

## マガキ凍結貯蔵中における脂質劣化とその防止

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## Lipid Deterioration and Its Inhibition of Japanese Oyster *Crassostrea gigas* during Frozen Storage

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Lipid deterioration and its inhibition in the Japanese oyster *Crassostrea gigas* during frozen storage were investigated. The shelled oyster were treated with antioxidants such as dibutylhydroxytoluene and natural vitamin E, and then stored at  $-20^{\circ}\text{C}$  for 12 months and the effect of deoxygenizer was examined similarly. Untreated oyster were also stored either at  $-20^{\circ}\text{C}$  or at  $-35^{\circ}\text{C}$ . During storage, changes in TBA value, POV, and fatty acid and class compositions of lipids were determined to evaluate the quality of oyster. The color and taste of cooked oysters were also evaluated.

POV in all the samples increased gradually with the duration of storage. Contents of phosphatidylcholine (PC) and triglyceride decreased in varied degrees, while those of free fatty acid, lyso-PC (LPC) increased. These changes in lipid classes proceeded at a higher rate in the samples stored at  $-20^{\circ}\text{C}$  than in the sample stored at  $-35^{\circ}\text{C}$ . Percentages of polyenoic acids in lipids decreased in all the samples during storage, whereas those of saturated and monoenoic acids increased. Decreasing rates in the percentages of polyenoic acids were highest in the untreated sample ( $-20^{\circ}\text{C}$ ) and lowest in the sample with enclosed deoxygenizer. Sensory scores of the untreated sample ( $-35^{\circ}\text{C}$ ) and the sample with enclosed deoxygenizer almost did not decrease during storage over 12 months. These results clearly indicated that the lipid deteriorations of oyster were inhibited effectively by storing at  $-35^{\circ}\text{C}$  as well as by storing with enclosed deoxygenizer at  $-20^{\circ}\text{C}$ .

It has been well known that oxidation and hydrolysis of lipids in fish and shellfish during frozen storage cause serious quality deterioration. During frozen storage, contents of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) decrease in the case of lean fish and those of triglyceride (TG), PC and PE decrease in fatty fish due to actions of endogenous lipolytic enzyme systems, resulting in the significant increase in free fatty acid (FFA) in the fish muscles.<sup>1-3)</sup> Lipid oxidation also occurs in fish during frozen storage, because fish lipids are rich in highly unsaturated fatty acids which are considerably susceptible to oxidation. Therefore, effects of various antioxidants on fish lipids have been investigated so far. In the previous studies, antioxidant potency of *tert*-butylhydroxy quinone (TBHQ),  $\alpha$ -tocopherol, tempeh oil, butylhydroxyanisole, and dibutylhydroxytoluene (BHT) was examined on lipid oxidations of mackerel<sup>4)</sup> and sardine.<sup>5)</sup> In recent years, the use of deoxygenizer has been

recommended by several workers for the purpose of inhibiting lipid oxidation in foods. They pointed out that the deoxygenizer exhibited strong antioxidative effects on the semi-dried fish,<sup>6)</sup> gonad of sea urchin<sup>7)</sup> and sardine<sup>8)</sup> lipids during storage.

However, limited information has been available on general aspects and the inhibition of lipid deterioration in shellfish during frozen storage. In the present study, Japanese oyster was investigated in regard with general aspect and inhibitions of oxidation and hydrolysis of lipids during frozen storage.

### Materials and Methods

#### Sample

Japanese oyster *Crassostrea gigas* (THUNBERG) cultured in the Hiroshima bay were harvested in March, 1988. The oyster samples were transported to a processing factory, where the total wet

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**Table 1.** Storage conditions of the oyster samples

Sample	Sample treatment	Storage temperature
C-20	Untreated	-20°C
S-35	Untreated	-35°C
S-Deoxy	Deoxygenizer	-20°C
S-BHT	0.02% BHT	-20°C
S-Toc	0.02% Natural Vitamin E + <i>dl</i> -alanine	-20°C

