid composition of lipids of sea and fresh water fishes are different (1). Therefore, the fatty acid composition of esters of sea and fresh water fishes may also be different. Little has been written on the fatty acid composition of various esters in fish except for triglycerides. Fatty acid composition of vitamin A_1 esters was studied by Kinumaki et al (2), Futterman and Andrews (3) and wax esters by Mori et al (4, 5). As yet, there seems to be nothing on the comparative studies of fatty acid composition of esters.

In this paper, fatty acid composition of triglycerides, cholesterol esters and vitamin A esters of fresh water fish liver are described. There are two vitamin A, A_1 and A_2, in fresh water fish liver, and it is very difficult to separate the vitamin A_1 ester from the vitamin A_2 ester. Therefore, the eel (*Anguilla japonica*) containing mostly vitamin A_1 as vitamin A and *Channa argus* containing mostly vitamin A_2 were studied as samples of fresh water fish.

Experimental

Materials and Procedures

Chemicals Reagents and solvents were obtained from commercial sources. Solvents were purified by distillation befor use.

Fish The eel, cultured at a fishfarm at Hamamatsu, Shizuoka pref., was obtained from commercial sources. A *Channa argus* was caught in Ninokura at Iwanuma, Miyagi pref., and kept in a pond until used.

Extraction and fractionation of lipids Liver and muscle were done. They were homogenized in a suitable blender in chloroform-methanol (2:1 v/v). The

homogenate was filtrated, and the residue was reextracted with the same solvent. After purification by washing with 0.88% KCl (6), the lipid extract was evaporated to dryness at reduced pressure, dissolved in petroleum ether (40–60°C), subjected on alumina column chromatography (weakend alumina with 10% water) and eluted with petroleum ether and diethyl ether.

The vitamin A esters fraction, fractionated by alumina column chromatography, was separated into vitamin A esters and cholesterol esters fractions by silica gel thin layer chromatography with an upper phase of solvent mixture containing petroleum ether, acetonitrile and acetic acid (190:10:1). The vitamin A esters fraction was subjected two times to alumina column chromatography and three times to silica gel thin layer chromatography.

Preparation of fatty acid The fatty acids of vitamin A_1 esters of the eel were prepared with the method employed in a previously reported experiment (7). The vitamin A esters of *Channa argus* is mostly vitamin A_2 ester. The vitamin A_2 ester dose not split the free fatty acid with the use of p-toluene sulphonic acid. The vitamin A esters are not isolated from the wax esters in this silica gel thin layer chromatography. But the vitamin A_2 esters fraction isolated from the liver of *Channa argus* was free from other ester form contaminants detectable by thin layer chromatography. Therefore, the fatty acid mxiture of vitamin A_2 esters was prepared by saponification in KOH-EtOH as were the other esters. Cholesterol esters, triglycerides, and muscle lipid were saponified in 1/2N KOH-EtOH, 70°C, 90 min. and mixtures of fatty acids were prepared with the ordinary method.

The preparation of fatty acid methyl ester and gas-liquid chromatography were the same as those described in the previous paper (7).

Results and Discussion

The lipid content of liver and muscle, and yields of each fractions of cholesterol esters, vitamin A esters and triglycerides in liver are given in Table 1. Because of the loss during the chromatography, the yields of each esters described on the table do not show the esters composition of liver accurately. But the silica gel thin layer chromatography suggests that the large part of muscle lipids are triglycerides

Table 1. Contents of lipid and fractions of esters

	Eel		*Channa argus*	
	Liver	Muscle	Liver	Muscle
Tissue weight (wet. g)	203	76	42	215
Extracted lipid (g)	8.15	14.96	3.9	1.3
Vitamin A esters fraction (mg)	106.8	—	10.6+	—
Cholesterol esters fraction (mg)	150.0	—	331.1+	—
Triglyceride (mg)	1563.7	—	312.8+	—

+Lipid 2.3953 g

Table 2. Fatty acid composition of esters in eel liver (%)

	Vitamin A esters	Cholesterol esters	Triglycerides	Muscle lipids
C 14:0	2.7	tr	6.0	4.9
C 15:0	0.8	tr	1.1	0.7
C 16:0	53.8	41.2	20.0	22.5
C 16:1	8.3	5.3	13.1	9.2
C 16:2	1.7	1.8	1.8	1.7
C 17:0	—	2.7	2.3	—
C 18:0	3.7	5.5	2.7	5.4
C 18:1	21.6	28.9	35.8	32.9
C 18:2	2.2	4.0	4.3	3.2
C 18:4	tr	tr	tr	1.5
C 20:0	tr	tr	tr	tr
C 20:1(18·3)	2.9	8.2	7.5	8.0
C 20:3	1.1	1.8	1.7	3.4
C 20:4	1.1	—	0.5	0.9
C 22:2	—	—	1.5	2.6
C 22:3	—	tr	—	—
C 22:5	tr	—	tr	0.9
C 22:6	—	—	1.6	2.1

