Kinetics of Antioxidant Loss in Mackerel Light and Dark Muscle

David Petillo, Herbert O. Hultin,* Judith Krzynowek,† and Wesley R. Autio‡

Department of Food Science, Massachusetts Agricultural Experiment Station, University of Massachusetts/Amherst, Marine Foods Laboratory, Marine Station, Gloucester, Massachusetts 01930

The loss of water- and lipid-soluble antioxidants of mackerel light and dark muscle was determined as a function of time of storage on ice to provide clues as to mechanisms of oxidation and to assess the use of antioxidants to measure and predict quality. Glutathione and ascorbate decreased faster initially than α-tocopherol and ubiquinone in both light and dark muscle of mackerel; in dark muscle, the rate of loss of ubiquinol was comparable to the loss of the water-soluble antioxidants. Antioxidants decreased more rapidly initially in dark muscle than in light muscle. Rates of sensory quality loss in the light and dark muscles were similar except that initial loss of quality in the dark muscle was greater than that in the light. Regression equations showed good relationships of tocopherol, ubiquinone-10, and TBARS in light and dark muscle with storage time. Ascorbate and glutathione gave the best correlation values with sensory scores for light muscle, while a good relationship was seen in dark muscle between sensory score and reduced or oxidized CoQ, ascorbate, and glutathione. The kinetic data suggest an important role of mitochondria in lipid oxidation in mackerel dark muscle.

Keywords: *Mackerel; antioxidants; ascorbate; glutathione; α-tocopherol; coenzyme Q; stability*

INTRODUCTION

Although there is general agreement that lipid oxidation makes a major contribution to the chemical spoilage of the post-mortem muscle tissue of fatty fish like Atlantic mackerel, there are many aspects of this process which are not well understood. Lipid oxidation occurs in the living animal (Ho et al., 1995). One of the clear indications for this is the presence of several supportive and interacting antioxidant systems in biological tissues (Sies, 1993). When a fish dies, several changes occur in the muscle which accelerate the rate of lipid oxidation (Hultin, 1992). These include the conversion of xanthine dehydrogenase to xanthine oxidase (Hille and Nishino, 1995), an increase in low molecular weight iron, oxidation of the iron in heme proteins from ferrous to higher oxidation states (Everse and Hsia, 1997), and the disintegration of membranes (Hultin, 1992, 1995).

The antioxidant systems in living organisms that combat these oxidative reactions are of two types. One is represented by enzymes which remove reactive oxygen species such as superoxide, hydrogen peroxide, and lipid peroxides and include superoxide dismutase, catalase, and the peroxidases. The other group of antioxidative compounds scavenge free radicals; these compounds are generally of low molecular weight and may be water- or lipid-soluble. Their activity may be mediated via enzymes, e.g., reduced ascorbate may be regenerated from the free radical, semidehydroascorbate by the enzyme semidehydroascorbate reductase using NADH as the electron donor (Navas et al., 1994). Examples of water-soluble free radical scavengers are ascorbate and glutathione, while tocopherol and ubiquinol (coenzyme Q, reduced) represent lipid-soluble low molecular weight free radical scavengers. There are proteins rich in sulfhydryls which also function as antioxidants, e.g., thioredoxin (Buchanan et al., 1994). These antioxidants reduce free radicals and are themselves oxidized. They can be reduced to their active forms by the reducing systems of the cells. Long term antioxidant function depends on metabolites such as NADH or NADPH and thus ultimately on ATP.

Lipid-soluble antioxidants function because they donate a hydrogen atom (H) to a fatty acid-based free radical more readily than does an unoxidized fatty acid. Water-soluble antioxidants can then interact with the lipid-soluble antioxidants to reduce the lipid-soluble free radical so they can continue to participate in these antioxidative reactions. The water-soluble antioxidants can interact with the lipid-soluble antioxidants since the reactive groups of the latter accumulate on the interface between the aqueous and lipid phases. In post mortem muscle tissue the ability to keep the antioxidants in the reduced state diminishes with time because of the loss of reducing compounds, the ability to stabilize lipid free radicals is lost, and the lipids will eventually oxidize. The time that this process takes is reflected in the "lag phase" of lipid oxidation.

The ability of an antioxidant to reduce another antioxidant or a lipid-derived radical is determined by their reduction potentials. This relationship has been described as a "pecking order" by Buettner (1993). Thus not only should the antioxidants of post-mortem muscle tissue be destroyed prior to fatty acid oxidation but also it could be predicted that the loss of antioxidants occurs in an orderly pattern determined by the respective oxidation reduction potentials of the compounds. The objectives of this research were to determine the loss of

* To whom correspondence should be addressed: H. O. Hultin, University of Massachusetts Marine Station, P.O. Box 7128, Gloucester, MA 01930 [telephone (508) 281-1930; fax (508) 281-2618; e-mail ummarine@tiac.net].

† Formerly associated with the National Marine Fisheries Service, Northeast Center, Gloucester Laboratory, Gloucester, MA 01930.

‡ Department of Plant and Soil Sciences, University of Massachusetts, Amherst, MA 01003.

10.1021/jf980364z CCC: $15.00
Published on Web 09/12/1998

Score	Description of Odor
7	fresh, seaweedy
6	seaweed odor disappears, low odor intensity
5	stale, peppery, earthy, sweet, rotting orange
4	sour, slight paint, tea
3	painty, mild ammonia, formaldehyde
2	fishy, fruity
1	putrid, ammonia

Figure 1. Description of sensory scores (from Kelleher et al., 1992).

the major water- and lipid-soluble antioxidants in mackerel light and dark muscle as a function of storage time and to evaluate whether the loss follows a predictable hierarchy based on their oxidation reduction potentials. It was also desired to compare the change in quality of the mackerel by both chemical and sensory quality measurements with the loss of the antioxidants.

MATERIALS AND METHODS

Materials. Whole Atlantic mackerel (*Scomber scombrus*) were obtained at a local fish distributor, Steve Connolly's Seafood in Gloucester, MA, and transported to the laboratory on ice. α-Tocopherol, dehydroascorbic acid, metaphosphoric acid, and triethanolamine were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). L-Ascorbic acid test kit and NADPH were purchased from Boehringer Mannheim Corporation (Indianapolis, IN). Disodium ethylenediamine tetraacetate (EDTA), hexane, methanol, potassium hydroxide, and 2-propanol were purchased from Fisher Scientific (Pittsburgh, PA). Coenzyme Q_{10} (ubiquinone 50), dimethyl sulfoxide, 5,5′-dithiobis(2-nitrobenzoic acid), DL-dithiothreitol, glutathione (reduced form), glutathione reductase, sodium dodecyl sulfate, 5-sulfosalicylic acid, and 1,1,3,3-tetraethoxypropane were purchased from Sigma Chemical Co. (St. Louis, MO).

Methods. Preparation of Mackerel Fillets. Mackerel were kept on ice (0−4 °C) throughout the entire process of filleting, mincing, extracting, and analyzing water- and lipid-soluble antioxidants. A paired fillet technique was used, immediately freezing one fillet "A" at −62 °C while storing its counterpart "B" for a specified time on ice (Xing et al., 1993). Six mackerel were used for each time period, which ranged from 12 h to 11 days. After that time, they were frozen as their counterpart "A" controls until the time of assay. After thawing, the fillets were skinned, and light muscle was separated from dark muscle by careful excision.

