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Oxidative stabilities of triacylglycerol and phospholipid fractions of cooked Japanese sardine meat during low temperature storage

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Abstract

Changes in triacylglycerols (TAG) and phospholipids (PL) compositions of cooked Japanese sardine meats as such or with prior addition of ethylene diaminetetracetic acid (EDTA) or a combination of nitrite and ascorbate were evaluated during chilled storage using gas chromatography and selected ion monitoring gas chromatography/mass spectrometry. The TAG molecular species compositions remained unchanged, while certain species of PL molecular species changed during storage at 2 °C for 14 days. The PL containing polyunsaturated fatty acids were highly susceptible to autoxidation. The PL fractions play an important role in oxidative rancidity and development of off-flavor in cooked sardine meat, while TAG fraction plays a minor role in the oxidative deterioration of the meat. Changes in peroxide and thiobarbituric acid values were also monitored. Added EDTA was not effective in controlling the decomposition of lipid hydroperoxide throughout the storage period. However, a combination of $NaNO_2$ and ascorbate not only suppressed the formation but also the decomposition of the primary oxidation products.
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1. Introduction

The intramuscular lipids in meat exist in close association with proteins and contain a large percentage of phospholipids (PL). Approximately 0.43–1.0% of the tissue weight in beef muscle is composed of PL (Hornstein, Crowe, & Heimberg, 1961). Although the percentage of PL in beef muscle tissue is quite low, the susceptibility to autoxidation and the development of oxidative rancidity make them important in governing the quality, i.e., off-flavor, off-odor and discoloration of beef tissue (Igene, Pearson, Dugan, & Price, 1980; Younathan & Watts, 1960). Convincing evidences for the involvement of PL in oxidized off flavors or warmed-over flavor (WOF) have been investigated by several researchers. Isolated PL from cooked turkey consumed oxygen at a faster rate than its non-polar lipid fraction (Acosta, Marion, & Forsythe, 1966). Hornstein et al. (1961) reported that upon exposure to air, the PL extracted from pork and beef tissues developed rancid off-flavors much faster than neutral fats. Pikul, Leszczynski, and Kummerow (1984) claimed that PL contribute to the formation of approximately 90% of thiobarbituric acid reactive substances (TBARS), which exist in total lipid fraction of chicken meat. In model systems, defatted chicken and beef muscle fibers with added PL had higher thiobarbituric acid (TBA) numbers and more pronounced WOF than those with added triacylglycerols (TAG) (Igene & Pearson, 1979). The PL extracted from pork and beef muscles developed rancid off-flavor much faster than neutral lipids upon exposure to atmospheric oxygen (Hornstein et al., 1961). The autoxidation of PL was primarily associated with the oxidative degradation of polyunsaturated fatty acids (PUFA). The decline of PUFA in PL fractions of a variety of meats suggests that the development of oxidized off-flavor in cooked sardine might be associated with the autoxidation of PUFA.

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The susceptibility of PL to oxidation is attributed to their higher concentration of PUFA. It has been believed that both TAG and PL may contribute to the rapid development of oxidative rancidity in fish meats. In sardine meat, the PL fractions contain the highest amount of PUFA compared to the total lipid and non-polar lipid fractions. Thus, the PL of sardine meat may play a very important role in flavor quality deterioration caused by lipid oxidation. However, the influence of PL or TAG in the development of lipid oxidation in fish meats has not yet been well understood.

This study was conducted to evaluate the role of each of the PL and TAG fractions in the lipid oxidation in cooked Japanese sardine meat during storage at 2 °C.

2. Materials and methods

2.1. Chemicals

Phospholipase C form *Bacillus cereus* was purchased from Boehringer Mannheim, GmbH-Yamanouchi Co. (Tokyo, Japan). Ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA) was procured from Wako Pure Chemical Industries, Co. Ltd. (Tokyo, Japan). Sodium nitrite ($NaNO_2$) and sodium L(+) ascorbate were from Kokusan Chemical Co. (Tokyo, Japan). *tert*-Butyldimethylsilyl ether imidazol reagent (*tert*-BDMS) was from GL Sciences Inc. (Tokyo, Japan). 1,2-Dihexadecanoyl-*sn*-glycerol (dipalmitin), 1,2-diacyl-*sn*-glycerol (diacylglycerol) and trimethylsilyl (TMS) ether derivatives were from Sigma–Aldrich Group Co. (Tokyo, Japan). 2-Thiobarbituric acid was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Organic solvents of analytical grade were purchased from Kokusan Chemical Co. (Tokyo, Japan) and used as obtained.

