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## Molecular Species of Fish Muscle Lipids

### II. Changes in triglyceride and phosphatidylcholine molecular species of sardine after frozen storage

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#### Abstract

Changes in the molecular species of triglyceride and phosphatidylcholine of sardine muscle after a two-month frozen storage at  $-20^{\circ}\text{C}$  was studied by probability calculation using a computer program reported in the previous paper.<sup>1)</sup> The results showed that the composition of molecular species of triglyceride did not change so much, whereas, that of phosphatidylcholine changed considerably, especially the combination of the  $\text{C}_{16:0}/\text{C}_{20:5}$ ,  $\text{C}_{16:0}/\text{C}_{22:6}$  and  $\text{C}_{16:0}/\text{C}_{18:1}$  types after frozen storage. The acid value, as well as the peroxide value, of sardine muscle lipids increased during frozen storage. It can be assumed that triglyceride molecules of the sardine muscle has a tendency to be decomposed randomly by hydrolysis and oxidation, and that of phosphatidylcholine specifically.

#### Introduction

Many workers<sup>2)-6)</sup> have studied on lipid changes of fish muscle during storage at a low temperature. However, these studies have been restricted mainly on the changes in lipid composition and in total fatty acid composition. Therefore, the present authors attempt to obtain a new interpretation in lipid decomposition during low temperature storage from the point of view of changes in molecular species. In this study, by applying a computer technique, the authors investigated changes in the molecular species of triglyceride (TG) and phosphatidylcholine (PC) using sardine muscle as a sample.

#### Materials and Methods

Sardine (*Sardinops melanosticta*) caught from the coast of Kamiiso, Hokkaido in Apr. 1978 (average body weight: 80 g), was randomly divided into two groups — one for the unfrozen sample, and another for the frozen stored sample. The lipids of the unfrozen sample were immediately extracted according to the method of Bligh and Dyer, and those of the frozen sample were extracted after a two-month storage at  $-20^{\circ}\text{C}$  in the same manner. Separation and purification of TG and PC were carried out by means of preparative thin layer chromatography (TLC) for the former, and column chromatography for the latter as reported in the previous paper.<sup>1)</sup>

Acid value (AV), peroxide value (POV), iodine value (IV) and saponification value (SV) were measured according to the standard analytical method of J.O.C.S.

Hydrolysis of the TG was carried out by Grignard's decomposition, and PC

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was hydrolyzed using phospholipase A (*Trimeresurus flavoviridis*) as described in the previous paper.<sup>1)</sup> The input data for the computer were also prepared in the same manner as described in the previous paper.<sup>1)</sup>

Lipid composition was estimated by TLC-Densitometric procedure using an Ozumor 82 densitometer. The developing solvents used were n-hexane:ethyl ether:acetic acid=75:25:1 (v/v) for the simple lipids, and chloroform:methanol:ammonia:water=70:30:2:3 (v/v) for the phospholipids. The ratio of phospholipid to simple lipid was calculated from the phosphorus content of the total lipids. The mean molecular weight of the PC was calculated from a formula given below, after the phosphorus determination of the PC.

$$\text{Mean Molecular Weight} = \frac{100 \times 30.974}{\text{phosphorus content (\%)}}$$

The mean molecular weight of the TG was calculated from the fatty acid composition of the TG determined by means of gas liquid chromatography (GLC). The analytical condition of GLC was as described in the previous paper.<sup>1)</sup>

### Results and Discussion

Lipid compositions are shown in Table 1. As shown in this table, TG and PC accounted for more than 60% of the lipids in unfrozen sardine. From this it was assumed that the changes in TG and PC significantly represent the changes in total lipids. Therefore, this study is centered basically on TG and PC changes, to represent changes in molecular species of total lipids in sardines.

Table 1. Changes in the lipid composition.

