Comparison of Quality Loss and Changes in the Glutathione Antioxidant System in Stored Mackerel and Bluefish Muscle

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Minced mackerel muscle was less stable at −20 °C than minced bluefish muscle as judged by odor evaluation but not by production of TBA-reactive substances. Minced mackerel muscle lost total glutathione faster than minced bluefish muscle at both −20 and 2 °C. Loss of glutathione was also more rapid in mackerel fillets than in bluefish fillets, but the differences were less. Sensory scores declined in both species after about two-thirds of the glutathione was lost. Neither soluble selenium nor glutathione peroxidase, oxidized glutathione reductase, or glutathione transferase activities appeared likely to account for the differences observed in glutathione losses. In a model system of washed, minced mackerel light muscle, glutathione/glutathione peroxidase was an effective antioxidant against lipid oxidation induced by an exogenous free radical-generating system. It seems possible that glutathione protected the fish muscle against quality loss in the early stages of storage.

Keywords: *Glutathione; antioxidant; mackerel; bluefish; quality loss and antioxidation*

INTRODUCTION

The glutathione system is an important antioxidant in the aqueous phase of many eukaryotic tissues (Halliwell and Gutteridge, 1989). The selenium-containing enzyme, glutathione peroxidase, reduces a number of peroxides, including fatty acid hydroperoxides and hydrogen peroxide, to the corresponding alcohols while oxidizing 2 mol of glutathione. The oxidized glutathione is reduced by an enzyme, glutathione reductase, at the expense of NAD(P)H. In living animals, the ratio of reduced glutathione (GSH) is high relative to the content of the oxidized form (GSSG).

It would be expected that the glutathione peroxidase system could function as an antioxidant system in post-mortem fish muscle by destroying both hydrogen peroxide and lipid hydroperoxides without producing radical products. Glutathione peroxidase has been found in the muscle tissue of a number of different fish species (Aksnes and Njaa, 1981; Nakano *et al*., 1992). Glutathione peroxidase activity can be enhanced in chicken muscle by increasing the level of selenium in the diet; the higher glutathione peroxidase activity obtained resulted in lowered TBA-reactive substances (TBARS) in post-mortem chicken muscle (DeVore *et al*., 1983).

Mackerel (*Scomber scombrus*) and bluefish (*Pomatomus saltatrix*) are two fatty fish that are readily subjected to lipid oxidation. It was observed that mackerel appeared to undergo spoilage at a more rapid rate than did bluefish, in handling these two fish in a number of experiments (Krzynowek, unpublished observations). The difference in stability of the two species could be due to a number of factors, including type and amount of specific antioxidants. The purpose of this work was to compare post-mortem quality losses in Atlantic mackerel and bluefish with the rate of change of the components of the glutathione system.

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MATERIALS AND METHODS

Materials. Atlantic mackerel and bluefish were obtained from local processors who received their fish from day boats. The fish were transported to the laboratory on ice and were either processed immediately or frozen at −40 °C until they were used.

Glutathione reductase (142 units/mg of protein), glutathione peroxidase (680 units/mg of protein), reduced glutathione (GSH), oxidized glutathione (GSSG), 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), hypoxanthine, and xanthine oxidase (0.4 unit/mg of protein) were obtained from Sigma Chemical Co., St. Louis, MO. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Boehringer Mannheim GMBH, Chicago, IL. 2-Vinylpyridine was obtained from Aldrich Chemical Co., Milwaukee, WI. All other chemicals were reagent grade.

Enzyme Assays. Fish which had been filleted and skinned, packaged, and stored at either −20 or −40 °C were used. Ten grams of frozen sample was thawed by tap water and homogenized in 90 mL of 50 mM potassium phosphate buffer (pH 7.0) at 2 °C for two 30 s intervals. Homogenates were centrifuged at 105000*g* for 60 min. The supernatant fraction was collected for the enzyme assays.

Glutathione peroxidase was determined according to the method of Paglia and Valentine (1967) which couples hydrogen peroxide reduction to oxidation of NADPH by glutathione reductase. Final concentrations of components in the assay medium were as follows: potassium phosphate buffer (50 mM, pH 7), sodium azide (1 mM), NADPH (0.15 mM), GSH (1 mM), and an excess of glutathione reductase (0.1 unit/mL). The reaction was started by addition of 0.15 mM hydrogen peroxide after a 5 min preincubation. The disappearance of NADPH was monitored at 340 nm at 37 °C. A molar extinction coefficient of $6.2 \times 10^3\ M^{-1}\ cm^{-1}$ was used to measure NADPH loss. An enzyme unit was defined as that activity which oxidized 1 μmol of NADPH per minute.