Sensory Determinations. Separate light and dark muscle samples (10 g) were randomly numbered and given sensory scores by a trained sensory panel of six according to an odor scale (Figure 1) developed for raw mackerel by monitoring the types of odors that Atlantic mackerel develop during refrigerated storage (Kelleher et al., 1992). Approximately 10 g of either light or dark muscle from each of six fish was placed in separate sterile polystyrene Petri dishes and put on a tray of ice. The six samples of either the light or dark muscle were presented to the panelists at one time.

Preparation of Samples for Chemical Analysis. After sensory evaluation, the separated muscle was minced with a KitchenAid grinder Model KSM90 (KitchenAid Inc., St. Joseph, MI) through a $^3/_{16}$ in. diameter sieve and the light and dark mince assayed separately for ascorbate ($AscH^-$), total glutathione (GSR), α-tocopherol (TOH), ubiquinol (QH_2), ubiquinone (Q), and thiobarbituric acid-reactive substances (TBARS).

Extraction Procedure for Ascorbate, Glutathione, and TBARS. Three grams of light or dark muscle mince was added to an amber glass vial containing 12 mL of chilled 5% 5-sulfosalicylic acid and homogenized in a Kinematic Polytron (Brinkmann Instruments, Westbury, NY) at setting 60 (30 s for white, 40 s for dark). The homogenate was then centrifuged in a chilled IEC Clinical Centrifuge Model 428 for 15 min at setting 7 (approximately 2250*g*). The supernate was then syringe-filtered using 0.45 μ polysulfone filters to remove particulate debris. The clarified supernate was then distributed among the following tests: 4 mL for ascorbate determination, 5 mL for glutathione, and 2 mL for TBARS determination.

Assays. Ascorbate was determined using an enzymatic test kit developed by the Boehringer Mannheim Corporation (Indianapolis, IN). Total glutathione (GSR) was determined according to the modification of Griffith (1980). Thiobarbituric acid-reactive substances (TBARS) were determined using a modification of the method by Vynke (1970) as described by Lemon (1975). The following modifications were used: (1) 5% 5-sulfosalicylic acid was used in place of the 7.5% trichloroacetic acid (TCA) as the extracting solution and (2) Pyrex tubes containing 2 mL of 0.02 M TBA and 2 mL of extract were capped and boiled for 20 min.

Extraction Procedure for α-Tocopherol, Ubiquinol, and Ubiquinone. Lipid-soluble antioxidants were extracted using a modification of Burton et al. (1985). This involved homogenizing 10 g of the prepared mince in three volumes (30 mL) of chilled 5 mM sodium phosphate buffer, pH 8.0. Forty milliliters of 0.1 M sodium dodecyl sulfate (SDS) was added to the homogenate and vigorously shaken for 2 min. Eighty milliliters of absolute ethanol was then added, and the mixture vigorously shaken for another 2 min. Twenty milliliters of hexane was added, and the mixture vigorously shaken for 2 min every 10 min for 30 min (three shakes for a total of 6 min). The capped tubes were centrifuged in a chilled Beckman Ultracentrifuge Model L5-65B for 15 min at 15000 rpm (35000*g*). The top hexane layer was removed and syringe-filtered with 0.45 μm polytetrafluoroethyl acetate (PTFE) filters to remove particulate debris. The total volume of hexane was measured with a Kimax volumetric tube. Two milliliters of hexane was dried for determining lipid content. The remaining hexane was evaporated to 1−3 mL under a stream of nitrogen.

Separation and Quantitation of α-Tocopherol, Ubiquinol, and Ubiquinone. Lipid-soluble antioxidants were separated and quantitated by a high-pressure liquid chromatography (HPLC) system incorporating a Dupont 870 Pump module, a Rheodyne model 7010 20 μL sample injection valve, a Rainin Microsorb 5 μM silica (25 cm bed and 1.5 cm guard module) column, and a Hitachi F-2000 fluorometric spectrophotometer. The normal-phase HPLC system used 1.5% 2-propanol in hexane at a flow rate of 1.0 mL/min at the waste outlet. Ubiquinol-10 standards were prepared from ubiquinone-10 according to the method of Lang et al. (1986). The effluent was monitored with the fluorescence spectrophotometer set at an excitation wavelength (λ_{ex}) of 295 nm and emission wavelength (λ_{em}) of 325 nm for α-tocopherol with a retention time of 271 s. An λ_{ex} of 271 nm and λ_{em} of 290 nm was used for ubiquinone-10 with a retention time of 282 s and an λ_{ex} of 294 nm and λ_{em} of 369 nm was used for ubiquinol-10 with a retention time of 353 s.

Determination of the Effect of Freezing and Time of Storage at −62 °C. The number of samples and analyses in this study necessitated the freezing and temporary storage of mackerel fillets in a still air freezer at −62 °C for such times as necessary to perform the analysis of sensory, TBARS, and antioxidant concentration determinations. This was done for the fillets stored on ice for periods up to 11 days and their time "0" control counterparts. There was a concern that the freezing/storage/thawing process could adversely affect these analyses.

The effect of freezing was determined by filleting six fresh Atlantic mackerel, placing them in polyethylene bags, and keeping the six fillets (from the six fish) in a still air freezer

Table 1. Initial Antioxidant Concentrations in Atlantic Mackerel Light and Dark Muscle[a]

	nmol/g wet weight muscle		nmol/g lipid	
	lt muscle	dk muscle	lt muscle	dk muscle
ascorbate	71 ± 9	68 ± 8		
glutathione	89 ± 21	79 ± 17		
α-tocopherol	53 ± 16	232 ± 54	460 ± 107	1498 ± 194
ubiquinone-10	14 ± 4	352 ± 108	130 ± 32	2307 ± 608
ubiquinol-10	2 ± 1	26 ± 8	19 ± 6	216 ± 78

[a] Light and dark muscle concentration data are represented as the mean ± deviation (n = 84).

at −62 °C for 1 day. Light and dark muscle were immediately separated from the remaining six fresh fish by careful excision. Sensory, TBARS, and water- and lipid-soluble antioxidant concentrations were determined as described previously. The six frozen fillets were thawed on ice overnight, and analyses were done as for the fresh fish.

The effect of the time of frozen storage at −62 °C was determined from the time "0" frozen controls of the fillet samples kept on ice. These samples were kept frozen for 1, 5, 11, 15, 23, 29, or 62 days in a still air freezer at −62 °C. When needed for analysis, a set of six frozen fillets from one time period was thawed overnight on ice and processed as described previously for sensory, TBARS, and antioxidant concentrations. This procedure was repeated for all other remaining time periods.

Replications and Statistical Methods. Two independent analyses were done on each fillet sample for all antioxidant concentration and TBARS determinations. Sensory scores were done only once for each muscle sample by six panelists. All data are represented as the mean ± standard deviation, unless otherwise noted.

The effect of freezing, time of frozen storage at −62 °C, and change over storage time on ice was evaluated for light and dark muscle using the analysis of variance (PROC ANOVA) procedure of the Statistical Analysis Systems (SAS Institute, Cary, NC, 1996). This was done for sensory scores, TBARS determinations, and for each water- and lipid-soluble antioxidant in terms of its concentration. The analysis of variance procedures was used to determine significant differences between light and dark muscle, fresh and frozen samples, and TBARS and antioxidant concentrations and sensory score differences between samples stored on ice and their time "0" controls. Regression procedures were used to determine suitable relationships between antioxidant concentrations (and TBARS) and time of storage on ice and between antioxidant concentrations (and TBARS) and sensory scores for both light and dark muscle. In addition, when significant differences were noted among regression data of antioxidant concentrations (and TBARS) and time of fish storage, (PROC REG) was used repeatedly on that regression data set eliminating one piece of data at a time to identify the data points that were the cause of the significant difference(s).