tissues of the oysters were removed and pooled. The sample preparations were summarized in Table 1; a portion of samples was treated either with dibutylhydroxytoluene (10% solution, S-BHT) or with natural vitamin E (10%) + *dl*-alanine (3%) solution (S-Toc), by spraying to give 0.02% of the sample weight. The composition of natural vitamin E used in the study was 5–10%  $\alpha$ -, 35–40%  $\gamma$ -, and 45–60%  $\delta$ -tocopherols. The remaining samples were not treated with antioxidant (C-20 and S-35). All the samples with or without antioxidants were frozen at -50°C in a spiral freezer and ice-glazed by dipping the frozen samples in water for 1 min. The ice-glazed samples were put into pouches of ethylene-vinyl acetate copolymer (20 cm  $\times$  15 cm, 60  $\mu$ m in thickness, O<sub>2</sub> transmittance of 2,500 ml/m<sup>2</sup>·atm·24 h at 25°C) and the openings were heat-sealed. In the case of deoxygenizer (AGELESS, S-200, Mitsubishi Gas Chemical Co., Inc., S-Deoxy) being examined, laminated polyvinylidene chloride-coated oriented nylon/polyethylene (15  $\mu$ m and 50  $\mu$ m, respectively, in thickness, 20 cm  $\times$  15 cm, O<sub>2</sub> transmittance of 6–10 ml/m<sup>2</sup>·atm·24 h at 20–25°C) was used. The samples were stored either at -20°C or at -35°C for 12 months. Duplicated samples (groups 1 and 2, consisting of 10–11 specimens and weighing about 200 g each) were subjected to analyses at intervals of three months during storage.

#### Lipid Extraction

Frozen sample was thawed at room temperature. Total lipid (TL) was extracted with chloroform/methanol according to the Bligh and Dyer procedure.<sup>9)</sup> Content of TL was determined gravimetrically.

#### Determinations of PL and NL Contents

The total phosphorus content of TL was determined spectrophotometrically according to the method of Bartlett,<sup>10)</sup> *i.e.*, after digestion with 10 N H<sub>2</sub>SO<sub>4</sub>, to the sample were added 5% am-

monium molybdenic acid, Fiske-SubbaRaw reagent and water, and the mixture was subsequently heated at 100°C for 15 min. Phospholipid (PL) content in TL was obtained by multiplying the phosphorus content by 25. Non-polar lipid (NL) content in TL was calculated from the difference between the TL and PL contents.

#### Determinations of TBA Value and POV

The TBA value was determined by the intact sample procedure of Sinnhuber and Yu.<sup>11)</sup> Peroxide value (POV) was determined according to the method of Buege and Aust.<sup>12)</sup> POV was calculated using a molar extinction coefficient of cumene hydroperoxide,  $1.73 \times 10^4 \text{ M}^{-1}$  and shown as mol/kg lipid.

#### Determination of Lipid Class Compositions of NL and PL

An aliquot of TL was submitted to preparative thin-layer chromatography (TLC) using precoated Silica Gel G plates (20 cm  $\times$  20 cm, 0.25 mm in thickness, E. Merck, Darmstadt, FRG) and a mixture of petroleum ether/diethyl ether/acetic acid (80:20:1, v/v/v) as developing solvent for analysis of lipid class composition of NL. For the lipid class composition of PL, a mixture of chloroform/methanol/acetic acid/water (65:45:1:2, by vol.) was used as a developing solvent. After spraying saturated K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>-70% sulfuric acid, the TLC plate was charred at 130°C for 5 min and subjected to densitometry at 570 nm by using a Shimadzu high speed TLC scanner CS-920. Apart from this, an aliquot of TL in C-20 was subjected to two dimensional TLC and phosphorus content of each of the separated PL class was determined, as described previously.<sup>13)</sup> From the phosphorus contents, percentages of PL classes were calculated. On the basis of these results, percentages of the PL classes in all samples obtained with densitometry were corrected.

#### Analysis of Fatty Acid Composition

TL was separated into PL and NL, using silicic acid cartridges (25 mm  $\times$  10 mm i.d., Sep-Pak, Waters Associates, Milford, Massachusetts, USA) according to the method of Juaneda and Rocquelin.<sup>14)</sup> Fatty acid compositions of TL, PL, and NL were analyzed by GLC as described previously.<sup>13)</sup>

#### Sensory Evaluation

Sensory evaluation was performed by 5-member semitrained panels. The frozen sample was

cooked in boiling water for 5 min and submitted to sensory evaluation. The panels evaluated color (yellowish discoloration and brightness) and taste (freshness and off-flavor) of the cooked samples. The samples were scored on 1–5 scales: 5, excellent; 4, good; 3, acceptable; 2, poor; and 1, unacceptable.

