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Quantification of Antioxidants in Channel Catfish during Frozen Storage

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Levels of ascorbic acid, α- and γ-tocopherol, glutathione, and volatile aldehydes were monitored over 6 months in frozen minced and intact fillets of channel catfish. For both samples, decline of glutathione occurred at a faster rate than that of ascorbic acid which in turn was faster than decline of α-tocopherol. Loss of α-tocopherol and formation of volatile aldehydes in mince were either similar or slightly less than in intact fillets throughout storage. γ-Tocopherol levels increased significantly during initial stages of storage and then declined for both mince and intact fillets. Ascorbic acid and glutathione levels in mince, on the other hand, decreased faster than levels in intact fillets during early stages of storage. It is conjectured that any enhancement of oxidation which might have been generated through tissue disruption is balanced either by enhancement of ascorbic acid or glutathione's antioxidant activity or by enhancement of some other unmeasured antioxidant system such as phospholipase. The possibility also exists that tissue levels of ascorbic acid and glutathione are insufficient to have a noticeable effect on the oxidation of lipids.

Keywords: *Tocopherol; glutathione; ascorbic acid; lipid oxidation; mince*

INTRODUCTION

The world's consumption of fish could more than double what it is today if the food industry could transform raw materials into stable, acceptable products (Flick et al., 1990). If an acceptable, marketable product for the United States could be developed out of minced fish, seafood processors could realize many advantages: First, through the use of mechanical deboning machines, 50−200 percent more usable meat could be obtained from a given fish than is now being utilized. Second, species that are currently not harvested for reasons of size or anatomy could be processed. Third, food-grade waste materials could be obtained from bone racks, minimizing waste costs and the associated environmental impact of previously discarded bone racks (Regenstein, 1986). Possible end uses of fish mince include fish blocks for production of fish sticks and patties, fish chowders, fish/soy blend products, fish-based snacks such as fish chips or fish jerky, and seafood rolls (Wheaton and Lawson, 1985). Unfortunately, aside from consumer acceptance, common knowledge holds that fish mince is more susceptible to oxidation, bacterial growth, and texture and color changes than whole or filleted fish (Flick et al., 1990; Wheaton and Lawson, 1985).

Frozen storage shelf-life deterioration of commercial channel catfish can occur within 4 months of storage (−18 °C) and results in many undesirable changes in the tissue, including development of rancid off-flavor, changes in texture, color, water-holding capacity, and nutritive properties, and increased safety risks associated with oxidized products (Thed et al., 1993). Related to shelf-life deterioration is degradation of endogenous lipid- and water-soluble antioxidants found in the tissue.

When present in low concentrations compared to those of an oxidizable substrate, antioxidants can delay

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or prevent the oxidation of that substrate (Halliwell, 1990; Nawar, 1985). Acting alone or together, antioxidants delay oxidative rancidity until a critical concentration of antioxidant protection is reached, and then propagation of lipid oxidation occurs unchecked. Many studies have attempted to increase levels of antioxidants in fish tissue by dietary supplementation or direct incorporation into the tissue to achieve control of oxidative rancidity. The role of added tocopherol has been studied, and researchers have shown that increased levels of dietary α-tocopherol have been effective in delaying oxidative rancidity in catfish (Gatlin et al., 1992; Johnsen and Dupree, 1991; O'Keefe and Noble, 1978). Similar findings were observed for supplementation with ascorbic acid (Thed and Erickson, 1994) and glutathione (Murai et al., 1988).

Several researchers have shown synergistic relationships between antioxidants in *in vitro* systems, such as regeneration of α-tocopherol by ascorbic acid (Yi et al., 1991; Sato et al., 1990; Mukai et al., 1989; Niki et al., 1984), a 30% increase in the induction period of soybean liposomes with the addition of glutathione and vitamin E compared to vitamin E only (Stocker and Peterhaus, 1989), and the induction period of oxidation increased by the addition of ascorbate and glutathione (Stocker and Peterhaus, 1989). Additionally, an *in vitro* experiment recently reported unequivocal evidence in support for the nonenzymatic regeneration of ascorbate from dehydroascorbate by glutathione (Winkler, 1992). These reports lend credence to the theory that antioxidant defense in tissue is a dynamic process requiring both biochemical and chemical interactions between and among antioxidants, enzymes, and other important cellular constituents. Quantification of antioxidants is essential for understanding the mechanism of oxidation and the protection afforded the tissue by endogenous and added antioxidants during storage. The purpose of this study was to compare antioxidant loss and concurrent formation of volatile aldehydes in mince and intact fillets of channel catfish during frozen storage.