Glutathione reductase activity was determined according to the method of Racker (1955) which measured the disappearance of NADPH. Final concentrations in the assay media were as follows: potassium phosphate buffer (50 mM, pH 7.6), NADPH (0.1 mM), and GSSG (3.3 mM). The temperature of

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the assay was 37 °C. The molar extinction coefficient used was the same as that for glutathione peroxidase measurement. An enzyme unit was defined as that activity which oxidized 1 μmol of NADPH per minute.

Glutathione transferase activity was measured by determining the rate of formation of a conjugate of glutathione and 1-chloro-2,4-dinitrobenzene at 340 nm using an $\epsilon_{340} = 10$ mM^{-1} cm^{-1} at 30 °C (Mannervik and Guthenberg, 1981). Final concentrations in the assay media were as follows: sodium phosphate buffer (0.1 M, pH 6.5), glutathione (1 mM), and 1-chloro-2,4-dinitrobenzene (1 mM). A unit was defined as the formation of 1 μmol of *S*-(2,4-dinitrophenyl)glutathione per minute.

Glutathione Assays. Ten grams of frozen fish samples was thawed as above and homogenized in 40 mL of 5% 5-sulfosalicylic acid for two 30 s intervals. The homogenates were centrifuged at 10000*g* for 5 min, and the soluble fraction was collected for the assays.

The total glutathione content of the tissue was determined by the method of Griffith (1980). This procedure uses DTNB to oxidize GSH and NADPH to reduce the oxidized glutathione. The amount of glutathione present is related to the reaction rate by following the production of 5-thio-2-nitrobenzoic acid (TNB) at 412 nm and 30 °C.

Oxidized glutathione was determined in the presence of 2-vinylpyridine using the method of Anderson (1985). 2-Vinylpyridine forms a complex with GSH that cannot be used as a substrate by oxidized glutathione reductase. After incubation of the sample for 60 min with 2-vinylpyridine, the assay is carried out as for total glutathione.

Throughout this report, results are expressed in terms of GSR where GS represents the glutathionyl moiety and R either a hydrogen or a second glutathionyl. Thus, GSH is equivalent to one GSR and GSSG to two.

Selenium Determination. Selenium concentrations were determined on microwave-digested samples using a Perkin-Elmer Model 5100 Atomic Adsorption Spectrophotometer equipped with Zeeman background correction and an AS-60 autosampler according to the method of Seaborn *et al*. (1993).

TBARS. Thiobarbituric acid-reactive substances (TBARS) were performed on fish muscle according to the procedure of Lemon (1975). Propyl gallate (0.1%) and EDTA (0.1%) were added to reduce lipid oxidation during the assay procedure. Measurement was at 532 nm using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. Results are reported in terms of malonaldehyde.

Sensory Evaluation. Panelists were trained to identify oxidative odors in fish muscle (Dethmers *et al*., 1981). Fish samples were put into small sample trays, warmed to room temperature, and sniffed by panelists under a red light. Sensory scores ranged from 9 to 1. The best was 9, fresh with no off-odor; 0 was strongly painty. A score of 6 represented a stale or rotting orange odor. Final sensory scores were the average of six panelists. Minced mackerel and bluefish muscle were used for the sensory tests.

Studies with the Model System. Mackerel fillets were minced and washed with 4 times their weight of cold (2 °C) distilled water. The insoluble fraction of the fish muscle was separated by straining the suspension through no. 80 mesh (0.18 mm) polyethylene. This process was repeated twice. After the final wash, the insoluble fraction of the fish muscle was centrifuged at 5000 rpm in the type 19 rotor of a Beckman L5-55B Ultracentrifuge at 5 °C for 20 min at 3700*g*. The insoluble fraction (washed mince) was treated with the free radical-generating system and/or components of the glutathione antioxidant system. Washed mince samples were incubated with and without the free radical-generating system for 2 h at 5 °C and then divided into two parts. One of the parts was incubated for 22 h at 5 °C (a total of 24 h at 5 °C), and the other was frozen at −10 °C for 20 h and thawed at room temperature for 2 h.

Each test was conducted on 20 g of the washed fish muscle. The free radical-generating system (FRGS) was composed of and added to the muscle in the following sequence: 6.0 mL of 50 mM sodium phosphate buffer (pH 7), 0.4 mL of 2 mM EDTA, 0.4 mL of 4 mM hypoxanthine, and 0.4 mL of a solution of xanthine oxidase containing 1 unit per milliliter. In the samples to which GSH and GSH-Px were added, these components were first added to the buffer, and then the other components of the FRGS were added. A total volume of 9 mL was added to the 20 g of minced washed mackerel muscle. In the 9 mL of solution added, the concentration of GSH was 14.48 mM and the activity of glutathione peroxidase was 1.1 units/mL. After thorough mixing and storage for an appropriate time at 2 °C, samples were evaluated for TBA-reactive substances and sensory score.