RESULTS

Antioxidants in Mackerel Light and Dark Muscle. Initial concentrations of water- and lipid-soluble antioxidants of mackerel light and dark muscle are shown in Table 1. The results for the lipid-soluble antioxidants α-tocopherol, ubiquinone-10, and ubiquinol-10 are expressed on both gram of wet weight and gram of lipid bases. The ascorbate and glutathione contents of the light and dark mackerel muscles were of the same order of magnitude. There were, however, significant differences between the glutathione contents in the light and dark muscle ($p < 0.01$), ascorbate concentration in light muscle compared to glutathione concentration in light muscle ($p < 0.01$), and ascorbate concentration in dark muscle compared to glutathione concentration in dark muscle ($p < 0.01$). The ascorbate content of light muscle was higher than the ascorbate content in the dark muscle ($p < 0.05$).

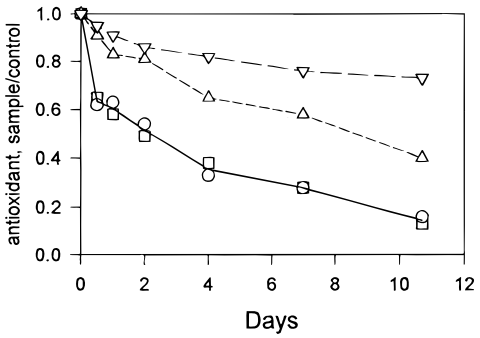

Figure 2. Antioxidants in mackerel light muscle stored on ice (0−4 °C). Results are expressed as fraction of the −62 °C control at a given time period: ○, ascorbate; □, total glutathione; △, α-tocopherol; ▽, ubiquinone-10.

The differences in the lipid-soluble antioxidants between the light and dark muscles were considerable. The greatest differences on a percentage basis occurred with coenzyme Q [ubiquinone-10 (Q) and ubiquinol-10 (QH_2)]. Coenzyme Q is found in a number of membranes and the lipoprotein particles of the blood, but it is particularly concentrated in mitochondria. Thus the great difference in these components between the light and dark muscle most likely reflects the high content of mitochondria in dark muscle (Love, 1980). Both forms of coenzyme Q were present in the dark muscle at concentrations greater than an order of magnitude than they were in the light muscle on a wet weight basis. The relative difference between the light and dark muscles in the case of tocopherol was 4.4-fold. When the results with the lipid-soluble antioxidants were expressed per gram of lipid, there was a decrease in differences between these components in the light and dark muscles compared to what was observed when the results were expressed on a gram wet weight basis. Nevertheless, the concentrations of the lipid-soluble antioxidants in dark muscle when expressed per gram of lipid still remained much higher than was observed for light muscle. The dark and light muscles used in this study had lipid contents of 11.5 ± 2.8% and 6.8 ± 2.1%, respectively.

Kinetics of Antioxidant Loss. The rate of change of the water- and lipid-soluble antioxidants in mackerel light muscle were followed over a period of approximately 11 days of storage on ice (Figure 2). The values were normalized to the corresponding −62 °C fillet so the data represent the proportion of each antioxidant remaining with time. The losses of ascorbate and glutathione were more rapid than the losses of the lipid-soluble antioxidants and were similar to each other. The loss of α-tocopherol was slower than that of the water-soluble antioxidants but more rapid than that of ubiquinone-10. Whereas both ascorbate and glutathione concentrations had dropped to 13−16% of their initial values, 40% of the initial α-tocopherol and almost three-quarters of the ubiquinone-10 were still present at the completion of storage. The data for ubiquinol-10 are not shown on the graph since its concentration was very low. Approximately one-third of the ubiquinol-10 was lost over the 11 days of iced storage.

The loss of antioxidants, including ubiquinol-10, is shown for dark muscle in Figure 3. A similar order of destruction of the antioxidants was observed with the

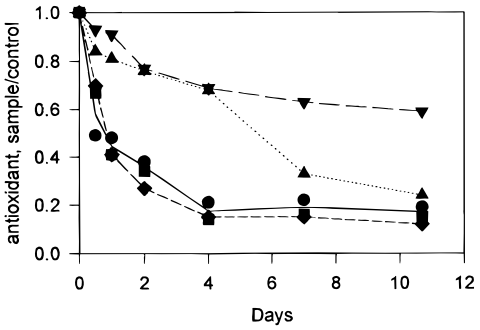

Figure 3. Antioxidants in mackerel dark muscle stored on ice (0−4 °C). Results are expressed as fraction of the −62 °C control at a given time period: ●, ascorbate; ■, total glutathione; ▲, α-tocopherol; ▼, ubiquinone-10; ◆, ubiquinol-10.

dark muscle samples as with the light. Ascorbate and glutathione decreased at approximately the same rate which was more rapid than the loss of α-tocopherol which in turn was more rapid than the loss of ubiquinone-10. In addition, the lipid-soluble antioxidant ubiquinol-10 (QH_2) decreased at approximately the same rate as that for the water-soluble antioxidants ascorbate and glutathione. The loss of ascorbate, total glutathione, and QH_2 initially was rapid and reached a minimum by day 4, whereafter there was little change. About three-quarters of the α-tocopherol was lost over 11 days but only about 40% of the ubiquinone-10.

In general, the antioxidants in the dark muscle decreased more rapidly than those in the light muscle. The one exception to this was the α-tocopherol content in the early stages of storage (up to 4 days) where losses were similar between the two types of muscles.

Sensory Changes. Sensory odor scores were obtained on the same raw samples that were used for antioxidant determinations. As was the case with the samples used for the chemical evaluations, comparisons were made between control fillets stored at −62 °C at 0 time and the counterpart paired fillets that had been stored for various periods of time on ice. Results of the sensory evaluations for light and dark muscles are shown in Figure 4a,b, respectively. In the case of the light muscle samples (Figure 4a), a loss of sensory quality occurred by the fourth day. A score of 6 indicates a very low odor intensity, whereas 5 is beginning to develop a variety of odors that were described by our expert panel as "stale, peppery, earthy, sweet, or rotting orange". A sensory score of 4 indicated the first detection of a clearly rancid odor. The light muscle retained a score of approximately 5 through the seventh day of storage. By the 11th day, the sensory score had dropped below 4, indicating a sample that had developed a rancid odor. The paired controls which had been stored at −62 °C maintained sensory scores of 6, i.e., low odor intensity, throughout most of the storage period.

The pattern of sensory changes in the dark muscle was similar to those that were observed in the light except that the absolute scores throughout the storage period rated approximately one unit less for the dark muscle than they did for the light. This was true even in the case of the control samples stored at −62 °C. A major loss of quality as judged by odor score occurred between 2 and 4 days as it had for the light muscle. There was little change between the fourth and seventh day, but another decrease occurred between 7 and 11 days of storage. Rancidity developed by the fourth day of storage in the case of the dark muscle.

