Compositional Parameters and Their Relationship to Oxidative Stability of Channel Catfish

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Two samples of channel catfish (AQUA and LSU) were subjected to frozen storage for 0, 3, 6, and 9 months under fluctuating temperatures (-6 to -18 °C). After 9 months of storage, levels of hydroperoxides, conjugated dienes, thiobarbituric acid reactive substances, and fluorescent pigments were higher in AQUA than in LSU. In contrast, individual volatiles were either similar or higher in LSU than in AQUA. α-Tocopherol equivalents, initially similar in the two samples, were lost slowly during the first 6 months of storage. Accelerated degradation of tocopherol after 6 months of storage coincided with increased generation of lipid oxidation products, indicating that the tocopherol concentrations of samples stored for 6 months were no longer sufficient to protect membrane lipids and prevent propagation. Higher activities of phospholipase and lipase, evident in LSU samples compared to AQUA samples, also appeared to have affected oxidative stabilities.

INTRODUCTION

Significant growth was experienced by the channel catfish industry during the 1980s. In the 1990s, a slower rate of growth occurred due in large part to the cyclical periods of excess supply and demand, which in turn resulted in misallocation of resources and price instability. Retailers point to these supply problems as one of the limiting factors for further expansion of the catfish industry (Zidack and Hatch, 1991). Attempts to circumvent these problems through frozen storage of the product have met with limited success due to the product's short shelf life (3–6 months).

The short shelf life of frozen catfish has been attributed to the formation of rancid odors and flavors which arise from oxidation of unsaturated fatty acids. While storage studies have been conducted to evaluate the effects of diet, cultural conditions, and processing treatments on lipid and flavor stability (Boggess et al., 1973; Gibson et al., 1977; Gibson and Worthington, 1977; O'Keefe and Noble, 1978; Fisher, 1983; Mitchell, 1986; Freeman, 1990; Gatlin et al., 1992), a clear relationship between muscle composition and its oxidative stability has not been established. Such information could be useful for shelf-life predictions and could also serve as a guide in feeding and breeding programs. This study, therefore, sought to monitor oxidation of the minced muscle from two samples of catfish during frozen storage and compare it to the muscle's composition.

MATERIALS AND METHODS

Sample Preparation and Storage. Two strains of 3-year-old channel catfish (*Ictalurus punctatus*), identified as LSU and AQUA, were obtained from the University of Georgia Cohutta Fisheries Research facility. Seven fish from each strain were commercially processed (electrically stunned, deheaded, gutted, and skinned with a Townsend skinner), weighed, and filleted. Fillets from each fish were pooled and minced to form a homogeneous sample.

Minced samples were subdivided into four portions with each portion being packaged in polyethylene bags (Product No. Curlon 550; Cryovac Co., Duncan, SC) before being subjected to frozen storage for 0, 3, 6, or 9 months. During storage, temperatures were fluctuated between -6 and -18 °C (five cycles/week) in an effort to simulate conditions encountered in commercial storage. Further storage at -100 °C was required until the various analyses could be performed on the samples. At the time of analysis, samples were removed from the -100 °C freezer and thawed in cold running water.

Moisture Analyses. Minced tissue (2.0 g) was analyzed in triplicate for moisture by drying in a microwave oven (Automatic Volatility Computer, Model AVC-80; CEM Corp., Indian Trail, NC) until constant weight was achieved.

Tocopherol Analyses. After saponification in the presence of ascorbic acid and ethanol, tocopherols were extracted from 1.0 g of minced muscle tissue ($n = 5$) with 10% (v/v) ethyl acetate in hexane (Erickson, 1992). The extract, evaporated to dryness under nitrogen, was reconstituted in 1.0 mL of the eluting solvent, methanol/water (98:2 v/v). Reversed-phase high-performance liquid chromatographic (HPLC) separation of tocopherols was conducted as described by Vatassery and Smith (1987) using a Zorbax ODS column (4.6 mm × 25 cm, 5-μm particle size) at a flow rate of 1.0 mL/min. The chromatographic system consisted of a Micromeritics 752 gradient programmer, Micromeritics 750 solvent delivery system, and a Brinkmann 656 electrochemical detector.