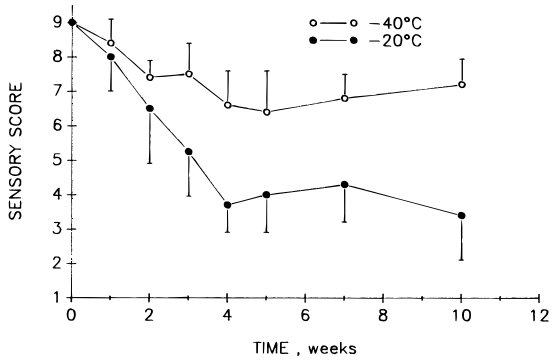

Figure 1. Sensory odor evaluations of minced mackerel stored at −20 and −40 °C. A 9-point scale was used; best quality was a score of 9.

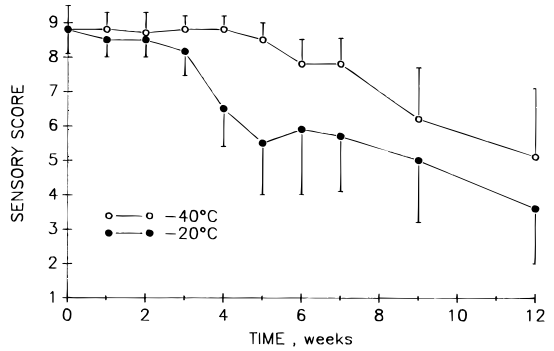

Figure 2. Sensory odor evaluations of minced bluefish stored at −20 and −40 °C. Sensory scoring was as indicated in Figure 1.

Statistical Analysis. All experiments were done at least in duplicate, and in each experiment, at least two replicates were done. Washed mince from the same fish was used for all direct comparisons. Results are reported as means ± standard deviations. In the figures, the standard deviations are shown with error bars. If no error bars are shown, the standard deviation is smaller than the symbol used. Analysis of variance was performed utilizing a general linear models procedure for the SAS system of personal computers (SAS, 1988). Mean separation was done with linear single degree of freedom comparisons.

RESULTS

Comparative Stabilities of Minced Mackerel and Bluefish Muscle. In comparisons of the stability of mackerel and bluefish, minced muscle tissue was used. This was done to ensure uniformity of samples. The sensory scores of minced mackerel and minced bluefish stored at −20 and −40 °C are shown in Figures 1 and 2. At −20 °C, the sensory score of mackerel decreased rapidly from the initial value and reached a sensory odor score of 4 within 4 weeks. Minced bluefish at that temperature was relatively stable for the first 3 weeks and then declined rapidly. Minced bluefish was rated higher than mackerel throughout storage at −20 °C.

Sensory scores for bluefish were higher than those for mackerel during the early stages of storage at −40 °C.

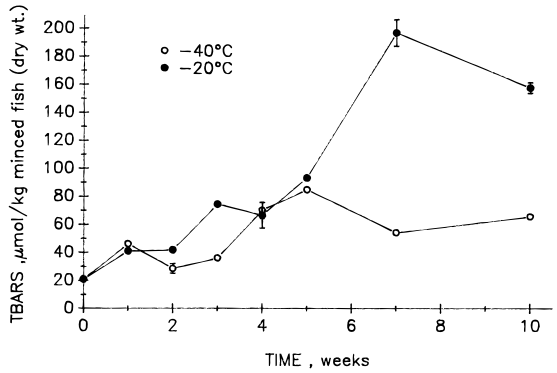

Figure 3. TBA-reactive substances formed during storage of minced mackerel at −20 or −40 °C.

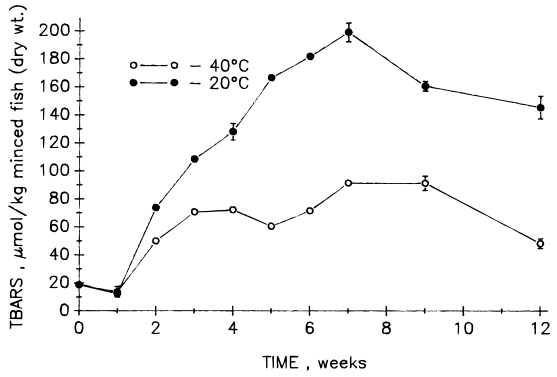

Figure 4. TBA-reactive substances formed during storage of minced bluefish at −20 or −40 °C.

Both minced bluefish and minced mackerel showed instability at this temperature. The patterns of sensory change of the two fish were quite different. Most of the decrease in the sensory scores of minced mackerel stored at −40 °C occurred during the first 4 weeks, after which no further decline was observed. The sensory scores of minced bluefish did not change very much through the first 5 weeks, but the scores declined steadily from the fifth through the twelfth weeks.

