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Effect of Deoxygenizer on the Suppression of Lipid Deterioration of Boiled and Dried-Anchovy *Engraulis japonica*

I. Changes in Lipid Class Compositions

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Anchovy *Engraulis japonica* boiled and dried was stored at 20℃ for 5 months after that treatment of sodium-erythorbate (Na-ery) or deoxygenizer (Deoxy). During storage, peroxide value (POV), thiobarbituric acid (TBA) value, lipid content, and lipid class compositions were determined to evaluate the quality of the samples.

POV was decreased rapidly for the first 3 months storage and its decrease was Deoxy group>Control group>Na-ery group in that order. TBA values increased for the first 4 months and then decreased rapidly, and it's increase was the highest in Control group, followed by Na-ery and Deoxy group. Total lipid contents in all samples declined during storage. Especially, phospholipid decreased mainly in Na-ery and Deoxy group, while neutral lipid mainly in Control group. Triglyceride (TG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) decreased, while free fatty acid (FFA) and lyso-PC (LPC) increased during storage. The decrease of TG was the highest in Control group and that of PE and PC was higher in Na-ery group than in other sample. The decrease of PE in all samples (except Deoxy group) was higher than that of PC. The increase of FFA and LPC were higher in Control and Na-ery group than in Deoxy group.

These results indicated that the lipid deterioration of the boiled and dried-anchovy was effectively suppressed by the enclosed deoxygenizer during storage at 20℃.

Key words : boiled and dried-anchovy, lipid deterioration, deoxygenizer, sodium erythorbate

Introduction

It has been well known that the boiled and dried-anchovy is an important marine dried food from the ancient in Korea. Anchovy are caught usually by a powered anchovy dragnet and its fisheries period is 8 months from July to February of the next year. Therefore, the boiled and dried-anchovy are commercialized in markets for about four to five months. During the distribution in markets. the anchovy often brings about serious quality deterioration, including lipid oxidation. Lipid oxidation of fish occurs during heating (Koizumi et al., 1986), drying (Nakamura et al., 1978: Lee et al., 1987; Takiguchi, 1986, 1987; Ro, 1988), and storage (Han et al., 1973; Yu and Lee, 1982; Takiguchi, 1992).

Because the fish, containing high levels of polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid and docosahexaenoic acid, are highly susceptible to oxidation (Cho et al, 1987; Fritsche and Johnston, 1988; Jeong et al., 1994). Therefore, effects of various antioxdants have been studied so far (Lee et al., 1965; Ke et al., 1977; Tsukuda, 1980). In recent year, the use of deoxygenizer has been recommended by several workers for the purpose of suppressing lipid oxidation in some foods. They reported that the deoxy-

genizer exhibited strong antioxidative effects on the semi-dried fish (Uchiyama et al., 1980), sardine (Suzuki et al., 1985), and oyster (Jeong et al., 1990) lipids during storage.

There is a little information on the the effects of antioxidants or packing materials on the lipid oxidation of the boiled and dried-anchovy during storage (Lee et al., 1985; Ro et al., 1987). In the present study, the boiled and dried-anchovy was investigated in regard with lipid oxidation and its suppression, using deoxygenizer and Na-erythorbate, during storage at 20℃.

Materials and Methods

Sample

Anchovy *Engraulis japonica* (body length, 6.5~8.3 cm; body weight, 1.07~2.45 g) were caught by a powered anchovy dragnet on the sea near Ulsan in Feb., 1993. The anchovy sample was immediately boiled with 5% NaCl solution on the ship and the boiled anchovy were transported to the laboratory, and then sun-dried for about 24 hrs. A portion of the anchovy sample was also transported in ice box to the laboratory and used as a raw sample. The sample preparations were summarized in Table 1; a portion of the boiled and dried-anchovy was treated with Na-erythorbate (Na-ery group), by spraying to give 0.02% of the sample weight and successively sun-dried for about 6 hrs. The remaining samples were not treated with antioxidant (Control and Deoxy group).