MATERIALS AND METHODS

Fish Supply. Live channel catfish (*Ictalurus punctatus*) were obtained from the Coastal Plains Experiment Station at Tifton, GA, on three consecutive weeks during the fall season. Fish were transported to the laboratory (3−3.5 h) in hauling units filled with pond water (25 °C) equipped with battery-operated air pumps. The hauling units were loaded at an approximate rate of 1 lb of fish/gal of water.

Sample Preparation. Once at the laboratory, fish were placed in aerated 55 gal tanks. Fish were asphyxiated with carbon dioxide-saturated water for 5−10 min and then immediately filleted. For each whole fish, each fillet was washed in water and then separated, with one of the fillets minced using a Black and Decker handy chopper (Shelton, CT) and the other half kept whole. For storage samples, five minced or five intact fillets were placed in Cryovac D955 packaging bags (oxygen transmission rate, 6000−8000 $cm^3 \cdot m^2 \cdot 24$ h (22.8 °C, 1 atm); W.R. Grace, Simpsonville, SC) and stored at −6 °C for the designated storage time to simulate potentially abusive storage conditions found in the industry. At each sampling time, bags were removed from storage and partially thawed under cold running water. Samples that were stored as mince were mixed thoroughly together, while samples that were stored as intact fillets were first minced and then thoroughly mixed together. The mince from both samples was then separately repackaged for each analysis in sterile, Whirl-Pac bags (Nasco, Fort Atkinson, WI) and stored at −100 °C until analysis could be undertaken.

For each month of frozen storage, two duplicate bags of five fillets of the mince (designated mince A and mince B) and five fillets of the intact fillets (designated whole A and whole B) were treated as above and repackaged for individual analysis. Three replicates of this scheme were undertaken in successive weeks.

Moisture Analysis. Minced tissue (1−2 g) was analyzed for moisture by drying in a microwave oven (Automatic Volatility Computer, Model AVC-80; CEM Corp., Indian Trail, NC) until constant weight was achieved. Triplicate moisture determinations were undertaken for each sample.

Fatty Acid Analysis. Chloroform/methanol (15 mL, 2:1, v/v) was used to extract lipids from 1.00 ± 0.01 g of catfish muscle tissue (Erickson, 1992a). Lipid class separation was achieved by applying a lipid aliquot to a thin-layer chromatography plate and developing in 80:20:1 (v/v/v) hexane/ethyl ether/glacial acetic acid. Phospholipid and triacylglycerol fractions were esterified using 4% H_2SO_4 in methanol (Erickson, 1992a). A glass capillary column (J&W DB-225; 30 m × 0.25 mm, 0.15 μm film) attached to an HP 5790A Series gas chromatograph (Wilmington, DE) was used for separation of the esterified fatty acids. The oven was held at 180 °C for 10 min, heated to 220 °C at 4 °C/min, and then held at 220 °C for an additional 20 min. The injector and detector temperatures were both 250 °C. The helium flow rate was 1.6 mL/min with a split ratio of 280:1. Relative retention times of fatty acid methyl ester standards (Supelco Inc., Bellefonte, PA) were used to identify peaks. Peak area was integrated with a HP 3390A integrator (Wilmington, DE) and compared to the response of an internal standard, behenic acid (22:0).

Tocopherol Analysis. Minced muscle tissue (1.0 g) was saponified with 60% KOH in the presence of ascorbic acid (3%) and ethanol (25%) and afterward extracted with 10% ethyl acetate in hexane as described by Erickson (1992a). The extract was evaporated to dryness under nitrogen and reconstituted in 0.5 mL of methanol. The amount of α- and γ-tocopherol was quantified using reverse-phase high-performance liquid chromatography (HPLC) (Vatassery and Smith, 1987) using a Shimadzu (Shimadzu Corp., Japan) Model C-45A data processor, a Model LC-6A solvent delivery module, and a RF-535 fluorescence detector. Quantification of tocopherols was accomplished by comparison to standards (Sigma, St. Louis, MO).