At both temperatures of frozen storage, mackerel seemed to lose its initial high quality, as indicated by its sensory score, more rapidly than bluefish. After the initial loss, the sensory properties of minced mackerel tissue changed little.

The patterns of TBARS production for the minced mackerel and bluefish at −20 and −40 °C are shown in Figures 3 and 4. The same samples were used for measuring TBARS as for sensory evaluation. There was an early increase of TBARS of minced mackerel which may relate to its rapid early decline in sensory odor. In general, however, the production of TBARS in the minced bluefish was at least as great as with the mackerel. This is true over the period of the first 4 weeks of storage where minced mackerel sensory scores declined more rapidly than did those of minced bluefish. The greatest increase in TBARS of minced mackerel at −20 °C occurred over a period of time (4−7 weeks) when there was essentially no change in the sensory scores of the mackerel. It would appear that TBARS was a poor measure of sensory odor quality between the two species processed and stored under these conditions.

Enzyme Activities. Bluefish muscle contained approximately 25% more activity of both glutathione peroxidase and oxidized glutathione reductase than did mackerel muscle. The activities of glutathione peroxidase were 0.24 μmol per minute per gram wet weight for mackerel and 0.30 for bluefish. The activity of oxidized glutathione reductase in mackerel was 0.16 μmol per minute per gram wet weight and 0.2 for bluefish. It does not seem that this relatively small difference in the activity of these enzymes could account for the greater stability of bluefish during the early stages of frozen storage. It must nevertheless be considered a possibility. There was no loss of enzymic activity in either of these enzymes during frozen storage at either −40 or −20 °C for up to 12 weeks. This is consistent with the observation of Aksnes *et al.* (1981), who observed that the activity of glutathione peroxidase activity in frozen mackerel was unchanged upon storage at −20 °C.

Table 1. Selenium Content of Total Soluble and Low Molecular Weight (LMW) Fractions from Bluefish and Mackerel Stored at −20 °C[a]

	selenium (μg/g)		
	0 weeks of storage	10 weeks of storage	12 weeks of storage
mackerel			
soluble	0.35 ± 0.05	0.31 ± 0.03	−
MW	0.18 ± 0.04c	0.14 ± 0.01b	−
bluefish			
soluble	0.34 ± 0.02	−	0.32 ± 0.02
LMW	0.16 ± 0.02c	−	0.12 ± 0.01a

[a] No significant difference ($p \leq 0.05$) in selenium content was found between any of the total soluble fractions. Low molecular weight selenium means with common letters (a−c) are not significantly different ($p \leq 0.05$).

The activity of glutathione transferase was determined in the light and dark muscles separately. The activity of this enzyme in the light muscle of mackerel ranged from 0.8 to 1.8 μmol of glutathione transferred per minute per gram wet weight and 4.1 to 7.7 for the dark muscle. Comparable samples of bluefish gave ranges of 5.5−9.9 μmol of glutathione transferred per minute per gram wet weight for light muscle and 20.5−24.1 for dark. Bluefish had approximately 5 times more GSH transferase activity than mackerel. To obtain these figures, nine fish were assayed at least in duplicate.

Soluble Selenium. Total soluble and low-molecular weight selenium were determined in samples of mackerel muscle before storage and after 10−12 weeks of storage at −20 °C (Table 1). Some 40−50% of the soluble selenium was present in the low-molecular weight fraction. No major changes in selenium content were observed in either fraction after storage. Presumably, the major form of the non-low-molecular weight soluble selenium is the selenium-containing glutathione peroxidase. There may be other selenium-containing proteins (Stadtman, 1991). There were no differences in either the total soluble or low-molecular weight soluble selenium between bluefish and mackerel.

Losses in Glutathione. Many factors control the rate of enzyme activity *in situ*; many of these factors are difficult to determine and are often unknown. However, the overall change in the substrates of enzyme-catalyzed reactions may be useful in evaluating changes that lead to quality degradation. For this reason, changes in total glutathione and oxidized glutathione were determined for mackerel and bluefish muscle stored both as mince and fillets at frozen (−20 °C) (Tables 2 and 3) and refrigerated (2 °C) (Figures 5 and 6) conditions. The amount of reduced glutathione can be determined from the difference between total and oxidized glutathione. The minced samples stored at

Table 2. Change in Total and Oxidized Glutathione of Minced Bluefish and Mackerel at − 20 °C[a]

time (weeks)	nanomoles of GSR per gram wet weight: total GSR	nanomoles of GSR per gram wet weight: oxidized GSR	fraction oxidized	fraction of initial level
bluefish				
0	183 ± 7	15 ± 3	0.08	1.00
1	129 ± 4	14 ± 3	0.11	0.70
2	122 ± 1	17 ± 1	0.14	0.66
3	67 ± 1	11 ± 1	0.16	0.37
4	50 ± 6	9 ± 1	0.18	0.27
5	58 ± 7	10 ± 5	0.17	0.32
6	49 ± 6	6 ± 4	0.12	0.27
mackerel				
0	230 ± 3	0	0	1.00
1	79 ± 3	2 ± 2	0.03	0.34
2.4	50 ± 3	1 ± 1	0.02	0.22

[a] GSR represents a glutathionyl moiety (GS) which may be linked to a H or to another GS; results are expressed on the basis of the moles of GS involved.