2.2. Sample preparation

Fresh Japanese sardine (*Sardinops melanostict*) were purchased from a local market. Ordinary muscles were removed, chopped twice using a meat chopper. The minced samples were divided into three groups, including a control, an EDTA-added group and a combined use of sodium nitrite and sodium ascorbate ($NaNO_2$–ascorbate) group. Each group consisted of 250 g of the minced meat. To the EDTA group was added 5 mL of EDTA solution to give a final concentration of 250 mg/100 g. A combination of $NaNO_2$ and ascorbate was used to prepare 5 mL of a mixed solution to afford a final concentration of 100 ppm (as NO_2^-) and 2000 ppm, respectively. To the control group was added 5 mL of water. The meat of each group was mixed thoroughly after the addition of the EDTA solution, the mixed solution of $NaNO_2$ and ascorbate, or water, then placed into petri dishes separately, and kept overnight at 2 °C in the dark to complete the reaction between $NaNO_2$ and myoglobin in the meats in the presence of ascorbate. Each group was autoclaved for 15 min at 100 °C and cooled to room temperature, mixed thoroughly, and stored at 2 °C in the dark for 14 days.

2.3. Lipid extraction

Total lipids (TL) were extracted from the cooked sardine meats by the Bligh and Dyer procedure (1959).

2.4. Fractionation of lipids

Phospholipids (PL) and non-polar lipids (NL) were separated from the TL by using Sep-pak silica cartridges (25 mm × 10 mm i.d., Water Associates Milford, MA, USA) as described by Juaneda and Rocquelin (1985). The NL and PL were eluted by chloroform and methanol, respectively.

2.5. Triacylglycerols analyses

Lipid fraction containing TAG of cooked Japanese sardine meat was isolated by silicic acid preparative thin layer chromatography on precoated silica gel 60 plate (20 × 20 cm, 0.25 mm, Merck, Darmstadt, Germany). After spotting the sample, the plate was developed in petroleum ether/diethyl ether/acetic acid (80:20:1, v/v/v). Then, TAG band on the plate was scrapped off and extracted with chloroform/methanol (1:2 v/v) for 30 min. Subsequently, the extract was filtered through a No. 1 filter paper and the solvent was evaporated using a rotary evaporator at 35 °C. The residue was dissolved in chloroform and stored at −30 °C. A small portion of TAG was hydrogenated in chloroform over a catalytic amount of platinum oxide with gentle stirring with a magnetic stirring bar at 0.2 kg/cm^2 of hydrogen pressure for 6 hr. The hydrogenated TAG was subjected to a model GC 12A gas chromatography (Shimadzu Corp. Kyoto, Japan) equipped with a Supercap methyl silicone fused silica open tubular column (0.25 mm i.d. × 25 m, 0.1 m thickness; Quadrex, New Haven, CT, USA). The column oven and injector temperatures were 325 and 390 °C, respectively. Helium was used as carrier gas under a column inlet pressure of 1.75 kg/cm^2.

2.6. Phospholipid molecular species analyses

An aliquot of chloroform solution containing 10 mg of PL was placed in 5 mL screw capped reaction vial and was dried under a nitrogen stream. To the sample were added 1 mL diethyl ether/ethanol (98:2, v/v), 2 mL of 5 mM $CaCl_2$–0.1 M Tris–HCl buffer (pH of 7.2) and 0.1 mL of phospholipase C (400 U/0.1 mL). The sample was incubated for 2 hr at 37 °C with gentle stirring using a magnetic stirring bar. After hydrolysis, the reactants were transferred into a separatory funnel, followed by the addition of 20 mL of diethyl ether and 20 mL of water to extract diacylglycerols (DAG) three times. All of the ether layers were pooled in a 100-mL

