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Effects of EDTA and a combined use of nitrite and ascorbate on lipid oxidation in cooked Japanese sardine (*Sardinops melanostictus*) during refrigerated storage

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Abstract

To elucidate the effects of EDTA and a combined use of nitrite and ascorbate on oxidative rancidity of cooked Japanese sardine (*Sardinops melanostictus*) stored at refrigeration temperature (2 °C), changes in peroxide values (PV), thiobarbituric acid (TBA) value and fatty acid composition were monitored. The PV and TBA values of the meat with and without EDTA (250 mg/100 g meat) increased gradually, while they remained unchanged when a mixture of sodium nitrite (10 mg NO_2/100 g meat) and sodium ascorbate (200 mg/100 g meat) was added. Total polyenoic acids in the nitrite and ascorbate mixture-treated meat decreased at a slower rate than in the control group. These changes in PUFA composition were due to differences in oxidative stability between the meats containing different antioxidants. In conclusion, a combined use of nitrite and ascorbate showed a greater antioxidativity in terms of the lower PV and TBA values than did EDTA and resulted in preventing the development of warmed-over flavour in cooked sardine during of 14 day refrigerated storage.

Keywords: EDTA; Sodium nitrite; Sodium ascorbate; Lipid oxidation; Warmed-over flavor

1. Introduction

The susceptibility of lipid to oxidation is one of the major causes of quality deterioration in many types of natural and processed foods. Lipid oxidation leads to changes in the quality of food, such as taste, texture, shelf life, appearance, and nutritional value (Ranken, 1994). Effects of lipid oxidation are also major causes of many pathological effects, such as cardiovascular disease, cancer, and brain dysfunction as well, as the aging process (Kinsella, 1987). Although, the annual landed amount of Japanese sardine (*Sardinops melanostictus*) has declined during the past decade, it is still one of most important fish resources for human consumption and it has been processed into a variety of products, including salted-dried and boiled-dried products, as well as fish sauce products. Fish lipids are characterized by a high degree of unsaturation in the form of multiple double bonds in the fatty acids and are generally susceptible to molecular oxygen (Olcott, 1962). Lipid oxidation is a rather complex process, whereby unsaturated fatty acids react with molecular oxygen via a free radical chain mechanism, and form fatty acyl hydroperoxides as primary products in the oxidation processes. Since lipid oxidation is a chain process, there are two mechanistically distinct classes of antioxidants which can be used to retard lipid oxidation. One group of the antioxidants controls the radical chain-breaking mechanism by inactivating alkyl peroxyl and alkyl radicals which are important in the chain-propagating step. The other

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group involves the prevention of the introduction of chain-initiating radicals, and this includes α-tocopherol (vitamin E), propyl gallate, BHA, BHT, BTHQ, ascorbic acid (vitamin C), a transition metal chelator, phosphate and citrate (Harris & Tall, 1994; Ladikos & Lougovois, 1990). Nitrite, usually used for a curing process of meat, is known to have powerful antioxidative effects (Gray & Pearson, 1994).

It has generally been accepted that the rapid flavour deterioration developing in cooked meats during storage, commonly referred to as warmed-over flavour (WOF), is attributed to autoxidation of lipids. The term of WOF was first introduced by Tims and Watts (1958) to describe the rapid development of oxidized flavour in cooked meat upon subsequent heating. The WOF has been recognized as one of the primary causes of quality deterioration of meat products during processing refrigerating as well as pre-cooking. It is, therefore, important to control lipid oxidation during processing and subsequent storage of food and foodstuffs to prevent development of WOF.

This paper deals with the influences of addition of EDTA, as well as a combination of use of nitrite and ascorbate, on the development of WOF in cooked sardine meat during storage at 2 °C to obtain information concerning the mechanism involved in oxidative rancidity of lipids in cooked sardine meat.

2. Materials and methods

2.1. Chemicals

Ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA) and 14% boron trifluoride in methanol (BF_3-MeOH) were purchased from Wako Pure Chemical Industries (Tokyo, Japan), sodium nitrite ($NaNO_2$) and sodium ascorbate from Kokusan Chemical (Tokyo, Japan). 2-Thiobarbituric acid was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Organic solvents of analytical grade were purchased from Kokusan Chemical (Tokyo, Japan).

Authentic lipid standard compounds were obtained from Sigma-Aldrich Group (Tokyo, Japan). They included 1-([*cis*,*cis*]-9-12-octadecadienoyl)-*rac*-glycerol (monoolein), 1,3-di-([*cis*,*cis*]-9,12-octadecadienoyl)-*rac*-glycerol monolinolein (dilinolein), 1,2,3-tri-(*cis*,*cis*,*cis*-9,12,15-octadecatrienoyl)-glycerol (trilinolein), *cis*,*cis*-9,12 octadecadienoic acid (linoleic acid), 1,2-diacyl-*sn*-glycero-3-phosphate sodium salt (phosphatidic acid), 1,2-diacyl-*sn*-glycero-3-phospho-[1-D-myo-inositol 4,5-bisphosphate] sodium salt (phosphatidylinositol), 1,2-diacyl-*sn*-glycero-3-phosphocholine (phosphatidylcholine), 1,2-diacyl-*sn*-glycero-3-phospho-L-serine (phosphatidylserine), and 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine (phosphatidylethanolamine).

2.2. Preparation of sample

Japanese sardine (*Sardinops melanostictus*, 2 years old, from Choshi, Chiba Prefecture) was purchased from a local market. Ordinary muscle was removed and minced twice with a meat chopper. The minced samples were divided into 3 groups, a control with added water, EDTA and $NaNO_2$-ascorbate. Each group contained of 250 g minced meat. To the EDTA group were added 5 ml of EDTA solution to give a final concentration of 250 mg/100 g. To the $NaNO_2$ group, were added 5 ml of a mixed solution of $NaNO_2$ and sodium ascorbate to give final concentrations of 100 ppm (as NO_2^-) and 200 mg/100 g, respectively. To the control were added 5 ml of distilled water. Each group was mixed thoroughly, put into petri dishes, separately, and kept overnight at 2 °C to complete the reaction between $NaNO_2$ and myoglobin in the presence of ascorbate in the $NaNO_2$-ascorbate group. All groups were autoclaved for 15 min at 100 °C and cooled to room temperature, mixed thoroughly, and subsequently stored at 2 °C for 14 days.

2.3. Moisture measurement

The moisture contents of samples were determined by weighing and drying at 105 °C according to the Official Method of Analysis of Official Analytical Chemistry (AOAC, 1980).

2.4. Lipid extraction

Lipid was extracted for lipid classification and the total lipid content determined following the procedures of Bligh and Dyer (1959).

2.5. Peroxide value measurement

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

2.6. TBA value measurement

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

2.7. Fractionation of lipids

Polar lipids (PL) and non-polar lipids (NL) were separated from the total lipids by using Sep-pak silica cartridges (25 mm×10 mm i.d., Waters Corp., Milford, MA, USA) as described by Juaneda and Rocquelin (1985). The non-polar and polar lipids were eluted by chloroform and methanol, respectively, in sequential order.

2.8. Lipid class analysis

The NL and PL fractions were separated by absorption thin-layer chromatography on precoated Kieselgel G F_{254} plates (0.25 mm in thickness, Merck, Darmstadt, Germany). For separation of lipid classes in the NL and PL, developing solvent systems of *n*-hexane–diethyl ether–acetic acid (80:20:1 v/v/v) and chloroform–acetone–methanol–acetic acid–water (65:20:10:10:3, v/v/v/v/v) were used, respectively. The plates were sprayed with 5% sulfuric acid and heated in an oven set at 100 °C for 15 min. Each spot was identified by authentic lipid standards, including monolinolein, dilinolein, trilinolein, linoleic acid, phosphatidic acid, phosphatidylinositol, phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine.

