ホタテガイ中腸腺のエイコサペンタエン酸含有トリグリセリド含量の季節的変化

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Seasonal Changes in the Contents of Eicosapentaenoic Acid-Containing Triglycerides in Hepatopancreas of Scallop

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Seasonal changes in the contents of eicosapentaenoic acid (20: 5ω3)-containing triglycerides from the hepatopancreas of the scallop *Patinopecten yessoensis* were investigated.

The content of hepatopancreas lipids varied considerably and seasonally, ranging from 1.2 to 8.2% of the tissue wet weight. Of the total lipids, the contents of the triglyceride class ranged from 14.5 to 87.4%. They had especially high values (mean: 75.8%) between April and October. Over an 11-month sampling period, the most predominant fatty acid in triglycerides isolated from the hepatopancreas lipids was 20: 5ω3. Due to seasonal changes, the percentages of this ω3 polyunsaturated fatty acid in triglycerides from the tissues from March to September were higher (32.3%) than those at other times (22.0%). In particular, the content of 20: 5ω3-rich triglycerides (4.7 g; 30.8% 20: 5ω3 in triglycerides) in the hepatopancreas of the scallops harvested between April and October was greater than that obtained during other months (0.6 g; 23.8%). This indicated that scallop hepatopancreas could be used as a potential natural source of 20: 5ω3-rich triglycerides in the production of nutritional or pharmaceutical products.

The ω3 series of polyunsaturated fatty acids, particularly eicosapentaenoic acid (20: 5ω3) and docosahexaenoic acid (22: 6ω3) in marine lipids, have attracted increased attention in recent years. Polyunsaturated fatty acids such as 20: 5ω3 are effective in reducing levels of serum cholesterol and triglyceride. It also tends to inhibit platelet aggregation and blood clotting and thereby lowers the risk of heart attacks.[1] Certain fish oil products containing mainly triglycerides are the current source for such fatty acids. It has also been shown that both glycolipids of marine chlorella[2] and phospholipids of marine bacteria[3] are rich in 20: 5ω3. We reported preliminarily that the triglyceride class in hepatopancreas lipids of the scallop *Patinopecten yessoensis* is characterized by a high level of 20: 5ω3.[4] Additionally, it was recognized that human absorption of 20: 5ω3 and 22: 6ω3 as triglycerides was more advantageous than those of ethyl esters.[5]

The aim of this study was to analyze the lipids of scallop hepatopancreas, an unutilized marine resource, in order to determine if this alternate source of 20: 5ω3-rich triglycerides may be of economic importance to the food or pharmaceutical industry.

Materials and Methods

Materials

Forty specimens of the scallop *P. yessoensis* were sampled monthly between July 1989 and May 1990, off the coast of Kikonai in Hokkaido, Japan. Hepatopancreas of the bivalve molluscs was separated from the shucked soft body, then weighed to process the lipid extraction.

Lipid Extraction and Analysis

Total lipids were extracted by the method of Bligh and Dyer.[6] Triglyceride class was fractionated from the tissue lipids by thin-layer chromatography (TLC). The triglycerides were saponified by refluxing with 1 N KOH-ethanol for 1 h. Following acidification with dilute HCl, fatty acids were recovered by diethyl ether extraction. Lipid class determination was performed using an Iatroscan TH-10 instrument with Chromarod S-III rods (Iatron Laboratories Inc., Tokyo). Two different solvent systems were used to separate the lipid classes: System I: hexane-diethyl ether-formic acid (92: 8: 0.5; v/v/v) for main class separation; and system II: chloroform-formic acid (100: 0.5; v/v) for separating free fatty acids from triglycerides. Chromatograms were recorded and integrated by a Shimadzu

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Chromatopack C-R1A (Shimadzu Co., Kyoto).

Derivatization

Fatty acids from triglycerides were methylated with 14% BF_3-methanol.[7]

Hydrogenation

An aliquot of fatty acid methyl esters fractionated with $AgNO_3$-TLC, each corresponding to their degree of unsaturation, was dissolved in hexane containing 5% palladium catalyst (Nakarai Chemicals Ltd., Kyoto) and treated with hydrogen at room temperature and under atmospheric pressure for 1 h.