flask and dehydrated with Na_2SO_4, followed by filtration and evaporation to dryness in vacuo. The residue was dissolved in a small volume of chloroform and spotted on TLC silica gel plate precoated silica gel 60 plates (20×20 cm, 0.25 mm, Merck, Darmstadt, Germany). Then, the plate was developed in petroleum ether/diethyl ether/acetic acid (170:30:2, v/v/v). For identification of DAG, dipalmitin was used as a standard. The DAG band was scrapped off and extracted with diethyl ether and the extract was filtered. The filtrate was washed with 20 mL of water, and dehydrated with Na_2SO_4 and evaporated to dryness in vacuo. The residue was subjected to reaction with 0.5 mL *tert*-BDMS for 1 h at 80 °C. The reaction products thus obtained were transferred to a separate funnel and extracted with 30 mL of *n*-hexane. The hexane layer was washed with 20 mL of water, dehydrated with Na_2SO_4, filtrated and evaporated. The residue was dissolved in a few drops of *n*-hexane for spotting on a TLC silica gel plate, which was subsequently developed to the same direction twice by toluene. *tert*-BDMS derivative of dipalmitin was used as a standard in TLC analysis. A band corresponding to *tert*-BDMS DAG was scrapped off and was extracted with diethyl ether and the extract was then filtered. The evaporated residue of the filtrate was dissolved in a small volume of *n*-hexane and transferred to a 5-mL screw capped reaction vial. A half portion of *tert*-BDMS DAG was subjected to hydrogenation in *n*-hexane with a catalytic amount of platinum oxide in a glass-tapered round bottom flask with stirring at 0.2 kg/cm^2 of hydrogen pressure for 6 h. The hydrogenated DAG derivative was subjected to gas chromatography using a model 12A gas chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with a Supercap methyl silicone fused silica open tubular capillary column (0.25 mm i.d. $\times$ 25 m, 0.1 μm thickness, Quadrex, New Haven, CT, USA) and flame ionization detector. The column oven and injector temperatures were 250 and 370 °C, respectively. Helium was used as carrier gas under a column inlet pressure of 1.75 kg/cm^2.

2.7. Selected ion monitoring of diacylglycerol tert-BDMS ether derivatives

Selected ion monitoring (SIM) in gas chromatography-mass spectrometry (GC-MS) of the 1,2-diacylglycerol *tert*-BDMS ether derivatives was carried out using a Shimadzu QP-1000 mass spectrometer equipped with MP65HT fused silica open-tubular column (0.25 mm i.d. $\times$ 15 m, 0.1 μm thickness, Quadrex, New Haven, CT, USA). Fragment ions $[M\text{-}57]^+$ generated by the cleavage of the *tert*-butyl group from the molecule and $[RCO + 74]^+$ due to fatty acyl groups of the DAG were selected as the characteristic fragment ions of the *tert*-BDMS ether derivatives of 1,2-diacylglycerol (Satouchi & Saito, 1979) in the SIM (Ohshima & Koizumi, 1991). Electron impact (EI) ionization was carried out at 310 °C ion source temperature and 70 eV ionization voltage. Helium was used as a carrier gas. The column oven and injector temperatures were 250 and 370 °C, respectively. Helium was used as carrier gas under a column inlet pressure of 1.75 kg/cm^2.

2.8. Peroxide value measurement

Peroxide value (PV) of the extracted lipid was measured following the procedure of Buege and Aust (1978) and expressed as milliequivalents of oxygen/kg of lipid (meq oxygen/kg lipid).

2.9. TBA value measurement

Thiobarbituric acid (TBA) value was measured and expressed as mg of malonaldehyde equivalents/kg of meat (mg MA eq/kg meat) according to Shinnhunber and Yu (1977).

2.10. Statistical analysis

Measurements excepting for TAG and PL distributions were carried out in triplicate and the results were expressed as means ± SD in the figures. Microsoft Excel 5.0 was used for all statistical analyses. Data were analyzed using one-way ANOVA, and mean values were compared using Student's *t* test. Differences were considered to be significant at $P < 0.05$.

3. Results

3.1. Lipid contents

Changes in total lipid contents of cooked Japanese sardine meats during storage are shown in Table 1. The amounts of the extracted lipids tended to increase slightly in all groups during the initial 2 days of storage time. This is probably due to the increased recovery of the extraction of lipids caused by changes in fish tissues. However, there were no significant differences in total lipid contents among the three groups.

Table 1
Changes in total lipid contents of cooked sardine meat during storage at 2 °C

Storage time (days)	Total lipid content (%)		
	Control group	EDTA group	$NaNO_2$–ascorbate group
0	2.30 ± 0.50	2.68 ± 0.30	2.22 ± 0.40
2	2.65 ± 1.00	2.68 ± 1.10	2.87 ± 0.80
4	2.61 ± 0.60	2.99 ± 1.20	2.74 ± 0.90
6	2.64 ± 0.50	2.61 ± 0.50	2.71 ± 1.00
8	2.80 ± 0.60	2.63 ± 0.40	2.73 ± 0.40
10	2.68 ± 0.70	2.67 ± 0.60	3.00 ± 0.50
12	2.72 ± 0.40	2.95 ± 0.40	3.06 ± 0.60
14	2.44 ± 0.60	2.66 ± 0.70	2.75 ± 0.80

Mean ± SD ($n = 3$).