Ascorbic Acid Analyses. Minced muscle tissue (4.0 g) was homogenized in duplicate with 20 mL of 40 mM sodium acetate buffer, pH 3.8, containing 15% (v/v) ethanol. An aliquot of the homogenate was applied to a series of filters [Whatman No. 5 filter paper, C_{18} PrepSep column (Fisher Scientific, Norcross, GA), and a Gelman nylon acrodisc, 0.2 μm (Ann Arbor, MI)] and the resulting filtrate subjected to quantification of the ascorbic acid by an ion-pairing HPLC method. The HPLC system consisted of the same apparatus as described for tocopherol analyses, while the mobile phase consisted of 40 mM sodium acetate and 1 mM decylamine in methanol/water (15:85 v/v), pH 4.0. The flow rate was maintained at 0.9 mL/min, while the oxidation potential of the electrochemical detector was set at 700 mV vs the Ag/AgCl reference electrode.

Extraction and Lipid Class Separation. Chloroform/methanol (2:1 v/v) was used to extract lipid from 1.00 ± 0.02 g of muscle tissue ($n = 5$) as described by Erickson (1992). Polar (phospholipids) and nonpolar (triacylglycerols, free fatty acids) fractions were obtained using silica Sep-Pak cartridges (Juaneda and Rocquelin, 1985). Free fatty acid fractions were obtained following application of an aliquot of the nonpolar lipid fraction to a thin-layer chromatography plate and development in 80:20:1 (v/v/v) hexane/ethyl ether/glacial acetic acid.

Esterification of Lipid Classes. Phospholipid and free fatty acid fractions were esterified using 4% H_2SO_4 in methanol (Erickson and Selivonchick, 1988). A glass capillary column (J&W DB-225; 30 m × 0.25 mm; 0.15-μm film) attached to a Hewlett-Packard 5790A Series gas chromatograph was used for separation of the esterified fatty acids. The oven was held for

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Table I. Oxidative Measurements of Frozen, Minced Channel Catfish Tissue[a]

	AQUA				LSU			
	storage, months							
measurement	0	3	6	9	0	3	6	9
peroxides, nmol/g dwb	8.9 a	9.4 a	18.6 a	1699.5 c	0.0 a	0.2 a	7.2 a	835.3 b
conjugated dienes, A_{232}	1.50 a	1.45 a	1.50 a	3.95 d	2.05 c	1.71 b	1.69 b	3.30 c
TBARS, nmol/g dwb	1.8 a	16.2 c	54.9 e	181.7 g	0.0 a	7.8 b	25.4 d	133.4 f
total volatiles, μg/g dwb	189.4 ab	171.3 ab	199.9 b	475.4 c	137.8 a	180.9 ab	202.7 b	524.6 c
aqueous fluorescent pigments, units/g dwb	473 a	636 c	666 c	1249 e	466 a	562 b	668 c	1030 d
organic fluorescent pigments, units/g dwb	942 a	1073 b	1133 b	2916 d	944 a	873 a	959 a	1669 c
α-tocopherol equivalents, nmol/g dwb	48.8 de	50.9 e	44.0 c	2.1 a	47.9 de	46.4 cd	34.7 b	3.7 a
ascorbic acid, nmol/g dwb	119.6 d	54.1 c	56.0 c	0.0 a	168.6 e	112.4 d	20.8 b	0.0 a
free fatty acids, μmol/g dwb	30.6 a	97.5 b	184.6 c	243.8 d	37.8 a	176.5 c	244.3 d	438.3 e
phospholipid FAME, μmol/g dwb	5.5 d	4.2 c	4.0 c	3.0 ab	5.6 d	3.3 b	3.3 b	2.7 a
expressible moisture, %	43.3 b	49.8 cd	54.9 e	51.5 d	37.8 a	48.4 c	50.3 cd	50.2 cd

[a] Values in a row followed by the same letter are not significantly different ($P > 0.05$).

10 min at 180 °C, then 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) subjected to the same conditions as experimental samples were used to identify chromatographic peaks while each peak's area was integrated with a Hewlett-Packard 3390A integrator and compared to the response of an internal standard, behenic acid (21:0).

Quantitation of Phospholipids. Aliquots of the phospholipid fraction were subjected to digestion and oxidation (Anderson and Davis, 1982). The inorganic phosphate released was measured using the procedure outlined by Bartlett (1959).

Hydroperoxide Measurement. The procedure of Buege and Aust (1978) was used for quantitation of hydroperoxides in an extracted lipid sample ($n = 5$) which had previously been washed with 0.88% KCl. The molar extinction coefficient of cumene hydroperoxide, 1.73×10^4/M, served to convert measured absorbances to hydroperoxide concentrations, and the data were expressed as nanomoles of peroxide per gram of sample on a dry weight basis (dwb).

Conjugated Dienes Measurement. A 5-mL aliquot of the lipid extract (25 mL total) was dispensed into a clean tube ($n = 5$) and washed with 1 mL of 0.88% KCl. Following aspiration of the upper layer, 2 mL of the lower layer was evaporated to dryness and reconstituted in 5 mL of isooctane, and the absorbance was read at 232 nm.