Ascorbic Acid Analysis. Ascorbic acid extraction and separation were performed according to the procedures described by Erickson (1993). The HPLC system (Micromeritics, Norcross, GA) used was equipped with a Brownlee Spheri-5 ODS analytical column (4.6 × 220 mm, 5 μm packing; Applied Biosystems Inc., San Jose, CA), an RP-C_{18} silica guard cartridge (Applied Biosystems Inc., San Jose, CA), an electrochemical detector (Model 656, Brinkmann Instruments Co., Westbury, NY), and an integrator (Hewlett Packard, Wilmington, PA). Detection of ascorbic acid and isoascorbic acid, added as an internal standard, was achieved at 0.75 V and 50 nA. Quantification of ascorbic acid was based on peaks for the internal standard.

Glutathione Analysis. Total (reduced + oxidized) glutathione analysis was performed according to a modified procedure described by Griffith (1980). Minced catfish tissue (1 g) was homogenized using a polytron mixer in 20 mM 5-sulfosalicylic acid (SSA) solution (5 mL). After centrifugation, an aliquot (0.2 mL) was added to a reaction vessel containing 0.3 mM nicotinamide adenine dinucleotide phosphate, reduced (NADPH), in 125 mM phosphate/6.3 mM ethylenediaminetetraacetic acid (EDTA), pH 7.5 (0.7 mL), and 6 mM 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB) in 125 mM phosphate/6.3 mM EDTA, pH 7.5 (0.1 mL), for a final reaction volume of 1 mL. Glutathione reductase (50 units/mL in 125 mM phosphate/6.3 mM EDTA, pH 7.5) was added (10 μL) and the solution vortexed, quickly transferred to a cuvette, and read spectrophotometrically in a HP 8451A diode array spectrophotometer (Wilmington, DE) at 412 nm for 300 s at 10 s intervals. A standard curve for the slopes of known concentrations of glutathione standards was generated, and total glutathione was quantified based on the slopes for the standard curve.

Oxidized glutathione (GSSG) analysis was performed similarly to the total glutathione assay except 97% 2-vinylpyridine

Table 1. Initial Fatty Acid Profile of Channel Catfish

	phospholipid fatty acids			triacylglycerol fatty acids		
FAME[a]	minced	whole	p^b	minced	whole	p
total[c]	26.89 ± 6.43	28.94 ± 4.38	NS	55.85 ± 15.34	47.85 ± 14.94	NS
sat[d]	28.67 ± 2.07	29.76 ± 2.38	NS	27.45 ± 0.76	27.91 ± 1.30	NS
14:0	0.28 ± 0.14	0.26 ± 0.11	NS	1.44 ± 0.08	1.27 ± 0.42	NS
16:0	18.57 ± 1.37	19.94 ± 1.59	0.01	21.67 ± 0.52	21.46 ± 1.61	NS
18:0	8.06 ± 0.57	8.24 ± 0.74	NS	4.18 ± 0.19	4.84 ± 0.95	NS
20:0	0.28 ± 0.25	0.24 ± 0.17	NS	0.16 ± 0.05	0.15 ± 0.13	NS
24:0	1.48 ± 0.30	1.08 ± 0.40	0.02	0.00 ± 0.007	0.19 ± 0.38	NS
mono[d]	35.01 ± 1.98	33.81 ± 2.99	NS	56.39 ± 1.52	53.16 ± 1.67	0.02
16:1 n-7	2.26 ± 0.15	2.25 ± 0.43	NS	2.09 ± 0.09	2.14 ± 0.08	NS
18:1 n-9	28.39 ± 1.81	27.32 ± 3.31	NS	50.38 ± 1.24	46.99 ± 3.87	NS
18:1 n-7	2.82 ± 0.22	2.70 ± 0.27	NS	2.48 ± 0.10	2.55 ± 0.18	NS
20:1 n-9	1.54 ± 0.15	1.54 ± 0.34	NS	1.44 ± 0.14	1.48 ± 0.16	NS
PUFA[d]	36.34 ± 1.84	36.43 ± 1.71	NS	16.88 ± 1.00	18.92 ± 1.22	NS
n-6[d]	23.08 ± 1.01	23.16 ± 1.27	NS	13.89 ± 0.81	15.22 ± 0.97	NS
18:2 n-6	15.15 ± 0.68	15.48 ± 0.80	0.04	13.03 ± 0.69	13.26 ± 0.88	NS
18:3 n-6	0.23 ± 0.11	0.20 ± 0.10	NS	0.17 ± 0.11	0.26 ± 0.03	0.05
20:3 n-6	3.85 ± 0.18	3.74 ± 0.47	NS	0.51 ± 0.33	1.02 ± 0.30	NS
20:4 n-6	3.85 ± 0.30	3.74 ± 0.41	NS	0.18 ± 0.06	0.68 ± 1.01	NS
n-3[d]	13.26 ± 0.86	13.27 ± 0.89	NS	2.99 ± 0.43	3.70 ± 0.34	NS
18:3 n-3	0.92 ± 0.08	0.90 ± 0.13	NS	1.20 ± 0.19	1.13 ± 0.18	NS
20:5 n-3	1.23 ± 0.14	1.28 ± 0.14	NS	0.15 ± 0.08	0.29 ± 0.31	NS
22:3 n-3	2.09 ± 0.48	2.23 ± 0.34	NS	0.10 ± 0.11	0.44 ± 0.65	NS
22:5 n-3	1.04 ± 0.26	1.00 ± 0.24	NS	0.08 ± 0.09	0.20 ± 0.30	NS
22:6 n-3	7.98 ± 0.29	7.86 ± 0.66	NS	0.74 ± 0.21	1.64 ± 2.12	NS