3.2. Changes in triacylglycerols

Changes in the compositions of hydrogenated TAG of cooked Japanese sardine meat during storage are shown in Table 2. The predominant TAG in cooked Japanese sardine meats had total acyl carbon numbers (TCN) of 46, 48, 50, 52, 54, 56, 58 and 60. The percentages of TAG molecular species in the control and EDTA groups remained unchanged, while certain molecular species of TAG with TCN 56 and 58 in the $NaNO_2$–ascorbate group slightly increased during storage time.

3.3. Estimation of phospholipids molecular species

A typical gas chromatogram of the hydrogenated DAG *tert*-BDMS ether derivatives obtained from cooked Japanese sardine meat is illustrated in Fig. 1. The hydrogenated DAG derivatives were separated to eight well-resolved

Table 2
Changes in compositions of triacylglycerol in cooked sardine meat during storage at 2 °C

Total acyl carbon numbers	Storage time (days)							
	0	2	4	6	8	10	12	14
Control								
48	7.91	8.17	8.18	8.22	7.80	7.91	7.86	7.79
50	12.92	13.30	13.49	13.02	12.86	13.24	13.93	13.57
52	19.78	17.22	17.11	16.76	17.1	16.98	16.81	16.68
54	17.10	17.04	17.17	17.28	17.14	17.07	16.83	16.87
56	14.59	14.21	14.21	14.21	14.52	14.34	14.55	14.47
58	9.86	9.73	9.69	9.69	9.95	9.93	9.90	10.3
EDTA								
48	7.90	7.99	8.08	8.17	7.94	7.72	7.85	8.09
50	12.96	13.05	13.45	13.11	13.36	12.90	13.69	12.88
52	16.83	16.92	16.95	16.98	16.71	16.73	16.89	17.18
54	16.84	16.95	16.87	16.97	16.67	16.68	17.04	16.83
56	14.23	14.25	14.34	14.28	14.22	15.19	14.54	14.43
58	10.06	9.86	9.66	9.74	9.86	10.24	10.00	9.88
$NaNO_2$–ascorbate								
48	8.07	8.24	8.12	7.96	7.87	7.71	7.45	7.48
50	13.25	13.41	13.08	13.24	12.82	13.00	12.42	13.09
52	16.95	17.12	16.98	17.27	16.75	16.88	16.53	16.79
54	16.77	16.86	16.74	17.19	16.64	16.93	16.96	17.34
56	14.18	14.08	14.25	14.26	14.28	14.34	15.22	14.85
58	9.70	9.58	9.75	9.94	10.00	9.97	10.72	10.07