Thiobarbituric Acid Reactive Substance (TBARS) Measurement. Quantification of TBARS for each sample ($n = 4$) was conducted according to the procedure of Vyncke (1970). Data were expressed as nanomoles of malonaldehyde equivalents per gram dwb.

Fluorescent Pigment Measurement. Aqueous and organic fluorescent pigments were determined on chloroform/methanol (2:1 v/v) lipid extracts ($n = 5$). Following washing of 10.0 mL of a lipid extract (25 mL total) with 2.5 mL of 0.88% KCl, diluted samples of both the aqueous and organic layers were taken, and their fluorescence was compared to that of a quinine sulfate standard (1×10^{-8} M) set equal to 100 units. Fluorescence measurements were taken on a Turner fluorometer, Model 112, with excitation and emission filters selected for wavelengths in the range 320–390 and 420–500 nm, respectively.

Headspace Volatile Analyses. Minced tissue (1.5 g), $n = 3$, was heated in a 5-mL conical vial for 15 min at 90 °C. Headspace volatiles (1.5 mL), removed with a gastight syringe, were subjected to the chromatographic conditions of Young and Hovis (1990). Tentative identification of volatile peaks was accomplished by subjecting volatile standards (Aldrich, Milwaukee, WI), 99% purity, to the same chromatographic conditions as the experimental samples, while quantification was based on the response of an internal standard, 4-heptanone, added to the experimental samples.

Metal Analyses. Wet ashing of minced tissue (5.0 g) was accomplished with concentrated nitric acid and hydrogen peroxide (McDaniel, 1991). The digested residue ($n = 3$ for AQUA and $n = 2$ for LSU) was brought to 50 mL with ultrapure deionized water and analyzed for iron, copper, manganese, and zinc content by an inductively coupled plasma atomic emission spectrometer (Thermo Jarrell Ash, Franklin, MA). Results were expressed as micrograms per gram dwb.

Expressible Moisture Measurements. Using the centrifugal method described by Jauregui et al. (1981), expressible moistures were determined on stored samples. Data were expressed on a percent basis.

Statistical Analyses. A StatGraphics software package (Rockville, MD) was used for statistical analysis of the data. Data were subjected to analysis of variance, and where statistical differences were noted for a measurement, differences among sample means were determined using the least significant difference test. Linear regression was employed to determine relationships among oxidative products and compositional parameters. The level of significance was set at $\alpha = 0.05$ for all tests unless otherwise noted.

RESULTS AND DISCUSSION

The two samples of channel catfish examined in this study were found to vary in their degree of lipid oxidation. While the primary products of lipid oxidation, hydroperoxides and conjugated dienes, did not increase during the first 6 months of frozen storage (Table I), elevated levels of these products were detected in the last 3 months of storage, with AQUA containing significantly greater quantities than LSU. Thiobarbituric acid reactive substances (TBARS), in contrast, increased throughout storage with a gradual rise occurring during the first 6 months and a sharp rise in production the last 3 months (Table I). Increases in aqueous fluorescent pigments were also noted in the first 6 months for both samples, whereas an increase in organic fluorescent pigments during this time period was only found in the AQUA sample (Table I). These oxidative product profiles (hydroperoxides, conjugated dienes, TBARS, fluorescent pigments) differ from those traditionally found in which hydroperoxides accumulated in the early stages of oxidation (Kim and LaBella, 1987; Cho et al., 1989; Fujimoto et al., 1990). The increased potential for interaction of iron ions with hydroperoxides that would have occurred upon mincing in these samples may account for the inability to detect primary products and the ability to detect secondary products of oxidation in the early stages of storage.

While the TBARS data indicated a greater susceptibility of the AQUA sample to oxidize than the LSU sample at all storage periods examined, total quantities of headspace volatiles were statistically similar for both samples throughout storage (Table I). Closer inspection of individual headspace volatile peaks in Table II, however, revealed some differences between the samples. Peaks corresponding to propanal, ethyl acetate, and pentanal for LSU 9-month samples had statistically larger amounts than