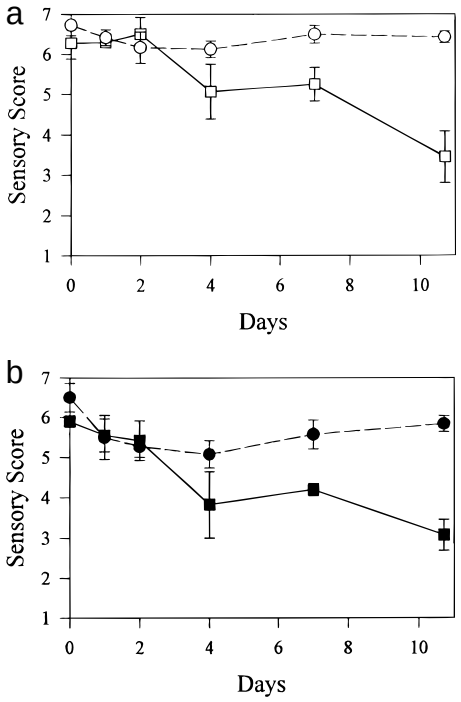

Figure 4. Sensory scores of light (a) and dark (b) mackerel muscle: ○, light and ●, dark controls (stored immediately at −62 °C). □, light and ■, dark paired fillets of controls that were stored on ice for the indicated times.

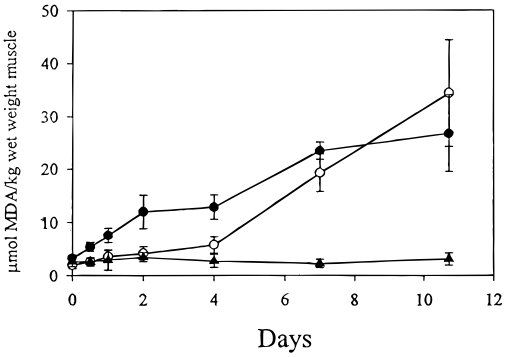

Figure 5. Comparison of the average TBARS values of Atlantic mackerel light (○) and dark (●) muscle and their −62 °C controls (▲). The same fish used for measuring changes in antioxidants in Figures 2 and 3 were used here.

There was very little difference in the scores between the light and dark muscles in the initial evaluation. The dark muscle, however, lost sensory odor quality within 12 h of storage. This difference in quality between the light and dark muscles remained constant throughout the rest of the storage period.

TBARS. There was a slow increase in TBARS in the acid extracts of light muscle up to 4 days, whereupon the rate increased considerably (Figure 5). With dark muscle, there was a relatively rapid increase in TBARS up to 2 days. The concentration was relatively constant between 2 and 4 days and then increased between 4 days and 11 days of storage. Throughout the first 4 days of storage TBARS of dark muscle were considerably higher than the TBARS values found in the light muscle ($p < 0.05$). This difference had disappeared by 7 days of storage ($p > 0.05$). No production of TBARS was observed in the control samples stored at −62 °C.

Oxidation at −62 °C. Due to the number of samples and assays that had to be performed on them, it was necessary to store both controls and samples stored on

Table 2. Antioxidant Concentrations in Atlantic Mackerel Light Muscle Stored Frozen at −62 °C[a]

	nmol/g wet weight muscle	
day	ascorbate	glutathione
1	80.9 ± 7.9[b]	112.9 ± 24.1[c]
5	64.9 ± 7.0	76.3 ± 6.6
11	68.6 ± 8.5	83.9 ± 19.9
15	67.3 ± 9.6	91.8 ± 23.6
23	77.1 ± 5.9	89.7 ± 16.4
29	72.8 ± 8.2	90.9 ± 14.6
62	66.1 ± 8.3	75.2 ± 15.1

[a] Data are from Atlantic mackerel, stage I, and each time period represents the average from six fillets, all frozen at time 0. Two independent analyses were done on each fillet sample. All data are represented as the mean ± standard deviation ($n = 12$). [b] Significantly different from other times of storage: ($p < 0.05$). [c] Significantly different from other times of storage: ($p < 0.01$).

Table 3. Antioxidant Concentrations in Atlantic Mackerel Dark Muscle Stored Frozen at −62 °C[a]

day	glutathione nmol/g wet weight muscle	ubiquinol-10 nmol/g lipid
1	102.7 ± 23.9[b]	167.3 ± 36.6
5	71.8 ± 9.0	248.4 ± 77.8[b]
11	71.6 ± 10.2	246.8 ± 47.6[b]
15	79.0 ± 11.6	188.3 ± 57.7
23	75.4 ± 13.5	185.5 ± 43.7
29	80.2 ± 10.6	192.2 ± 45.2
62	73.9 ± 17.6	282.4 ± 139.8

[a] Data are from Atlantic mackerel, stage I, and each time period represents the average from six fillets, all frozen at time 0. Two independent analyses were done on each fillet sample. All data are represented as the mean ± standard deviation ($n = 12$). [b] Significantly different from other times of storage: ($p < 0.01$).

ice at various periods of time at −62 °C before analysis. It was possible that a temperature of −62 °C was not sufficiently low to completely retard oxidative processes in the muscle tissue. To evaluate this possibility, a comparison was made between antioxidant concentrations in light and dark muscle samples that had been stored for various periods of time at −62 °C. The samples that were used for these studies were the zero time controls, that is, those samples that were immediately frozen and stored at −62 °C without any storage time at 0−4 °C. Ascorbate and glutathione were the only antioxidants in light muscle that showed significant changes on storage at −62 °C (Table 2). Ascorbate ($p < 0.05$) and glutathione ($p < 0.01$) decreased significantly between the first and the fifth and subsequent days of frozen storage. This represents an average drop of 14% in ascorbate and 25% in glutathione between the first day of frozen storage and the other time periods. There were no significant differences among the other time periods.

The only dark muscle antioxidants to show significant changes during time of frozen storage at −62 °C were glutathione and ubiquinol-10 (Table 3). Glutathione decreased 27% ($p < 0.01$) between the first day of frozen storage and all other time periods. Unlike light muscle, ascorbate in dark muscle did not show any significant loss. Ubiquinol-10 increased 18% ($p < 0.01$) on the fifth and 11th days of storage compared to all other time periods.

The "day 1" samples in the above experiments were frozen for 1 day before analysis. To determine if the process of freezing and thawing caused a decrease in any of the antioxidants, the antioxidant contents of fresh, unfrozen samples were compared to their paired fillets that were frozen overnight at −62 °C before thawing and assaying. Seven sets of paired fillets with duplicate samples from each fillet ($n = 28$) were used in these studies. No significant differences were found between the fresh or frozen fillet pairs for any of the antioxidants in either the light or dark muscle.

DISCUSSION

Post-mortem metabolism in muscle tissue is critical in controlling and maintaining quality. Enzymic and chemical reactions continue to occur after the death of the animal although they may be significantly modified by conditions existing post-mortem. Among the more important of the post-mortem conditions in the muscle tissue is the loss of the oxygen supply and the concurrent decrease in ATP, the principal chemical energy source in the cell. Not only has interest been focused on post mortem metabolism because of its effect on the quality of the product but the possibility has been explored that the extent of some of these post-mortem reactions may be used to estimate the quality of the muscle food. Determination of the products of a given metabolic sequence might also allow a prediction to be made as to the potential shelf life of the muscle tissue. Perhaps the most studied example of the use of metabolic products to predict quality and potential shelf life of muscle foods is the post-mortem breakdown of ATP. ATP is metabolized in post-mortem muscle into a series of compounds which are formed in a time/temperature-dependent manner (Botta, 1995). The relative amounts of the various metabolites formed can indicate quality and predict shelf life. The products of the breakdown of ATP have some direct effect on product quality as well, e.g., inosine monophosphate contributes a "meaty" flavor while hypoxanthine has a bitter taste (Foegeding et al., 1996).