## Results and Discussion

### Changes in Moisture and pH Value

The moistures of the oyster were 83.2–83.5% before storage. There was no remarkable change in the moisture of samples during storage for 12 months. The pH values of the samples before storage were 6.45 and also remained almost unchanged during storage.

### Changes in TBA Value and POV

As shown in Fig. 1, TBA values increased slightly in all the samples with duration of storage, but no noticeable differences in increasing rates of TBA values after 12 months were found among the samples. Changes in POV of the samples during storage are shown in Fig. 2. The POV increased remarkably in all samples during storage for up to 3 months, then slowly until the end of storage period. C-20 showed the highest rate of increase in POV, while S-Deoxy the slowest. The rates of increases in POV of the oyster samples were estimated to be high in the following order; C-20>S-35>S-BHT>S-Toc>S-Deoxy. That S-Deoxy showed the lowest rate of increase in POV indicated clearly that the enclosed deoxygenizer

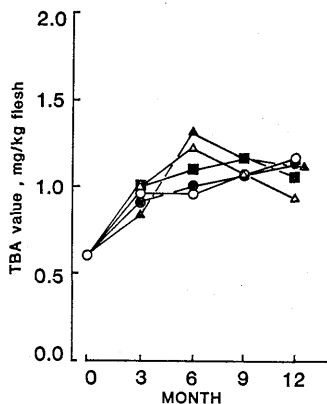


Fig. 1. Changes in the TBA values of Japanese oyster during storage. The largest standard deviation of TBA values was  $\pm 0.08$  ( $n=6$ ) for S-35 of 6-month storage. Symbols: ○, C-20; ●, S-35; △, S-Deoxy; ■, S-BHT; ▲, S-Toc.

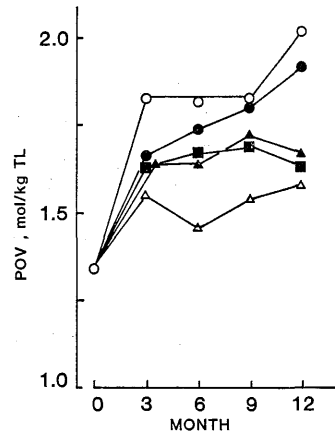


Fig. 2. Changes in the POV of TL in Japanese oyster during storage. The largest standard deviation of POV was  $\pm 0.27$  ( $n=6$ ) for C-20 of 6-month storage. Symbols are the same as in Fig. 1.

was highly effective in inhibiting lipid oxidation. In the case of samples with added vitamin E and BHT, oxidations of the lipids were also inhibited to some extent. These results agreed well with those reported by Ke *et al.*<sup>4)</sup> on mackerel skin lipids and Tsukuda<sup>5)</sup> on sardine lipids.

### Changes in Lipid Contents

Changes in TL, PL, and NL contents during storage are shown in Table 2. The contents of TL, PL, and NL of the oyster before storage were 2.05%, 0.55%, and 1.50%, respectively, as reported in the previous paper.<sup>13)</sup> In all the samples, the PL content decreased and the NL content increased during storage. The decrease in the PL content was considered due to the endogenous lipolytic enzyme systems as will be mentioned later. On the other hand, the increase in the NL content seemed to be due to increase in FFA released from PL during storage. The content of the PL of S-35, stored at  $-35^{\circ}\text{C}$ , decreased only slightly during storage, differing from those of the other samples stored at  $-20^{\circ}\text{C}$ . These results suggest that the enzymatic hydrolysis of PL in the oyster progresses slowly at as low temperature as  $-35^{\circ}\text{C}$ , as in the case of certain fish fishes reported previously.<sup>3,15,16)</sup>

### Changes in Lipid Class Compositions of NL and PL

Changes in the lipid class compositions of NL and PL of the samples during storage are shown in Figs. 3 and 4. The NL consisted of 6 lipid classes; TG, diglyceride (DG)+free sterol (ST),

Table 2. Changes in the lipid contents of Japanese oyster during storage (%)