2.9. Fatty acid analysis

Fatty acids of the TL were transesterified to methyl esters, using a base-catalyzed transesterification, followed by a BF_3-MeOH-catalyzed esterification, according to the official method of AOCS Ce 1b-89 (AOCS, 1994), to obtain fatty acid methyl ester (FAMEs). The FAMES were dissolved in *iso*-octane and injected in to a model GC 14B gas chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with a Supelcowax-10 fused silica wall-coated open tubular column (0.25 mm i.d. × 30 m, 0.25 μm in film thickness; Supelco, Bellefonte, PA, USA) and a flame-ionization detector. The column oven and injection port temperature were held

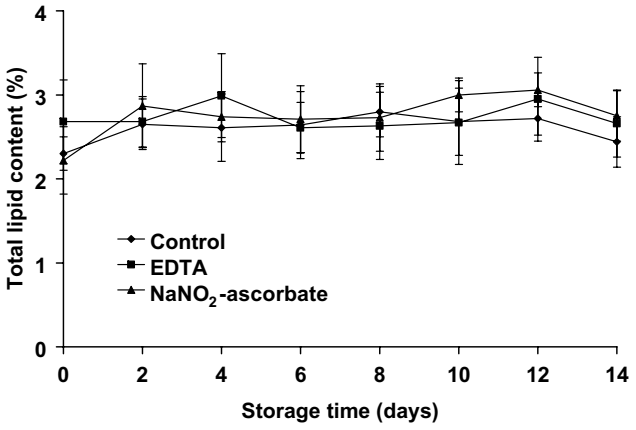

Fig. 1. Changes in total lipid contents of cooked Japanese sardine meat during storage at 2 °C. EDTA, ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid-treated group; $NaNO_2$-ascorbate, sodium nitrite and ascorbic acid-treated group; Control, Japanese sardine meats treated with water.

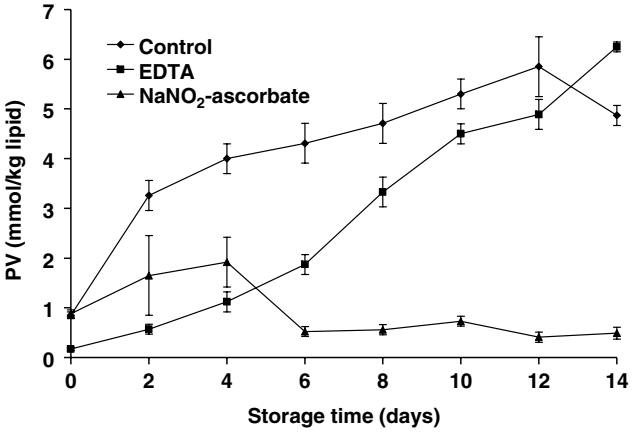

Fig. 3. Changes in peroxide values of total lipid of cooked Japanese sardine meat during storage at 2 °C. EDTA, ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid-treated group; $NaNO_2$-ascorbate, sodium nitrite and ascorbic acid-treated group; Control, Japanese sardine meats treated with water.

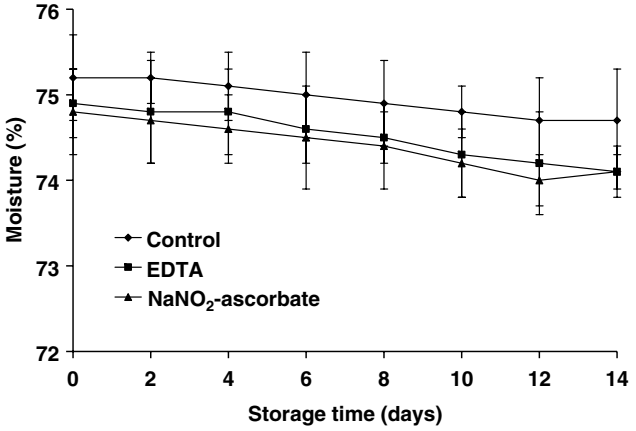

Fig. 2. Changes in moisture contents of cooked Japanese sardine meat during storage at 2 °C. EDTA, ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid-treated group; $NaNO_2$-ascorbate, sodium nitrite and ascorbic acid-treated group; Control, Japanese sardine meats treated with water.

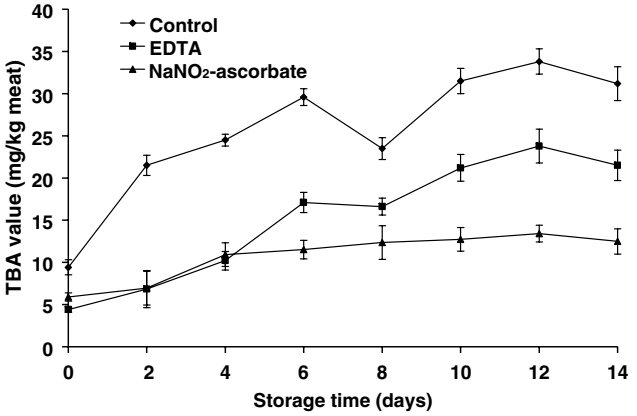

Fig. 4. Changes in thiobarbituric acid values of total lipid of cooked Japanese sardine meat during storage at 2 °C. EDTA, ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid-treated group; $NaNO_2$-ascorbate, sodium nitrite and ascorbic acid-treated; Control, Japanese sardine meats treated with water.

initially at 150 °C for 2 min, then programmed to 195 °C at a rate of 5 °C/min, from 195 °C to 250 °C at a rate of 1.2 °C/min, and finally held at 250 °C for 35 min. Helium was used as a carrier gas with an inlet pressure of 2.0 kg/cm^2.

2.10. Statistical analysis

All measurements carried out in triplicate and the results expressed as means ± standard deviation 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 the Student's *t*-test. Differences were considered to be significant at $P<0.05$.

3. Results

3.1. Changes in total lipid contents

Changes in total lipid (TL) contents of cooked Japanese sardine meat stored at 2 °C for 14 days are shown in Fig. 1. The mean contents of TL ranged between 2.2% and 3.0%. The TL contents of the control, EDTA and $NaNO_2$-ascorbate groups were not significantly different ($P<0.05$) from each other at any of the storage times.

3.2. Changes in moisture contents

Changes in moisture contents of the cooked sardine meat during storage at 2 °C are shown in Fig. 2. Mois-

Table 1
Changes in fatty acid compositions of total lipid in cooked sardine meat (control group) during storage at 2 °C