[a] Fatty acid methyl ester. [b] Significance level for Student's *t*-test. [c] Total: mg of fatty acids/g of tissue DW; all others expressed as percent of total. [d] Sat: total of saturated fatty acids. Mono: total of monounsaturated fatty acids. PUFA: total of polyunsaturated fatty acids. n-6: total of n-6 fatty acids. n-3: total of n-3 fatty acids.

Table 2. Muscle α- and γ-Tocopherol Levels in Frozen (−6 °C) Mince and Intact Fillets of Channel Catfish[a]

	α-tocopherol (μmol/g of dry wt)		γ-tocopherol (μmol/g of dry wt)	
month	mince	intact fillet	mince	intact fillet
0	60.54 ± 7.16 A	59.47 ± 7.06 AB	9.05 ± 1.49 B	8.68 ± 1.50 B
1	59.07 ± 7.43 AB	57.60 ± 7.00 AB	9.34 ± 1.25 B	9.50 ± 1.79 B
2	55.49 ± 6.96 BC	52.88 ± 7.34 CD	10.48 ± 1.56 A	10.67 ± 1.99 A
3	50.07 ± 9.38 DE	47.29 ± 9.22 E	9.41 ± 1.12 B	9.10 ± 1.00 B
4	53.23 ± 10.99 CD	48.20 ± 7.13 E	6.59 ± 1.74 C	6.86 ± 1.33 C
5	38.47 ± 7.64 F	42.03 ± 10.95 FG	6.52 ± 2.06 C	7.33 ± 1.49 C
6	35.23 ± 3.04 G	35.71 ± 3.44 G	5.13 ± 0.46 D	5.05 ± 0.53 D

[a] Values within α- or γ-tocopherol data sets followed by a different letter are significantly different ($p < 0.05$).

(10 μL) was added to the sample/SSA supernatant (0.5 mL). Triethanolamine (30 μL) was added, the solution was vortexed, and an aliquot (0.2 mL) was treated by the procedure described for total glutathione analysis. Reduced glutathione (GSH) concentrations were determined as the difference between total glutathione and oxidized glutathione.

Volatile Analysis. For quantification of headspace volatiles, minced tissue (1.5 g) was placed in a 5 mL conical vial and heated for 20 min at 90 °C. Headspace (1.5 mL) was removed from the vial with a gas-tight syringe and subjected to the gas chromatographic conditions described by Erickson (1993). Identification of peaks was accomplished by subjecting volatile standards (Aldrich, Milwaukee, WI), 99% purity, to the same chromatographic conditions as the mince tissue. Quantification of volatiles was based on the response of an internal standard, 4-heptanone, added to the mince.