Table 3. Fatty acid composition of esters in *Channa argus* liver (%)

	Vitamin A esters	Cholesterol esters	Triglyceride	Muscle lipids
C 14:0	0.8	0.3	0.3	0.2
C 15:0	1.4	0.5	0.5	0.4
C 16:0br	0.7	0.4	0.3	1.1
C 16:0	63.4	16.5	20.6	31.0
C 16:1	13.8	5.4	12.0	9.8
C 16:2	2.8	2.3	2.4	1.6
C 17:0				2.1
C 18:0	4.1	5.3	2.4	7.6
C 18:1	7.0	23.8	45.4	15.5
C 18:2	2.2	3.2	6.9	4.3
C 18:4	—	tr	1.4	—
C 20:0	1.4	1.6	3.1	0.4
C 20:1(18:3)	—	3.2	6.9	4.3
C 20:2	0.3	tr	0.6	0.6
C 20:3	0.6	—	0.8	9.5
C 22:1	—	—	tr	—
C 22:3	—	—	0.2	—
C 22:4	—	—	—	1.3
C 22:5	—	—	tr	2.2
C 22:6	—	—	0.4	8.8
C 24:0	—	29.6	0.4	1.5
X	—	3.7	—	—
Y	—	1.4	—	—

and triglycerides of liver lipids are somewhat smaller (approximating 50% or less).

The fatty acid composition of esters is given in Table 2 and Table 3. The differences of fatty acid composition among the esters were distinctly observed.

Vitamin A ester The vitamin A_1 esters of eel contained a slightly higher

proportion of saturated acids than unsaturated ones. The major acids were C 16:0 54%, C 16:1 8% and C 18:1 22%. There were no signfiicant amounts of polyunsaturated acids. The reported fatty acid composition of vitamin A esters of fish livers shows a slightly high proportion of saturated acids such as C 16:0, C 18:0 and small amounts of polyunsaturated acids (2, 3). The vitamin A_1 esters of eel showed similar results.

In *Channa argus*, the vitamin A_2 esters contained a higher proportion of saturated fatty acids than eel. The major acids were C 16:0 63%, C 16:1 14% and there were no little sign of polyunsaturated acids. The C 16 acid content 77% is much larger than that shown in other results (2,3). Goodman, Huang and Shiratori (8) reported the metabolism of newly absorbed C-14 labeled vitamin A_1 esters in rat. They found that the absorbed vitamin A_1 esters were stored in liver rapidly in the form vitamin A_1 palmitate as predominant form. In their experiments, the ratio of C 16:0/C 18:0 in chylomicron and plasma was 1.7–1.9 and C 16:0 was 42%–44%. In liver, however, the ratio C 16:0/C 18:0 changed to 5–6 and C 16:0 was 71%–76%. Their results suggest that the selective metabolism of vitamin A_1 esters occures in a shrot time after absorption. The remarkable differences of the ratio C 16:0/C 18:0 between vitamin A esters and muscle lipids observed in our experiments, such as 14.5 and 4.2 in eel and 15.5 and 4.1 in *Channa arugs*, may be suggest that, probably in fish, similar selective metabolism of vitamin A esters exists.