In fatty fish species, a major loss of quality is due to the oxidation of the highly unsaturated fatty acids that are found in the tissue. Although certain oxidative reactions in lipids have important physiological functions and occur in the living animal (Smith, 1989), much of fatty acid oxidation that occurs in post-mortem muscle tissue is the result of random oxidations by a system that has lost its ability to adequately contain its oxidative potential. The usual focus in estimating quality in fatty-type fish muscle tissue has been to look for early signs of chemical changes in the lipids themselves. Our work in this study was undertaken based on the supposition that the antioxidants which are present in muscle tissue should also deteriorate with time post-mortem since they are destroyed by oxidative reactions, as are the lipids. There is a potential advantage of measuring changes in the antioxidants rather than changes in the lipids themselves. Since antioxidants function by protecting lipids from oxidation by being preferentially oxidized, it can be hypothesized that loss of antioxidants should precede oxidation of lipids, thus giving a warning of incipient oxidation. In addition, it would be expected that cellular antioxidants undergo a sequential destruction among themselves since they have different subcellular localizations and different oxidation reduction potentials. This "pecking order" has been described in detail by Buettner (1993). Important water-soluble antioxidants in muscle foods are ascorbate and glutathione, while tocopherol and coenzyme Q are major lipid-soluble antioxidants. These antioxidants most likely act in a concerted way to protect sensitive molecules such as the unsaturated fatty acids from oxidation.

The ability of ascorbate and tocopherol to suppress oxidation longer than the sum of their individual effects demonstrated that there was a cooperative effect of these antioxidants in suppressing lipid oxidation of membrane phospholipids (Leung et al., 1981). Barclay (1988) showed that glutathione could trap peroxyl radicals in the aqueous phase and thus indirectly spare tocopherol in lipid bilayers; glutathione did not regenerate tocopherol directly from the tocopheroxyl radical. Winkler et al. (1994) showed that glutathione reduced dehydroascorbate to ascorbate, which then reduced the tocopheroxyl radical. Xu and Wells (1996) stated that ascorbate can scavenge oxygen radicals produced by mitochondria and can also reduce α-tocopheroxyl radicals. Roginsky et al. (1996) showed that reduction of oxidized coenzyme Q by ascorbate was rapid at low oxygen concentrations, while May et al. (1996) demonstrated that in red blood cells the reduction of dehydroascorbate to ascorbate was largely dependent on glutathione by both enzymic and nonenzymic modes.

The results obtained in the work reported here are consistent with a sequential loss of antioxidants. In both mackerel light and dark muscle, ascorbate was lost before α-tocopherol. This would result from a sequence whereby tocopherol (TOH) donated hydrogen to a lipid peroxyl radical and the ascorbate ($AscH^-$) then interacted with the tocopheroxyl radical to regenerate tocopherol producing the semidehydroascorbate radical (eqs 1 and 2). The semidehydroascorbate radical could then be further oxidized to dehydroascorbate (DHA) by reacting with another tocopheroxyl radical or by another radical such as a peroxyl radical in the aqueous phase (eqs 3 and 4). The dehydroascorbate could be directly reduced by glutathione (eq 5). Winkler et al. (1994) concluded from their studies that a direct interaction between glutathione and dehydroascorbate is the most likely physiological reaction to cause the reduction of dehydroascorbate to ascorbate in mammalian tissues.

$$LOO^\bullet + TOH \rightarrow LOOH + TO^\bullet \quad (1)$$

$$TO^\bullet + AscH^- \rightarrow TOH + Asc^{\bullet -} \quad (2)$$

$$TO^\bullet + Asc^{\bullet -} \rightarrow TOH + DHA \quad (3)$$

$$ROO^\bullet + Asc^{\bullet -} \rightarrow ROOH + DHA \quad (4)$$

$$DHA + 2GSH \rightarrow AscH^- + GSSG \quad (5)$$

It is doubtful that this last reaction (eq 5) had a significant role in regenerating dehydroascorbate since we have observed little build up of oxidized glutathione in stored mackerel muscle (Jia et al., 1996). The total amount of glutathione, i.e., oxidized plus reduced, in the study reported here decreased as rapidly as did the ascorbate concentration in both the light and dark muscles. Thus, it seems likely that something other than a simple cycling of glutathione between its oxidized and reduced states is involved. Glutathione can donate a hydrogen to several species of free radicals such as hydroxyl, alkoxyl, and peroxyl to produce the glutathiyl radical ($GS^\bullet$) under the conditions in stored mackerel muscle, i.e., initial intracellular glutathione concentrations of roughly 0.1 mM and the low concentrations of molecular oxygen in the interior of the fillet. The glutathiyl radical can react with another GSH to form the oxidized glutathione radical ($GSSG^{\bullet -}$) (eq 6) (Buettner, 1993). This radical is a very potent reducing agent and could reduce the semidehydroascorbate radical to ascorbate (eq 7).

$$GS^\bullet + GSH \xrightarrow{+H^+} GSSG^{\bullet -} + HA \quad (6)$$

$$GSSG^{\bullet -} + Asc^{\bullet -} \xrightarrow{+A^-} AscH^- + GSSG \quad (7)$$

The glutathiyl radical could also react with oxygen. The expected relatively low concentration of oxygen in the tissue would suggest that this reaction would not be very competitive with the reaction with glutathione (eq 6). However, our data in this paper showed a loss in total glutathione during storage of the mackerel muscle, while in an earlier study it was demonstrated that the loss in reduced glutathione was not accompanied by an increase in the oxidized form (Jia et al., 1996). Thus it would appear that reactions other than oxidation may have accounted for our observations. The high activities of glutathione transferase found in mackerel muscle (Jia et al., 1996) could have been responsible for the loss in total glutathione. If this were the case, then it may be that the close correlation between glutathione and ascorbate losses observed in this study were coincidental.

In addition to its role as an electron carrier in the inner membrane of the mitochondria, ubiquinol (reduced coenzyme Q) can inhibit lipid oxidation in many membrane systems (Ernster and Dallner, 1995). The great majority of the coenzyme Q determined in the light and dark mackerel muscle of this study was in the oxidized form where it was unlikely to function directly as an antioxidant. Nevertheless, with storage time there was a relatively slow decrease in the concentration of ubiquinone-10 in both the light and dark muscle as well as a rapid decrease in ubiquinol-10 in the dark muscle. The decrease of the ubiquinol-10 in dark muscle was as rapid as was the loss of ascorbate and glutathione. There was little loss of ubiquinol-10 in the light muscle, but the concentration to begin with was very low and may not have played a major role in the antioxidative processes. The ubiquinol-10 in the dark muscle could have reacted directly with any oxygen radicals produced or it could have regenerated tocopherol from the α-tocopheroxyl radical (Buettner, 1993).