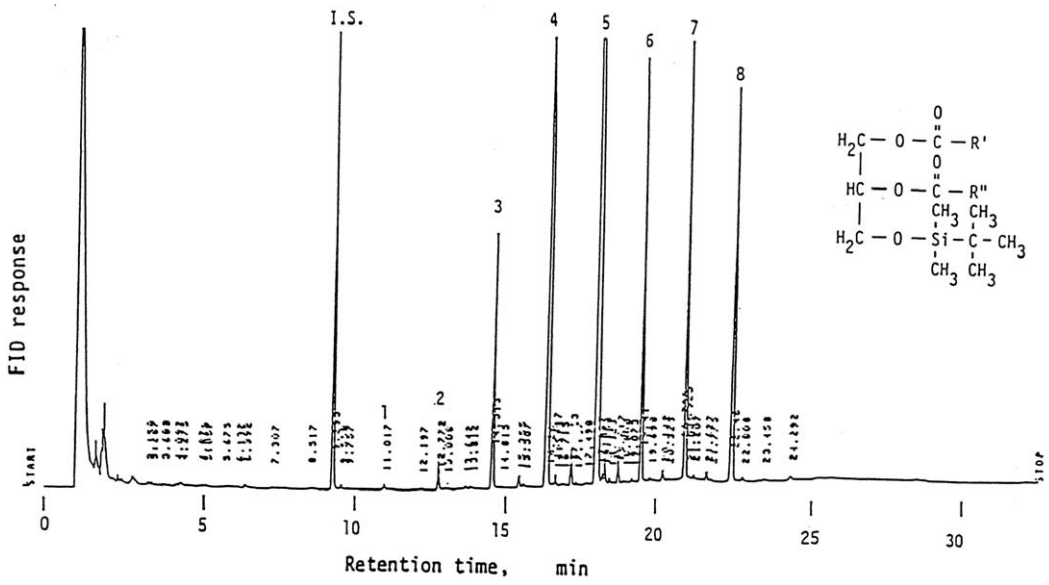

Fig. 1. A typical gas chromatogram of hydrogenated diacylglycerol *tert*-butyldimethylsilyl ether derivatives from polar lipids of cooked Japanese sardine meat.

peaks. In Fig. 2, a typical SIM profile of the same sample as in Fig. 1 is provided. The DAG *tert*-BDMS ether derivatives yielded $[M\text{-}57]^+$ due to a molecular ion and $[RCO + 74]^+$ due to the fatty acyl residue. The number of peak components appeared in the SIM profiles were the same as those in the typical gas chromatogram. As shown in the SIM profile in Fig. 2, peak No. 8 yielded the fragment ions of *m/z* 397 due to C22:0 fatty acyl residue and *m/z* 794 due to $[M\text{-}57]^+$ of hydrogenated DAG *tert*-BDMS ether derivatives with TC = 44 at the same retention time. According to Ohshima and Koizumi (1991), peak No. 8 was certainly identified as C22:0–C22:0 molecular species. In a similar manner, peak Nos.7 and 6 were assigned to C22:0–C22:0 and C18:0–C20:0, respectively. From the fatty acid compositions of the PL fraction of the cooked Japanese sardine meat, TC = 44 species seemed to contain predominantly C22:6*n*−3–C22:6*n*−3. Thus, all peak components were estimated as follows; peak No. 1, C14:0–C16:0; peak No. 2, C16:0–C16:0; peak No. 3, C16:0–C18:0; peak No. 4, C16:0–C20:5*n*−3; peak No. 5, C16:0–C22:6*n*−3; peak No. 6, C18:1*n*−9–C22:6*n*−3 and C20:5*n*−3–C20:*n*−3; peak No. 7, C20:5*n*−3–C22:6*n*−3; peak No. 8 C22:6*n*−3–C22:6*n*−3.

3.4. Changes in phospholipid molecular species

Changes in DAG *tert*-BDMS ether derivative compositions of PL in cooked sardine meat during storage are shown in Table 3. The predominant DAG *tert*-BDMS of cooked Japanese sardine were of TC = 34, 36, 38, 40, 42 and 44. In the control group, the percentages of molecular species of DAG consisting of saturated fatty acids, including TC = 30 (C14:0–C16:0); TC = 32 (C16:0–C16:0) and TC = 34 (C16:0–C18:0) increased during storage time, while those of DAG containing unsaturated fatty acids such as TC = 36 (C16:0–C20:5*n*−3); TC = 38 (C16:0–C22:6*n*−3); TC = 40 (C18:1*n*−9–C22:6*n*−3 and/or C20:5*n*−3–C20:5*n*−3); TC = 42 (C20:5*n*−3–C22:6*n*−3) and TC = 44 (C22:6*n*−3–C22:6*n*−3) significantly decreased. In the EDTA group, the percentages of DAG having TC of 30, 32, 34 and 36 increased, while those of TC of 38, 40, 42 and 44 slightly decreased. In the $NaNO_2$–ascorbate group, the percentages of all DAG species remained fairly constant during storage time.

3.5. Changes in peroxide values

Changes in PV of cooked Japanese sardine meats are shown in Fig. 3. The PV of the control group rapidly increased from 0.78 meq oxygen/kg lipid to 4.47 meq oxygen/kg lipid during the initial 4 days of storage and then increased gradually up to 7.11 meq oxygen/kg lipid. In the EDTA treated group, the PV slowly increased from 0.26 meq oxygen/kg lipid to 2.44 meq oxygen/kg lipid during the initial 10 days of storage. In the $NaNO_2$–ascorbate group, the PV slightly increased from 1.22 meq oxygen/kg

