Effect of Washing with or without Antioxidants on Quality Retention of Mackerel Fillets during Refrigerated and Frozen Storage

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Unwashed fillets from stage I rigor mackerel deteriorated extensively compared to unwashed fillets from stage III mackerel; this was likely related to the greater amount of blood contaminating the fillet surface of the fresher fish. Washing improved the quality of fillets from rigor fish but not stage III fish, which was attributed to the greater amount of blood removal from the fresher fish by washing. Having antioxidants in the washing solution doubled the shelf life of fillets compared to water-washed fillets. Filleting in an antioxidant solution slightly but significantly improved quality compared to cutting in air and rinsing with antioxidant solution 1 min after filleting with frozen but not refrigerated samples. Improvements with antioxidants were seen with fillets from stage I but not stage III mackerel. The pro-oxidative activity in extracts prepared from fillet surface tissue using linoleic acid as substrate was stimulated by lipid hydroperoxides. The heme proteins present in tissue extracts had the potential to account for much of the lipid oxidation observed.

Keywords: *Washing mackerel fillets; blood, role in quality; antioxidants, effect on mackerel quality; mackerel; quality of washed mackerel*

INTRODUCTION

Many of the pro-oxidants and antioxidants in fish muscle are found in the aqueous phase (Koizumi et al., 1987; Decker and Hultin, 1990b; Han and Liston, 1989; Benzie and Strain, 1997). Removing pro-oxidants is desirable while retaining antioxidants would aid in maintaining quality during storage of the muscle. Washing muscle will remove some pro-oxidants but will at the same time remove tissue factors that protect against lipid oxidation. One way to compensate for this is by washing with an antioxidant solution. Kelleher et al. (1992) found that washing minced mackerel with an antioxidant solution best inhibited lipid oxidation during storage when added at the grinding step compared to waiting until the washing step or just before storage. Samples washed with water were consistently of lower quality. These results suggest not only that having antioxidants in the washing solution improved quality but also that washing was most effective if done at the moment of cellular breakage.

In frozen surimi prepared from mackerel, filleting and mincing under water improved gel strength compared to the normal procedure (Katoh et al., 1989). Since the normal method of making surimi involves filleting and mincing in air before washing, the key point appeared to be washing the muscle at the moment of cellular disruption. Oxygen concentration in water is about 30 times less than in air. This lower oxygen concentration could cause less damage. For example, mincing reintroduces oxygen into postmortem muscle; the loss of anaerobic conditions along with tissue disruption allows xanthine oxidase to produce $O_2^{\bullet -}$ and H_2O_2 (Halliwell and Gutteridge, 1989). The lower oxygen concentration in water compared to air could limit the production of $O_2^{\bullet -}$ and H_2O_2. Instantaneous removal of aqueous prooxidants by washing may be critical since tissue breakage due to grinding in air can allow previously segregated reactants to mix causing various reactions to occur including oxidation of lipid (Virtanen, 1962). Like grinding and mincing, filleting a fish causes tissue disruption, albeit less.

Table 1. Quality Characteristics of Mackerel

attribute	stage I	stage II	stage III
eyes	very clear	slight cloudiness	cloudy
color dorsal fin	dark blue/black	dark blue/black	turning pale
gills	cherry red	maroon	dark brown
flesh	firm, shiny	firm shiny	firm, dull
fiber bundles	tightly adhered	tightly adhered	separate easily
odor of skin	seaweedy	no odor	slight off-odors

Our primary objective was to determine whether washing would improve the quality and thereby extend the shelf life of mackerel fillets. Further, we wanted to examine the role that exogeneously added antioxidants had on the quality of fillets.

MATERIALS AND METHODS

Materials. Atlantic mackerel (*Scomber scombrus*) were obtained as fresh as possible (nonfrozen, nonbled) from fish wholesalers in Gloucester, MA or Narrangansett, RI. The quality characteristics of stage I, II, and III mackerel are listed (Table 1). Stage I fish were processed immediately, while a second group from the same lot were kept on ice until judged to be stage III. They were then processed in an identical manner to the stage I group. It took 5−7 days for iced stored stage I mackerel to be downgraded to stage III.

Sodium ascorbate, sodium tripolyphosphate, bovine hemoglobin, horse heart myoglobin, Tween 20, 99% linoleic acid,

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10.1021/jf980311h CCC: $15.00
Published on Web 09/16/1998

Table 2. Number of Fish Used in Regards to Paired Fillet Treatments, Stage Fish Used, and Storage Temperature

stage	air vs H_2O	air (H_2O) vs H_2O	air (H_2O) vs AO	air (AO) vs AO
I	{7}	(6) {7}	(8) {7}	(12) {8}
III	{7}	(2) {4}	(2) {2}	not determined

[a] Number in ( ) is number of fish used for each set of treatments (2 °C storage of fillets). Number in { } is number of fish used for each set of treatments {−20 °C storage of fillets}. These values are the number of fish used for data in Figures 1 and 2 and Tables 5−8.

tetraethoxypropane, cumene hydroperoxide, and menhaden fish oil were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium dithionite was obtained from Fisher Scientific (Pittsburgh, PA). All other chemicals were reagent grade. Oxygen electrode membranes were obtained from Yellow Springs Instrument Co., Inc. (Yellow Springs, OH).

A solution of antioxidants consisting of 0.2% sodium ascorbate and 0.2% sodium tripolyphosphate was used for the antioxidant treatments. The antioxidant (AO) solution was used to rinse fillets after 1 min exposure to air or as a medium in which fish were submerged and filleted. Fish were submerged around 30 s. Fillets that were rinsed were held under the liquid for 10 s. The flow rate of the rinsing solution was around 75 mL/s.

Paired Fillet Technique. We used a paired fillet technique in which a treatment applied to a fillet from one side of a fish is compared to a control which is the fillet from the other side of the same fish. This was done to reduce variability among samples (Xing et al., 1993).