Thin-Layer Chromatography

Fractionation of triglycerides from the total lipids was accomplished on silicic acid plates. A portion of the fatty acid methyl esters was fractionated on silicic acid plates impregnated with 15% $AgNO_3$. Thin layer plates of silicic acid, 0.25 mm thick (20×20 cm), were used for analytical and preparative purposes. The system of hexane-diethyl ether-acetic acid (85: 15: 1; v/v/v) was used as the developing solvent. After development, the plates were sprayed with 50% sulfuric acid or 0.05% alcoholic rhodamine-6-GO as the visual reagent.

Gas-Liquid Chromatography (GLC)

Analysis by GLC was carried out using a Shimadzu model GC 8APF gas chromatograph equipped with a dual hydrogen flame ionization detector. The fatty acid methyl esters were analyzed on two different glass columns, each paeked with 10% DEGS on Chromosorb W AW (80/100 mesh) and with Unisole 3000 on Uniport C (80/100 mesh), respectively. Column temperatures were 185°C for the former column and 210°C for the latter one. Nitrogen was used as a carrier gas. The components' identification was made by comparison of retention times of available standards, argentation-TLC followed by GLC of the bands separated by degree of unsaturation, and GLC of the hydrogenated fatty acid methyl esters. A quantitative analysis was performed on the basis of the peak areas calculated using a Shimadzu model C-R3A Chromatopack.

Results and Discussion

Tissue Weight of Hepatopancreas

Throughout the 11-month period, the weights of each hepatopancreas examined ranged from 3.6 to 8.3 g (mean: 4.9 g). The weight of the tissues accounted for 5.5 to 8.7% (7.3%) of the soft body wet weight of the scallop. Due to seasonal changes the hepatopancreas mass was the largest during March through July, ranging from 5.2 to 8.3 g (6.2 g). Outside of this time period its mass was observed to average 4.2 g.

Fatness of the hepatopancreas tissues during spring may be correlated closely with the blooming of phytoplankton and/or detritus causing an increase in available dietary sources for the mollusc.

Lipid Content of Hepatopancreas

Lipid contents of the hepatopancreas tissues examined appear in Table 1. The percentages of

Table 1. Seasonal changes in lipid content and class composition of hepatopancreas of scallops

	Sampling season										
	1989						1990				
	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Lipid content %*1	8.2	6.9	6.4	4.4	2.2	1.4	1.2	1.5	3.2	5.1	5.9
Lipid class %*2											
SE	2.2	3.4	4.6	6.9	11.4	16.0	12.7	9.3	2.8	0.9	0.8
DAGE	0.2	0.1	0.1	0.3	0.2	tr*3	0.1	tr	tr	0.1	0.1
TG	85.1	87.4	81.3	71.3	54.9	31.6	17.3	14.5	34.3	61.2	68.6
FFA	2.4	0.3	0.5	1.7	0.9	5.6	10.1	18.9	29.0	10.9	11.5
ST	1.3	1.0	1.5	2.5	3.9	5.4	9.3	7.7	2.5	1.8	1.5
PG	0.5	0.2	0.6	0.5	0.4	0.3	0.5	0.5	2.2	2.3	1.9
PL	8.3	7.6	11.4	16.8	28.3	41.1	50.0	49.1	29.2	22.8	15.6

*1 % on wet wt. basis of tissue.
*2 % to total lipid.
*3 Trace (less than 0.05%).
SE, steryl ester; DAGE, diacyl glyceryl ether; TG, triglyceride; FFA, free fatty acid; ST, sterol; PG, partial glycerol; PL, phospholipid.

the total lipids varied considerably at different times during the year, ranging from 1.2 to 8.2% (4.2%) on wet weight basis of the tissue. The total lipids in the months between April and September had relatively high values, ranging from 5.1 to 8.2% (6.5%) when compared to other months (2.3%). Throughout the sampling period, tissue lipid weighing 50 to 430 mg (210 mg) was obtained from the individual hepatopancreas. The tissues of the scallops harvested from March through October had approximately fourfold higher lipid level, ranging from 180 to 430 mg (290 mg), than those collected during other months (68 mg).

A significant increase in hepatopancreas lipids obtained between spring and summer was observed. Appreciable amounts of tissue lipids were considered to be related to the accumulation of the dietary lipids by ingestion of phytoplankton and/or detritus during the spring bloom.