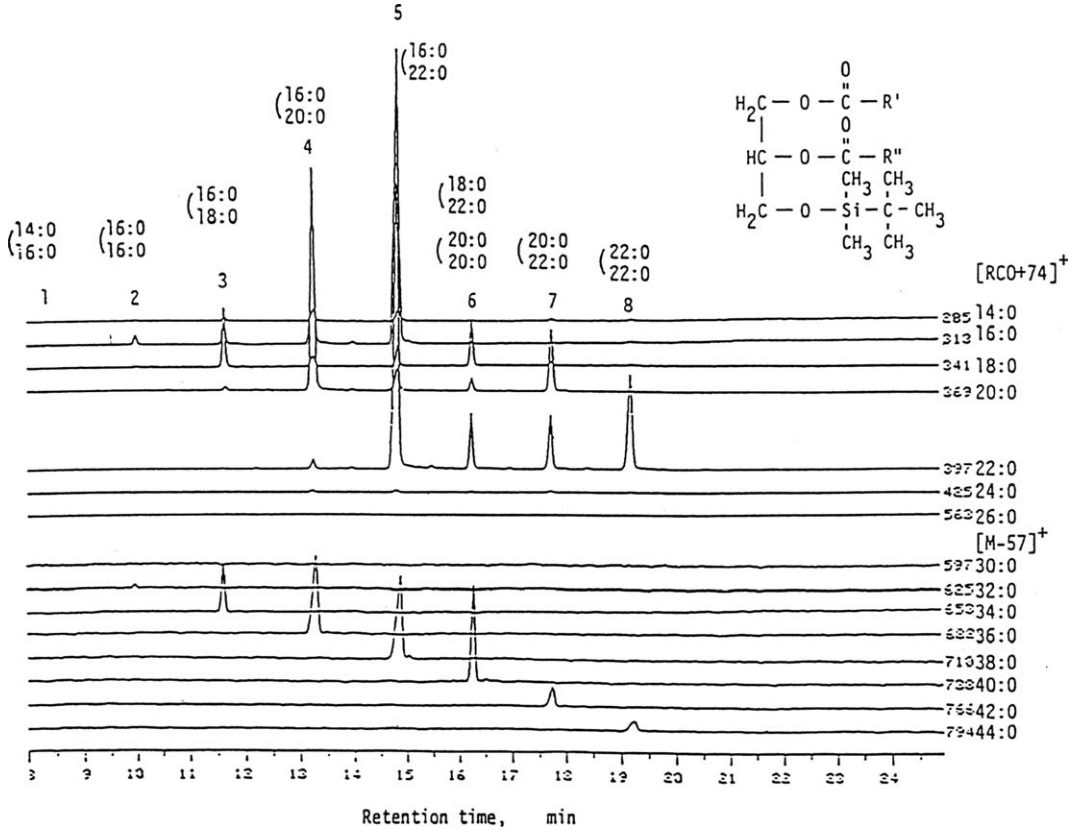

Fig. 2. A typical selected ion monitoring profile of hydrogenated diacylglycerol *tert*-butyldimethylsilyl ether derivatives from polar lipids of cooked Japanese sardine meat.

Table 3
Changes in compositions of diacylglycerol *tert*-butyldimethylsilyl ether derivative of polar lipid in cooked sardine meat storage at 2 °C

Total acyl carbon numbers	Storage time (days)							
	0	2	4	6	8	10	12	14
Control								
34	7.25	8.64	8.61	8.54	8.94	8.82	9.50	9.64
36	22.15	23.36	23.31	23.10	23.60	23.41	22.85	23.04
38	37.01	36.90	36.97	37.06	36.99	36.81	38.03	36.38
40	10.03	9.48	9.25	9.52	8.95	9.34	8.63	9.12
42	8.81	7.90	8.13	8.06	7.86	7.86	7.37	7.56
44	9.56	8.06	8.13	8.28	7.80	7.89	7.27	7.63
EDTA								
34	7.09	7.41	7.21	7.08	7.57	7.72	7.96	8.07
36	21.84	21.91	22.06	22.69	22.34	22.48	22.73	22.83
38	37.28	36.86	36.90	37.03	36.85	36.98	36.96	36.95
40	10.52	10.24	10.07	9.73	9.82	9.79	9.65	9.51
42	8.72	8.77	8.95	8.82	8.78	8.62	8.50	8.38
44	9.56	9.43	9.63	9.41	9.32	9.08	8.80	8.72
$NaNO_2$–ascorbate								
34	8.53	8.77	7.72	7.18	7.60	8.09	7.92	8.47
36	22.89	22.97	23.21	22.16	22.36	23.24	22.97	23.48
38	36.72	38.18	37.31	37.09	37.13	36.89	36.76	37.35
40	9.66	9.18	9.29	9.87	9.90	9.45	9.37	9.16
42	8.32	7.61	8.51	8.90	8.54	8.40	8.57	7.93
44	8.58	7.92	8.73	9.52	9.12	8.46	8.92	8.04