Sample	Storage period, month																		
	0			3			6			9			12						
	TL	PL	NL	TL	PL	NL	TL	PL	NL	TL	PL	NL	TL	PL	NL				
C-20	Group 1	2.08	0.55	1.53	—	—	—	—	—	2.23	0.47	1.76	2.26	0.42	1.84	2.27	0.40	1.87	
	Group 2	2.02	0.54	1.48	2.01	0.48	1.53	2.14	0.47	1.67	2.29	0.44	1.85	2.29	0.44	1.85	2.39	0.46	1.93
	Mean	2.05	0.55	1.51	2.01	0.48	1.53	2.19	0.47	1.72	2.28	0.43	1.85	2.28	0.43	1.85	2.33	0.43	1.90
S-35	Group 1	2.08	0.55	1.53	2.05	0.48	1.57	2.24	0.47	1.77	2.27	0.48	1.79	2.27	0.48	1.79	2.31	0.47	1.84
	Group 2	2.02	0.54	1.48	2.15	0.50	1.65	2.06	0.51	1.55	2.27	0.48	1.79	2.27	0.48	1.79	2.37	0.49	1.88
	Mean	2.05	0.55	1.51	2.10	0.49	1.61	2.15	0.49	1.66	2.27	0.48	1.79	2.27	0.48	1.79	2.34	0.48	1.86
S-Deoxy	Group 1	2.08	0.55	1.53	1.92	0.38	1.54	2.20	0.45	1.75	2.04	0.43	1.61	2.04	0.43	1.61	2.31	0.43	1.81
	Group 2	2.02	0.54	1.48	—	—	—	2.46	0.46	2.00	2.16	0.45	1.71	2.16	0.45	1.71	2.31	0.44	1.87
	Mean	2.05	0.55	1.51	1.92	0.38	1.54	2.33	0.46	1.88	2.10	0.44	1.66	2.10	0.44	1.66	2.31	0.44	1.88
S-BHT	Group 1	2.08	0.55	1.53	2.33	0.48	1.85	2.29	0.48	1.81	2.48	0.42	2.06	2.48	0.42	2.06	2.35	0.40	1.95
	Group 2	2.02	0.54	1.48	2.20	0.46	1.74	2.23	0.43	1.80	2.39	0.48	1.91	2.39	0.48	1.91	2.42	0.46	1.96
	Mean	2.05	0.55	1.51	2.27	0.47	1.80	2.26	0.46	1.81	2.44	0.45	1.99	2.44	0.45	1.99	2.39	0.43	1.96
S-Toc	Group 1	2.08	0.55	1.53	2.32	0.48	1.84	2.63	0.50	2.13	2.25	0.47	1.78	2.25	0.47	1.78	2.52	0.42	2.10
	Group 2	2.02	0.54	1.48	2.34	0.47	1.87	2.64	0.47	2.17	2.44	0.47	1.97	2.44	0.47	1.97	2.44	0.43	2.01
	Mean	2.05	0.55	1.51	2.33	0.48	1.86	2.64	0.49	2.15	2.35	0.47	1.88	2.35	0.47	1.88	2.48	0.43	2.06

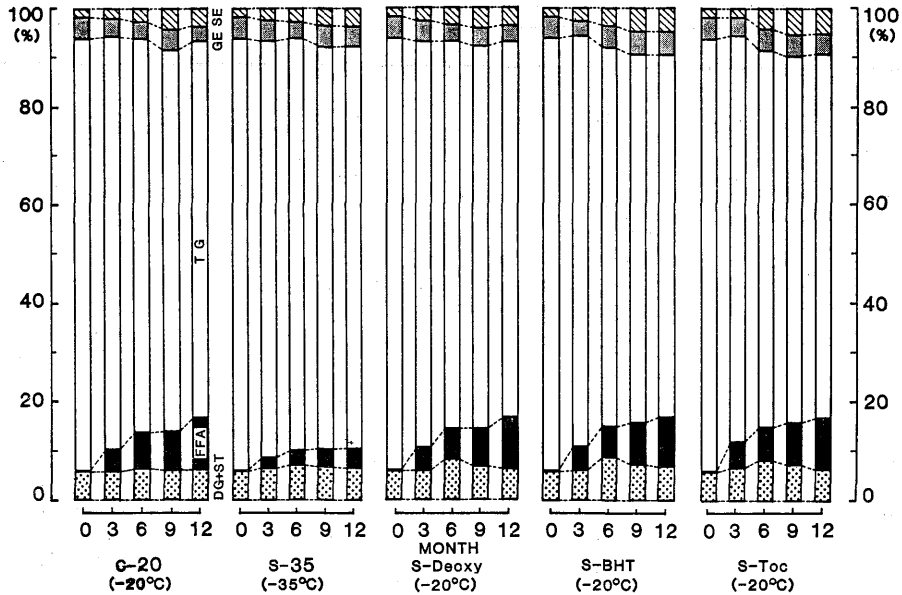


Fig. 3. Changes in the NL class compositions of Japanese oyster during storage.