Statistics. Paired fillets from different fish were grouped. A paired t-test determined if there were significant differences between the control and the treatment (Tilling et al., 1994). The variability among fish is partially accounted for by the paired t-test, so differences between treatments can be detected without being hampered as much by the fish to fish variation.

Paired fillets which were stored at 2 °C from 4 to 9 days were grouped. Samples stored at −20 °C from 3 to 9 weeks were grouped as well. This grouping system gave us a relatively high number of replications for statistics and condensed the data (see Tables 5−8). Grouping data this way will increase standard deviations which decreases the likelihood of significant differences being obtained between two treatments that were actually different.

Treatments. The treatments performed on sets of paired fillets were as follows: (1) One fillet removed in air that was not rinsed was compared to its paired fillet removed while the fish was submerged under water [air vs H_2O] (2) A fillet removed in air that was rinsed with water after 1 min exposure to air was compared to the fillet cut under water [air (H_2O) vs H_2O]. (3) A fillet removed in air that was rinsed with water after 1 min was compared to the fillet cut in AO solution [air (H_2O) vs AO]. (4) A fillet removed in air that was rinsed with AO solution after 1 min was compared to the fillet cut in AO solution [air (AO) vs AO]. Randomization of paired fillets was done by alternating the order in which the fillet was removed by cutting under water or by cutting in air ± rinsing. Distilled, deionized water that was chilled to 6 °C was used. Water or AO solution was placed into large plastic vessels that allowed for the manual removal of the fish fillets while submerged. After washing fillets, they were placed skin-side down on one layer of paper towel; then the fillet surface was blotted for 1−2 s to remove excess liquid. After processing, the fillets were placed into oxygen permeable bags. They were stored at 2 °C or −20 °C. The number of fish used for each set of paired fillet treatments is listed (Table 2).

Sensory Evaluation. Sensory analysis was performed on iced stored fillets and frozen stored fillets that were thawed for approximately 1 h at ambient temperature. Fillets (skin-side down) were placed in 11 cm diameter × 5.3 cm depth glass bowls with lids. Samples were marked with random two digit numbers. Bowls were partially submerged in ice to control temperature during evaluation. The overall quality of paired fillets were judged by five to eight panelists. Panelists were familiarized with the onset and degree of spoilage of mackerel by daily examination of iced stored mackerel fillets and trained to recognize the odors that developed with storage. Panelists with poor sensitivity were removed. A reference that was slightly painty was prepared by blending oxidized menhaden fish oil and a nearly odorless vegetable oil. After bubbling with nitrogen the reference was stored frozen at −40 °C until needed. One-half milliliter of the reference was placed in a disposable Petri dish and presented with the fillets. Recalibration of panelists entailed using frozen stored mackerel fillets of varying degrees of painty odor. The odor score sheet was modified from Kelleher et al. (1992) and was as follows: 8: fresh seaweedy, 7: low odor, 6: stale, earthy, 5: sour, fishy, rotting orange, 4: slight paint, 3: moderate paint, 2: strong paint, 1: putrid ammonia. A red light in a sensory booth was used to remove the influence of the varying color of different fillets. A maximum of four samples were examined at one time to lessen fatigue. Scores were averaged. The averaged values of samples from different fish are grouped based on treatment in Tables 5−8. The average of the grouped values and the standard deviations are presented.

Removal of Surface Tissue. After sensory analysis fillets that had been on ice were frozen at −40 °C overnight, fillets that had been stored at −20 °C were thawed for sensory analysis and then frozen overnight at −40 °C. Overnight storage at −40 °C was done so that after a 40 min thaw at room temperature, a thin surface layer could be removed from nearly the entire length of the fillet using a standard filleting knife. The tissue (1−2 mm in depth) was taken from the meat side opposite the skin. Surface tissue was taken from fillets to determine thiobarbituric acid-reactive substances (TBARS) and peroxide values or to prepare extracts from the surface tissues to measure heme pigment content and assess the pro-oxidative activity of the muscle sections removed.

TBARS. Thiobarbituric acid-reactive substances (TBARS) were determined using the muscle extraction procedure of Lemon (1975) in which EDTA and propyl gallate were added to the extraction solution to lessen development of TBARS during the analytical procedure. There was one modification. Around 4 g of surface tissue was blended with 16 mL of extracting solution. The 4:1 ratio was used instead of the 2:1 ratio of the Lemon procedure. The 4:1 ratio gave more uniform blending in the Waring minicontainer blenders (capacity 12−37 mL) (Fisher, Pittsburgh, PA). A ratio of 5:1 was used with mackerel muscle by Vyncke (1975). For the standard curve an extinction coefficient of 1.28×10^5 $M^{-1}cm^{-1}$ was determined using tetraethoxypropane standard. The TBARS data are expressed as μmol of malonaldehyde per kg of tissue.

Lipid Extraction and Peroxide Value. Peroxide values were determined on the surface tissue of mackerel fillets held 4 days at 2 °C. Total lipid was extracted from surface tissue by modifying the method of Burton et al. (1985). Around 5 g of surface tissue was homogenized for 1 min 30 s (rheostat set at 75) in 15 mL of distilled deionized water using a Polytron Type PT 10/35 (Brinkman Instruments, Westbury, NY). Homogenizing was done in Nalgene centrifuge bottles. Twenty milliliters of 0.1 M sodium dodecyl sulfate was added, and the bottles were shaken for 2 min by hand. Forty milliliters of 100% ethanol were added, and the bottles again were shaken for 2 min by hand. Twenty milliliters of hexane was added, and the bottles were shaken for 1 min 30 s, three times at 10 min intervals. The mixture was centrifuged for 20 min at 35 000*g* in a chilled No. 19 rotor in a Beckman Ultracentrifuge Model L5-65B (Beckman Instruments Inc., Palo Alto, CA). An aliquot of the hexane layer was dried with nitrogen gas. The peroxide value was then determined in the oil using the method of Shantha and Decker (1994). There were two modifications. The oil was solubilized in dichloromethane/methanol (2:1) instead of chloroform/methanol (7:3) because chloroform gave high blank readings. Cumene hydroperoxide was used as a standard instead of iron powder. An extinction coefficient of 3.7×10^4 $M^{-1}cm^{-1}$ was determined using cumene

hydroperoxide standard. Peroxide value was expressed as milliequivalents of peroxide per kilogram of oil.