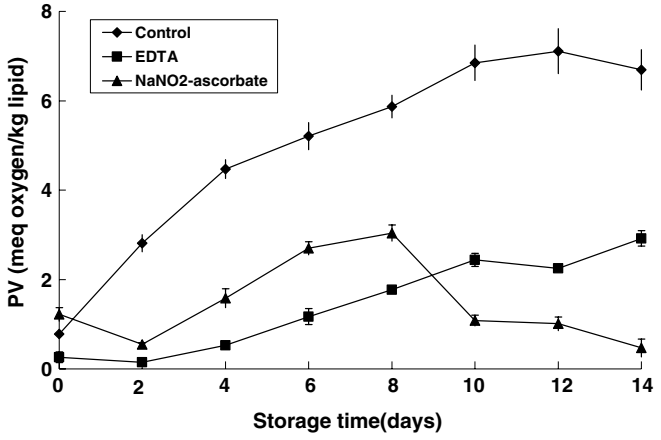

Fig. 3. Changes in peroxide values of total lipid of cooked Japanese sardine meat during storage at 2 °C. EDTA, ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid-added group; $NaNO_2$–ascorbate, sodium nitrite and ascorbic acid-added group; Control, Japanese sardine meats added with water. meq /kg lipid, milliequivalents of iodine/kg of lipid.

lipid to meq oxygen/kg lipid through 8 days of storage, and thereafter decreased to 0.47 meq oxygen/kg lipid after 14 days of storage. Among the initial 8 days of storage, EDTA group showed the lowest PV, followed by the $NaNO_2$–ascorbate group. However, EDTA group had higher PV compared to $NaNO_2$–ascorbate group at the end of the 14-day storage. The control group had the highest PV throughout the storage period.

3.6. Changes in TBA values

The effects of a combination of nitrite and ascorbate as well as EDTA on the TBA values of the cooked Japanese sardine meat are shown in Fig. 4. Measurements of TBA values were carried out during 10 days of storage due to sample availability. The TBA values of the control group increased rapidly from 9.14 mg MA eq/kg meat to 21.5 mg MA eq/kg meat during initial 2 days of storage time and then increased gradually up to 31.5 mg MA eq/kg meat after 10 days of storage. In the EDTA group, the TBA values once decreased for the initial 2 days and then increased gradually from 6.83 mg MA eq/kg meat to 21.2 mg MA eq/kg meat during 10 days of storage. In the $NaNO_2$–ascorbate group, the TBA values remained unchanged, varying

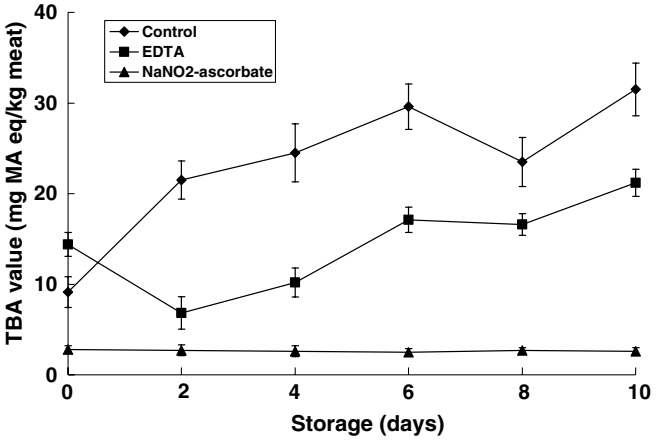

Fig. 4. Changes in thiobarbituric acid values of total lipid of cooked Japanese sardine meat during storage at 2 °C. EDTA, ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid-added group; $NaNO_2$–ascorbate, sodium nitrite and ascorbic acid-added group; Control, Japanese sardine meats added with water. mg MA eq/kg meat, mg of malonaldehyde equivalents/kg of meat.

between 2.5 and 2.8 mg MA eq/kg meat throughout the 10 days of storage.