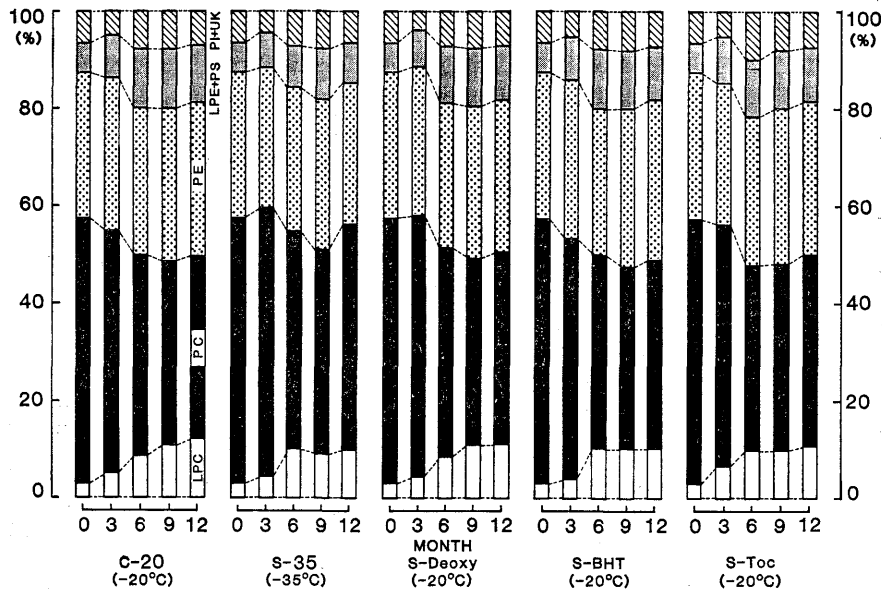


Fig. 4. Changes in the PL class compositions of Japanese oyster during storage.

diacyl glycerylether (GE), steryl ester (SE), and FFA. Before storage, the TG was most prominent lipid class in the sample, accounting for 87.8% of the NL, while DG+ST (mostly ST), GE, SE, and FFA were minor components. Percentages of the TG decreased in all samples during storage for 12 months and those of FFA increased. The decreasing rate of TG in S-35 was lowest among the samples throughout the storage period.

Percentage of the accumulated FFA in S-35 after 12-month storage was about one third of the percentages of samples stored at  $-20^{\circ}\text{C}$ . The lipid classes other than TG and FFA did not show noticeable changes in their percentages during storage.

The PL consisted of 7 lipid classes; PC, PE, LPC, PS+lyso-PE (LPE), PI, and unidentified component (UK). The PC and PE were pro-