Na-ery and Control group were put into pouches of polyethylene film (PE, 60 μm in thickness) and the openings were heat-sealed.

In the case of deoxygenizer (Ageless, ZD-100, Mitsubishi Gas Chemical Co., Inc.), OPP/Al/PE film (20/7/50 μm, in thickness) was used. Duplicated samples (groups 1 and 2, and weighting about 80 g each) were stored at 20℃ for 5 months. All results showed as the mean value of four different analyses (two group x two times).

Lipid extraction

Total lipid (TL) was extracted with chloroform/methanol mixture solution according to the Bligh and Dyer procedure (1959). Content of TL was determined gravimetrically.

Determination of TBA value and POV

TBA value was determined according to the intact sample procedure of Sinnhuber and Yu (1977). POV was determined by A. O. C. S. official method Cd 8-53 (1990).

Determination of lipid class compositions of PL and NL

Lipid class compositions of phospholipid (PL) and neutral lipid (NL) were determined according to the method described previously (Jeong et al., 1993), using Chromarod S-III and an Iatroscan MK-5 TLC/FID analyzer (Iatron Laboratories Inc., Tokyo, Japan).

Briefly, an aliquot of the chloroform solution of TL was spotted on the Chromarods with a single spotting action, using Drummond Microcap disposable pipets (1 μl, Drummond Scientific Co., Broomall, Pa., USA).

Table 1. Storage conditions of the boiled and dried-anchovy

Sample group	Sample treatment	Packing material	Storage Temp.
Control	Untreated	PE(60μm)	20℃
Na-Ery	0.02% Na-erythorbate	PE(60μm)	20℃
Deoxy	Deoxygenizer (Ageless Z, D-100)	OPP/Al/PE (20/7/50μm)	20℃

The Chromarods were placed in a constant humidity tank over saturated sodium chloride solution for 10 min and then immediately transferred to the developing tank. The solvent system for NL was a mixture of n-hexane/diethyl ether/formic acid (97 : 3 : 1, v/v/v).

In the case of PL, acetone and a mixture of chloroform/methanol/water (65 : 35 : 4, v/v/v) were used as the solvent system. The Chromarods were heated for 2 min in an oven at 115℃ and were scanned using an Iatroscan MK-5. The air and hydrogen flow rates for the Iatroscan analyzer were 2,000 ml/min and 160 ml /min, respectively, and the scan speed was set at 30 sec/scan. Each peak component that appeared on a Iatrocoder TC-II (Iatron Laboratorries Inc., Tokyo, Japan) was calculated from standard curves previously obtained using authentic lipids.

Results and Discussion

Lipid components of raw, and the boiled and dried-anchovy

The lipid contents, lipid class compositions, POV, and TBA values of raw, and boiled and dried-anchovy are summarized in Table 2. The contents of TL, PL, and NL in raw anchovy were 1.38%, 1.02%, and 0.36 %, respectively. In the case of the boiled and dried-anchovy, 6.06%, 3.77% and 2.29% for TL, PL and NL, respectively. PL classes of raw anchovy were found to be PC (57.6%), PE (32.3%), cardiolipin+phosphatidic acid (CA+PA, 4.39%), sphingomyelin (SPM, 3.87%) and LPC (1.59%). Comparing with raw anchovy, the percentages of PE and PC in the boiled and dried-anchovy were low level, while those of CA+PA, LPC and SPM were high level. Particulary, PE was small about 10% compared with that of raw anchovy, while CA+PA was large about 10%. This might be due to generation of PA by heat during boiling process (Koizumi et al., 1986).

Table 2. Lipid compositions of raw, and boiled and dried-anchovy (%)

Lipid	Raw	Dried
TL	1.38	6.06
PL	1.02(74%)*1	3.77(62%)
CA+PA	4.39*2	14.9
PE	32.3	22.5
PC	57.6	55.6
SPM	3.87	4.13
LPC	1.59	2.93
NL	0.36(26%)	2.29(38%)
SE	5.40*2	3.92
TG	23.2	54.0
FFA	20.3	6.05
ST	51.0	36.1
POV	21.9meq/kg	95.2meq/kg
TBA value	4.25meq/kg	9.44mg/kg

*1 Figures in parentheses show the percentages in TL content.
*2 Values are presented as the percentages of PL and NL contents.