Table II. Headspace Volatiles (Micrograms per Gram Dry Weight Basis) of Channel Catfish Muscle Tissue Subjected to Various Periods of Frozen Storage[a]

retention time, min	volatile[b]	AQUA storage, months 0	AQUA 3	AQUA 6	AQUA 9	LSU 0	LSU 3	LSU 6	LSU 9
1.03	acetaldehyde	82.7 b	116.8 c	132.0 cd	138.6 d	48.4 a	112.3 c	113.3 c	151.2 d
1.91	propanal	9.5 ab	17.6 abc	21.4 bcd	143.9 e	7.2 a	24.8 cd	32.9 d	159.5 f
2.61	butanal	0.2 a	1.4 b	1.8 cd	1.9 de	0.0 a	1.4 b	1.4 bc	2.2 e
2.94	ethyl acetate	0.0 a	1.9 b	2.9 bc	11.6 d	0.0 a	3.1 bc	3.9 c	14.7 e
4.02	pentanal	0.0 a	0.0 a	1.0 a	3.6 b	0.0 a	0.3 a	1.4 a	10.2 c
5.10	hexanal	4.8 a	16.8 b	20.2 b	122.9 c	2.6 a	18.3 b	25.6 b	113.1 c
6.44	heptanal	0.0 a	0.3 a	0.9 a	3.7 c	0.0 a	2.2 b	1.8 b	5.2 d

[a] Values in a row followed by the same letter are not significantly different ($P > 0.05$). [b] Tentative identification.

Table III. Fatty Acid Composition (Milligrams per Gram Dry Weight Basis) in Phospholipid Fraction of Channel Catfish Muscle Tissue Subjected to Various Periods of Frozen Storage[a]

FAME	AQUA storage, months 0	AQUA 3	AQUA 6	AQUA 9	LSU 0	LSU 3	LSU 6	LSU 9
sum sat.	3.71 d	2.53 b	2.81 c	2.41 ab	3.60 d	2.29 a	2.41 ab	2.73 c
14:0	0.11 bc	0.13 d	0.13 d	0.09 a	0.10 b	0.13 d	0.12 cd	0.10 b
16:0	2.48 e	1.70 ab	1.93 c	1.70 ab	2.44 e	1.68 a	1.79 b	2.07 d
18:0	1.09 g	0.67 d	0.73 e	0.60 c	1.02 f	0.46 a	0.48 a	0.53 b
sum mono	4.62 e	4.01 d	4.15 d	2.74 b	4.54 e	2.95 bc	3.00 c	2.46 a
16:1	0.28 d	0.26 cd	0.25 c	0.15 a	0.28 d	0.22 b	0.21 b	0.16 a
18:1 *n*-9	3.68 e	3.27 d	3.41 d	2.25 b	3.69 e	2.43 bc	2.51 c	2.05 a
18:1 *n*-11	0.42 f	0.31 d	0.33 d	0.23 c	0.35 e	0.20 b	0.19 b	0.16 a
20:1	0.24 e	0.16 c	0.17 c	0.12 b	0.22 b	0.10 a	0.09 a	0.09 a
sum pufa	4.98 g	3.24 e	3.20 e	1.94 b	4.62 f	2.34 d	2.11 c	1.57 a
sum *n*-6	3.23 g	2.19 e	2.14 e	1.29 b	2.92 f	1.50 d	1.36 c	0.98 a
18:2 *n*-6	1.93 f	1.48 e	1.44 e	0.83 b	1.89 f	1.08 d	0.99 c	0.70 a
18:3 *n*-6	0.03 a	0.02 a	0.03 a	0.02 a	0.03 a	0.02 a	0.02 a	0.02 a
20:3 *n*-6	0.48 e	0.26 d	0.25 d	0.15 b	0.48 e	0.19 c	0.16 b	0.11 a
20:4 *n*-6	0.79 f	0.43 d	0.42 d	0.29 c	0.53 e	0.21 b	0.19 ab	0.15 a
sum *n*-3	1.76 e	1.06 d	1.07 d	0.65 ab	1.70 e	0.84 c	0.75 bc	0.60 a
18:3 *n*-3	0.14 d	0.11 c	0.10 c	0.06 a	0.12 c	0.08 b	0.07 b	0.05 a
20:5 *n*-3	0.26 e	0.14 c	0.14 c	0.09 b	0.24 d	0.10 b	0.09 b	0.07 a
22:5 *n*-3	0.17 d	0.11 c	0.12 c	0.06 a	0.24 e	0.13 c	0.10 bc	0.08 b
22:6 *n*-3	1.19 d	0.70 c	0.71 c	0.44 ab	1.10 d	0.53 b	0.48 ab	0.39 a

[a] Values in a row followed by the same letter are not significantly different ($P > 0.05$).