**Table 3.** Fatty acid compositions of TL, PL, and NL of Japanese oyster\*1 (%)

Fatty acid	TL	PL	NL
14:0	4.90±0.06	4.06±0.11	5.63±0.06
15:0 iso	0.12±0.01	0.13±0.03	0.14±0.00
15:0 anteiso	0.05±0.01	0.16±0.03	0.06±0.01
15:0	0.60±0.01	0.71±0.03	0.66±0.01
16:0 iso	0.12±0.01	0.21±0.03	0.12±0.01
16:0	18.5 ±0.20	15.7 ±0.52	20.7 ±0.53
17:0 iso	0.43±0.04	0.45±0.02	0.49±0.05
17:0	1.14±0.03	1.17±0.03	1.19±0.02
18:0 iso	0.34±0.01	0.25±0.01	0.35±0.01
18:0	3.46±0.14	5.75±0.10	3.28±0.10
19:0	tr*2	tr	tr
20:0	0.12±0.03	0.69±0.01	0.12±0.02
Σ Saturated	29.8	29.3	32.7
14:1n-5	0.08±0.00	0.05±0.02	0.09±0.02
15:1n-8	0.02±0.01	0.10±0.04	0.05±0.01
16:1n-7	4.18±0.15	2.11±0.09	4.71±0.26
16:1n-5	0.22±0.01	0.22±0.02	0.27±0.04
17:1n-8	0.21±0.12	0.41±0.03	0.45±0.01
18:1n-9	2.68±0.02	1.62±0.04	3.07±0.11
18:1n-7	6.15±0.05	5.26±0.25	6.36±0.08
20:1n-11	1.33±0.05	1.11±0.01	1.31±0.03
20:1n-9	0.37±0.04	0.97±0.02	0.22±0.06
20:1n-7	2.89±0.08	4.64±0.05	2.38±0.10
22:1n-9	0.20±0.04	tr	0.22±0.10
Σ Monoenoic	18.3	16.5	19.1
16:2n-7	0.32±0.06	1.23±0.03	0.24±0.02
16:2n-4	0.78±0.02	0.46±0.09	0.87±0.02
16:3n-3	0.11±0.01	tr	0.11±0.00
16:4n-1	0.16±0.02	tr	0.15±0.00
18:2n-7	0.11±0.07	0.08±0.01	0.07±0.03
18:2n-6	1.26±0.07	0.83±0.02	1.35±0.06
16:2n-4	0.70±0.01	0.43±0.01	0.74±0.02
18:3n-6	0.06±0.01	0.05±0.04	0.26±0.01
18:3n-4	0.38±0.02	0.22±0.04	0.47±0.03
18:3n-3	1.16±0.03	0.74±0.03	1.19±0.03
18:3n-1	0.09±0.01	tr	0.11±0.01
18:4n-3	3.39±0.14	1.66±0.02	3.53±0.05
18:4n-1	0.15±0.01	tr	0.16±0.00
19:3n-6	0.32±0.04	1.66±0.06	tr
20:2NMID (5, 11)	0.18±0.06	0.22±0.01	0.27±0.05
20:2NMID (5, 13)	1.19±0.21	0.62±0.04	1.38±0.04
20:2NMID	0.08±0.02	tr	0.12±0.06
20:2n-6	0.14±0.01	0.15±0.04	0.12±0.02
20:3n-6	0.16±0.02	0.15±0.01	0.14±0.02
20:4n-6	2.00±0.04	3.02±0.12	1.65±0.01
20:3n-3	0.02±0.01	tr	0.12±0.03
20:4n-3	0.67±0.10	0.61±0.01	0.54±0.00
20:5n-3	22.4 ±0.76	19.8 ±0.18	21.3 ±0.35
22:2NMID (7, 13)	0.41±0.06	1.08±0.03	0.24±0.07
22:2NMID (7, 15)	2.35±0.04	4.49±0.20	1.79±0.44
21:5n-3	1.17±0.05	1.30±0.03	1.19±0.06
22:5n-6	0.11±0.07	0.52±0.12	0.27±0.04
22:5n-3	1.03±0.21	1.60±0.10	0.76±0.08
22:6n-3	11.0 ±0.16	13.4 ±0.48	8.96±0.46
Σ Polyenoic	51.9	54.3	48.1

\*1 The data are presented as the mean ± standard deviation of 4 (2 groups × 2) determinations.

\*2 'tr' denotes &lt;0.01%.

minent lipid classes, accounting for 54.3% and 29.9% of PL, respectively, whereas LPC, PS+LPE, and PI+UK were minor lipid classes, as already shown previously.<sup>13)</sup> In C-20, percentages of PC decreased markedly during storage for 6 months. The LPC increased considerably throughout the storage period, although its initial content was very low. The PS+LPE also increased during storage for up to 6 months mainly due to increase in LPE, and thereafter decreased. The percentage of PE remained almost unchanged during storage. However, PE seemed to be hydrolyzed gradually during storage because of the increase in LPE. It has been known that the affinities of lipolytic enzymes to ether phospholipids are lower than those to ester phospholipids<sup>17)</sup> and that PE of the Japanese oyster is rich in ether phospholipids.<sup>13)</sup> This may be one of the reasons for the lower decreasing rate of PE observed in the present study. The tendency of changes in the lipid class compositions of PL in the other samples was similar to that of C-20. However, decreasing rate in the percentage of PC was markedly lower in S-35 than in C-20 during storage. Many workers<sup>18-20)</sup> observed that LPC and LPE do not accumulate in fish fleshes during low temperature storage, in spite of the gradual decreases in PC and PE. On the basis of these observations, they postulated that the activity of lysophospholipase in fish fleshes was higher than that of phospholipase

A<sub>2</sub>. However, Braddock and Dugan<sup>21)</sup> and Ohshima *et al.*<sup>22-25)</sup> reported that LPC and LPE accumulated in the fleshes of certain species of fishes such as coho salmon, skipjack, and sardine during low temperature storage. In the present study on the oyster, decreases in PC and increases in LPC were observed in all of the samples. The percentages of FFA increased in all of the samples in varied degrees with the duration of storage and the increases were considered due to hydrolyses of both the PL and TG; PC seemed to be hydrolyzed at higher rate than TG. This suggests that, when oyster is stored at -35°C, the activity of lipase in the oyster is suppressed much more than that of phospholipase.