On the other hand, NL classes found in raw anchovy were free sterol (ST, 51.0%), TG (23.3%), FFA (20.3%) and steryl ester (SE, 5.40%). After boiling and sun-drying, the percentage of TG was 54.0%, while those of FFA and ST were 6.05% and 36.1%, respectively. It was of specific that FFA existed to a great extent in raw anchovy. This is uncertain that either a lot of FFA inherently exists in the fish (mainly in viscera) or the lipid was hydrolyzed by endogenous enzyme system during transportation and/or fishery work. Koizumi et al. (1986) reported that the contents of PE and PC in the minced bigeye tuna decreased during thermal processing at 115℃, while those of LPC and TG increased without change of FFA. Therefore, they pointed out that PE and PC of bigeye tuna were hydrolyzed on thermal processing and that the level of FFA unchanged was due to simultaneous oxidation and heat decomposition of FFA and PL during thermal processing. A similar result was also

reported in dried marckerel (Shimizu and Kaneda, 1969).

Changes in POV and TBA value

POV indicating primary oxidation product was 4.3-folds higher in the boiled and dried-anchovy than that in raw anchovy, and TBA value indicating secondary oxidation product, malonaldehyde, was also 2.2-folds higher in the former (Table 2). Takiguchi (1986, 1987) also reported that POV of the boiled and dried-anchovy increased rapidly after drying for 20 hrs. These results indicate that boiling and sun-drying processing for raw anchovy influenced strongly against its lipid deterioration.

As shown in Fig. 1, POV decreased rapidly in Control and Deoxy group and slowly in Na-ery group with duration of storage. In general, POV in dried fish is rapidly increased during drying (Takiguchi, 1986, 1987; Lee et al, 1987; Ro, 1988). This is considered due to the production of peroxides, which are mainly generated in the early stage of lipid oxidation. In the present study, high POV after drying was estimated due to lipid oxidation occurred in boiling and sun-drying processing. The decrease of POV during storage was seemed to be resultant of the decomposition of peroxides more than production of them.

However, a decrease of POV were high in the following order; Deoxy group>Control group>Na-ery group. Therefore, deoxygenizer suppressed effectively the increase of POV of the lipid by removing the oxygen in the package during storage (Uchiyama et al., 1980; Suzuki et al., 1995). On the other hand, Control group showing the lower POV than Na-ery group is seemed to be proceeded much fast decomposition of peroxides compared with the latter. This result might be elucidated from the theory that peroxide-decomposing property of TL depend upon PL content (Lee et al., 1981; Lee, 1984). In fact, Control group has been kept higher PL content during storage than Na-ery group.

On the other hand, TBA values increased up to

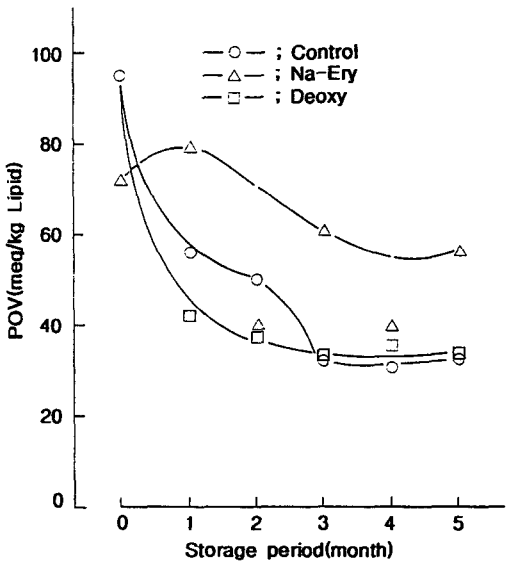

Fig. 1. Changes in POV of TL in the boiled and dried-anchovy during storage.