Measuring Pro-Oxidative Activity of Tissue Extracts. The method of measuring pro-oxidative activity in mackerel was modified from that of Harris and Tall (1994). Four grams of surface tissue was added to 50 mL of sodium phosphate buffer (10 mM, pH 6.5). The tissue was homogenized for 60 s using the Polytron Type PT 10/35 (rheostat set at 75). The pH was adjusted to 6.5, if necessary, and centrifuged at 110 000*g* for 40 min using a No.35 rotor. The supernate was filtered through Whatman #1 filter paper and used as the final extract.

A fatty acid substrate was prepared in 10 mM sodium phosphate buffer (pH 7.4) containing 36.5 mM linoleic acid and 10 mg/mL of Tween 20. A lipid hydroperoxide (LHP) concentration of around 0.30 mM was routinely used. The LHP concentration was based on the absorbance of the substrate at 234 nm using a molar extinction coefficient of 2.5×10^4 M^{-1} cm^{-1} for hydroperoxylinoleate (Johnston et al., 1961). A 0.30 mM lipid hydroperoxide concentration was reached by allowing freshly prepared substrate to sit at room temperature for approximately 18 h. Another way this was accomplished was by adding preformed LHP. A substrate solution with a LHP concentration of approximately 3 mM (which sat at room temperature for 66 h) was diluted with freshly prepared substrate so the final LHP concentration was standardized to 0.30 mM. The latter method was most often used because it offered better control over the final LHP concentration. During analysis, the substrate was kept on ice to prevent increases in hydroperoxides. Hydroperoxide levels of the substrate by itself were monitored, and there were negligible increases during the analysis time.

Pro-oxidative activity was assayed by monitoring oxygen uptake. The reaction was initiated by adding 0.8 mL of substrate to 1.0 mL of tissue extract in a water jacketed cell (20 °C) equipped with an electrode connected to the Gilson K-IC Oxygraph (Gilson, Middleton, WI). Activity was expressed as μmol of oxygen consumed per min per g of wet weight and nmol oxygen consumed per min per μg of heme pigment. Gram wet weight basis was used since moisture contents in surface tissues varied by less than 1.3% between paired fillet treatments (data not shown).

Determination of Heme Content of Extracts. A portion of the extract was mixed with around 1 mg of sodium dithionite in a cuvette. The sample was immediately scanned spectrophotometrically against a reference with no sodium dithionite using a Hitachi U-3110 double beam spectrophotometer (Hitachi Instruments, Inc., San Jose, CA). The difference in absorbance of the peak (432 nm) and valley (410 nm) of the spectrum was used as a measure of total heme pigments. This method was a modification of Hudzik (1990). A standard curve was constructed using bovine hemoglobin.

RESULTS

Fillets Cut in Air and Not Rinsed Compared to Fillets Cut under Water (−20 °C Storage). Quality deterioration was determined in muscle of stage I and stage III mackerel that were cut in air and not rinsed at any stage of processing. The paired fillets of these samples were cut under water. Those cut in air or water from a single fish were directly compared. Fillets from stage I fish cut in air had moderate to strong painty odors throughout −20 °C storage, whereas their paired fillets cut under water did not develop painty odors (Figure 1). With stage III fish, in some cases there was essentially no difference between the sample cut in air and sample cut under water, and in other cases the difference was marked. When stage I rigor mackerel were cut in air, blood readily appeared and soon spread over the whole surface of the fillet. For the stage III mackerel cut in air, there was little or no blood flow when the samples were filleted. When fillets of stage I

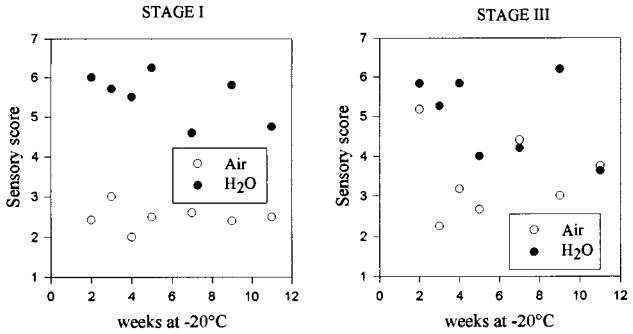

Figure 1. Sensory scores for paired fillets from stage I and stage III mackerel stored at −20 °C. One fillet was cut in air and was not rinsed; its pair was cut under water. A score of 4 or lower indicates rancidity.

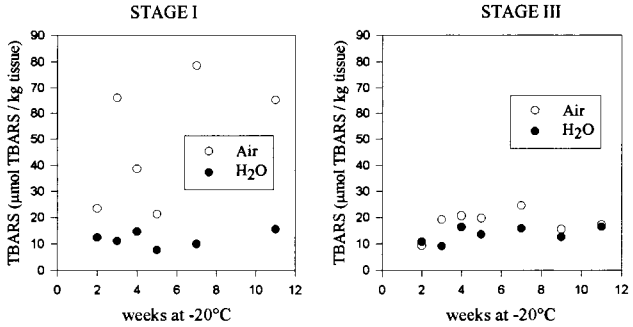

Figure 2. TBARS values for paired fillets from stage I and stage III mackerel stored at −20 °C. One fillet was cut in air and was not rinsed; its pair was cut under water.

fish were cut under water, the water rapidly became cloudy and red with blood. The development of turbidity and red color was far less when stage III mackerel were filleted in a similar manner. These results suggested that the amount of blood left on the surface might be a critical factor in quality deterioration.