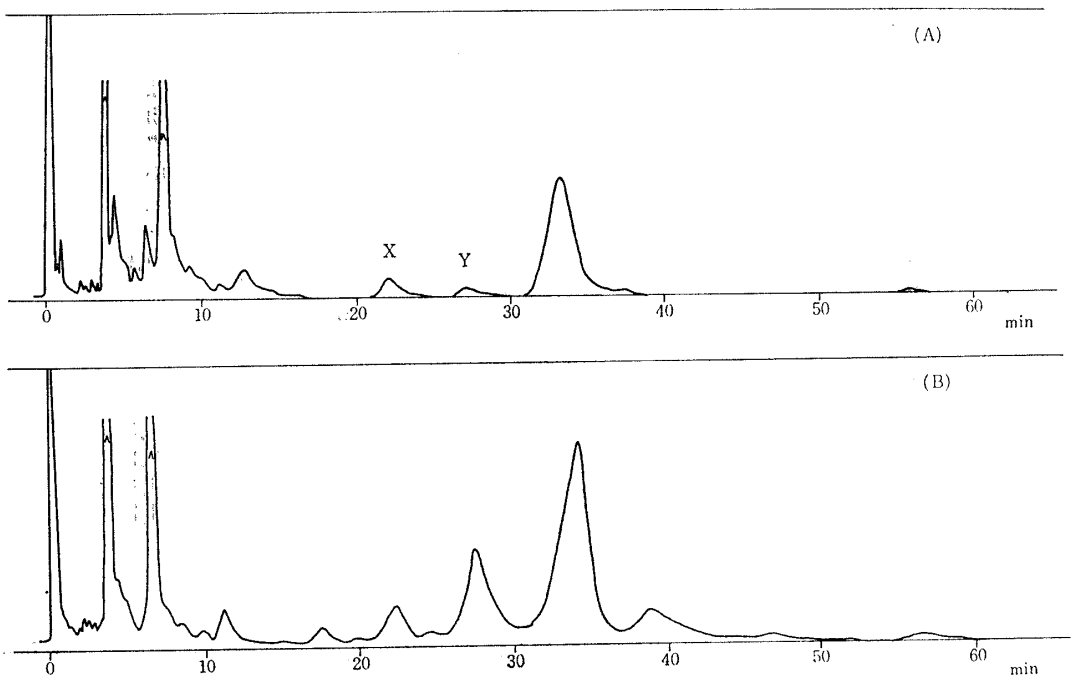

Fig. 1. Gas-liquid chromatogram of faffy acid methyl esters of cholesterol esters of *channa argus* liver

A: before hydrogenation B: after hydrogenation

Cholesterol ester The cholesterol esters of eel were similar to the vitamin A_1 esters, but slightly more unsaturated. the major acids were C 16:0 41%, C 18:1 29% and C 20:1 (18:3) 8%.

In *Channa argus*, the cholesterol esters composition was very peculiar. Fig. 1 shows the gas-liquid chromatogram of fatty acid methyl esters of cholesterol esters of *Channa argus*.

The large last peak was identified as C 24:0 by hydroganation and relative retention time. The major acids were C 24:0 30%, C 18:1 24% and C 16:0 17%. In general, cholesterol esters composition of liver are somewhat unsaturated (9). But in *Channa argus*, cholesterol esters are considarably saturated because of the large amounts of C 24:0 acid. Small amounts of C 24:0 acid were found in the triglyceride and the muscle lipids. The existence of C 24 acid in fish has been reported by many workers (4, 5, 10–14), but the reported amounts are below 2%. In mammals, such as rat, dog, man, rabbit, cow and guinea pig, the existence of C 24:0 acid has not ever been reported (9).

Recent works of cholesterol esters metabolism with use of C–14, H–3 labeled cholesterol and mevalonic acid in rat (15) and man (16) showed that the formation of a high proportion of cholesterol esters occured after injection. Although cholesterol esters composition are affected by diet lipids, in general, cholesteryl oleate is usually the major ester (9). The fatty acid composition of cholesterol esters of the eel and cray fish (17) shows the same results as that of mammals. But in *Channa argus*, it is very peculiar. Therfore, there may be large differences in the fatty acid composition of cholesterol esters among fish.

Further study is neccessary to elucidate the accumulation of C 24:0 acid in cholesterol ester of *Channa argus* liver.

Triglyceride and muscle lipids In the eel, the triglycerides were similar to the muscle lipids and contained a high proportion of unsaturated acids approximating more than 70%.

The major acids were C 18:1 36%, C 16:0 20%, C 16:1 13%, C 20:1 (18:3) 8% and C 14:0 6%. Polyunsaturated acids existed in slightly quantities than that of the vitamin A_1 and cholesterol esters. The muscle lipids were very similar to the triglycerides of liver.

In *Channa argus*, the triglycerides of liver were more unsaturated and contained a high proportion of oleic acid as much as 45%. The other major acids were C 16:0 21% and C 16:1 12%. The muscle lipids were also more unsaturated and major acids were C 16:0 31%, C 18:1 16%, C 16:1 10% and C 20:3 10%.

In general, the differences of fatty acid composition between triglycerides of liver and muscle are small. However, the triglycerides of liver of *Channa argus* contained somewhat more oleic acid and smaller amounts of polyunsaturated acids than that of the muscle lipids.

From these results, though the fish lipid composition is changed by the diet

lipid (1, 18), it seems that the accumulation of fatty acids in esters are respectively different.

Summary

The fatty acid composition of cholesterol esters, vitamin A esters and triglycerides of the eel liver and *Channa argus* liver were studied. The differences of fatty acid composition among these esters were distinctly observed.

Eel liver The vitamin A esters contained a slightly high proportion of saturated acid and the major acids were C 16:0, C 18:1 and C 16:1. The cholesterol esters contained a slightly high proportion of unsaturated acids and the major acids were C 16:0, C 18:1 and C 20:1 (18:3). The triglycerides were similar to the muscle lipids, and contained a high proportion of unsaturated acids and the major acids were C 18:1, C 16:0 and C 16:1.

Channa argus liver The vitamin A_2 esters contained a high proportion of saturated acids and the major acids were C 16:0, C 16:1 and C 18:1. The cholesterol esters were considerably saturated and contained unusual large amounts of C 24:0 acid as much as 30%. The triglycerides were more unsaturated and the major acids were C 18:1 and C 16:0. Polyunsaturated acids were very small in quntity. The muscle lipids of the eel and *Channa argus* were more unsaturated and showed the usual composition of fish oil.

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