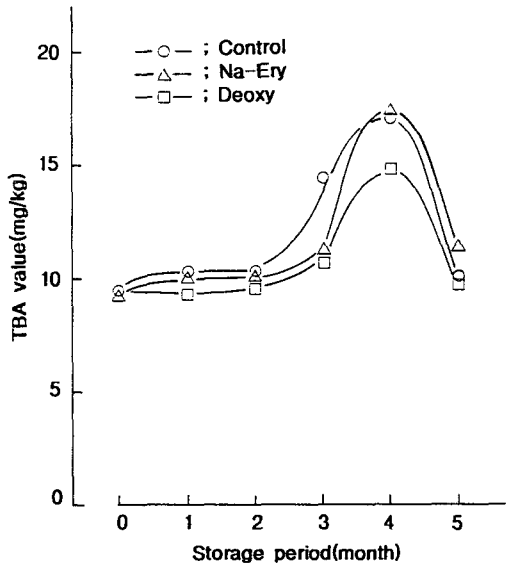

Fig. 2. Changes in TBA value of the boiled and dried-anchovy during storage.

4 months, but after that, the values decreased in all samples (Fig. 2). This indicates that malonaldehyde was generated successively up to 4 months of storage. The increase rate of TBA value was the fastest in Control group, followed by Na-ery and Deoxy group.

From these results, the degree of lipid oxidation was the lowest in Deoxy group of all samples. Therefore, it showed that the deoxygenizer suppressed most effectively in lipid oxidation of the boiled and dried-anchovy during storage.

Changes in lipid contents

Changes in TL, PL and NL contents during storage are shown in Table 3. The contents of TL, PL and NL before storage were 6.06%, 3.77% and 2.29%, respectively, in Control and Deoxy group, and 5.78%, 3.66% and 2.12%, respectively, in Na-ery group. TL contents decreased in all samples during storage, and its decreased level was 12% for Control group, 7% for Na-ery group, and 3% for Deoxy group after 5 months of storage. PL contents also decreased in all samples with duration of storage.

The decrease of PL was 13% for Na-ery group, 10 % for Control group, and 8% for Deoxy group. On the other hand, NL content of Control group decreased 19% compared to the before storage, while those of Deoxy and Na-ery groups unchanged almostly. These results, therefore, indicate that deoxygenizer inhibited effectively for lipid deterioration compared with sodium-erythorbate.

Table 3. Changes in the lipid contents of the boiled and dried-anchovy (%)

Storage months	Lipid class	Sample		
		Control	Na-Ery	Deoxy
0	TL	6.06	5.78	6.06
	PL	3.77	3.66	3.77
	NL	2.29	2.12	2.29
1	TL	5.89	5.79	5.99
	PL	3.78	3.84	3.71
	NL	2.11	1.95	2.28
2	TL	5.48	5.47	5.58
	PL	3.66	3.39	3.60
	NL	1.82	2.08	1.98
3	TL	5.40	5.37	5.44
	PL	3.54	3.18	3.26
	NL	1.86	2.19	2.21
4	TL	5.34	5.32	5.62
	PL	3.48	3.09	3.55
	NL	1.86	2.23	2.07
5	TL	5.31	5.35	5.86
	PL	3.45	3.18	3.48
	NL	1.86	2.17	2.38

Changes in PL class compositions

As shown in Iatroscan chromatograms (Fig. 3), PL classes were changed significantly in all samples during storage. The major components, PE and PC were decreased, while the major components such as LPC, CA+PA, and SPM were increased slightly.

Changes in the prominent components are shown in Fig. 4. The percentage of PC apparently seems to be unchanged in all samples, but the amounts practically decreased because of decreasing of PL contents; decreased 349 mg in Na-ery group, 231 mg in Deoxy group and 192 mg in Control group after storage for 5 months. The percentage of PE also decreased in all samples and the amount decreased after 5 months storage was 375 mg in Na-ery group, 310 mg in Control group and 20 mg in Deoxy group.