Statistical Analysis. SAS (Cary, NC) was used to analyze data using the general linear model (PROC GLM), regression (PROC REG), and simple statistics (PROC MEANS). The level of significance for all tests was set at $\alpha = 0.05$. Mean separations were achieved according to Duncan's multiple range test.

RESULTS

Moisture and Lipid Content. Initially, moisture levels in mince and intact fillets were 77.94 ± 0.79% and 78.16 ± 0.70%, respectively. Moisture levels did not significantly decrease over 6 months of frozen storage (data not shown), indicating that packaging in moisture barrier bags avoided sample dehydration.

Total lipids in mince and intact fillets were not significantly different with levels ranging from 1.7%−1.8%. Fatty acid analysis of the major lipid classes also revealed no significant differences between mince and intact fillets with saturated, monounsaturated, and polyunsaturated fatty acids accounting for 29, 35, and 36 wt % of the phospholipid fraction, respectively, and 28, 54, and 18 wt % of the triacylglycerol fraction, respectively (Table 1).

Tocopherol. Tocopherol degradation was monitored over 6 months of frozen storage for both mince and intact fillets (Table 2). α-Tocopherol content gradually decreased over 6 months of frozen storage, exhibiting linear degradation for both mince ($r = -0.84$) and intact fillets ($r = -0.89$). Mean comparisons of mince versus intact fillets up to 3 months of storage were not significantly different, while at 4 months of frozen storage mince was significantly higher in α-tocopherol content. This difference was not observed at 5 or 6 months of storage.

In contrast to the losses observed in α-tocopherol in early stages of storage, γ-tocopherol levels increased significantly. The maximum increase in γ-tocopherol over initial values was observed at 2 months of storage for both mince (115.5%) and intact fillets (122.2%) with

Table 3. Muscle Ascorbic Acid Levels in Frozen (−6 °C) Mince and Intact Fillets of Channel Catfish[a]

	ascorbic acid (μg/g of tissue)	
month	mince	intact fillet
0	14.75 ± 2.82 A	14.61 ± 2.58 A
1	11.86 ± 2.86 B	12.42 ± 2.09 BC
2	8.58 ± 1.74 D	10.82 ± 1.70 C
3	4.71 ± 3.10 F	6.50 ± 2.50 E
4	1.94 ± 1.77 GH	4.24 ± 0.99 F
5	1.76 ± 2.05 GHI	2.67 ± 1.69 G
6	0.63 ± 0.51 I	1.19 ± 0.70 HI

[a] Values followed by a different letter are significantly different ($p < 0.05$).

subsequent degradation occurring in both samples over the remaining storage. Overall, there were no significant differences observed between mince and intact fillets.

Ascorbic Acid. Concentrations of ascorbic acid in mince and intact fillets stored over 6 months are listed in Table 3. Loss of ascorbic acid can be described by a first-order regression equation for both mince ($r = -0.89$) and intact fillets ($r = -0.92$) with loss of ascorbic acid in the mince occurring at a faster rate over the 6 month storage period than for the intact fillets. This effect was especially apparent through the first 4 months of storage as the degradation of ascorbic acid in the mince exhibited a significantly more negative slope (−4.51 ppm/month) than for the whole samples (−2.24 ppm/month).

Glutathione. Loss of reduced glutathione as well as formation of oxidized glutathione was measured over the 6 month storage period (Table 4). Exponential degradation was observed for reduced glutathione ($r = -0.91$, mince; $r = -0.95$, intact fillets). Formation of oxidized glutathione occurred but could not be explained by any type of regression.

The greatest loss of reduced glutathione was observed between 0 and 2 months of storage for both mince and intact fillets, but the decline occurred at a slightly faster rate for mince (−5.65 nmol/month) than for intact fillets (−4.60 nmol/month). By 4 months of storage, however, no significant differences in the levels of reduced glutathione were found between mince and intact fillets.