TBA-reactive substances (TBARS) (Figure 2) were determined on the same samples as those in Figure 1. In this case, the samples that were cut in water, whether from stage I or stage III fish, were uniformly low. The samples of the fresh stage I fish that were cut in air showed a wide range of TBARS values, both in absolute numbers and in terms of the difference from their controls. On the other hand, the TBARS values of the stage III fish were only slightly higher than the control samples that were cut in water, and none showed the high TBARS values that were attained by some of the samples of stage I fish that were cut in air. These results suggested that washing improved the quality of the fillet from stage I fish by removing blood components, while washing had less of an effect with older stage III fish because the blood had coagulated in the muscle and hence could not flow out of the fish when filleting.

To evaluate the quantities of blood that collected on unrinsed fillet surfaces, mackerel at different quality stages (stages I, II, and III) were filleted in air with no special treatment or rinsing and were immediately placed in oxygen permeable bags and frozen overnight at −40 °C. The cut surface layer (around 2 mm in depth) was removed from each fillet and analyzed for heme pigment to estimate blood content (Table 3). It was presumed that the content of myoglobin would be relatively constant so that differences in heme content would reflect differences in blood content. The tissue removed was from near the backbone; most of the

Table 3. Effect of Quality Stage on Heme Pigment Extracted from the Fillet Surface of Stages I, II, and III Mackerel Filleted in Air and Not Rinsed[a]

stage	mg of heme pigment/g of tissue
I	3.80 ± 0.53
II	1.23 ± 0.33
III	0.61 ± 0.10

[a] The stage I fish were in rigor. Two fish were used from each stage.

Table 4. Zero-Time TBARS Values of Surface Tissue Samples from Washed Fillets of Stage I and Stage III Mackerel[a]

stage	TBARS
I	6.8 ± 1.6 (12)
III	21.7 ± 7.4 (8)

[a] Difference between stage I and stage III samples was significant using an unpaired t-test ($p < 0.00001$). Number in parentheses represent total number of fillets analyzed.

myoglobin-rich red muscle is on the skin side of mackerel. Thus, it is likely that the myoglobin content was low. The amount of heme pigment in the surface tissue was greater in stage I fish than it was in fish that was less fresh. The amount of heme pigment in stage II fish was only about one-third of that in the stage I fish. Only half as much was found in the stage III fish compared to the stage II fish. The reduced amount of blood contaminating the fillet surface of the older fish may explain why stage III fillets cut in air were less deteriorated than stage I fillets cut in air (Figure 2). Washed, minced mackerel muscle prepared from stage III fish had better sensory attributes than that prepared from stage I mackerel (Kelleher et al., 1992).

Washed Fillets from Stage I Fish Compared to Stage III Fish. Zero-time, i.e., initial TBARS values, were compared between stage I and stage III fish that had been cut either under water or cut in air and then rinsed with water (Table 4). This was done to assess the quality deterioration that had occurred in the muscle while the fish was intact. Averaged TBARS values were significantly greater in the stage III samples ($p < 0.00001$). This suggests stage III fish had undergone considerable oxidation prior to preparing the fillets.

Fillets Cut Directly in Liquid Compared to Air-Cut and Rinsed Fillets (2 °C Storage). Previously, fillets that were cut in air were not rinsed to illustrate what could happen under abusive conditions. Commercial practice includes a rinse at some time after filleting. The effectiveness of filleting under water or in a solution of antioxidants consisting of 0.2% sodium ascorbate and 0.2% sodium tripolyphosphate was compared to rinsing with distilled deionized water or rinsing with the antioxidant solution 1 min after filleting. The paired fillets evaluated were as follows: (1) filleted in air and then rinsed with water compared to filleted under water [air (H_2O)/H_2O]; (2) filleted in air and then rinsed with water compared to filleted in antioxidant solution [air (H_2O)/AO]; and (3) filleted in air and then rinsed with antioxidant solution compared to filleted in antioxidant solution [air (AO)/AO]. Averages of the TBARS values of the fillets from stage I fish stored from 4 to 9 days are listed (Table 5). Significant differences between pairs were only seen with samples filleted in air and rinsed with water compared to their pairs filleted in antioxidant solution ($p < 0.01$). Sensory scores paralleled TBARS results. There were significant differences only between the air (H_2O)/AO pairs ($p < 0.001$). It should be noted that on average antioxidant treated samples were superior to samples treated with water based on sensory score and TBARS values (Table 5).

Peroxide values showed a trend in the same direction as TBARS values and sensory scores, i.e., the largest differences between pairs were those that were cut in air and rinsed with water vs those that were cut directly in antioxidant solution. However, because of the variability of the data and the relatively small number of samples, the differences were not statistically significant. The procedure of using only the surface tissue to measure the chemical oxidative changes did not furnish enough sample to do both TBARS and peroxides on the same fish. We usually measured TBARS rather than peroxide values as an indication of oxidation since TBARS gave larger differences between pairs that showed significant sensory differences (see Table 5). Possible microbial contributions to the changes observed were not investigated.

Fillets Cut Directly in Liquid Compared to Air-Cut and Rinsed Fillets (−20 °C storage). Similar studies were carried out on stage I mackerel treated and analyzed the same way as those in the section above except that storage was at −20 °C. Averages of the TBARS values of the paired fillets stored from 3 to 9 weeks are listed (Table 6). The samples with the greatest difference in TBARS values were those where one of the fillet pairs was cut in air and rinsed with water, while the other was cut directly in the antioxidant solution ($p < 0.05$, Table 6). However, on storage at −20 °C, there was a significant difference in TBARS values between the sample cut in air and washed with the antioxidant solution vs the sample that was cut directly in the antioxidant solution ($p < 0.05$). There was no difference in sensory scores or TBARS values for samples cut in air and rinsed in water compared to those cut directly in water.

Significant differences in sensory scores were observed in both of the sets of pairs that showed significant differences in TBARS values (Table 6). Greater and more significant differences in average sensory scores were observed in sample pairs in which the fillets cut in air and rinsed in water were compared with those cut directly in the antioxidant solution than with the sample pairs rinsed in antioxidant vs cut directly in antioxidant solution.