Table 3. Change in Total and Oxidized Glutathione of Bluefish and Mackerel Fillets at −20 °C[a]

time (weeks)	nanomoles of GSR per gram wet weight: total GSR	nanomoles of GSR per gram wet weight: oxidized GSR	fraction oxidized	fraction of initial left
bluefish				
0	183 ± 7	15 ± 3	0.08	1.00
1	180 ± 4	14 ± 3	0.08	0.98
2	169 ± 3	11 ± 1	0.07	0.92
4	123 ± 9	12 ± 3	0.09	0.67
6	105 ± 3	11 ± 0	0.10	0.57
mackerel				
0	230 ± 3	0	0	1.00
1	183 ± 2	16 ± 4	0.09	0.79
2.4	175 ± 8	11 ± 3	0.06	0.76

[a] GSR represents a glutathionyl moiety (GS) which may be linked to a H or to another GS; results are expressed on the basis of the moles of GS involved.

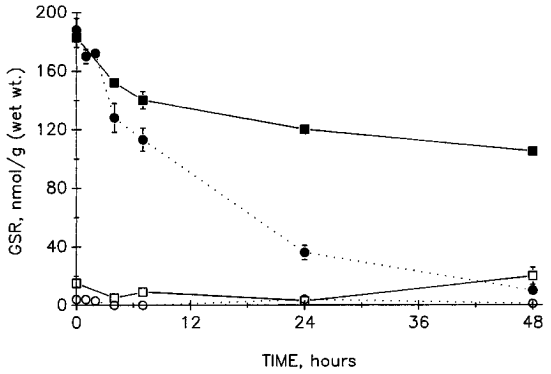

Figure 5. Change in total and oxidized glutathione of minced mackerel and bluefish at 2 °C. GSR represents a glutathionyl moiety (GS) which may be linked to H or to GS; results are expressed on the basis of the moles of GS involved. Mackerel: (●) total GSR and (○) oxidized GSR. Bluefish: (■) total GSR and (□) oxidized GSR.

−20 °C were the same as were used for TBARS and sensory measurements (Figures 1−4).

Evaluating minced muscle has the advantages that the rate of oxidation is accelerated and the samples are uniform.

The rate of loss of total glutathione was more rapid with minced mackerel than with minced bluefish (Table 2). After 2 weeks of storage, the bluefish lost approximately one-third of its glutathione, while the mackerel lost two-thirds in 1 week. After 3 weeks, the loss of total glutathione in minced bluefish at −20 °C was approximately two-thirds, and it decreased only slightly over the next 3 weeks. The loss of total glutathione in minced mackerel was almost 80% after 17 days. The fraction of total glutathione that was in the oxidized form was very low in the case of minced mackerel (only up to 3%) and less than 20% in minced bluefish muscle at −20 °C.

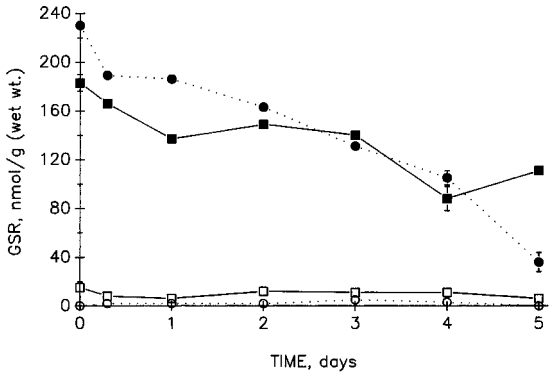

Figure 6. Change in total and oxidized glutathione of mackerel and bluefish fillets at 2 °C. GSR represents a glutathionyl moiety (see Figure 5). Mackerel: (●) total GSR and (○) oxidized GSR. Bluefish: (■) total GSR and (□) oxidized GSR.

When mackerel and bluefish fillets were stored at −20 °C (Table 3), the rate of loss of glutathione was less than it had been with the minced tissue. This result is not unexpected since mincing of muscle tissue is known to accelerate many of the reactions occurring in the tissue, including those related to oxidation. Mincing could lead to loss of glutathione by a number of mechanisms that are not mutually exclusive. Incorporation of oxygen into the minced tissue and mixing of reactants, e.g., enzymes and substrates, that were maintained in separate compartments in the intact tissue could have produced higher reaction rates in minced muscle than in fillets. Another possibility is that extracellular pro-oxidants, such as those in the blood, were brought into closer contact with susceptible intracellular molecules when the tissue was minced.