The oxidized coenzyme Q (ubiquinone) could possibly have participated as an antioxidant in the mackerel muscle of this study. The semidehydroascorbate radical could reduce ubiquinone to the semiubiquinone while being oxidized to dehydroascorbate (eq 8). The semiubiquinone radical could then either reduce a tocopheroxyl radical regenerating the tocopherol (eq 9) or two molecules of the semiubiquinone could dismutate to one oxidized and one reduced molecule of the coenzyme (eq 10). The rapid loss of reduced coenzyme Q suggests that it may have functioned to regenerate tocopherol from the tocopheroxyl radical directly (eq 11).

$$Asc^{\bullet -} + Q \rightarrow DHA + QH^\bullet \quad (8)$$

$$QH^\bullet + TO^\bullet \rightarrow Q + TOH \quad (9)$$

$$2QH^\bullet \rightarrow Q + QH_2 \quad (10)$$

$$QH_2 + TO^\bullet \rightarrow QH^\bullet + TOH \quad (11)$$

In general, for all antioxidants, the decrease was faster initially in dark muscle than in light muscle. This is not surprising since dark muscle is known to turn rancid faster than light muscle. This is probably due to the higher content of pro-oxidants in the dark tissue than in the light (Hultin, 1994). There was also more lipid in the dark muscle (11.5 ± 2.8%) versus that in the light (6.8 ± 2.1%). The difference in stability between light and dark muscles was not due to a lack of antioxidants. Although there were significantly higher glutathione ($p < 0.01$) and ascorbate ($p < 0.05$) concentrations in the light muscle compared to the dark, the absolute differences were small and not likely to be a major factor. The dark muscle had much higher contents of the lipid-soluble antioxidants α-tocopherol and coenzyme Q than did the light muscle. On a wet weight basis, it was not surprising to see this since there was more lipid in the dark muscle. However, even when expressed on a gram of lipid basis, the lipid-soluble antioxidants were much higher in the dark muscle than in the light, e.g., over 3-fold for α-tocopherol, almost 18-fold for ubiquinone, and 11-fold for ubiquinol (Table 1). Thus it seems most likely that the greater oxidation that occurs in dark muscle is due to the higher content of the pro-oxidants in the dark muscle compared to the light.

Brannan and Erickson (1996) studied the loss of ascorbate, α- and γ-tocopherol, and glutathione in frozen mince and fillets of channel catfish over a 6 month storage period. They observed a more rapid decrease in glutathione than ascorbate in this species under these conditions. The decline in α-tocopherol was slower than either of the water-soluble antioxidants. Initially the proportion of glutathione in the oxidized form in their studies was small, but it increased somewhat with storage time. The major change in glutathione, however, was a loss in total glutathione.

The effect of time of storage at −62 °C on control samples that were not stored on ice but frozen immediately at −62 °C showed that two antioxidants in both the light and dark muscle showed changes with time of frozen storage at −62 °C. In the case of light muscle, both ascorbate and glutathione decreased significantly from the first day of frozen storage to the fifth. After that, there was no further significant decrease in either of these antioxidants throughout the rest of the 62 days of storage at −62 °C. These data suggest that there is a fraction of these antioxidants which is more labile than the rest. Results from a separate experiment indicated that there was no difference in ascorbate or glutathione between an unfrozen control and a control that had been frozen for 1 day at −62 °C. Ascorbate and glutathione showed themselves to be the most sensitive of the antioxidants in mackerel light muscle when stored on ice.

A similar observation was observed with glutathione in Atlantic mackerel dark muscle that was stored at −62 °C. There was a significant decrease between the first and fifth day of storage but none thereafter. This was not seen in the case of ascorbate, which showed no significant change over the 62 days of storage at −62 °C. However, the ascorbate content of the dark muscle was at a value commensurate with its concentration in light muscle after the latter had undergone the loss which occurred between the first and fifth days of storage. It may be that the ascorbate in the dark muscle was more sensitive to destruction than ascorbate in light muscle, the decrease occurred faster, and the loss had already occurred by the first day.

The ubiquinol-10 content in the dark muscle was the only antioxidant that was seen to increase with time having significantly higher concentrations at the fifth and 11th days of storage at −62 °C compared to the other time periods both before and after the fifth and 11th days. It is not clear why this antioxidant should increase. It may represent a reduction from oxidized coenzyme Q (ubiquinone) to reduced coenzyme Q (ubiquinol) in the muscle tissue due to unknown factors. Ubiquinone was present at a concentration 1 order of magnitude higher than that of the reduced form of coenzyme Q. Thus a relatively small amount of reduction of the oxidized coenzyme could cause the increase in the reduced form.

Storage at −62 °C prevented any increase in TBARS. Also, an essentially constant sensory score was maintained over the storage period.

In the early stages of storage, TBARS values increased considerably faster in dark muscle tissue than in light, but after 7 days there was no significant difference between the two. Significant changes took place in the sensory scores of both light and dark muscles between 2 and 4 days ($p < 0.01$ for both). Over that same period of time, TBARS changes in both the light and dark muscle were not significant. TBARS values of the light muscle were still in the lag phase portion of the oxidation curve at 4 days of storage. It has been found in our laboratory that mackerel muscle fillets develop rancid odors when TBARS values reach the range of 10−20 nmol TBARS calculated as malonaldehyde per gram of wet weight of muscle. TBARS values for dark muscle were essentially the same after 2 and 4 days of iced storage even though the muscle had developed sensory scores indicating rancidity (sensory scores of 4 or less) at day 4 but not at day 2. Light muscles did not develop rancidity until day 11 when their TBARS score went to over 30. Dark muscle became rancid at a TBARS value of about 13, while light muscle was not rancid at a TBARS value of around 19. This suggests that rancidity in mackerel dark muscle is associated with lower TBARS values than is rancidity in mackerel light muscle.

Two types of information are required if the antioxidant data is to be used to determine quality and/or predict shelf life. First, it is necessary to know the antioxidant concentrations in the fresh muscle tissue. Not surprisingly, variability in the data was considerable (Table 1). Ascorbate concentrations in both the light and dark muscle, and α-tocopherol in the dark muscle, had lower variabilities than other antioxidants in either tissue. Second, a mathematical expression is needed that describes the change in an antioxidant concentration as a function of some other variable. This other variable could either be time of storage or a measure of quality of the fish. Regression equations and correlation values for antioxidant ratios with respect to storage time are shown in Tables 4 and 5 for light and dark muscle, respectively. Changes in α-tocopherol, ubiquinone-10, and TBARS of light muscle were closely related ($r^2 = 0.97$ to 0.98) with changes in storage time (Table 4). Changes in α-tocopherol ($r = 0.97$), ubiquinone-10 ($r^2 = 0.99$), and TBARS ($r = 0.98$) of dark muscle had good relationships to storage time (Table 5).

Table 4. Regression Equations and Correlation Values for Light Muscle Antioxidant Ratios with Respect to Storage Time[a]

antioxidant	regression equation	r^2 or r
ascorbate ($AscH^-$)	$[AscH^-] = -0.15(days) + 0.0085(days)^2 + 0.82$	0.87
glutathione (GSR)	$[GSR] = -0.14(days) + 0.0075(days)^2 + 0.81$	0.86
α-tocopherol (TOH)	$[TOH] = -0.08(days) + 0.0027(days)^2 + 0.95$	0.97
ubiquinol-10 (QH_2)	$[QH_2] = -0.02(days) + 0.77$	0.56
ubiquinone-10 (Q)	$[Q] = -0.05(days) + 0.0028(days)^2 + 0.97$	0.97
TBARS	$[TBARS] = -0.0087(days) + 0.094(days)^2 + 0.87$	0.98

[a] The regression equations for each antioxidant are based on the ratio of the antioxidant concentration at any given time at 0−4 °C to the concentration in its paired fillet that had been immediately frozen at −62 °C. Water-soluble ascorbate and glutathione ratios were calculated on a nmol/g wet weight basis. Lipid-soluble ubiquinol-10, α-tocopherol, and ubiquinone-10 ratios were calculated on a nmol/g lipid basis. Thiobarbituric acid-reactive substances (TBARS) are expressed as nmol MDA/g muscle. Time of storage (0−11 days on ice). r^2 is the coefficient of determination for polynomial equations; r is the correlation coefficient for linear equations.