#### Changes in Fatty Acid Compositions

Table 3 shows the fatty acid compositions of TL, PL, and NL in fresh oyster used in the present study. Prominent fatty acids of the PL were similar to those of the NL; 20:5n-3, 16:0, and 22:6n-3. The PL, however, was rich in longer fatty acids such as 22:6n-3, 18:0, 20:1n-7, and 22:2NMID and the NL shorter fatty acids such as 16:0, 14:0, and 16:1n-7. The NL was rich in 20:5n-3 compared with the PL. Figure 5 shows changes in the percentages of saturated, monoenoic, and polyenoic acids of TL, PL, and NL in C-20, S-35, S-Deoxy, S-BHT, and S-Toc during storage.

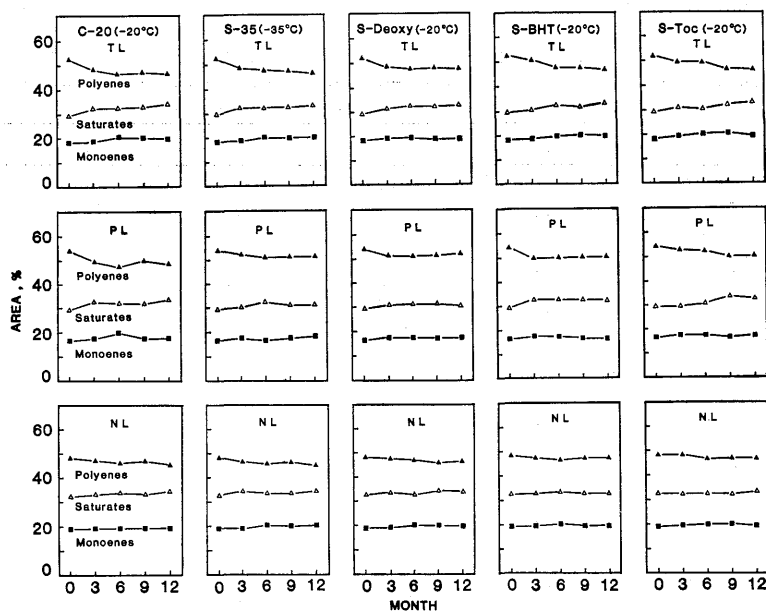


Fig. 5. Changes in the saturated, monoenoic, and polyenoic fatty acids of TL, PL, and NL in Japanese oyster during storage.



The percentages of the saturated and monoenoic acids of the oyster samples before storage were higher in the following order; NL>TL>PL, differing from those of polyenoic acids; PL>TL>NL. During storage, in all the sample percentages of the polyenoic acids in PL, NL, and TL decreased and those of the saturated acids increased. The decrease of the total polyenoic acids was due to decrease in 20:5n-3 and 22:6n-3 during storage. The increase in saturated acids was mainly due to the increase in 16:0. These changes in the fatty acid compositions during storage were more remarkable in the PL than in the NL. Decreases in the percentages of polyenoic acids and increases in those of saturated acids occurred at higher rates in C-20 than in S-35 and S-Deoxy. These changes in percentages of S-BHT and S-Toc were intermediate between those of C-20 and S-35. These results show that the antioxidants, such as natural vitamin E and BHT, are effective to some extent on inhibition of lipid oxidation. Percentages of polyunsaturated acids (PUFA, 20:5n-3+22:6n-3) in PL decreased readily from 33.2% to 26.6% in C-20 and slowly from 33.2% to 30.6% in S-35 during storage. Decreasing rates in percentages of PUFAs in these samples were high in the following order; C-20>S-Toc>S-Deoxy>S-BHT>S-35. However, the percentages of PUFAs (20:5n-3 and 22:6n-3) in NL remained almost unchanged throughout the storage period. Therefore, the PUFAs released from PL seemed to be oxidized to some extent

during storage. Ratios of the percentages of 20:5n-3+22:6n-3 to 16:0, which were used as a measure of lipid oxidation, decreased at a higher rate in the C-20 than in the S-Deoxy (Fig. 6). The rate of decrease in the ratio in the S-35 was intermediate between those of C-20 and S-Deoxy.