Stage III fish were subjected to similar comparative washing procedures as described above for stage I fish. No significant differences were observed between the washing techniques with or without antioxidants in terms of sensory score or TBARS for any of the samples from stage III fish (Table 7 for the 2 °C data, Table 8 for the data obtained after storage at −20 °C).

Pro-Oxidative Activities of Mackerel Muscle Extracts. The possibility that removal of blood may be a contributing factor to the improvement in quality retention was tested. The heme pigment content of the tissue extract was used as an indicator of the amount of blood that was in the extract. The oxidation of linoleic acid by the tissue extract was dependent upon a species in the substrate that absorbed light at around 234 nm (Table 9). This species was probably the conjugated diene of linoleic acid hydroperoxide. If hydroperoxides of linoleic acid were added from a sample separately prepared or if the linoleic acid in the model system was allowed to age for several hours to produce peroxides,

Table 5. Sensory Scores, TBARS, and Peroxide Values of Stage I Mackerel Stored at 2 °C from 4 to 9 Days after Varying Treatments[a]

paired fillets	sensory score		TBARS		peroxide value	
air (H_2O)	4.88 ± 1.60	(6)	67.0 ± 27	(6)	1.23 ± 0.49	(2)
H_2O	4.95 ± 1.80		61.7 ± 23		1.20 ± 0.29	
air (H_2O)	3.34 ± 0.63[b]	(8)	86.5 ± 38[c]	(6)	1.79 ± 0.92	(4)
AO	6.21 ± 0.42[b]		9.8 ± 4.4[c]		0.75 ± 0.20	
air (AO)	5.82 ± 1.03	(12)	22.2 ± 18	(8)	0.63 ± 0.25	(4)
AO	6.01 ± 0.88		25.0 ± 25.2		0.83 ± 0.47	

[a] Number in parentheses represents total pairs of fillets analyzed. All peroxide values were determined after 4 days of storage at 2 °C. AO: antioxidant solution consisted of 0.2% sodium ascorbate and 0.2% sodium tripolyphosphate. As sensory score decreased, off-odor increased (scale 8−1). Four was slight painty. TBARS: μmol of malondialdehyde/kg of tissue. Peroxide value: mequiv of cumene hydroperoxide/kg of oil. [b] $p < 0.001$. [c] $p < 0.01$. [d] $p < 0.05$ (significant difference between grouped paired fillets).

Table 6. Sensory Scores and TBARS of Stage I Mackerel Stored at −20 °C from 3 to 9 Weeks after Varying Treatments[a]

paired fillets	sensory score		TBARS	
air (H_2O)	5.55 ± 0.62	(7)	16.6 ± 8	(5)
H_2O	5.55 ± 0.93		16.2 ± 8	
air (H_2O)	4.17 ± 0.94[c]	(7)	23.8 ± 19[d]	(7)
AO	5.89 ± 0.75[c]		7.3 ± 4[d]	
air (AO)	5.21 ± 1.02[d]	(8)	11.1 ± 4[d]	(8)
AO	6.03 ± 0.50[d]		8.7 ± 2[d]	

[a] Number in parentheses represents total pairs of fillets analyzed. AO: antioxidant solution consisted of 0.2% sodium ascorbate and 0.2% sodium tripolyphosphate. As sensory score decreased, off-odor increased (scale 8−1). Four was slight painty. TBARS: μmol of malondialdehyde/kg of tissue. [b] $p < 0.001$. [c] $p < 0.01$. [d] $p < 0.05$ (significant difference between grouped paired fillets).

Table 7. Sensory Scores and TBARS of Stage III Mackerel Stored at 2 °C from 4 to 9 Days after Varying Treatments[a]

treatment	sensory score		TBARS	
air (H_2O)	4.11 ± 0.20	(6)	153 ± 28	(2)
H_2O	3.90 ± 0.39		163 ± 39	
air (H_2O)	5.18 ± 0.67	(6)	87.5 ± 57	(2)
AO	4.83 ± 1.22		125.5 ± 67	

[a] Number in parentheses represent total pairs of fillets analyzed. AO: antioxidant solution consisted of 0.2% sodium ascorbate and 0.2% sodium tripolyphosphate. As sensory score decreased, off-odor increased (scale 8−1). Four was slight painty. TBARS: μmol of malondialdehyde/kg of tissue.

Table 8. Sensory Scores and TBARS of Stage III Mackerel Stored at −20 °C from 3 to 9 Weeks after Varying Treatments[a]

treatment	sensory score		TBARS	
air (H_2O)	4.08 ± 1.09	(6)	31.8 ± 8	(4)
H_2O	4.36 ± 1.25		23.3 ± 3	
air (H_2O)	4.05 ± 0.35	(2)	39.3 ± 11	(2)
AO	3.90 ± 0.00		26.3 ± 3	

[a] Number in parentheses represent total pairs of fillets analyzed. AO: antioxidant solution consisted of 0.2% sodium ascorbate and 0.2% sodium tripolyphosphate. As sensory score decreased, off-odor increased (scale 8−1). Four was slight painty. TBARS: μmol of malondialdehyde/kg of tissue.

the oxidation was increasingly stimulated over the range examined.

The pro-oxidative activity was determined in tissue extracts prepared from stage I rigor mackerel that was either cut in air without a rinse or was filleted under water by measuring oxygen uptake. The results showed that there was almost 10 times as much pro-oxidative activity in the sample that was cut in air and not rinsed compared to the sample that was cut under water when based on sample weight (Table 10). When the results were expressed on the basis of heme pigment in the extract, the pro-oxidative activity was nearly the same in the samples that were cut in air compared to the samples that were cut in water. These results imply that much of the pro-oxidative activity was caused by blood components.