Loss of total and oxidized glutathione were determined in mince (Figure 5) and fillets (Figure 6) of mackerel and bluefish stored at 2 °C. The rate of loss of glutathione was more rapid at 2 than at −20 °C. Minced mackerel stored at 2 °C lost almost all its glutathione within 48 h, whereas bluefish under the same conditions lost around 40−50% of its initial glutathione. The loss of total glutathione in minced mackerel muscle at 2 °C followed first-order kinetics with a pseudo-first-order rate constant of 0.0625 h^{-1} and a correlation coefficient of 0.998. The kinetics of total glutathione loss from minced bluefish muscle under the same conditions were complex. This suggests that the mechanisms of destruction of glutathione in the minced muscle tissue of these two species were different.

The large differences between mackerel and bluefish observed with the minces stored at 2 °C (Figure 5) were not so apparent in the case of the fillets (Figure 6). The rate of loss of total glutathione appeared to be somewhat less in bluefish than in mackerel fillets. At the end of 5 days, the bluefish retained over 50% of its initial content, whereas the total glutathione in the mackerel fillets had decreased by about 80%. The overall rate of loss of total glutathione in mackerel fillets was significantly greater than that in bluefish fillets ($p < 0.05$). The data have to be viewed with caution, however, since

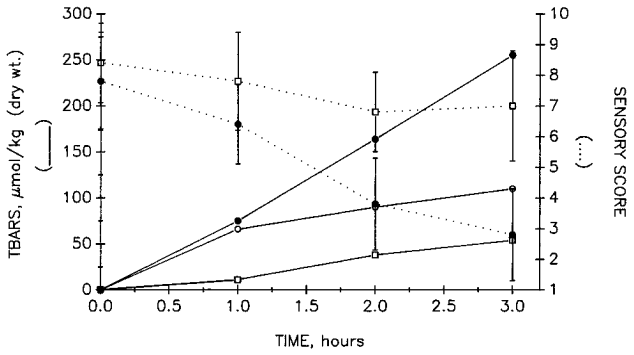

Figure 7. Change in sensory odor score and TBA-reactive substances (TBARS) in a model system of washed, minced mackerel light muscle treated with an exogenous free radical-generating system of xanthine oxidase, hypoxanthine, and $FeCl_3$ at 2 °C with and without the antioxidant system of glutathione and glutathione peroxidase: sensory score (9 is best) (···) TBARS (—). Control, washed minced mackerel (○); washed minced mackerel + the free radical-generating system (●); washed minced mackerel + the free radical-generating system + glutathione and glutathione peroxidase (□).

problems of sampling fillets make it difficult to reproduce results exactly.

Functioning of the Glutathione System as an Antioxidant. The rationale for studying the enzymic activities and stabilities and the rate of change of glutathione was the premise that the glutathione system could function as an antioxidant in post-mortem fish muscle. Irrespective of the relative rates of glutathione loss with post-mortem storage time between mackerel and bluefish as a function of fish form and time and temperature of storage, it is clear that all samples of fish showed a significant decrease in glutathione content with storage time post-mortem. If this system functioned as an antioxidant, this would mean a loss in the capacity of the system to protect the tissue.

To determine the efficacy of the glutathione system as an antioxidant in post-mortem fish muscle, a model system using washed minced mackerel muscle was investigated. The minced muscle was washed to remove pro- and antioxidants. This allowed control of the rate of oxidation of the samples and removed antioxidants which would interfere with interpretation of the effectiveness of the glutathione system. Lipid oxidation was initiated using a free radical-generating system consisting of ferric chloride, hypoxanthine, and xanthine oxidase (Srinivasan and Hultin, 1995). Evaluation of the inhibition of lipid oxidation by the glutathione system was then carried out by adding glutathione and glutathione peroxidase. The rate of production of TBA-reactive substances in the untreated washed sample was greatly accelerated when the free radical-generating system was added (Figure 7). The rate of oxidation in either the control or the sample with the free radical-generating system was decreased by the addition of glutathione and glutathione peroxidase. This indicates that the glutathione system has the potential to function as an antioxidant in post-mortem mackerel muscle.

Sensory scores of washed minced mackerel muscle that had been exposed to the free radical-generating system and to this system plus glutathione and glutathione peroxidase indicated that the glutathione system could inhibit sensory deterioration as well as that measured by TBARS (Figure 7). The sensory tests were limited to two samples because of the quantity of material that was required and also to reduce the number of samples that the sensory panel had to evaluate. Caution must be exercised, of course, in extrapolating results in this washed minced muscle system to what might occur in unwashed minced muscle, where the balance of pro-oxidants and antioxidants would be much different.