Table 5. Regression Equations and Correlation Values for Dark Muscle Antioxidant Ratios with Respect to Storage Time[a]

antioxidant	regression equation	r^2 or r
ascorbate ($AscH^-$)	$[AscH^-] = -0.18(days) + 0.012(days)^2 + 0.74$	0.74
glutathione (GSR)	$[GSR] = -0.22(days) + 0.015(days)^2 + 0.79$	0.83
α-tocopherol (TOH)	$[TOH] = -0.07(days) + 0.91$	0.97
ubiquinol-10 (QH_2)	$[QH_2] = -0.22(days) + 0.015(days) + 0.79$	0.82
ubiquinone-10 (Q)	$[Q] = -0.14(days) + 0.017(days)^2 - 0.00076(days)^3 + 1.0$	0.99
TBARS	$[TBARS] = 0.75(days) + 1.55$	0.98

[a] All conditions are as described in Table 4.

Table 6. Regression Equations and Correlation Values for Sensory Scores with Respect to Light Muscle Antioxidant Ratios[a]

antioxidant	regression equation	r^2 or r
ascorbate ($AscH^-$)	sensory score $= 12.9[AscH^-] - 8.6[AscH^-]^2 + 1.8$	0.94
glutathione (GSR)	sensory score $= 12.1[GSR] - 7.9[GSR]^2 + 2.1$	0.92
α-tocopherol (TOH)	sensory score $= 4.9[TOH] + 1.9$	0.91
ubiquinol-10 (QH_2)	sensory score $= 2.7[QH_2] + 3.6$	0.42
ubiquinone-10 (Q)	sensory score $= 116.9[Q] - 62.5[Q]^2 - 48.3$	0.83
TBARS	sensory score $= 6.4 - 0.2[TBARS]$	0.90

[a] Sensory scores are described in Figure 1. All other conditions are as described in Table 4.

Regression equations and correlation values for antioxidants and TBARS with sensory scores are shown in Tables 6 and 7 for light and dark muscle, respectively. Ascorbate ($r^2 = 0.94$) and glutathione ($r^2 = 0.92$) had the best relationships with respect to sensory scores in light muscle (Table 6), while the same two antioxidants plus coenzyme Q (Q, $r = 0.91$; QH_2, $r^2 = 0.90$) were the best in dark muscle (Table 7). These regression equations indicate that measurement of some antioxidants can be good indicators of the sensory odor quality of raw mackerel light and dark muscle. Plots of the regression equations for sensory scores versus ascorbate concentrations in light and dark mackerel muscle show that the curves for the two types of tissue are similar (Figure 6). Fewer data points for the dark muscle are recorded at high sensory scores than are seen for the light muscle. This is due to the more rapid initial decrease of the antioxidant and sensory score in the dark muscle compared to the light. The first indication of rancidity (sensory score of 4) occurs when the content of ascorbate in either type of muscle tissue decreases to about 20% of its initial value.

Table 7. Regression Equations and Correlation Values for Sensory Scores with Respect to Dark Muscle Antioxidant Ratios[a]

antioxidant	regression equation	r^2 or r
ascorbate ($AscH^-$)	sensory score $= 14.1[AscH^-] - 9.4[AscH^-]^2 + 1.2$	0.90
glutathione (GSR)	sensory score $= 12.3[GSR] - 8.5[GSR]^2 + 2.1$	0.90
α-tocopherol (TOH)	sensory score $= 3.3[TOH] + 2.5$	0.87
ubiquinol-10 (QH_2)	sensory score $= 13.0[QH_2] - 9.3[QH_2]^2 + 2.1$	0.90
ubiquinone-10 (Q)	sensory score $= 6.3[Q] - 0.2$	0.91
TBARS	sensory score $= 6.2 - 0.3[TBARS]$	0.88

[a] Conditions are as described in Table 6.

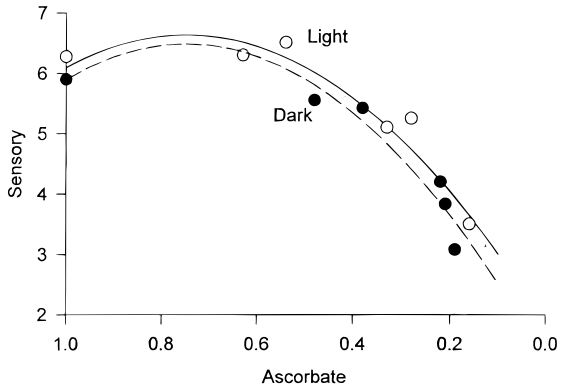

Figure 6. Plots of regression equations for sensory scores as a function of ascorbate concentration ratios for light (○) and dark (●) mackerel muscle.

The regression equations showed better relationships when the fraction of antioxidant remaining was evaluated with respect to storage time compared to sensory score. This may reflect the fact that the general level of antioxidants decreases with time of storage but that specific losses of antioxidants in specific areas of the muscle cell are more important to sensory quality than the general loss of antioxidants. The two water-soluble antioxidants, ascorbate and glutathione ($r^2 = 0.90$) and coenzyme Q (reduced, $r^2 = 0.90$ and oxidized, $r = 0.91$), had the highest correlations with sensory scores in dark muscle. Although found in a wide variety of membranes, coenzyme Q is located primarily in the mitochondrial inner membrane. We found the concentration of coenzyme QH_2 to be 11 times greater in the dark muscle than in the light on a nanomoles per gram of lipid basis, while the content of phospholipid in the dark flesh of mackerel is only about 3 times that of the light flesh (Ackman and Eaton, 1971). Most of the phospholipid of the tissue is found in membranes. These data reflect the high content of mitochondria in dark muscle. It has been reported that the number of mitochondria is some 12 times greater in the dark muscle of Atlantic pollock (*Pollachius virens*) compared to that in the light (Love, 1980).

Thus, the major source of coenzyme Q in dark muscle tissue is the mitochondrial inner membrane. It has

been established that this membrane activates much of the oxygen which passes through biological tissue (Skulachev, 1996). A side effect of this activation is the release of hydrogen peroxide and superoxide radical into the cellular milieu. The release may be accelerated by changes in the tissue after death of the fish. These reactive oxygen species may be responsible for much of the oxidation of lipid which occurs. The anaerobic conditions after death allows the build up of reduced components of the electron transport chain. Thus, an electron transport component such as coenzyme Q should be reduced under anaerobic conditions. In fact, we observed that over 90% of the coenzyme Q in mackerel dark muscle was initially in the oxidized form (Table 1). We suggest that coenzyme Q was oxidized because it had reduced molecular oxygen to superoxide and hydrogen peroxide. As was seen with the water-soluble antioxidants ascorbate and glutathione, rancidity occurred in mackerel dark muscle only when QH_2 had been reduced to 20% of its initial value. In contrast, rancid odors developed in mackerel dark muscle, while there was still 70% of the original α-tocopherol remaining. This level of tocopherol expressed on a lipid basis is approximately equal to 450 ppm. This value is 50% higher than the amount of tocopherol that is allowed by the FDA to be added to food products. Thus rancidity developed in mackerel dark muscle in the presence of high concentrations of tocopherol. The content of tocopherol in the mitochondrial inner membrane was not determined.