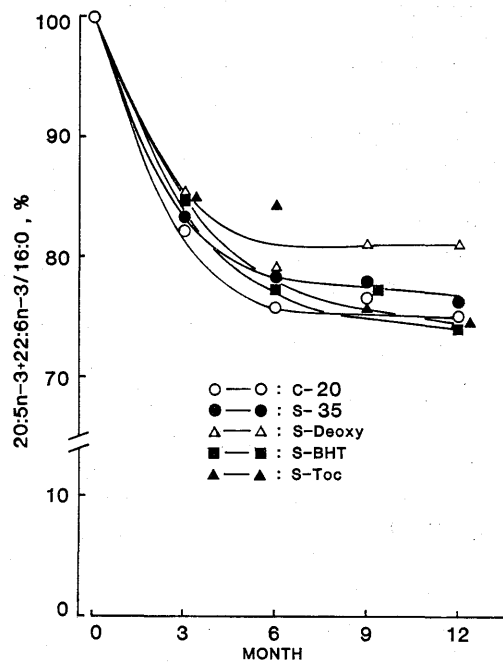


Fig. 6. Decreasing rates in the ratios of percentage of the 20:5n-3+22:6n-3 to that of 16:0 of TL in Japanese oyster during storage.

Table 4. Changes in the sensory scores of Japanese oyster during storage

Sample		Storage period, month				
		0	3	6	9	12
C-20	Color	5.0	4.0	3.8	3.5	3.0
	Flavor	5.0	4.5	4.0	3.5	3.3
	Sum	10.0	8.5	7.8	7.0	6.3
S-35	Color	5.0	4.5	4.5	4.5	4.5
	Flavor	5.0	4.5	4.5	4.3	4.0
	Sum	10.0	9.0	9.0	8.8	8.5
S-Deoxy	Color	5.0	4.8	4.5	4.5	4.5
	Flavor	5.0	4.8	4.8	4.5	4.0
	Sum	10.0	9.6	9.3	9.0	8.5
S-BHT	Color	5.0	4.5	4.0	4.0	3.5
	Flavor	5.0	4.5	4.0	3.5	3.3
	Sum	10.0	9.0	8.0	7.5	6.8
S-Toc	Color	5.0	4.5	4.0	4.0	3.5
	Flavor	5.0	—*	—	—	—
	Sum	10.0	—	—	—	—

\* See the text.

In the case of the samples with added antioxidants, S-Toc and S-BHT, the decreasing rates were similar to the decreasing rate in C-20 in advanced stage of storage. These results suggested that lipid oxidation progressed at higher rate in C-20; lipid in S-Deoxy was relatively stable for oxidation, though the both samples, C-20 and S-Deoxy, showed similar stabilities for lipid hydrolysis.

From these results, it was suggested that quality of the Japanese oyster stored at  $-35^{\circ}\text{C}$  was kept highest in regard to lipid hydrolysis during storage. Furthermore, deoxygenizer more effectively prevented lipid oxidation of the oyster than antioxidants such as S-Toc and BHT during storage at  $-20^{\circ}\text{C}$ . S-Toc acted effectively as an antioxidant on the oyster lipids compared with BHT during storage for up to 6 months.

#### Sensory Evaluation

Table 4 shows the changes in sensory score of the samples during storage. The scores of the samples after storage for 12 months were 8.5 for S-35 and S-Deoxy, 6.8 for S-BHT, and 6.3 for C-20 sample. No sensory test could be done on S-Toc sample in this time, because of interference of the added antioxidant, *dl*-alanine and natural vitamin E. Quality of the sample rated high in the sensory evaluation was also evaluated high from the lower degree of lipid deterioration, *i.e.*, hydrolyses of PC and TG and oxidation of PUFAs. Consequently, the quality of the Japanese oyster in regard to lipid deterioration might be warranted for at least 12 months, when they were frozen, ice-glazed, packed in air-tight film, and stored either at  $-35^{\circ}\text{C}$  or at  $-20^{\circ}\text{C}$  after enclosing deoxygenizer.

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