Thus, the decreasing rates of PE in Control and Na-ery group were higher than those of PC. Similar results have been reported in halibut (Koizumi et al., 1986), salted-dried mackerel (Shimizu and Kaneda, 1969) and salted-dried yellow corvenia (Ro, 1988). On the other hand, the amount of LPC increased in all samples during storage. The increasing rate of LPC showed differences in the samples; Deoxy group was the lowest and Na-ery group was similar to Control group. In general, LPC is generated from PC by phospholipase. In the present study, the increase of LPC suggested the possibility of presence of phospholipase, in spite of inactivity of enzyme by boiling. The increase of LPC was also observed in the previous works (Takiguchi, 1987; Ro, 1989).

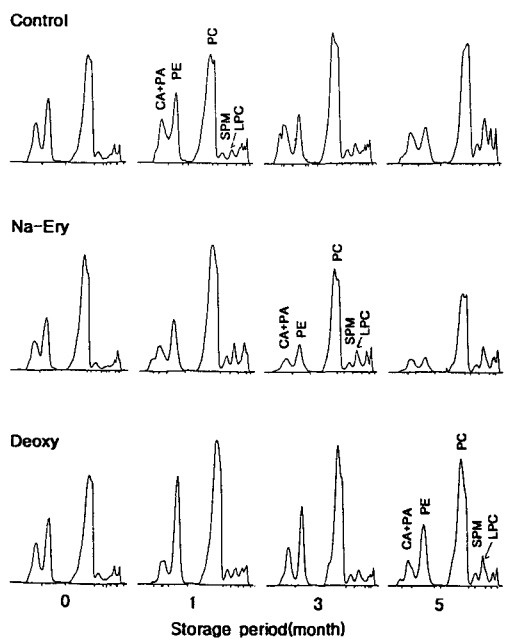

Fig. 3. Changes in Iatroscan chromatograms of PL in the boiled and dried-anchovy during storage.

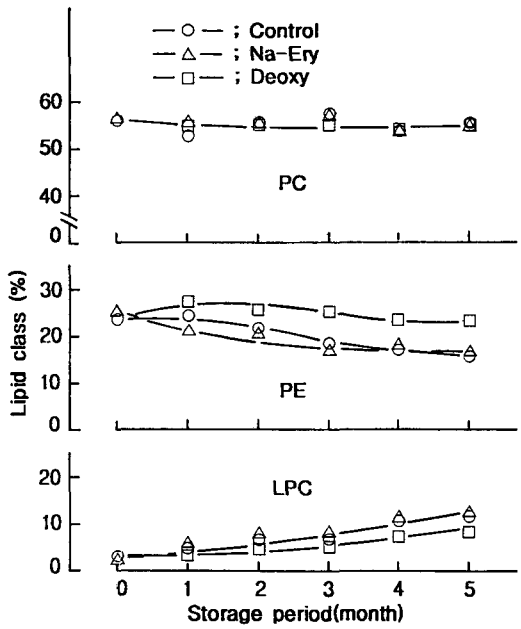

Fig. 4. Changes in PC, PE and LPC of PL in the boiled and dried-anchovy during storage.