Fatty acid	Storage time (days)							
	0	2	4	6	8	10	12	14
C14:0	8.73	8.70	9.42	8.83	9.51	7.99	7.82	9.43
C15:0 I	0.18	0.20	0.18	0.18	0.21	0.18	0.18	0.20
C15:0 A	0.07	0.08	0.11	0.08	0.10	0.08	0.09	0.09
C15:0	0.72	0.74	1.16	0.76	0.82	0.73	0.75	0.90
C16:0 I	0.12	0.13	0.33	0.13	0.15	0.14	0.15	0.17
C16:0	27.4	27.9	31.7	28.5	29.8	27.9	28.4	32.4
C17:0 I	0.50	0.54	0.73	0.57	0.58	0.58	0.61	0.64
C17:0 A	0.29	0.32	0.37	0.33	0.32	0.35	0.37	0.33
C17:0	0.66	0.67	0.85	0.78	0.74	0.70	0.75	0.83
C18:0	4.73	4.94	5.65	4.78	5.15	5.12	5.51	5.72
C19:0	0.36	0.36	0.41	0.38	0.40	0.36	0.37	0.38
C20:0	0.55	0.58	0.68	0.51	0.61	0.56	0.64	0.61
Saturated	44.3	45.2	51.6	45.8	48.4	44.7	45.6	51.7
C16:1 n7	8.05	7.85	7.43	8.30	8.09	8.13	7.92	7.41
C16:1 n5	0.13	0.22	0.12	0.25	0.20	0.23	0.23	0.21
C18:1 n9	8.18	8.34	7.63	8.45	8.22	8.81	8.90	7.82
C18:1 n7	3.28	3.24	2.92	3.39	3.26	3.40	3.39	3.00
C18:1 n5	0.24	0.42	1.69	0.22	0.83	0.31	0.58	1.91
C20:1 n11	1.53	1.47	1.69	1.31	1.47	1.39	1.65	1.45
C20:1 n9	0.19	0.18	0.22	0.16	0.19	0.17	0.19	1.06
C22:1 n11	0.36	0.43	0.49	0.55	0.40	0.43	0.45	0.29
Monoenoic	22.0	22.2	22.2	22.6	22.7	22.9	23.3	23.2
C16:2 n4	1.31	1.24	1.21	1.24	1.29	1.30	1.25	1.10
C16: 3n4	1.13	1.11	0.99	1.19	1.09	1.14	1.08	1.00
C17: 2n8	0.19	0.20	0.29	0.21	0.21	0.22	0.23	0.24
C17:2	0.14	0.14	0.12	0.14	0.12	0.13	0.14	0.10
C18:2 n6	1.10	1.09	0.89	1.11	1.16	1.10	1.08	0.93
C18:2n4	0.40	0.39	0.34	0.40	0.46	0.37	0.38	0.33
C18:3 n4	0.34	0.34	0.29	0.36	0.28	0.36	0.31	0.30
C18:3 n3	0.72	0.69	0.60	0.72	0.64	0.75	0.69	0.59
C18:4 n3	1.79	1.70	1.49	1.71	1.64	1.72	1.68	1.60
C18:4 n1	0.25	0.21	0.14	0.22	0.22	0.22	0.20	0.16
C20:2	0.29	0.32	0.53	0.25	0.36	0.30	0.33	0.34
C20:2 n6	0.22	0.21	0.20	0.11	0.20	0.14	0.16	0.19
C20:3 n6	0.17	0.19	0.24	0.18	0.15	0.18	0.19	0.14
C20:4 n6	1.44	1.45	1.25	1.36	1.28	1.48	1.43	1.19
C20:4 n3	0.77	0.73	0.55	0.72	0.66	0.73	0.73	0.60
C20:5 n3	11.1	10.7	8.04	10.6	9.41	11.0	10.4	8.17
C21:5 n3	0.55	0.42	0.29	0.40	0.36	0.43	0.40	0.29
C22:5 n3	1.89	1.90	1.38	1.76	1.57	1.76	1.68	1.28
C22:6 n3	10.3	9.64	7.34	8.88	7.87	9.15	8.76	6.60
Polyenoic	34.1	32.7	26.2	31.6	29.0	32.4	31.1	25.2

ture contents, in all groups, ranged between 74.0% and 75.2%. No significant differences ($P<0.05$) in the moisture contents were observed among the three groups.

3.3. 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 0.85 to 3.26 mmol/kg during the initial 2 days of storage and then increased gradually up to 5.85 mmol/kg. In the EDTA group, the PV slowly increased from 0.17 to 1.87 mmol/kg during the initial 6 days of storage, followed by a gradual increase upto 14 days of storage. In the $NaNO_2$-ascorbate group, the PV slightly increased from 0.88 to 1.92 mmol/kg through day 4 of storage, and thereafter decreased and remained the same at day 6 through to the end of storage.

Among the three groups, the $NaNO_2$-ascorbate group showed the lowest PV throughout storage time, followed by the EDTA group. However, the EDTA group had the highest PV at the end of 14 days of storage. The control group had the highest PV in the initial and middle stages of storage. These results suggest that nitrite was more effective in suppressing the oxidation of cooked Japanese sardine meat than was EDTA.

Table 2
Changes in fatty acid compositions of total lipid in cooked sardine meat with added ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA group) during storage at 2 °C

Fatty acid	Storage time (days)							
	0	2	4	6	8	10	12	14
C14:0	8.41	8.98	8.11	8.64	9.24	8.90	8.58	8.52
C15:0 I	0.13	0.15	0.15	0.16	0.20	0.20	0.20	0.20
C15:0 A	0.10	0.07	0.06	0.08	0.08	0.08	0.09	0.07
C15:0	0.71	0.77	0.70	0.76	0.79	0.81	0.77	0.75
C16:0 I	0.23	0.13	0.11	0.13	0.14	0.15	0.14	0.14
C16:0	27.4	29.0	26.8	29.0	28.8	30.9	28.9	29.0
C17:0 I	0.48	0.54	0.54	0.56	0.51	0.62	0.59	0.58
C17:0 A	0.30	0.34	0.33	0.30	0.29	0.31	0.34	0.32
C17:0	0.70	0.72	0.67	0.86	0.75	0.80	0.71	0.74
C18:0	4.60	4.85	4.74	5.10	4.69	5.68	5.01	5.21
C19:0	0.36	0.36	0.34	0.36	0.36	0.40	0.36	0.37
C20:0	0.50	0.49	0.54	0.59	0.56	0.71	0.57	0.58
Saturated	43.9	46.4	43.1	46.5	46.5	49.6	46.3	46.5
C16:1 n7	8.36	8.05	8.00	7.68	8.35	7.45	8.23	7.69
C16:1 n5	0.09	0.24	0.19	0.21	0.12	0.15	0.25	0.22
C18:1 n9	7.91	8.04	8.45	8.13	7.93	7.96	8.64	8.34
C18:1 n7	3.28	3.19	3.33	3.15	3.15	3.09	3.32	3.24
C18:1 n5	0.14	0.55	0.15	0.81	0.47	1.50	0.42	0.85
C20:1 n11	1.40	1.41	1.37	1.42	1.50	1.75	1.52	1.53
C20:1 n9	0.17	0.17	0.17	0.20	0.17	0.22	0.21	0.18
C22:1 n11	0.39	0.44	0.41	0.49	0.40	0.42	0.38	0.36
Monoenoic	21.7	22.1	22.1	22.1	22.1	22.5	23.0	23.4
C16:2 n4	1.28	1.23	1.29	1.05	1.27	1.12	1.31	1.21
C16: 3n4	1.15	1.14	1.14	1.04	1.14	0.98	1.14	1.10
C17: 2n8	0.20	0.19	0.20	0.21	0.20	0.22	0.21	0.22
C17:2	0.14	0.13	0.15	0.13	0.13	0.10	0.14	0.14
C18:2 n6	1.02	1.03	1.10	1.01	1.20	0.99	1.09	1.05
C18:2n4	0.36	0.36	0.40	0.35	0.47	0.38	0.39	0.37
C18:3 n4	0.33	0.32	0.32	0.29	0.30	0.30	0.34	0.33
C18:3 n3	0.67	0.68	0.74	0.67	0.65	0.63	0.72	0.69
C18:4 n3	1.68	1.77	1.77	1.62	1.70	1.55	1.77	1.80
C18:4 n1	0.22	0.22	0.24	0.20	0.21	0.17	0.24	0.21
C20:2	0.32	0.34	0.26	0.35	0.30	0.50	0.34	0.32
C20:2 n6	0.23	0.27	0.15	0.14	0.15	0.23	0.19	0.15
C20:3 n6	0.26	0.21	0.20	0.26	0.14	0.17	0.18	0.23
C20:4 n6	1.68	1.45	1.53	1.47	1.31	1.29	1.40	1.53
C20:4 n3	1.66	0.72	0.79	0.68	0.67	0.65	0.70	0.75
C20:5 n3	11.2	10.3	11.5	10.2	10.2	8.85	10.2	10.3
C21:5 n3	0.42	0.51	0.48	0.41	0.41	0.34	0.39	0.39
C22:5 n3	1.78	1.67	1.97	1.77	1.71	1.55	1.62	1.56
C22:6 n3	9.87	9.19	10.6	9.72	9.34	7.91	8.38	8.74
Polyenoic	34.4	31.7	34.8	31.6	31.5	27.9	30.8	31.1