Simple lipid			Phospholipid		
Component	Unfrozen %	Frozen %	Component	Unfrozen %	Frozen %
MG	0.8	2.1	Lyso PC	14.7	6.4
DG		5.7	SM	Trace	8.9
ST	5.1	8.5	PC	68.9	41.7
FFA	1.6	8.5	PS	12.4	24.3
TG	89.4	69.5	PE	2.7	14.0
Others	3.1	5.7	Others	1.2	4.7

Each in relative percentage determined by a densitometer.

Abbrev.: MG: Monoglyceride

SM: Sphingomyelin

DG: Diglyceride

PC: Phosphatidylcholine

ST: Sterol

PS: Phosphatidylserine

FFA: Free fatty acid

PE: Phosphatidylethanolamine

TG: Triglyceride

Table 2 shows the changes in molecular species in TG and a summarized form of the output data is shown in Table 3. As shown in these tables, no remarkable differences in molecular species were observed between the TG of unfrozen sardine and that of frozen stored sardine.

Table 2. Changes in molecular species of triglyceride.

	Position 1, 3	Position 2	Unfrozen	Frozen
Di	16:0* or 20:5	20:5	1.6	1.4
	14:0 or 16:0	14:0	0.8	0.8
	16:1 or 16:0	16:1	0.8	0.6
	18:1 or 16:0	18:1	0.8	0.6
	20:5 or 18:1	20:5	0.8	0.6
	22:6 or 16:0	22:6	0.8	1.0
	.	.	.	.
	.	.	.	.
	.	.	.	.
	16:0	22:6	2.2	1.9
	16:0	20:5	1.9	1.7
	16:0	14:0	1.1	0.9
	16:0	16:1	1.0	0.8
	16:0	18:2	0.9	0.4
	16:0	18:1	0.8	0.6
	14:0	22:6	0.4	0.3
.	.	.	.	
.	.	.	.	
.	.	.	.	
Tri	16:0 or others	22:6	9.2	9.0
	16:0 or others	20:5	7.2	7.2
	16:0 or others	14:0	4.4	4.2
	16:0 or others	18:2	3.8	1.6
	16:0 or others	16:1	3.6	3.4
	20:5 or others	22:6	3.2	2.0
	16:0 or others	18:1	2.4	1.2
	18:1 or others	22:6	2.0	3.6
	14:0 or others	20:5	1.6	0.8
	.	.	.	.

in mole %

Abbrev.: Tri : Tricomponent combinational type

Di: Dicomponent combinational type

\* Number of carbon atoms and double bonds of fatty acid.

On the other hand, considerable differences were observed in PC molecular species between the unfrozen and the frozen stored as shown in Table 4. For example, the PC combinational groups such as  $C_{16:0}/C_{22:6}$ ,  $C_{16:0}/C_{20:5}$  and  $C_{16:0}/C_{18:1}$  decreased remarkably in the frozen sample, which in effect increased relatively the amount of some other combinational groups of molecular species in this table. Although there are increases in the relative amount of some kinds of molecular species in this table, this does not necessarily imply an increase in the absolute amount. For example, the increase in the relative amount of  $C_{16:1}/C_{22:6}$  in this table was found to be unchanged by calculating the absolute amount from Tables 4 and 5.

Table 5 shows the recovery of the lipids. The results shown in Table 6 were

Table 3. *Combinational type of triglyceride printed.*

Combinational type	Unfrozen		Frozen	
	Mole %	Printed component number	Mole %	Printed component number
$\begin{matrix} \text{—A} \\ \text{—A} \\ \text{—A} \end{matrix}$	1.0	4	1.3	3
$\begin{matrix} \text{—A} \\ \text{—A} \\ \text{—B} \end{matrix} + \begin{matrix} \text{—A} \\ \text{—B} \\ \text{—A} \end{matrix}$	25.0	67	23.9	69
$\begin{matrix} \text{—A} \\ \text{—B} \\ \text{—C} \end{matrix}$	43.8	126	42.0	132
Sum of the probability printed	69.8	—	67.2	—
Sum of the printed component number	—	197	—	204

in mole % ( $\geq 0.2\%$ )

calculated from Tables 1 and 5. As shown in this table, the amount of free fatty acid increased inversely to the decrease of TG and PC. This implies a hydrolytic degradation of these two lipids during frozen storage.