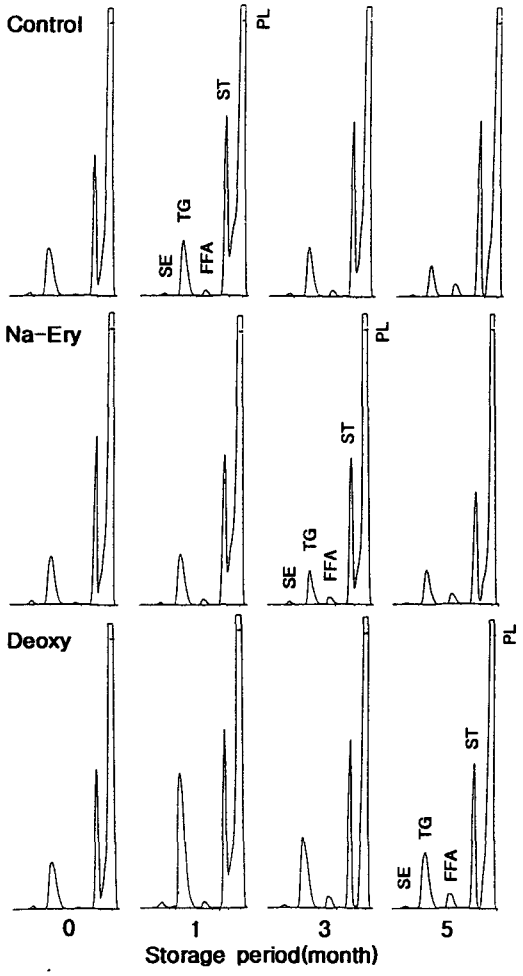

Fig. 5. Changes in Iatroscan chromatograms of TL (NL) in the boiled and dried-anchovy during storage.

Changes in NL class compositions

Fig. 5 shows Iatroscan chromatograms of NL during storage. TG decreased in all samples during storage, while FFA and sterol increased. Changes in TG and FFA during storage are shown in Fig. 6. During storage, the percentage of TG decreased rapidly in all samples, except that of Deoxy group. The percentage of FFA, however, increased in all samples with duration of storage. The TG in Control group decreased a great amount after 5 months of storage. However,

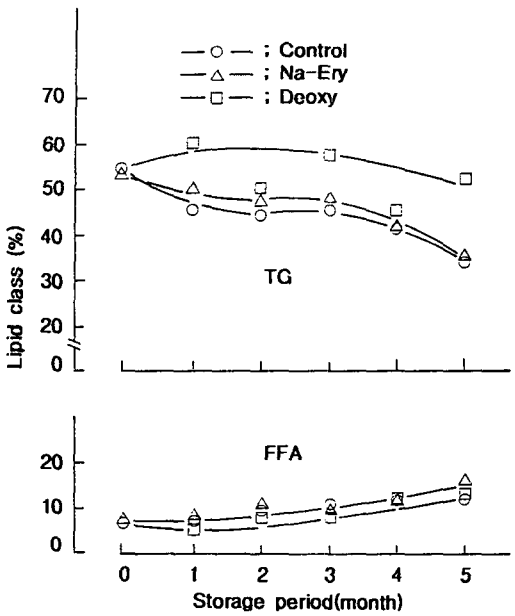

Fig. 6. Changes in TG and FFA of NL in the boiled and dried-anchovy during storage.

the TG of Deoxy group remained unchanged with duration of storage. On the other hand, the increasing level in FFA was apparently low in Deoxy group compared with Na-ery and Control groups. However, the increasing amount of FFA in the samples were as follows; 80 mg for Control group, 219 mg for Na-ery group and 164 mg for Deoxy group after 5 months of storage. Therefore, these results showed that Control group was lost a great amount of FFA by oxidation during storage, while Deoxy and Na-ery groups were suppressed the loss of FFA compared with Control group.

In general, lipid oxidation and hydolysis occurs simultaneously during heating, drying and storing of fish. In a microscopic observation on the anchovy meat (Takiguchi, 1986), it was suggested that the TG distributed on the surface portion of the fatty anchovy meat was oxidized in an early stage of drying and that the oxidized TG penetrated into the inner portion of the muscular tissue to come in contact with the PL and participated in its oxidation. In the present study, lipid oxidation in Control group showed a similar pattern to the case of the fatty anchovy reported by Takiguchi (1986), because the decrease of PL was observed after drying, and that of TG and PL was also observed during storage.

From these results, lipid deterioration of the boiled and dried-anchovy occurred at a great extent in drying process and the early stage of storage, and then slowly during storage. The lipid deterioration of the boiled and dried-anchovy was effectively suppressed by the enclosed deoxygenizer during storage at 20℃ and slightly suppressed by treatment of sodium-erythorbate.

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