3.4. Changes in TBA values

The effect of a combined use of nitrite and ascorbate, as well as a single use of EDTA on the TBA values of the cooked Japanese sardine meat is shown in Fig. 4. The TBA values of the control group increased rapidly from 9.41 to 21.5 mg/kg during the initial 2 days of storage time and then increased gradually up to 31.3 mg/kg. In the EDTA group, the TBA values increased slowly from 4.39 to 21.0 mg/kg during 14 days of storage. In the $NaNO_2$-ascorbate group, the TBA values increased slightly from 5.88 to 11.52 mg/kg for the initial time of storage and then steadied after 8 days of storage.

3.5. Changes in fatty acid compositions

Changes in fatty acid compositions of the TL of cooked Japanese sardine meat during storage at 2 °C are shown in Tables 1–3. For the control group (Table 1), the predominant fatty acids were C14:0, C16:0, C16:1 n-7, C18:0, C18:1 n-9, C20:5 n-3 and C22:6 n-3 which together accounted for 78% of the total fatty acids. Total fatty acids were composed of 44% of saturated, 22% of monoenoic and 34.5% of polyenoic acids. During 14 days of storage, C14:0, C16:0, C18:0 and C18:1 n-9 increased and C16:1n-7, C20:5n-3 and C22:6n-3 decreased in the control

Table 3
Changes in fatty acid compositions of total lipid in cooked sardine meat with added sodium nitrite and sodium ascorbate ($NaNO_2$-ascorbate group) during storage at 2 °C

Fatty acid	Storage time (days)							
	0	2	4	6	8	10	12	14
C14:0	7.70	7.75	8.13	9.11	8.61	8.92	7.44	8.49
C15:0 I	0.15	0.15	0.17	0.16	0.21	0.20	0.17	0.19
C15:0 A	0.07	0.06	0.08	0.07	0.09	0.09	0.08	0.07
C15:0	0.68	0.71	0.74	0.77	0.73	0.75	0.71	0.78
C16:0 I	0.11	0.12	0.14	0.13	0.13	0.11	0.13	0.14
C16:0	27.1	27.9	27.8	28.9	27.8	28.0	27.9	29.7
C17:0 I	0.51	0.54	0.54	0.55	0.57	0.60	0.57	0.60
C17:0 A	0.28	0.30	0.31	0.31	0.33	0.37	0.31	0.31
C17:0	0.68	0.69	0.71	0.82	0.69	0.74	0.74	0.76
C18:0	5.21	5.02	4.90	4.70	4.84	4.68	5.63	5.33
C19:0	0.36	0.34	0.35	0.37	0.36	0.35	0.42	0.46
C20:0	0.58	0.53	0.52	0.45	0.53	0.47	0.70	0.70
Saturated	43.5	44.1	44.4	46.3	44.9	45.3	44.8	47.6
C16:1 n7	7.14	7.82	8.19	8.10	7.73	8.16	7.11	7.25
C16:1 n5	0.21	0.21	0.20	0.22	0.24	0.27	0.17	0.22
C18:1 n9	8.10	8.59	8.63	7.96	8.05	8.09	8.33	7.83
C18:1 n7	3.22	3.33	3.35	3.20	3.15	3.25	3.18	3.06
C18:1 n5	0.50	0.39	0.20	0.44	0.44	0.29	1.01	1.17
C20:1 n11	1.65	1.46	1.31	1.42	1.47	1.42	1.70	1.62
C20:1 n9	0.16	0.17	0.14	0.15	0.17	0.16	0.18	0.22
C22:1 n11	0.43	0.47	0.39	0.37	0.41	0.37	0.51	0.43
Monoenoic	21.4	22.4	22.4	21.9	21.7	22.0	22.2	21.8
C16:2 n4	1.20	1.24	1.27	1.19	1.29	1.32	1.18	1.18
C16: 3n4	1.02	1.11	1.16	1.18	1.11	1.21	1.00	1.02
C17: 2n8	0.19	0.20	0.21	0.21	0.24	0.23	0.25	0.21
C17:2	0.13	0.14	0.15	0.14	0.18	0.17	0.15	0.12
C18:2 n6	1.04	1.10	1.10	1.08	1.03	1.08	1.06	1.04
C18:2n4	0.34	0.38	0.37	0.38	0.36	0.39	0.41	0.43
C18:3 n4	0.32	0.34	0.32	0.32	0.31	0.31	0.33	0.36
C18:3 n3	0.71	0.73	0.73	0.70	0.71	0.72	0.71	0.74
C18:4 n3	1.78	1.81	1.76	1.76	1.74	1.73	1.74	1.91
C18:4 n1	0.22	0.23	0.22	0.20	0.22	0.22	0.20	0.42
C20:2	0.30	0.31	0.30	0.30	0.30	0.28	0.36	0.41
C20:2 n6	0.14	0.21	0.15	0.16	0.15	0.15	0.14	0.20
C20:3 n6	0.27	0.20	0.22	0.23	0.19	0.17	0.18	0.17
C20:4 n6	1.59	1.47	1.47	1.47	1.50	1.44	1.49	1.37
C20:4 n3	0.72	0.74	0.78	0.73	0.70	0.73	0.75	0.69
C20:5 n3	11.3	11.1	11.1	10.4	10.8	10.7	10.7	9.73
C21:5 n3	0.45	0.41	0.35	0.39	0.42	0.41	0.43	0.37
C22:5 n3	2.08	1.85	1.86	1.67	1.91	1.74	1.90	1.64
C22:6 n3	11.3	9.82	9.69	9.36	10.3	9.68	10.1	8.65
Polyenoic	35.2	33.4	33.2	31.9	33.4	32.7	33.1	30.7

Table 4
Changes in fatty acid compositions of non-polar lipids in cooked sardine meat (control group) during storage at 2 °C