Table 9. Effect of Peroxides in Substrate on Pro-Oxidative Activity of Mackerel Surface Tissue Extracts[d]

linoleic acid substrate	conjugated dienes (mM)[a]	O_2 consumption (μmol/min/gww)
freshly prepared	0.05	0.30 ± 0.03
added peroxides[b]	0.29	2.08 ± 0.05
incubated 15 h[c]	0.30	3.45 ± 0.53
incubated 24 h	0.89	8.97 ± 1.31

[a] This concentration represents approximately the concentration of lipid peroxide in the substrate since the molar extinction coefficient ϵ_{233} of conjugated dienes is 2.52×10^4 $M^{-1}cm^{-1}$ (Buege and Aust, 1978) and the ϵ_{234} hydroperoxylinoleate is 2.50×10^4 M^{-1} cm^{-1} (Johnston et al., 1961). [b] A portion of freshly prepared substrate that was incubated at ambient temperature for 66 h was diluted with freshly prepared substrate so that the conjugated diene concentration of the mixture was 0.29 mM. [c] Freshly prepared substrate was incubated at ambient temperature for 15 h to allow lipid peroxides to form in the substrate to attain a conjugated diene concentration of 0.30 mM. [d] Extracts were prepared from stage I fillets that were rinsed with deionized distilled water.

Extracts of surface tissue from fillets that were cut in air and then rinsed with water 1 min after cutting were compared with samples obtained by cutting directly in water. In other words, the blood was washed away from both fillet pairs before the extracts were prepared (Table 11). When the samples cut in air were washed in water, the pro-oxidative activity on a wet weight basis was reduced considerably over those samples described in Table 10 in which the samples that were cut in air were not rinsed. However, these samples had greater pro-oxidative activity than those cut directly in water when the activities were expressed per g wet weight. When pro-oxidative activity was expressed on a heme pigment basis, the ratios obtained were close to 1, indicating again that blood contamination may be responsible for the results observed.

The next series of experiments were designed to determine the potential contribution of the heme proteins of the sample to lipid oxidation. Bovine hemoglobin and horse heart myoglobin were the sources for the standard heme proteins in these experiments. The activity of pure hemoglobin was compared with the activity of the surface tissue extracts (Table 12). The ratio of the activities of the tissue extract to hemoglobin was 1.37 in one experiment and 1.29 in another. The results indicate that there was pro-oxidative activity in the tissue extract that was somewhat greater than that

Table 10. Oxygen Uptake and Heme Pigment Content of Stage I Mackerel Surface Tissue Extracts from Fillets Cut in Air and Not Rinsed or Cut under Water[a]

treatment	mg of heme pigment/g of tissue	O_2 consumption (μmol /min/gww)	ratio air/ H_2O	O_2 consumption (nmol/min/μg of pigment)	ratio air/ H_2O
air (no rinse)	4.23	67.6 ± 1.92	9.7	16.0 ± 0.45	0.98
H_2O	0.44	7.0 ± 0.33		16.3 ± 0.63	

[a] One milliter of substrate was added to 0.8 mL of extract. Data represent two fish.

Table 11. Oxygen Uptake and Heme Pigment Content of Stage I Mackerel Surface Tissue Extracts from Fillets Cut in Air and Rinsed with Water or Cut under Water[a]

treatment	mg of heme pigment/g of tissue	O_2 consumption (μmol /min/gww)	ratio air(H_2O)/ H_2O	O_2 consumption (nmol/min/μg of pigment)	ratio air(H_2O)/ H_2O
air (H_2O)	1.83	16.9	2.60	9.1	1.04
H_2O	0.73	6.5		8.8	
air (H_2O)	0.93	10.8	4.03	11.2	1.20
H_2O	0.29	2.7		9.3	
air (H_2O)	1.15	6.9	2.17	6.0	0.96
H_2O	0.52	3.2		6.2	
air (H_2O)	0.60	6.5	1.21	10.8	1.22
H_2O	0.61	5.4		8.0	

[a] Experimental conditions described in Materials and Methods. Data represent four fish. One milliter of extract was added to 0.8 mL of substrate.

Table 12. Oxygen Uptake of Extracts from Stage I Mackerel Surface Tissue, Dark Muscle, Bovine Hemoglobin and Horse Myoglobin[a]

	O_2 consumption					
	μmol/min/gww		nmol/min/μg of pigment		ratio:: muscle extract/heme protein	
treatment	I	II	I	II	I	II
mackerel surface tissue extract	2.00	3.58	2.32	3.73		
					1.37	1.29
bovine hemoglobin			1.69	2.89		
dark muscle	8.81	13.6	1.24	1.90		
					1.03	1.09
horse heart myoglobin			1.20	1.74		

[a] I and II indicate two different experiments. Fillets were rinsed with water prior to removal of tissue.

which could be accounted for by bovine hemoglobin. Blood components other than hemoglobin which have the potential to contribute to the pro-oxidative activity observed include myeloperoxidases (Kanner and Kinsella, 1983; Folkes et al., 1995) and lipoxygenases (Pettitt et al., 1989).

The pro-oxidative activity of myoglobin was compared with extracts of dark muscle, a source of myoglobin (Table 12). The pro-oxidative activity of extracts of dark muscle was not very different from that of horse heart myoglobin; the ratio was 1.03 in one experiment and 1.09 in another. This suggests that the heme proteins in dark muscle have the potential to cause the oxidations observed in the linoleate model system. Caution must be used in comparing activities of the extracts with the pure proteins since they were from different species.

DISCUSSION

It is not clear which specific factors cause quality deterioration of mackerel fillets. As expected, increasing post mortem age of the whole fish prior to filleting had a negative impact on initial quality of fillets (Table 4). However, when unwashed fillets from stage I fish were compared with unwashed fillets from stage III fish, the fillets from stage I fish were of poorer quality as indicated by sensory and TBARS values (Figures 1 and 2). This was likely related to the fact that more blood collected on the surface of fillets from stage I fish than stage III fish (Table 3). Connell (1975) reported that fish blood in vessels and organs remains fluid for up to 30 min at chill temperatures but tends to clot rapidly after this time and sooner at higher temperatures. Thus, coagulation processes should be more advanced in stage III mackerel compared to stage I mackerel which can explain the lesser amount of blood on the unwashed fillet surface of the older fish. Much of the blood could be removed from the fillet surface of stage I fish by either filleting under water or rinsing the fillet surface (Tables 10 and 11). Removal of blood by washing may be the reason for the better storage quality of washed fillets compared to unwashed fillets from stage I fish (Figures 1 and 2).