Increase in AV and decrease in SV also imply hydrolytic degradations as shown in Table 7. It was suggested that though the TG does not change in the composition of molecular species, it is hydrolyzed to a considerable amount. Since the increase in the amount of free fatty acid was not so large compared with that of the decrease in TG and PC, the results shown in Table 6 suggested not only hydrolytic degradation, but also oxidative degradation as well.

As shown in Table 7, increase in POV and decrease in IV also support this assumption. From Table 6, it was also assumed that the rate of decomposition of PC is faster than that of TG, since the decreasing ratio was 75% in PC and approximately 49% in TG after two-month frozen storage. These respective decreasing ratios were obtained using the following formula.

$$\text{Decreasing Ratio} = 100 - \frac{\text{abs. amt. in frozen sample}}{\text{abs. amt. in unfrozen sample}} \times 100$$

To summarize the authors' interpretation of the results, it could be said that the TG molecules of sardine muscle have a tendency to be decomposed randomly, and that of PC to be decomposed specifically during frozen storage—especially the molecules which combined highly unsaturated fatty acids with palmitic acid such as  $C_{16:0}/C_{22:6}$ ,  $C_{16:0}/C_{20:5}$  and  $C_{16:0}/C_{18:1}$  molecules. A decrease in the mean molecular weight in PC from 1187 to 1050, and unchangeability in that of TG supported the results mentioned above.

Table 4. Changes in molecular species of phosphatidylcholine.

Position 1 \ Position 2	Position 2												
	14:0	15:0	16:0	16:1	18:1	18:2	20:1	20:2	20:5	22:1	22:4	22:5	22:6
12:0*													0.2
14:0			0.3		0.2 0.4				0.4 0.6				0.8 0.9
15:0									0.2				0.3 0.4
16:0	0.4 0.5	0.2	4.3 4.5	1.3 1.3	9.1 6.6	0.6 0.4	0.4 0.2	0.4 0.2	17.3 9.5	3.0 1.9	0.2	1.7 0.8	30.8 15.9
16:1	0.4		0.3 3.5	1.0	0.5 5.3	0.3			1.6 7.5	1.5		0.6	1.8 12.6
18:0			0.2		0.4 0.3				0.7 0.5				1.3 0.8
18:1			0.2 0.5		0.5 0.7				0.9 1.0	0.2			1.6 1.7
20:4					0.3				0.4				0.6
20:5			0.3		0.2 0.4				0.4 0.5				0.8 0.9
22:1									0.2				0.4
22:3					0.3 0.2				0.6 0.3				1.1 0.6
22:6			0.7 0.7	0.2	1.5 1.0				2.9 1.5	0.3 0.5		0.3	5.1 2.5

Upper: Unfrozen, Lower: Frozen, Probability  $\geq 0.2\%$

\* Number of carbon atoms and double bonds of fatty acid.

Table 5. Recovery of the lipids.

	Unfrozen	Frozen
Total lipid	10.3	6.3
Phospholipid	1.1	0.4
PC	0.8	0.2

Percentage of lipids per 100 grams fish muscle.

Table 6. *The absolute amount of main lipids in 100 grams of sardine muscle.*

Compondnt	Unfrozen (g)	Frozen (g)
TG	8.2	4.2
PC	0.8	0.2
FFA	0.1	0.5
MG & DG	0.1	0.1

Table 7. *Changes in acid value, peroxide value, saponification value and iodine value.*

	Unfrozen	Frozen
AV	2.9	9.2
POV	0.0	19.6
SV	136.0	131.1
IV	184.6	182.7

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