Fatty acid	Storage time (days)							
	0	2	4	6	8	10	12	14
C14:0	9.55	9.76	9.37	9.17	9.07	8.74	8.51	9.54
C15:0 I	0.24	0.27	0.26	0.24	0.22	0.24	0.24	0.23
C15:0 A	0.07	0.09	0.08	0.08	0.07	0.08	0.09	0.08
C15:0	0.79	0.83	0.80	0.76	0.74	0.76	0.76	0.82
C16:0 I	0.13	0.14	0.14	0.13	0.13	0.14	0.16	0.14
C16:0	28.0	28.6	28.3	26.8	26.5	28.0	26.8	28.9
C17:0 I	0.65	0.65	0.67	0.63	0.62	0.65	0.68	0.65
C17:0 A	0.25	0.21	0.28	0.22	0.21	0.27	0.61	0.22
C17:0	0.70	0.71	0.70	0.67	0.66	0.72	0.69	0.72
C18:0	5.18	5.43	5.24	4.92	4.89	5.49	5.18	5.43
C19:0	0.19	0.20	0.15	0.24	0.18	0.17	0.21	0.16
C20:0	0.71	0.76	0.69	0.63	0.66	0.72	0.70	0.72
Saturated	46.4	47.7	46.7	44.5	43.9	46.0	44.6	47.6
C16:1 n7	8.34	8.20	8.74	8.88	8.71	8.50	9.74	8.40
C16:1 n5	0.21	0.19	0.22	0.21	0.23	0.21	0.20	0.20
C18:1 n9	8.98	8.96	9.39	9.41	9.38	9.64	9.47	9.30
C18:1 n7	3.46	3.48	3.61	3.60	3.58	3.71	3.61	3.59
C18:1 n5	0.79	0.57	0.37	0.17	0.17	0.33	0.45	0.44
C20:1 n11	1.59	1.64	1.64	1.60	1.62	1.78	1.74	1.68
C20:1 n9	0.17	0.15	0.19	0.19	0.19	0.17	0.19	0.17
C22:1 n11	0.65	0.60	0.60	0.69	0.54	0.61	0.70	0.62
Monoenoic	24.2	23.8	24.8	24.8	24.4	25.0	26.1	24.4
C16:2 n4	1.33	1.30	1.37	1.45	1.42	1.35	1.40	1.34
C16: 3n4	1.16	1.10	1.15	1.23	1.23	1.09	1.15	1.09
C17: 2n8	0.22	0.22	0.22	0.21	0.20	0.24	0.25	0.22
C17:2	0.09	0.09	0.10	0.11	0.10	0.09	0.10	0.09
C18:2 n6	1.13	1.07	1.12	1.15	1.14	1.08	1.10	1.05
C18:2n4	0.37	0.36	0.36	0.42	0.39	0.36	0.38	0.36
C18:3 n4	0.27	0.30	0.30	0.33	0.34	0.29	0.26	0.30
C18:3 n3	0.69	0.67	0.71	0.76	0.80	0.70	0.70	0.67
C18:4 n3	1.61	1.54	1.61	1.81	1.82	1.54	1.69	1.56
C18:4 n1	0.20	0.19	0.20	0.25	0.24	0.20	0.22	0.19
C20:2	0.33	0.30	0.33	0.34	0.35	0.31	0.36	0.30
C20:2 n6	0.15	0.15	0.15	0.14	0.16	0.15	0.14	0.15
C20:3 n6	0.17	0.16	0.16	0.17	0.18	0.16	0.17	0.20
C20:4 n6	1.33	1.20	1.18	1.25	1.30	1.22	1.20	1.26
C20:4 n3	0.77	0.74	0.69	0.74	0.82	0.67	0.63	0.72
C20:5 n3	9.87	9.59	9.76	10.62	10.9	9.88	9.75	9.42
C21:5 n3	0.45	0.42	0.44	0.46	0.48	0.47	0.45	0.42
C22:5 n3	2.05	1.98	1.86	1.98	2.11	2.02	1.98	1.86
C22:6 n3	7.19	7.13	6.81	7.34	7.66	7.26	7.35	6.77
Polyenoic	29.4	28.5	28.5	30.8	31.6	29.1	29.3	28.0

group. The saturated fatty acids and monoenoic acids increased and total polyenoic acids decreased. For the EDTA group (Table 2), C14:0, C16:0, C18:0 and C18:1n-9 in the EDTA group increased and C16:1n-7, C20:5n-3 and C22:6n-3 decreased. The total saturated fatty acids and monoenoic acids increased and total polyenoic acids decreased at a slower rate than those in the control group. For the $NaNO_2$-ascorbate group (Table 3), C14:0 and C16:0 decreased. C18:0, C16:1n-7 and C18:1n-9 remained unchanged, while C20:5n-3 and C22:6n-3 decreased. Total saturated fatty acid and monoenoic acids in the $NaNO_2$-ascorbate group increased while total polyenoic acids decreased at a slower rate than those in the EDTA and control groups.

Changes in fatty acid compositions of the NL of cooked sardine meat during storage at 2 °C are shown in Tables 4–6. As shown in Table 4, the predominant fatty acids of the NL were C16:0, C16:1n-7, C18:0, C18:1n-9, C20:5n-3 and C22:6n-3, accounting for over 70% of total fatty acids. Total fatty acids were composed of 43–47% of saturated fatty acids, 23–26% of monoenoic and 27–31% of polyenoic acids. For the control group (Table 5), C16:0, C18:0, C16:1n-7 and C18:1n-9 increased while C20:5n-3 and C22:6n-3 decreased. Total saturated fatty acids and monoenic acids increased,

Table 5
Changes in fatty acid compositions of non-polar lipids in cooked sardine meat with added ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA group) during storage at 2 °C

Fatty acid	Storage time (days)							
	0	2	4	6	8	10	12	14
C14:0	9.22	9.60	8.84	8.82	9.15	9.01	8.19	9.14
C15:0 I	0.25	0.25	0.22	0.24	0.22	0.22	0.24	0.24
C15:0 A	0.08	0.08	0.06	0.08	0.07	0.07	0.09	0.08
C15:0	0.84	0.80	0.74	0.76	0.79	0.76	0.74	0.78
C16:0 I	0.14	0.14	0.13	0.14	0.15	0.14	0.16	0.14
C16:0	27.5	28.2	26.7	26.6	28.8	27.9	27.2	27.7
C17:0 I	0.67	0.65	0.64	0.65	0.66	0.64	0.69	0.64
C17:0 A	0.24	0.25	0.27	0.36	0.25	0.25	0.57	0.23
C17:0	0.71	0.70	0.68	0.68	0.72	0.70	0.72	0.69
C18:0	5.33	5.21	4.98	4.96	5.53	5.35	5.41	5.23
C19:0	0.22	0.17	0.16	0.16	0.17	0.16	0.21	0.15
C20:0	0.76	0.71	0.66	0.70	0.75	0.73	0.72	0.70
Saturated	45.9	46.8	44.1	44.1	47.2	45.9	45.0	45.7
C16:1 n7	8.08	8.35	8.67	8.88	8.25	8.48	9.29	8.55
C16:1 n5	0.23	0.19	0.22	0.21	0.25	0.21	0.20	0.22
C18:1 n9	9.21	8.88	9.32	9.22	9.57	9.35	9.61	9.43
C18:1 n7	3.51	3.44	3.57	3.54	3.71	3.60	3.68	3.63
C18:1 n5	0.65	0.87	0.28	0.30	0.54	0.40	0.57	0.30
C20:1 n11	1.74	1.30	1.64	1.63	1.80	1.69	1.81	1.71
C20:1 n9	0.21	0.57	0.18	0.18	0.20	0.16	0.20	0.18
C22:1 n11	0.74	0.71	0.64	0.60	0.63	0.63	0.66	0.64
Monoenoic	24.4	24.3	24.5	24.6	25.0	24.5	26.0	24.7
C16:2 n4	1.33	1.30	1.41	1.40	1.30	1.36	1.34	1.37
C16: 3n4	1.12	1.12	1.18	1.19	1.06	1.12	1.05	1.13
C17: 2n8	0.22	0.22	0.21	0.22	0.23	0.22	0.26	0.22
C17:2	0.09	0.09	0.10	0.10	0.09	0.10	0.09	0.10
C18:2 n6	1.12	1.09	1.12	1.12	1.08	1.09	1.10	1.09
C18:2n4	0.40	0.40	0.42	0.39	0.35	0.35	0.37	0.38
C18:3 n4	0.33	0.30	0.29	0.30	0.29	0.30	0.24	0.31
C18:3 n3	0.70	0.67	0.72	0.74	0.67	0.70	0.66	0.70
C18:4 n3	1.62	1.62	1.75	1.76	1.49	1.63	1.51	1.64
C18:4 n1	0.20	0.19	0.23	0.23	0.18	0.21	0.19	0.21
C20:2	0.33	0.36	0.32	0.33	0.32	0.30	0.35	0.31
C20:2 n6	0.16	0.15	0.15	0.15	0.16	0.15	0.16	0.15
C20:3 n6	0.18	0.16	0.18	0.17	0.17	0.16	0.17	0.17
C20:4 n6	1.26	1.23	1.31	1.28	1.22	1.24	1.26	1.23
C20:4 n3	0.77	0.73	0.80	0.79	0.68	0.68	0.72	0.71
C20:5 n3	10.0	9.73	10.8	10.8	9.33	10.2	9.66	10.1
C21:5 n3	0.45	0.41	0.48	0.48	0.42	0.44	0.46	0.45
C22:5 n3	2.04	1.94	2.09	2.07	1.93	1.99	2.03	2.00
C22:6 n3	7.44	7.16	7.89	7.81	6.85	7.40	7.39	7.34
Polyenoic	29.8	28.9	31.4	31.3	27.8	29.6	29.0	29.6