Headspace Volatiles. Volatile aldehydes are formed as secondary breakdown products from oxidation of n-6 and n-3 fatty acids. While hexanal is a major volatile aldehyde formed from oxidation of n-6 fatty acids (Tamura et al., 1991), propanal, a secondary product of n-3 fatty acid oxidation (Boyd et al., 1992), would also be a major volatile in fish due to the presence of high concentrations of n-3 fatty acids (Erickson, 1993b,c). In this study, formation of volatile aldehydes was measured over 6 months of frozen storage for both mince and intact fillets (Table 5). Initially, hexanal was not detected for mince or intact fillets; however, propanal was detected in small concentrations. Other minor volatiles, including pentanal, when detected during storage were at concentrations less than 0.01 μg/g of tissue (data not shown). Formation of propanal and hexanal over the 6 month storage period for both mince and intact fillets followed a logarithmic trend that can be described by three distinct phases: (1) a lag phase exhibited during the first 2 months of storage, (2) a slow increase in volatile formation observed from 2 to 4 months, and (3) a subsequent burst of aldehyde formation occurring from 4 to 6 months of storage.

At 5 months of storage, no differences in hexanal content were observed between treatments, but these samples were significantly higher in hexanal than samples stored up to 4 months. At 6 months of frozen storage, however, mean hexanal levels for the intact fillets were significantly higher than for the mince. Propanal levels increased significantly after 4 months of storage, but no differences were observed between treatments.

DISCUSSION

Initial levels of antioxidants in channel catfish for this study were much higher than previously reported. Specifically, α-tocopherol levels were 1.5−3.5 times higher than previously reported (Erickson, 1992b,c). Absence of supplemental feeding to fish in the other studies is likely given the time of year they were harvested, whereas recent application of feed had occurred to fish from the present study and may be the primary factor responsible for the higher antioxidant level. In contrast, it is contended that lower glutathione levels found in catfish by Gatlin and Bai (1993) compared to this study were due to the fact that their tissue was stored at −20 °C for up to 6 months before analysis, during which time degradation of glutathione could have occurred.

Loss of glutathione occurred at a faster rate than loss of ascorbic acid for both the mince and intact fillets in early stages of storage. After 1 month of frozen storage, 48% and 40% of the glutathione had been depleted in mince and intact fillets, respectively, compared to losses of only 20% and 15% for ascorbic acid. By 4 months of storage, glutathione losses approached those of ascorbic acid in minced samples, while this effect was not observed until 5 months of storage in intact fillets. Degradation of α-tocopherol, in turn, occurred at a much slower rate than either of the water-soluble antioxidants although the pattern of α-tocopherol loss was similar throughout storage for both the mince and the intact fillets. The relative rates of antioxidant degradation observed in these tissues are in agreement with the antioxidant's pecking order of oxidative activity (Buettner, 1993).

Only 20% of the initial α-tocopherol had been lost before volatile levels increased suggesting that the concentration of tocopherol at that point was no longer sufficient to protect the tissue lipids. Whether there is any relevance to the observation that the increase in volatiles occurred concurrently with the decline of γ-tocopherol remains to be seen. Similarly, the increase in γ-tocopherol during the initial stages of frozen storage may only be the subject of speculation at this point. α-Tocopherol, a 5,7,8-trimethyl-substituted tocopherol, could in theory be converted to the 7,8-dimethyl-γ-tocopherol form through a demethylation pathway similar to that involved in the conversion of lanosterol to 7-dehydrocholesterol (Voet and Voet, 1990). In this pathway, hydroxylation of methyl groups by a NADH microsomal system previously found in channel catfish (Eun et al., 1994) would be followed by oxidation to a carboxylic acid and then a ketone, after which efficient decarboxylation could occur. The fact that γ-tocopherol accumulation has not been documented previously through such a pathway may be due to the reported preferential removal of γ-tocopherol from membranes (Parker, 1989), a mechanism which would be inoperative in postmortem tissue. It is to be noted, however, that the increases observed in γ-tocopherol do not account for all the α-tocopherol losses, implying that oxidation in addition to demethylation of α-tocopherol was occurring during the first 2 months of storage.