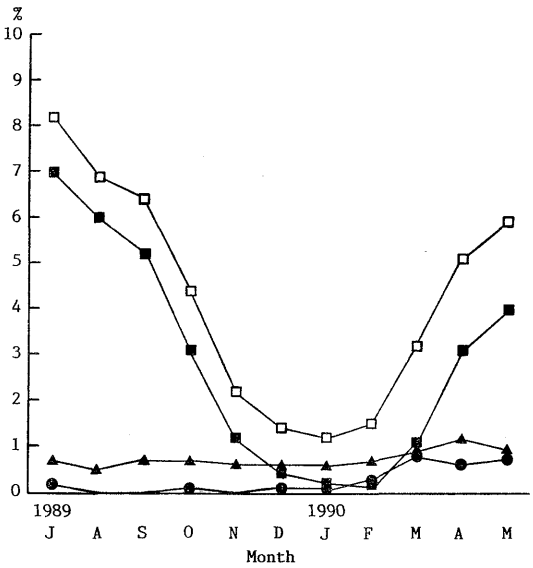

Fig. 1. Seasonal changes in the levels of total lipids and of the lipid constituents of hepatopancreas of scallops.

Percentage on the wet wt. basis of tissue: □, Total lipid; ■, Triglyceride; ▲, Phospholipid; ●, Free fatty acid.

Triglyceride Content of Hepatopancreas Lipid

Lipid class compositions of the hepatopancreas lipids from the scallops are presented in Table 1. Of the total lipids during an entire year, triglycerides varied considerably between 14.5 and 87.4% (55.2%). This lipid class in the months

Table 2. Seasonal changes in fatty acid composition of triglyceride class of hepatopancreas of scallops

(Peak area %)

	Sampling season										
	1989						1990				
Fatty acid	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
14:0	4.5	4.3	4.0	3.4	5.4	5.0	5.0	7.9	6.7	5.7	5.9
16:0	12.1	12.1	14.8	15.1	15.3	14.1	15.5	13.5	10.5	10.0	10.7
18:0	2.1	2.0	2.1	2.9	2.6	2.7	3.0	3.2	3.3	2.2	2.3
16:1	11.6	12.4	12.8	11.8	12.8	11.3	10.6	10.3	8.9	10.2	10.7
18:1	8.9	9.6	10.1	11.3	11.2	10.5	11.2	7.3	4.8	5.4	6.7
20:1	2.3	2.3	2.6	3.3	3.1	3.1	3.4	3.8	2.4	1.8	1.7
16:2	1.5	1.5	1.3	1.5	1.5	1.5	1.4	1.9	2.4	1.8	1.8
16:4	tr*1	tr	tr	tr	tr	tr	0.2	2.5	3.3	1.0	1.1
18:2ω6	1.9	3.2	1.6	2.4	2.3	3.1	2.8	2.2	0.7	0.6	0.9
18:3ω3	1.8	1.9	1.2	2.0	1.6	2.0	1.7	2.4	1.6	0.8	0.9
18:4ω3	4.6	4.3	3.6	3.7	3.7	4.8	4.5	8.5	5.8	2.9	3.8
20:5ω6	1.2	1.4	1.5	1.6	1.5	1.6	1.6	1.3	1.6	3.3	3.1
20:5ω3	29.4	28.4	28.2	24.2	23.0	22.9	22.7	17.2	33.0	39.2	35.4
21:5ω3	5.0	4.1	3.5	3.5	2.8	3.6	3.0	2.2	4.2	6.1	5.0
22:6ω3	7.5	7.8	6.9	7.3	7.6	8.0	8.2	7.8	4.5	3.7	4.9
Saturates	19.9	19.6	22.0	22.7	24.8	23.1	24.8	26.4	22.0	19.2	19.9
Monoenes	24.6	25.3	27.1	28.5	29.0	26.7	26.8	23.4	18.1	19.2	20.5
Polyenes	55.1	54.8	50.5	48.6	45.9	49.9	48.2	50.1	59.8	61.3	59.4

*1 Trace (less than 0.05%).

from April to October had relatively high values, ranging from 61.2 to 87.4% (75.8%) when compared to other months (30.5%). Other lipid constituents such as phospholipids (ranging from 7.6 to 50.0%: 25.5%), free fatty acids (from 0.3 to 29.0%: 8.3%), steryl esters (from 0.9 to 16.0%: 6.5%), sterols (from 1.0 to 9.3%: 3.5%), partial glycerides (from 0.2 to 2.3%: 0.9%), and diacyl glyceryl ethers (from trace amount to 0.3%: 0.1%) were also present in the hepatopancreas.