while total polyenoic acids decreased. For the EDTA group (Table 6), C16:0, C16:1n-7, C18:1n-9 and C20:5n-3 increased, while C18:0 and C22:6n-3 decreased. In the $NaNO_2$-ascorbate group, C16:0 and C20:5n-3 increased while C18:0, C16:1n-7, C18:1n-9 and C22:6n-3 decreased.

Changes in fatty acid compositions of the PL of cooked Japanese sardine meat are presented in Tables 7–9. For the control group, shown in Table 7, the predominant fatty acids of PL were C16:0, C16:1n-7, C18:0, C18:1n-9, C20:5n-3 and C22:6n-3, which together accounted for more than 70% of total fatty acids. Total fatty acids were composed of 44–58% of saturated fatty acids, 10–12% of monoenoic and 33–46% of polyenoic acids. C16:0, C18:0, C16:1n-7 and C18:1n-9 increased while C20:5n-3 and C22:6n-3 decreased. Total saturated fatty acids and monoenic acids increased, while total polyenoic acids decreased. For the EDTA group (Table 8), C16:0, C18:0, C16:1n-7 increased, while C18:1n-9, C20:5n-3 and C22:6n-3 decreased. Total saturated fatty acids and monoenic acids increased, while total polyenoic acids decreased. For the $NaNO_2$-ascorbate group (Table 9), C16:0, C16:1n-7, C20:5n-3 and C22:6n-3 increased, while

Table 6
Changes in fatty acid compositions of non-polar lipids in cooked sardine meat with added sodium nitrite and sodium ascorbate during ($NaNO_2$-ascorbate group) storage at 2 °C

Fatty acid	Storage time (days)							
	0	2	4	6	8	10	12	14
C14:0	8.55	9.57	9.00	8.90	8.75	8.95	9.79	9.53
C15:0 I	0.23	0.26	0.23	0.22	0.22	0.23	0.28	0.23
C15:0 A	0.07	0.08	0.07	0.07	0.07	0.08	0.10	0.08
C15:0	0.72	0.80	0.76	0.74	0.73	0.76	0.83	0.77
C16:0 I	0.13	0.14	0.13	0.13	0.13	0.14	0.18	0.14
C16:0	26.7	28.0	27.7	26.8	26.7	27.3	27.9	26.9
C17:0 I	0.65	0.66	0.64	0.64	0.64	0.65	0.65	0.62
C17:0 A	0.36	0.24	0.23	0.23	0.26	0.31	0.20	0.23
C17:0	0.70	0.70	0.70	0.68	0.68	0.69	0.68	0.65
C18:0	5.22	5.21	5.24	5.09	5.06	5.11	4.89	4.78
C19:0	0.18	0.16	0.15	0.16	0.21	0.15	0.14	0.18
C20:0	0.70	0.71	0.70	0.69	0.66	0.66	0.60	0.61
Saturated	44.2	46.5	45.6	44.3	44.1	45.0	46.3	44.7
C16:1 n7	8.78	8.44	8.57	8.40	8.74	8.60	8.66	8.74
C16:1 n5	0.21	0.21	0.22	0.21	0.23	0.21	0.23	0.23
C18:1 n9	9.47	9.13	9.41	9.29	9.53	9.27	8.88	9.01
C18:1 n7	3.62	3.54	3.62	3.57	3.65	3.55	3.42	3.46
C18:1 n5	0.41	0.46	0.30	0.58	0.14	0.38	0.42	0.25
C20:1 n11	1.70	1.66	1.67	1.66	1.66	1.66	1.40	1.50
C20:1 n9	0.17	0.17	0.17	0.19	0.18	0.17	0.12	0.16
C22:1 n11	0.70	0.69	0.62	0.65	0.56	0.60	0.47	0.52
Monoenoic	25.1	24.3	24.6	24.6	24.7	24.4	23.6	23.9
C16:2 n4	1.37	1.33	1.39	1.36	1.45	1.36	1.44	1.42
C16: 3n4	1.16	1.12	1.15	1.16	1.24	1.12	1.23	1.25
C17: 2n8	0.24	0.22	0.22	0.21	0.22	0.22	0.20	0.20
C17:2	0.10	0.09	0.10	0.10	0.11	0.10	0.10	0.11
C18:2 n6	1.14	1.10	1.12	1.11	1.17	1.08	1.08	1.11
C18:2n4	0.41	0.37	0.37	0.42	0.41	0.37	0.39	0.38
C18:3 n4	0.28	0.30	0.30	0.31	0.31	0.27	0.27	0.32
C18:3 n3	0.70	0.69	0.71	0.74	0.75	0.71	0.74	0.78
C18:4 n3	1.68	1.60	1.66	1.73	1.80	1.65	1.79	1.82
C18:4 n1	0.21	0.20	0.22	0.23	0.24	0.20	0.22	0.23
C20:2	0.33	0.33	0.30	0.30	0.31	0.29	0.29	0.29
C20:2 n6	0.16	0.15	0.14	0.15	0.14	0.15	0.13	0.14
C20:3 n6	0.18	0.17	0.18	0.18	0.19	0.17	0.16	0.17
C20:4 n6	1.27	1.24	1.26	1.30	1.28	1.25	1.27	1.28
C20:4 n3	0.78	0.71	0.73	0.78	0.80	0.73	0.71	0.76
C20:5 n3	10.5	9.87	10.2	10.7	10.7	10.4	10.6	11.0
C21:5 n3	0.47	0.43	0.45	0.47	0.48	0.47	0.45	0.48
C22:5 n3	2.12	1.99	1.99	2.11	2.04	2.10	1.90	2.02
C22:6 n3	7.74	7.27	7.35	7.76	7.57	7.87	7.13	7.69
Polyenoic	30.8	29.2	29.9	31.1	31.2	30.5	30.1	31.4

C18:0 and C18:1n-9 decreased. Total saturated fatty acids and monoenic acids decreased, while total polyenoic acids increased.