4. Discussion

Changes in hydrogenated TAG compositions and DAG *tert*-BDMS ether derivative compositions of PL of the cooked Japanese sardine meat found in this study clearly indicate that DAG containing PUFA were highly susceptibile to oxidation during storage; the TL contents of the control, EDTA and $NaNO_2$–ascorbate groups increased and resulted in an increase in PL molecular species of DAG containing PUFA in the $NaNO_2$–ascorbate treated group, but not in the control group. These results correspond to those reported by Acosta et al. (1966), who found that TAG did not influence significantly the development of WOF in the cooked meat. The PL fraction rather than TAG was found responsible for oxidative deterioration in cooked turkey meat. Lea (1957) suggested that decreases in the PL component may be due to either autoxidation, hydrolytic decomposition, lipid browning reactions or lipid-protein co-polymerization. The high susceptibility of PL to autoxidation in cooked meat is attributed to the presence of high concentrations of polyunsaturated fatty acids (Pearson, Love, & Shorland, 1977; Sato & Herring, 1973). The rate of autoxidation of fatty acids significantly increased with an increase in the number of double bonds. Although TAG comprises the major portion of TL in fatty meats, their PUFA are mainly due to C18:0 with significant amount of C18:3*n*−3, but small quantities of C22:6*n*−3. Contrary to this, PL represent only 0.5%–1% of the tissue content; however, the PL fraction contains not only C18:2*n*−6 and a significant amount of C18:3*n*−3, but also high concentrations of C20:3*n*−3 and other PUFA (Hornstein et al., 1961; Igene, Pearson, & Gray, 1981; Lee & Dawson, 1973; O'Keefe, Wellington, Mattick, & Stouffer, 1968; Pearson et al., 1977; Pikul et al., 1984; Sinclair, Slattery, & O'dea, 1982). The autoxidation of PL is primarily associated with degradation of PUFA. The reduction of PUFA in the PL fractions suggests that autoxidation of PUFA was associated with the development of oxidized off-flavors including WOF in cooked meat. Oxidation and decomposition of unsaturated fatty acids of NL and PL have been reported in a variety of cooked meats kept at different temperatures, and it was revealed that the PUFA of PL with more than four double bonds show the greatest oxidative degradation (Gokalp, Ockerman, Pilmpton, & Harper, 1983; Igene et al., 1981; Lee & Dawson, 1973; Willemont, Poste, Salvador, & Wood, 1985). Younathan and Watts (1960) showed that the PL fraction was more susceptible to oxidation than the TAG fraction in tissue lipids of cooked pork. The most pronounced loss of PL was found in phosphatidylethanolamine followed by phosphatidylcholine during refrigerated or frozen storage of cooked chicken meat (Acosta et al., 1966; Igene & Pearson, 1979; Lee & Dawson, 1976; Sharma, Kowale, & Joshi, 1982).

Changes in TBA values obtained in the present study are in good agreement with the previous results in which the TBA values increased in the cooked and subsequently stored mackerel meat (Ohshima, Wada, & Koizumi, 1988). Generally, EDTA inhibits the catalytic behavior of nonheme iron, but has no influence on heme iron catalysis. Non-heme iron catalysis appeared to be related in part to lipid oxidation in the cooked meat. In the present study, addition of EDTA seemed more effective to suppress lipid oxidation when PV was measured as a lipid oxidation index. Contrary to this, the TBA values of $NaNO_2$–ascorbate added meat remained always smaller than those of EDTA-added meat. This means addition of EDTA did not control decomposition of lipid hydroperoxide throughout the storage period, and a combined use of $NaNO_2$ and ascorbate suppressed not only the formation of the primary oxidation product of oxidation, but also the decomposition of lipid hydroperoxide to secondary oxidation products. It could be concluded from these results that nitric oxide ferrohemochromogen formed from myoglobin and added nitrite in the presence of ascorbate in sardine meat acted as a metal chelator, thus exhibiting antioxidant activity. The PL fraction plays an important role in oxidative deterioration and development of off-flavor in cooked sardine meat. The TAG fraction plays a minor role in oxidative deterioration although its content is much higher than PL in fatty sardine meats. The different oxidative stabilities of PL and NL are probably due to the existing differences in the contents of PUFA in the two lipid fractions. The Japanese Regulation for Food Additives does not allow the use of EDTA or sodium nitrite for the processing of fish meat; however, nitrite can be used for curing of salmon and Alaska pollock roes. The results of the present study will provide valuable information for better understanding of lipid oxidation in sardine meat.

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