Using heme pigment to estimate blood content is rapid but has its disadvantages. Any myoglobin contaminating the extract would give errant high estimates of hemoglobin and hence blood content. However, the differences in heme pigment contents between washed and unwashed samples were so large (Tables 3 and 10) that it seems unlikely that myoglobin could have been a major contaminant in the extracts. In fish muscle a secondary vascular system carrying only plasma and white cells has been described (Steffensen and Lomholt, 1992). This system runs parallel to the primary vascular system which carries all the blood components. Thus, any measurement based on hemoglobin content will underestimate plasma and white cell concentrations.

The strong and rapid development of rancid odor in unwashed fillets from very fresh mackerel indicated

that blood had a high pro-oxidative potential. Removing as much blood as possible from the fish makes sense. There is evidence that the window of time after capture to get the maximal amount of blood out of the fish is very brief. Tretsvan and Patton (1981) stated less blood was removed from trout if there was a 20 min delay between capture and bleeding compared to a 1 min delay. These authors found bleeding by cutting the caudal vein 1 min after capture reduced rancidity development in trout fillets during frozen storage compared to unbled controls. Botta et al. (1986) reported that mean sensory quality of Atlantic cod was more affected by the time on board prior to bleeding/gutting than by the actual bleeding/gutting procedure. When fish were bled immediately, 74% received a grade A from the inspector. After 4 h of delay, only 30% were grade A.

One factor that separates migratory species such as mackerel from ground fish is the presence of a large portion of dark muscle in the migratory species. Blood is more concentrated in dark muscle than light due to greater capillary density in dark muscle compared to light muscle (Mathieu-Costello, 1993). Failure to remove blood components from mackerel may be more of a factor compared to ground fish because the amount of blood is greater in mackerel.

Surface tissue of fillets was used to prepare extracts from which the pro-oxidative activity was determined. Surface tissue would demonstrate the effect of washing better than using the entire fillet because the percentage of sample in contact with the washing solutions decreases as the depth of the sample increases. When comparing extracts from washed and unwashed fillets using an emulsified linoleic acid substrate, the substantially different pro-oxidative activities observed on a g wet weight basis were much reduced when compared on a μg heme pigment basis (Tables 10 and 11). This relationship between heme content and oxidative ability suggests blood components were responsible for the pro-oxidative activity in unwashed and washed fillets.

The fish tissue extracts required preformed lipid peroxides to be active (Table 9). Preformed peroxides are required for activity of both heme proteins and lipoxygenases. It is difficult to distinguish between hemoglobin and lipoxygenase on the basis of their lipid peroxidative activities. Hemoglobin can behave as a lipoxygenase, i.e., a pseudolipoxygenase (Everse and Hsia, 1997). Beef hemoglobin and reticulocyte lipoxygenases were exposed to a linoleic acid substrate seeded with 1.5% hydroperoxylinoleic acid (Kühn et al., 1981). Hemoglobin and lipoxygenase formed 68 and 122 μM hydroperoxylinoleic acid per min per μM protein, respectively. These similar rates of lipid peroxidation may be especially relevant since hemoglobin is many orders of magnitude more concentrated in blood than lipoxygenases. The hemoglobin concentration in blood of Atlantic mackerel is 131 mg/mL (Eisler, 1965). Another piece of evidence that hemoglobin was likely an important contributor to the lipid peroxidative activity in this study was that the pro-oxidative activities of the surface tissue extracts and a bovine hemoglobin solution were remarkably similar on a heme pigment basis despite the compositional differences between the two samples and the fact that a fish hemoglobin was compared to a mammalian hemoglobin (Table 12). The redox state of heme proteins can influence the catalysis of lipid peroxidation, yet Baron et al. (1997) found using a linoleic acid emulsion system that the presence of lipid hydroperoxides was the crucial factor rather than the redox state of the protein.

Since hemoglobin appeared likely to be a major pro-oxidant in the surface tissue extracts, ascorbate was used as an antioxidant in the wash or cutting solutions to keep hemoglobin reduced during storage of fillets. Tripolyphosphates (TPP) were also in the antioxidant solution to act as chelators for iron ions that were present. Kelleher et al. (1994) used the same combination of antioxidants in the wash water to delay lipid oxidation in preparing mackerel surimi.

The antioxidant solution improved the quality of fillets only with fresh stage I mackerel. Immediately after capture there is a reservoir of endogenous antioxidant substances such as ascorbate and tocopherol in muscle that decreases with storage time (Thed and Erickson, 1994). With increasing time of storage there is an increase in pro-oxidative substances such as hemin and low molecular weight iron (Decker and Hultin, 1990a), lipid peroxides (Hardy and Smith, 1976), and H_2O_2 (Harel and Kanner, 1985). Thus, the antioxidant/pro-oxidant ratio should be higher in stage I fish than stage III fish. The antioxidant solution should function best when pro-oxidative substances in the muscle are low since ascorbate has been found to have pro-oxidant effects in the presence of low molecular weight iron (Yamamoto et al., 1987) and lipid peroxides (Kanner and Mendel, 1977). Our data suggests that added ascorbate might have been a prooxidant when used in the solutions to wash fillets from stage III mackerel based on lower TBARS values of water washed fillets compared to their paired fillets washed with antioxidant solution (Table 7). Since there were only two samples, future work is needed to confirm this.