DISCUSSION

The purpose of these experiments was to compare the potential antioxidative capacity of the glutathione system in mackerel and bluefish muscle and to determine whether it was related to the loss of quality in the muscle tissue as determined by sensory odor evaluation. Sensory odor evaluation was determined on muscle tissue that was minced and stored at −20 °C. Mincing was performed to allow good uniformity in the sample. Freezing was the storage method of choice since it would eliminate most bacterial decomposition problems, thus highlighting autolytic changes in the muscle, and provide an appropriate time frame over which the deterioration could occur. Results indicated that the loss of total glutathione, which was almost completely in the reduced form, occurred more rapidly in minced mackerel than in minced bluefish at −20 °C. Two-thirds of the glutathione of mackerel muscle was lost after storage for 1 week, twice that which was lost from minced bluefish under the same conditions. Comparable amounts of glutathione were lost from bluefish after 3 weeks of storage. Concomitantly with the rapid loss of glutathione from minced mackerel, there was a rapid decrease in sensory score through the first 4 weeks of storage. Minced frozen bluefish maintained a high score through 3 weeks but declined rapidly between the third and fifth weeks. The data suggest the possibility that deterioration proceeds relatively fast once approximately two-thirds of the glutathione is lost in either species. The loss of glutathione in the samples is probably a reflection of the oxidative stress to which the tissue has been subjected, as well as its ability to respond to this stress. Differences between the species could be caused by either lower concentrations of other antioxidants (Buettner, 1993) or more active pro-oxidative systems.

It is difficult to judge enzymic activity *in situ* on the basis of the activity of extracts. The extracted activities of glutathione peroxidase and oxidized glutathione reductase in both bluefish and mackerel were roughly 0.2−0.3 μmol of substrate converted per minute per gram wet weight. The total amount of glutathione in the two species was similar, being around 0.2 μmol per gram wet weight. Thus, there was sufficient enzymic activity to convert all of the substrate (either reduced or oxidized form) to the other every minute. Even accounting for the difference in temperature of storage (−20 °C) versus that of the standard assay procedure (37 °C), over the period of storage there would have been considerable excess of enzymic activity even at −20 °C, and certainly at 2 °C, to rapidly cycle the glutathione between its oxidized and reduced forms. Thus, it appears rather unlikely that the relatively small (20−25%) differences in these activities between the two fish species could have played an important role in the slower development of lipid oxidation and greater retention of glutathione observed in minced bluefish versus that in minced mackerel muscle. Storage at 2 or −20 °C over the period of time evaluated in these experiments did not lead to any significant losses in the extractable activities of these enzymes. The similarities in concentrations of high-molecular weight soluble

selenium in the two species (Table 1) support this perspective; glutathione peroxidase is a selenium-containing enzyme.

Our average for glutathione peroxidase activity of 0.24 μmol per minute per gram wet weight in mackerel muscle was similar to the value of 0.23 μmol per minute per gram wet weight observed by Aksnes and Njaa (1981) in fresh mackerel muscle. This enzymic activity was the highest value observed in nine different species of fish that the latter authors evaluated. More recently, Nakano *et al*. (1992) determined the activity of glutathione peroxidase in five species of fish, evaluating ordinary muscle, dark muscle, skin, and liver. Their results showed an activity of at least 1 order of magnitude greater than ours and those of Aksnes and Njaa (1981). In the conversion of their data to the units we used, it was assumed that the fish muscle contained 18% protein. This assumption may lead to some error, but it seems unlikely to account for the large differences observed between their samples and ours. Nakano *et al*. (1992) did not examine either bluefish or mackerel.

The glutathione/glutathione peroxidase system is considered a primary antioxidant in a wide variety of organisms (Halliwell and Gutteridge, 1989). Presumably, it could perform this role in post-mortem fish muscle. A model system was used to test the potential of this system as an antioxidant in mackerel muscle. The mackerel muscle was minced and washed to remove water soluble antioxidants that would normally be present. This was done to make interpretation of the results simpler. Likewise, it would remove a lot of the pro-oxidants, which are mostly water soluble (Hultin, 1988) while keeping intact many of the basic components and structures of the cell. In our model system, the rate of lipid oxidation was increased by the addition of a free radical-generating system that produces both superoxide and hydrogen peroxide (Fridovich, 1985). The results of adding glutathione and glutathione peroxidase to the minced mackerel muscle that had been treated with the free radical-generating system showed that both the production of TBA-reactive substances and the reduction in sensory odor scores were inhibited in the presence of the glutathione/glutathione peroxidase system. Although the results only indicate a potential to prevent lipid oxidation, it can be considered evidence that the glutathione system could play an important role in the stability of muscle tissue post-mortem. This antioxidant system could produce quite different effects in whole mackerel muscle due to the presence of pro-oxidants and other water soluble antioxidants.