ABBREVIATIONS USED

$AscH^-$, ascorbate monoanion; $Asc^{\bullet -}$, semidehydroascorbate radical; DHA, dehydroascorbate; GSR, reduced plus oxidized glutathione expressed as glutathiyl moiety; GSH, reduced glutathione; GSSG, oxidized glutathione; $GS^\bullet$, glutathiyl radical; Q, ubiquinone-10; QH_2, ubiquinol-10; $QH^\bullet$, semiubiquinone radical; α-TOH, α-tocopherol; α-$TO^\bullet$, α-tocopheroxyl radical.

LITERATURE CITED

Ackman, R. G.; Eaton, C. A. Mackerel lipids and fatty acids. *Can. Inst. Food Technol. J.* **1971**, *4*, 169−174.

Barclay, L. R. C. The cooperative antioxidant role of glutathione with a lipid-soluble and a water-soluble antioxidant during peroxidation of liposomes initiated in the aqueous phase and in the lipid phase. *J. Biol. Chem.* **1988**, *263*, 16138−16142.

Botta, J. R. *Evaluation of Seafood Freshness*; VCH Publishers, Inc.: New York, 1995.

Brannan, R. G.; Erickson, M. C. Quantification of antioxidants in channel catfish during frozen storage. *J. Agric. Food Chem.* **1996**, *44*, 1361−1366.

Buchanan, B. B.; Schurmann, P.; Decottignies, P. T.; Lozano, R. M. Perspectives in biochemistry and biophysics: thioredoxin: a multifunctional regulatory protein with a bright future in technology and medicine. *Arch. Biochem. Biophys.* **1994**, *314*, 257−260.

Buettner, G. R. The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate. *Arch. Biochem. Biophys.* **1993**, *300*, 535−543.

Burton, G. W.; Webb, A.; Ingold, K. U. A mild, rapid, and efficient method of lipid extraction for use in determining vitamin E/lipid ratios. *Lipids* **1985**, *20*, 29−39.

Ernster, L.; Dallner, G. Biochemical, physiological and medical aspects of ubiquinone function. *Biochim. Biophys. Acta* **1995**, *1271*, 195−204.

Everse, J.; Hsia, N. The toxicities of native and modified hemoglobins. *Free Radical Biol. Med.* **1997**, *22*, 1075−1099.

Foegeding, E. A.; Lanier, T. C.; Hultin, H. O. Characteristics of edible muscle tissue. In *Food Chemistry*; Fennema, O. R., Ed.; Marcel Dekker, Inc.: New York, 1996; pp 879−931.

Griffith, O. W. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal. Biochem.* **1980**, *106*, 207−212.

Hille, R.; Nishino, T. Xanthine oxidase and xanthine dehydrogenase. *FASEB J.* **1995**, *9*, 995-1003.

Ho, R. Y. N.; Liebman, J. F.; Valentine, J. S. Biological reactions of dioxygen: an introduction. In *Active Oxygen in Biochemistry*; Valentine, J. S., Foote, C. S., Greenberg, A., Liebman, J. F., Eds.; Blackie Academic & Professional: London, 1995; pp 1−36.

Hultin, H. O. Biochemical deterioration of fish muscle. In *Quality Assurance in the Fish Industry*; Huss, H. H., Jakobsen, M., Liston, J., Eds.; Elsevier: Amsterdam, 1992; pp 125−138.

Hultin, H. O. Role of membranes in fish quality. In *Fish Quality − Role of Biological Membranes*; Jessen, F., Ed.; Nordic Council of Ministers: Copenhagen, 1995; pp 13−35.

Jia, T.-d.; Kelleher, S. D.; Hultin, H. O.; Petillo, D.; Maney, R.; Krzynowek, J. Comparison of quality loss and changes in the glutathione antioxidant system in stored mackerel and bluefish muscle. *J. Agric. Food Chem.* **1996**, *44*, 1195−1201.

Kelleher, S. D.; Silva, L. A.; Hultin, H. O.; Wilhelm, K. A. Inhibition of lipid oxidation during processing of washed, minced Atlantic mackerel. *J. Food Sci.* **1992**, *57*, 1103−1108, 1119.

Lang, J. K.; Gohil, K.; Packer, L. Simultaneous determination of tocopherols, ubiquinols, and ubiquinones in blood, plasma, tissue homogenates, and subcellular fractions. *Anal. Biochem.* **1986**, *157*, 106−116.

Lemon, D. W. Improved TBA test for rancidity. In *New Series Circular No. 51*; Woyewoda, A. D., Ke, P. J., Burns, B. G., Eds.; Department of Fisheries and Oceans: Halifax, NS, 1975; pp 65−72.

Leung, H.-W.; Vang, M. J.; Mavis, R. D. The cooperative interaction between vitamin E and vitamin C in suppression of peroxidation of membrane phospholipids. *Biochim. Biophys. Acta* **1981**, *664*, 266−272.

Love, R. M. *The Chemical Biology of Fishes, Advances 1968−1977*; Academic Press: London, 1980; Vol. 2, p 85.

May, J. M.; Qu, Z.-C.; Whitesell, R. R.; Cobb, C. E. Ascorbate recycling in human erythrocytes: role of GSH in reducing dehydroascorbate. *Free Radical Biol. Med.* **1996**, *20*, 543−551.

Navas, P.; Villalba, J. M.; Cordoba, F. Ascorbate function at the plasma membrane. *Biochim. Biophys. Acta* **1994**, *1197*, 1−13.

Roginsky, V. A.; Mohr, D.; Stocker, R. Reduction of ubiquinone-1 by ascorbic acid is a catalytic and reversible process controlled by the concentration of molecular oxygen. *Redox Rep.* **1996**, *2*, 55−62.

SAS. *The SAS system for windows, release 6.11*; SAS Institute, Inc.: Cary, NC, 1996.

Sies, H. Stategies of antioxidant defense. *Eur. J. Biochem.* **1993**, *215*, 213-219.

Skulachev, V. P. Role of uncoupled and noncoupled oxidations in maintenance of safely low levels of oxygen and its one-electron reductants. *Q. Rev. Biophys.* **1996**, *29*, 169−202.

Smith, W. L. The eicosanoids and their biological mechanisms of action. *Biochem. J.* **1989**, *259*, 315−324.

Vyncke, W. Direct determination of the thiobarbituric acid value in trichloroacetic acid extracts of fish as a measure of oxidative rancidity. *Fette Seifen Anstrichm.* **1970**, *72*, 1084−1087.

Winkler, B. S.; Orselli, S. M.; Rex, T. S. The redox couple between glutathione and ascorbic acid: a chemical and physiological perspective. *Free Radical Biol. Med.* **1994**, *17*, 333−349.

Xing, Y.; Yoo, Y.; Kelleher, S. D.; Nawar, W. W.; Hultin, H. O. Lack of changes in fatty acid composition of mackerel and cod during iced and frozen storage. *J. Food Lipids* **1993**, *1*, 1−14.

Xu, D. P.; Wells, W. W. α-Lipoic acid dependent regeneration of ascorbic acid from dehydroascorbic acid in rat liver mitochondria. *J. Bioenerg. Biomembrane* **1996**, *28*, 77−85.

Received for review April 9, 1998. Revised manuscript received July 28, 1998. Accepted July 30, 1998. This work was supported in part by the Massachusetts Agricultural Experiment Station and by a grant from the National Oceanic and Atmospheric Administration through the UMASS/NOAA Cooperative Marine Education and Research Program. The views expressed herein are those of the authors and do ot necessarily reflect the views of NOAA or any of its subagencies.

JF980364Z