4. Discussion

Changes in TBA values obtained in the present study are in good agreement with the previous results in which the in TBA values increased in the cooked and subsequently stored meats, including beef, turkey, pork, chicken, mackerel, as well as sardine (Keller & Kinsella, 1973; Johnson, Cunningham, & Bowers, 1974; Younthana, Marjan, & Arshad, 1980; Younthana, Oon, & Yusof, 1983). The increased rancidity, based on the TBA value is due mainly to the formation of malonaldehyde decomposed from hydroperoxides of fatty acids which contain three or more double bonds (Sikorsky, Olley, & Buisson, 1984). Generally, EDTA inhibits the catalytic behaviour of non-heme iron, but has no influence on heme iron catalysis. It is well known that heme and non-heme iron accelerate lipid oxidation of cooked beef and pork (Liu & Watts, 1970) as well as cooked beef and chicken (Igene, King, Pearson, & Gray, 1979). The added EDTA suppresses the non-heme iron-catalyzed lipid oxidation by

Table 7
Changes in fatty acid compositions of polar lipids in cooked sardine meat (control group) during storage at 2 °C

Fatty acid	Storage time (days)							
	0	2	4	6	8	10	12	14
C14:0	2.23	2.84	2.94	2.14	2.71	2.43	2.70	2.51
C15:0 I	0.36	0.39	0.37	0.11	0.10	0.10	0.12	0.09
C15:0 A	0.34	0.34	0.48	0.10	0.08	0.07	0.08	0.08
C15:0	0.85	0.91	1.03	0.58	0.68	0.64	0.84	0.73
C16:0 I	0.40	0.45	0.53	0.20	0.18	0.14	0.15	0.14
C16:0	36.5	39.5	41.3	36.9	43.3	38.0	40.9	41.9
C17:0 I	0.42	0.55	0.57	0.30	0.35	0.31	0.35	0.31
C17:0 A	0.28	0.41	0.42	0.15	0.15	0.15	0.15	0.13
C17:0	0.78	1.03	1.04	0.69	0.85	0.87	0.94	0.93
C18:0	6.22	6.61	6.57	5.74	6.67	6.80	6.64	6.45
C19:0	0.14	0.19	0.21	0.25	0.30	0.25	0.23	0.20
C20:0	0.10	0.12	0.13	0.13	0.12	0.12	0.15	0.13
Saturated	48.6	53.3	55.5	47.3	55.5	49.9	53.2	53.6
C16:1 n7	2.81	3.02	3.11	3.16	3.44	3.55	4.07	3.83
C16:1 n5	0.36	0.38	0.40	0.31	0.29	0.21	0.27	0.28
C18:1 n9	4.49	4.46	4.47	4.67	4.53	4.64	4.92	4.73
C18:1 n7	2.30	2.29	2.30	2.56	2.35	2.98	2.58	2.62
C18:1 n5	0.31	0.57	0.55	0.06	0.57	0.70	0.54	0.06
C22:1 n11	0.27	0.26	0.26	0.37	0.26	0.27	0.34	0.31
Monoenoic	10.5	11.0	11.1	11.1	11.4	12.4	12.7	11.8
C16:2 n4	0.70	0.79	0.94	0.59	0.75	0.65	0.62	0.71
C16: 3n4	0.36	0.44	0.43	0.28	0.28	0.30	0.27	0.26
C17: 2n8	0.30	0.37	0.35	0.17	0.21	0.21	0.20	0.17
C17:2	0.47	0.46	0.42	0.43	0.32	0.34	0.30	0.36
C18:2 n6	0.96	0.86	0.86	0.99	0.86	0.96	0.92	0.89
C18:2n4	0.16	0.14	0.15	0.17	0.22	0.14	0.18	0.18
C18:3 n4	0.21	0.19	0.17	0.15	0.17	0.17	0.23	0.18
C18:3 n3	0.37	0.32	0.32	0.39	0.31	0.36	0.40	0.37
C18:4 n3	0.59	0.55	0.51	0.66	0.52	0.56	0.51	0.50
C20:2 n6	0.12	0.07	0.08	0.07	0.09	0.11	0.12	0.08
C20:3 n6	0.18	0.14	0.13	0.17	0.14	0.15	0.20	0.16
C20:4 n6	2.38	2.10	1.96	2.33	2.00	2.31	2.12	2.11
C20:4 n3	0.38	0.31	0.32	0.37	0.28	0.32	0.34	0.35
C20:5 n3	10.5	9.06	8.65	10.3	8.84	9.92	8.94	8.96
C21:5 n3	0.32	0.38	0.23	0.36	0.30	0.37	0.19	0.23
C22:5 n3	1.21	1.06	0.97	1.36	0.94	1.19	1.19	1.13
C22:6 n3	21.7	18.5	16.9	22.9	16.9	20.3	17.6	18.2
Polyenoic	40.9	35.7	33.4	41.7	33.2	38.4	34.3	34.8

chelation, but not the heme-catalyzed oxidation. EDTA also inhibits lipid oxidation in cooked mackerel meat. Non-heme iron catalysis appears to be related, in part, to lipid oxidation in the cooked meat. The addition of nitrite and ascorbate resulted in a significant inhibition of lipid oxidation (Ohshima, Wada, & Koizumi, 1988). It could be concluded from these results that nitric oxide ferrohemochromogen formed from myoglobin and added nitrite in the presence of ascorbate in mackerel meat, acted as a metal chelator, resulting in antioxidant activity. Kanner, Shegalovich, Harel, and Hazan (1988) suggested that both nitric oxidemyoglobin and the cooked cured- turkey meat heme proteins have those iron atoms in the ferrous oxidation state and their coordination sites are occupied. Nitric oxidemyoglobin compounds probably act in the early stage of the reaction to neutralize substrate-free radicals and thus inhibit lipid oxidation (Kanner, Ben-Gera, & Berman, 1980). MacDonald, Gray, and Gibbins (1980) investigated the antioxidative role of nitrite in cured meat products using a model lipid system and demonstrated that nitrite acted a prooxidant toward linoleate itself, while it acted as an antioxidant in the linoleate oxidation which was catalyzed by Fe^{2+}, Fe^{2+}-EDTA and an aqueous extract of pork, suggesting that nitrite functions as a metal chelator to tie up trace metals present in meat. The formation of malonaldehyde as a secondary product of lipid oxidation results in an increase of TBA value of stored muscle food from early through to the mid stage of storage, but TBA value declines toward the late stage, due mainly to the reaction of malonaldehyde with protein in cooked meat and fishery products (Benedict, Strange, & Swift, 1975; Butt-

Table 8
Changes in fatty acid compositions of polar lipids in cooked sardine meat with added ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA group) during storage at 2 °C