Table 4. Muscle Levels of Reduced Glutathione (GSH) and Oxidized Glutathione (GSSG) in Frozen (−6 °C) Mince and Intact Fillets of Channel Catfish[a]

	GSH (nmol/g of tissue)		GSSG (nmol/g of tissue)	
month	mince	intact fillet	mince	intact fillet
0	639.39 ± 83.14 A	644.30 ± 70.77 A	9.01 ± 3.41 A	10.88 ± 4.77 A
1	330.28 ± 74.10 C	391.12 ± 40.78 B	29.57 ± 7.61 CDE	26.61 ± 6.45 CD
2	191.75 ± 27.77 E	254.73 ± 38.21 D	20.92 ± 8.18 B	31.76 ± 14.52 DEF
3	148.69 ± 22.51 F	199.86 ± 28.79 E	24.36 ± 6.44 BC	34.23 ± 13.12 EFG
4	102.74 ± 24.58 GH	132.59 ± 21.29 FG	32.32 ± 5.65 DEF	29.63 ± 6.61 CDE
5	86.69 ± 25.30 HI	105.05 ± 17.71 GH	36.88 ± 9.68 FG	36.38 ± 6.75 FG
6	64.68 ± 20.22 I	88.47 ± 22.72 HI	38.47 ± 6.35 G	34.47 ± 7.51 EFG

[a] Values within GSH and GSSG data sets followed by a different letter are significantly ($p < 0.05$).

Table 5. Levels of Hexanal and Propanal in Frozen (−6 °C) Mince and Intact Fillets of Channel Catfish[a]

	propanal (μg/g of tissue)		hexanal (μg/g of tissue)	
month	mince	intact fillet	mince	intact fillet
0	0.06 ± 0.03 F	0.08 ± 0.03 EF	0.00 ± 0.00 F	0.00 ± 0.00 F
1	0.09 ± 0.04 DEF	0.13 ± 0.04 CDE	0.06 ± 0.04 EF	0.06 ± 0.04 EF
2	0.09 ± 0.09 DEF	0.09 ± 0.05 DEF	0.06 ± 0.05 EF	0.07 ± 0.05 EF
3	0.15 ± 0.09 CD	0.17 ± 0.07 C	0.12 ± 0.09 DE	0.12 ± 0.10 DE
4	0.15 ± 0.06 CD	0.18 ± 0.07 C	0.16 ± 0.10 D	0.14 ± 0.10 D
5	0.42 ± 0.16 B	0.39 ± 0.13 B	0.41 ± 0.28 C	0.37 ± 0.25 C
6	0.80 ± 0.37 A	0.86 ± 0.45 A	0.78 ± 0.42 B	0.95 ± 0.46 A

[a] Values for mince and intact fillets in the same response followed by a different letter are significantly different ($p < 0.05$).

The process of mincing modifies the tissue microenvironment by introducing increased interaction among many of the cellular constituents of the tissue, damage to the membrane layer, and increased exposure to oxygen. These modifications would be expected to enhance oxidation, and the enhanced losses of ascorbic acid and glutathione are in line with these expectations. Over the time periods examined, however, volatile accumulation in mince was either comparable to or less than levels found in intact fish, agreeing with the results of Wada et al. (1977) who found that oxidation of lipids in cod was prevented to some extent in the early stages of storage by mincing. As displacement of ascorbic acid or glutathione from an intracellular environment to an intercellular matrix would be expected to increase their interaction with and reduction of metal ions and/or decrease their reduction of tocopherol through a dilution effect, enhanced activity of a prooxidant nature by ascorbic acid and glutathione should also be presumed. That such a response was not recorded and no differences in lipid contents existed between the samples suggest that mincing may also activate some unmeasured antioxidant system, such as phospholipase (Shewfelt et al., 1981; Shewfelt and Hultin, 1983) or aldehyde dehydrogenase. Alternatively, any enhancement of ascorbic acid's or glutathione's prooxidant reactions in mince may be counterbalanced by a simultaneous enhancement of their antioxidant activity. It is also possible that at the levels present in the tissue, ascorbic acid's and glutathione's activities are minor compared to the overall oxidative activity taking place.

This study has demonstrated the dynamic and complex role antioxidants play in tissue oxidative stability. While degradation of water-soluble antioxidants was accelerated by mincing, degradation of fat-soluble antioxidants was not. Since generation of oxidative volatiles was also not accelerated by mincing, it is not possible to conclusively identify the mechanism responsible for stabilizing the lipids against increased oxygen exposure. Several explanations were presented, but further study will be required to pinpoint those factors of major concern.

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