Glutathione functions as an antioxidant in tissue by donating one hydrogen atom from each of two molecules of glutathione to a peroxide with the production of one molecule of oxidized glutathione and two hydroxyl derivatives. This reaction is catalyzed by the enzyme glutathione peroxidase. In turn, the oxidized glutathione is reduced by obtaining hydrogen from NAD(P)H in a reaction catalyzed by oxidized glutathione reductase. The cyclic oxidation/reduction of glutathione occurs much faster than the synthesis of new molecules of glutathione or their degradation. In tissues, the ratio of reduced to oxidized glutathione is generally high (Halliwell and Gutteridge, 1989).

With the high cost of glutathione making it impractical to add this directly to fish muscle as an antioxidant, the possibility of finding a way to recycle oxidized glutathione to the active reduced form was initially seen as an interesting possibility. It was based on the assumption that with time of storage post-mortem the total concentration of glutathione would remain relatively constant or decrease slowly, but there would be an increase in the ratio of oxidized to reduced forms. This would occur as the reducing components in the post-mortem muscle were used up. The data (Tables 2 and 3; Figures 5 and 6) showed a quite different story. The total amount of glutathione decreased rapidly, while there was relatively little change in the high ratio of the reduced to oxidized forms of glutathione with time of storage at either 2 or −20 °C.

If the reduced glutathione was not simply converted to oxidized glutathione with storage time, the question is what happened to it? Two possibilities seem likely to have contributed to the loss in total glutathione. The activity of glutathione transferase was 1−2 orders of magnitude greater than the activities observed for glutathione peroxidase and oxidized glutathione reductase. Thus, it seems possible that some of the glutathione could be tied up through disulfide bonds to components such as proteins or sulfur amino acids. Transfer of the glutathionyl moiety to oxidation products of lipids can also occur and has been suggested to be a means of detoxifying some of these components (Mosialou and Morgenstern, 1989). The activity of the transferase enzymes was about 5 times greater in bluefish muscle than in mackerel muscle. On the basis of these relative activities, it might be expected that there would be a greater loss of glutathione in bluefish than in mackerel. In general, we found the opposite. This would argue against the transferase reaction being a major factor involved in loss of glutathione in fish muscle post-mortem.

It is also possible that glutathione could become involved in free radical chain reactions. It can give up its sulfhydryl hydrogen relatively easily to form the glutathionyl radical (eq 1) which can further react as shown in eqs 2−4 [adapted from Buettner (1993)]:

$$HO^{\bullet}, RO^{\bullet}, \text{ or } ROO^{\bullet} + GSH \rightarrow \underset{\text{glutathionyl radical}}{GS^{\bullet}} + H_2O, ROH, \text{ or } ROOH \quad (1)$$

$$GS^{\bullet} + O_2 \rightarrow GSOO^{\bullet} \text{ thiol peroxyl radical} \quad (2)$$

$$GSOO^{\bullet} + GSH \rightarrow \underset{\text{sulfinyl radical}}{GSO^{\bullet}} + \underset{\text{sulfenic acid}}{GSOH} \quad (3)$$

$$GSO^{\bullet} + GSH \rightarrow GSOH + GS^{\bullet} \quad (4)$$

Thus, reduced glutathione would become a casualty of the general oxidative processes during post-mortem storage of fish muscle.

A general loss of cellular compartmentation could account for both of these reaction pathways, i.e., glutathione transferase activity or radical attack, that would cause reduced glutathione to be converted to something other than oxidized glutathione. This decompartmentation could be the result of either physical treatment such as mincing of tissue or the normal loss of membrane structure which occurs through the catabolic reactions in the tissue after death of the animal (Hultin, 1995). In the case of the enzymic reaction, such decompartmentation could allow the enzyme to interact with its substrates to an extent that might not be possible in the living animal with its cellular structures intact. Free radical reactions would be favored by an

increase in oxygen concentration which would be important at cut surfaces or in minced muscle. Both mixing of enzymes and substrates and increase in oxygen concentrations would be more favored in minced muscle than in fillets. While losses of glutathione would be expected to occur in both tissue forms, minced muscle should lose this component at a greater rate, as was observed in these experiments.

In any case, metabolism of glutathione in post-mortem fish muscle appears to be rapid and may be related to quality maintenance, particularly in the early stages of post-mortem storage. Evidence indicates that this early time period is very important in the development of oxidative reactions in stored fish muscle (Kelleher *et al*., 1991). Thus, although glutathione may not provide long term protection to stored fish muscle, it is potentially important in protecting against oxidation in the early stages of processing.

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