Figure 1 illustrates seasonal changes in the contents of total lipids, triglycerides, phospholipids, and free fatty acids in each hepatopancreas examined. Triglyceride class showed the largest coefficient of variation in the total lipids.

The triglyceride contents of each hepatopancreas ranged from 9 to 363 mg (139 mg) during all seasons. In particular, the contents of these compounds found in the tissues during the period from April to October were much higher values, ranging from 132 to 363 mg (224 mg), than those observed during other months (37 mg).

Fatty Acid Composition of Triglyceride from Hepatopancreas

The changes in the major component fatty acids of triglycerides in the hepatopancreatic tissues from the scallops are shown in Table 2. Over an 11-month sampling period, the most predominant component found was 20: 5ω3, ranging from 17.2 to 39.2% (27.6%), followed by 16: 0 (ranging from 10.0 to 15.1%: 13.1%), 16: 1 (from 8.9 to 12.8%: 11.2%), 18: 1 (from 5.4 to 11.3%: 8.8%), and 22: 6ω3 (from 3.7 to 8.2%: 6.7%), indicating a high content of polyunsaturates (from 45.9 to 61.3%: 53.1%). In addition, a maximum level of 20: 5ω3 in triglycerides from the spring samples had a slight tendency to decrease during the period from summer to winter. An inverse change was observed in the fatty acids such as 16: 0, 16: 1, 18: 1, and 22: 6ω3 during the same period. The percentages of 20: 5ω3 in triglycerides from the hepatopancreas tissues obtained between March and September were relatively higher (32.3%) than those of other months (22.0%). On the other hand, 22: 6ω3 levels remained essentially unchanged, ranging from 3.7% to 8.2% (6.7%). Therefore, over the 11-month study period, the ratio of 20: 5ω3 to 22: 6ω3 increased 4-fold.

The characteristic component fatty acids described above were in agreement with those found in the triglyceride class in each scallop hepatopancreas described previously.[4]

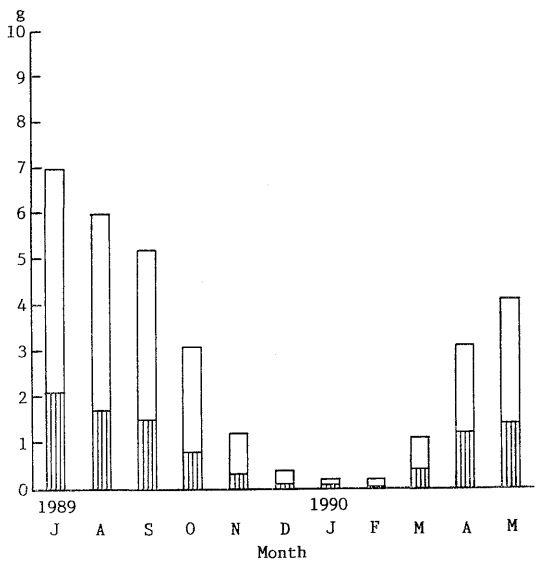

Fig. 2. Seasonal changes in the contents of triglycerides and 20: 5ω3 in triglycerides in hepatopancreas (100 g tissues) of scallops. □, Triglyceride; ▥, 20: 5ω3 level in triglyceride.

20: 5ω3-Containing Triglyceride Content in Hepatopancreas

Throughout the sampling period, seasonal changes in the contents of triglycerides and 20: 5ω3 in triglycerides in the 100 g samples of the scallop hepatopancreas are presented in Fig. 2. As seen in Fig. 2, the contents of the 20: 5ω3-containing triglycerides varied seasonally, ranging from 0.21 to 7.00 g (2.88 g) in absolute value. These triglyceride fractions also contained considerable amounts of 20: 5ω3, accounting for 0.04 to 2.05 g (0.86 g). In particular, the yield of 20: 5ω3-rich triglycerides (4.7 g; 30.8% 20: 5ω3 in triglycerides) of the hepatopancreas from the scallops harvested during the period between April and October was greater than those obtained during other months (0.6 g; 23.8%).

The waste products of hepatopancreatic tissues originating from food processing such as boiled dried adductor muscles of scallops amounted to several thousand t per year in Hokkaido. This indicated that scallop hepatopancreas could be used as a potential natural source of 20: 5ω3-rich triglycerides for nutritional or pharmaceutical products.

Acknowledgements

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