Initial TBARS values of washed fillets from stage I and III mackerel were around 3 times higher in the older fish (Table 4). Postmortem age had no detrimental effect on initial odor quality of washed mince from mackerel (Kelleher et al., 1992). Components responsible for off-odor of deteriorating mackerel flesh seemed to be removed by washing. A number of tissue components could have been removed by this washing procedure. Blood was likely among the materials removed since blood is an extracellular component of muscle tissue. Coagulated blood should be more difficult to remove from fillets than a washed mince product. This can help explain why postmortem aging lowered the quality of fillets but was not a factor with washed minced mackerel.

Fillets cut directly in the antioxidant solution were statistically of better quality than fillets rinsed with antioxidant solution 1 min after cutting if the fillets were stored at -20 °C but not 2 °C (Table 6). At 2 °C putrid odor from microbial growth could interfere with lipid oxidation odors at the tail-end of storage. During frozen storage microbial reactions are suppressed which may allow for lipid oxidation reactions to be more clearly expressed. Filleting directly in antioxidant solution may have been more effective in inhibiting lipid oxidation during -20 °C storage than rinsing with antioxidant solution after 1 min because of blood removal. To determine if blood was more easily removed by filleting in liquid than rinsing with liquid, extracts were prepared from surface tissue of fillets rinsed with water and fillets cut under water. The heme pigment content of the extracts (Table 11) was used as an estimate of

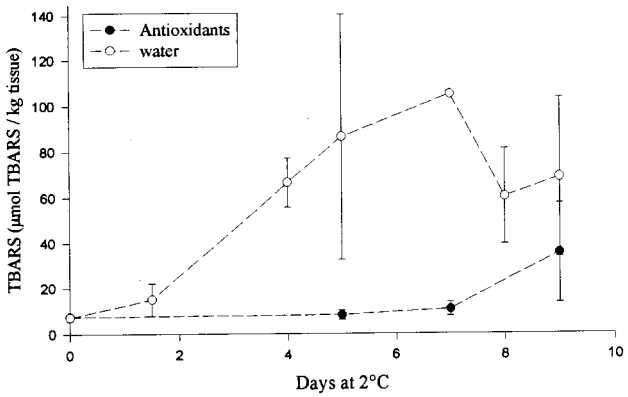

Figure 3. Summary data of all paired fillets from stage I mackerel treated either with water or antioxidant solution (whether rinsed or submerged) and stored at 2 °C.

blood content. The amount of heme pigment was generally greater in the rinsed fillet compared to its paired fillet cut under water ($p = 0.10$). The greater amount of blood (as indicated by heme pigment content) in the rinsed fillets compared to the fillets cut under water may have lowered quality. One interesting observation was that the amount of heme pigment varied over a 2-fold range in both the samples that were cut in air followed by a water rinse and those that were cut directly in water. The flow of blood when filleting mackerel appeared to decrease around the time the fish came out of rigor. The stage I mackerel used in this experiment had varying amounts of rigor. It is possible that the blood of these individual stage I fish was at different stages of coagulation at the time of filleting. This could affect how easily the blood could be removed.

Surface tissue was used to measure TBARS and peroxide values. Using the entire fillet was avoided since oxygen from the atmosphere penetrates only 1−4 mm into muscle tissue (Lawrie, 1974). Poor oxygen penetration can explain why the interior of herring fillets had less lipid oxidation products formed during storage than surface tissues (Undeland et al., 1998). A sample for chemical analysis most representative of the sensorial quality changes that occurred during storage of fillets was desired. Since oxygen penetration into muscle foods is limited so too should be the release of odor volatiles from the muscle. Odor volatiles the nose detects should come from the surface of the fillet. Since there was a significant difference in TBARS values between sets of paired fillets whenever there also was a significant difference in sensory score (Tables 5 and 6), it would seem that analyzing surface tissue is a satisfactory procedure.

A composite graph was made of all the data from all paired fillets from all stage I fish that were treated either with water or with antioxidant solution stored at 2 °C. No distinction was made between those samples which were cut directly in the water or antioxidant solution and those which were rinsed with these liquids 1 min after cutting. The improvement in shelf life by the addition of antioxidants can be immediately observed (Figure 3). It was found by sensory evaluation that the first rancid odors were detected at TBARS values between 12 and 20 μmol per kg of tissue. This level of TBARS was not reached until after 7 days when the washing or cutting solution contained the antioxidant. On the other hand, samples that were washed with or cut under water showed high TBARS values by 4 days. Thus it appeared that the antioxidant treatment doubled the storage life of the fillets compared to those that were not so treated.

We observed another interesting phenomenon with these data. When the TBARS values were low, i.e., below the values indicating rancidity by sensory measurement, the range of TBARS values was narrow. However, once the TBARS values rose well above the point at which the samples became rancid, the range of these values became very large. In addition, they no longer consistently increased with time after they reached this high level (Figure 3).

The incorporation of antioxidants into the rinsing water would represent a considerable expense and probably would not make it an appropriate treatment for a relatively low value fish such as the Atlantic mackerel used in this study. It is also likely that filleting the fish under water may present a challenging engineering problem. In addition, when fresh fish are used, the amount of blood that accumulates on the surface is considerable. It would quickly contaminate the wash water and require frequent changes. A practical and effective approach might be to rinse the cut fillets immediately with water to be followed by a dip in antioxidant solution. This would remove most of the blood proteins that would contaminate the antioxidant dip solution while allowing the rapid application of antioxidants to the cut flesh. If the product was to be frozen, our results indicate that a 1 min time gap between the cutting and the washing will lead to a shorter shelf life. This may be further compounded if the initial contact with the fluid is with water rather than the antioxidant solution. From our results it seems clear that there are some reactions taking place during the first minute after the tissue has been cut. Further research is needed to determine how much this time has to be reduced to eliminate the lower quality obtained when the fish is not cut directly in the aqueous medium.

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Received for review March 25, 1998. Revised manuscript received July 29, 1998. Accepted July 30, 1998. This work was supported in part by the Massachusetts Agricultural Experiment Station and by Grant NA46FD0323 of the Saltonstall-Kennedy program of the National Oceanic and Atmospheric Administration. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies.

JF980311H