Fatty acid	Storage time (days)							
	0	2	4	6	8	10	12	14
C14:0	2.17	2.67	1.83	2.00	2.68	2.22	2.39	2.52
C15:0 I	0.33	0.37	0.12	0.09	0.08	0.14	0.09	0.08
C15:0 A	0.30	0.34	0.07	0.09	0.06	0.09	0.08	0.06
C15:0	0.78	0.84	0.50	0.54	0.94	0.61	0.74	0.82
C16:0 I	0.36	0.40	0.12	0.10	0.15	0.23	0.17	0.16
C16:0	33.3	40.3	33.8	36.0	43.7	42.6	39.5	42.1
C17:0 I	0.36	0.49	0.25	0.30	0.42	0.32	0.32	0.31
C17:0 A	0.18	0.33	0.12	0.15	0.17	0.12	0.12	0.12
C17:0	0.73	0.93	0.63	0.74	0.77	1.07	0.96	0.95
C18:0	5.90	6.77	5.32	5.45	5.88	7.79	6.94	6.49
C19:0	0.16	0.19	0.24	0.22	0.29	0.23	0.25	0.19
C20:0	0.10	0.11	0.10	0.10	0.09	0.17	0.17	0.12
Saturated	44.6	53.8	43.1	45.7	55.2	55.6	51.8	54.0
C16:1 n7	2.91	2.87	2.84	2.72	3.53	2.22	2.38	3.12
C16:1 n5	0.31	0.34	0.30	0.29	0.32	0.84	0.00	0.23
C18:1 n9	4.61	4.07	4.12	4.56	3.88	4.16	4.45	4.51
C18:1 n7	2.38	2.14	2.63	2.44	2.03	2.00	2.23	2.22
C18:1 n5	0.08	0.59	0.00	0.20	0.53	0.96	0.60	0.50
C22:1 n11	0.28	0.19	0.28	0.34	0.20	0.26	0.32	0.28
Monoenoic	10.6	10.2	10.2	10.6	10.5	10.4	9.98	10.9
C16:2 n4	0.61	0.78	0.58	0.63	0.58	0.45	0.57	0.70
C16: 3n4	0.28	0.35	0.25	0.29	0.24	0.23	0.29	0.26
C17: 2n8	0.21	0.27	0.15	0.18	0.16	0.26	0.21	0.18
C17:2	0.36	0.34	0.45	0.46	0.28	0.29	0.29	0.31
C18:2 n6	1.03	0.84	0.98	1.01	0.75	0.76	0.85	0.88
C18:2n4	0.16	0.11	0.16	0.16	0.11	0.18	0.17	0.17
C18:3 n4	0.17	0.17	0.12	0.15	0.14	0.20	0.18	0.18
C18:3 n3	0.41	0.32	0.39	0.40	0.33	0.27	0.35	0.37
C18:4 n3	0.64	0.52	0.66	0.66	0.81	0.44	0.56	0.59
C20:2 n6	0.07	0.00	0.15	0.08	0.00	0.00	0.11	0.13
C20:3 n6	0.17	0.13	0.16	0.17	0.12	0.14	0.17	0.15
C20:4 n6	2.57	2.12	2.49	2.53	1.95	2.05	2.25	2.19
C20:4 n3	0.38	0.28	0.40	0.40	0.29	0.31	0.35	0.37
C20:5 n3	11.7	9.31	11.4	11.6	8.59	8.70	9.56	9.44
C21:5 n3	0.31	0.37	0.40	0.38	0.27	0.26	0.36	0.18
C22:5 n3	1.32	1.01	1.47	1.28	0.99	0.99	1.27	1.00
C22:6 n3	24.5	19.11	26.6	23.3	18.7	18.5	20.3	18.3
Polyenoic	44.9	36.0	46.8	43.7	34.3	34.0	37.8	35.4

kus, 1967; Tarladgis, Watts, Younathan, & Dugan, 1960).

Fresh sardine, with a fat content over 6% (Mai, Shimp, Weihrauch, & Kinsella, 1978; Beamonte & Castrillon, 1989), usually has high content of C16:0, C18:1n-9, C22:5n-3 and C22:6n-3 and the levels of PUFA are higher than those of saturated fatty acids. The ratio of 4:1 in n-3: n-6 fatty acid indicates that sardine oils are desirable for preventing cardiovascular diseases (Kinsella, 1987; Sanchez-Muniz, Viejo, & Medina, 1992). In the present study, total polyenoic acids in TL, NL and PL of the $NaNO_2$-ascorbate group decreased at a slower rate than the control group, suggesting that in the $NaNO_2$-ascorbate group, lipid oxidation decreased slowly as is also shown in the PV changes. These different behaviours between the three experimental groups in total polyenoic acid changes must be due to differences in oxidative stabilities. The declines of PUFA in the control group are consistent with higher susceptibility to autoxidation. Rapid oxidations of PUFA, within several days of refrigerated storage of cooked beef, pork and chicken, have been reported (Hornstein, Crowe, & Heinberg, 1961; Sato, Hegarty, & Herring, 1973; Pearson, Love, & Shordland, 1977). The PUFA may be involved in the heat-induced degradation of lipids which results in off flavour (Younatha & Watts, 1960; Keller & Kinsella, 1973). Therefore, the high susceptibility of cooked sardine meat to lipid oxidation could be controlled by a combined use of $NaNO_2$ and ascorbate, thereby preventing the development of WOF during refrigerated as well as frozen storage.

Table 9
Changes in fatty acid compositions of polar lipids in cooked sardine meat with added sodium nitrite and sodium ascorbate ($NaNO_2$-ascorbate) during storage at 2 °C

Fatty acid	Storage time (days)							
	0	2	4	6	8	10	12	14
C14:0	2.73	2.55	2.26	2.32	2.09	2.45	2.39	2.12
C15:0 I	0.53	0.32	0.27	0.27	0.07	0.09	0.12	0.08
C15:0 A	0.42	0.31	0.28	0.24	0.07	0.09	0.08	0.09
C15:0	0.85	0.83	0.75	0.73	0.62	1.23	0.62	0.55
C16:0 I	0.46	0.36	0.26	0.26	0.12	0.24	0.20	0.13
C16:0	35.5	39.8	35.0	36.6	38.2	44.3	42.8	37.9
C17:0 I	0.51	0.46	0.37	0.46	0.35	0.30	0.32	0.29
C17:0 A	0.37	0.22	0.24	0.36	0.16	0.10	0.11	0.14
C17:0	0.97	0.82	0.83	0.91	0.79	0.88	1.02	0.83
C18:0	6.77	6.81	6.31	6.31	6.47	7.97	7.45	5.98
C19:0	0.20	0.11	0.21	0.22	0.25	0.41	0.20	0.30
C20:0	0.12	0.11	0.12	0.08	0.29	0.15	0.17	0.10
Saturated	49.4	52.7	46.9	48.7	49.5	58.2	55.5	48.5
C16:1 n7	3.06	3.17	3.00	3.22	3.15	3.69	2.72	3.28
C16:1 n5	0.42	0.35	0.34	0.40	0.29	0.00	0.19	0.28
C18:1 n9	5.04	4.79	4.98	4.63	4.45	3.56	4.53	4.40
C18:1 n7	2.73	2.53	2.79	2.69	2.49	1.74	2.23	2.32
C18:1 n5	0.09	0.22	0.10	0.14	0.27	1.04	0.63	0.14
C22:1 n11	0.32	0.30	0.31	0.27	0.26	0.34	0.32	0.28
Monoenoic	11.7	11.4	11.5	11.4	10.9	10.4	10.6	10.7
C16:2 n4	0.86	0.76	0.73	0.79	0.63	0.64	0.60	0.63
C16: 3n4	0.42	0.33	0.37	0.42	0.27	0.22	0.25	0.27
C17: 2n8	0.41	0.28	0.34	0.38	0.20	0.20	0.26	0.22
C17:2	0.50	0.35	0.56	0.60	0.42	0.22	0.33	0.45
C18:2 n6	1.08	0.94	1.01	1.07	0.96	0.57	0.82	0.99
C18:2n4	0.20	0.16	0.15	0.16	0.15	0.08	0.15	0.19
C18:3 n4	0.19	0.18	0.15	0.15	0.18	0.13	0.20	0.14
C18:3 n3	0.43	0.36	0.39	0.40	0.39	0.27	0.36	0.39
C18:4 n3	0.58	0.55	0.44	0.62	0.61	0.92	0.53	0.63
C20:2 n6	0.11	0.08	0.10	0.10	0.10	0.12	0.09	0.08
C20:3 n6	0.18	0.16	0.19	0.16	0.15	0.12	0.14	0.19
C20:4 n6	2.33	2.23	2.35	2.41	2.37	1.83	2.10	2.41
C20:4 n3	0.41	0.36	0.42	0.38	0.37	0.30	0.37	0.37
C20:5 n3	10.3	9.86	10.6	10.5	10.4	7.39	8.97	10.6
C21:5 n3	0.33	0.32	0.34	0.30	0.28	0.24	0.27	0.27
C22:5 n3	1.19	1.10	1.70	1.12	1.18	0.88	1.05	1.21
C22:6 n3	19.5	18.0	21.8	20.4	21.1	17.4	17.7	22.0
Polyenoic	39.0	36.0	41.6	40.0	39.7	31.5	34.2	41.0

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