AQUA 9-month samples, whereas levels of hexanal, a noted marker of lipid oxidation (Jeon and Bassette, 1984; Shahidi et al., 1987; St. Angelo et al., 1987; Hu et al., 1990; Boyd et al., 1992), were similar for both samples at 9 months. The different responses displayed by these assays designed to measure secondary products agree with the lack of correlation previously seen between TBARS and the perception of oxidized odor (Satomi et al., 1988) and emanates from the assay's selectivity. In the case of the TBA assay, malonaldehyde is the main compound measured, although aldehydes such as hexanal also react with TBA to form less intense chromogens (Kosugi and Kikugawa, 1986). These results demonstrate the limitations in using only one oxidative assay to compare tissue stabilities. Caution should also be taken in relying solely on one storage time, such as the 9-month storage time, to assess oxidative stabilities of samples. For example, if a sample exhibited a shorter induction phase as well as a slower rate of propagation compared to another sample, the sample might be considered less stable than the other in the early stages of storage and more stable in later stages of storage.

Quantities of phospholipid (PL) fatty acids extracted from the 3-, 6-, and 9-month samples were lower than those from the 0-month samples (Table I). Losses exhibited by LSU during the first 3 months were 1.5 times larger than losses exhibited by AQUA. Free fatty acids (FFA) extracted from samples, on the other hand, increased during storage, LSU exhibiting higher recoveries than AQUA at 3, 6, and 9 months. By 9 months of storage, the increases in FFA were 100–200 times the levels of PL fatty acids which had been lost.

Detailed profiles of the fatty acids in the PL and FFA fractions are found in Tables III and IV, respectively. The major polyunsaturated fatty acids (PUFA) in the PL fractions were 18:2 *n*-6, 20:4 *n*-6, and 22:6 *n*-3, while the major PUFA in the FFA fractions were 18:2 *n*-6, 18:3 *n*-3, and 22:6 *n*-3. Although losses in both saturated (sat.) and unsaturated fatty acids occurred in the PL fraction, PUFA constituted the largest proportion of those losses. Over 85% of the total PL sat. fatty acid losses occurred during the first 3 months of storage compared to 66% of the total PL PUFA losses. The largest loss that occurred over the entire storage period for an individual PL fatty acid was for 18:2 *n*-6. In turn, the largest individual increase of a FFA was for 18:2 *n*-6. Approximately half of the total increase in the AQUA's PUFA FFA occurred during the first 3 months of storage, whereas nearly half of LSU's increases in PUFA FFA occurred primarily in the last 3 months of storage. Losses of PL PUFA, together with the increased level of PUFA in the FFA fraction, would indicate that phospholipase activity was occurring in both samples, but at a higher rate in LSU than in AQUA over the first 6 months of storage. Differential quantities of phospholipases released during mincing would have influenced the loss in PL fatty acids. Increased hydrolysis of phospholipids in LSU samples, in turn, could also explain the reduced levels of TBARS and organic fluo-

Table IV. Composition (Milligrams per Gram Dry Weight Basis) of Free Fatty Acid Fractions Isolated from Channel Catfish Muscle Tissue Subjected to Various Periods of Frozen Storage[a]

	AQUA				LSU			
	storage, months							
FAME	0	3	6	9	0	3	6	9
sum sat.	1.20 a	3.34 b	6.60 c	9.54 d	1.52 a	5.90 c	8.44 d	16.92 e
14:0	0.05 a	0.26 b	0.56 c	0.73 d	0.07 a	0.54 c	0.75 d	1.38 e
16:0	0.84 a	2.26 b	4.40 c	6.53 d	1.10 a	4.09 c	5.82 d	12.43 e
18:0	0.31 a	0.82 ab	1.58 c	2.21 d	0.36 a	1.24 bc	1.78 cd	3.00 e
sum mono	4.19 a	12.77 b	24.81 c	33.17 d	4.98 a	24.66 c	33.46 d	60.67 e
16:1	0.13 a	0.88 b	1.63 c	2.09 d	0.27 a	1.80 c	2.37 e	3.89 f
18:1 *n*-9	3.90 a	10.59 b	20.83 c	28.12 d	4.53 a	20.82 c	28.45 d	52.58 e
18:1 *n*-11	0.06 a	0.86 b	1.48 cd	1.75 d	0.08 a	1.40 c	1.70 d	2.56 e
20:1	0.07 a	0.44 b	0.88 d	1.21 e	0.11 a	0.64 c	0.94 d	1.64 f
sum PUFA	1.60 a	6.17 b	10.74 c	12.96 d	2.14 a	9.83 c	13.91 d	22.53 e
sum *n*-6	1.39 a	5.24 b	9.21 c	11.24 d	1.78 a	8.37 c	11.70 d	18.50 e
18:2 *n*-6	1.33 a	4.62 b	8.23 c	10.11 d	1.63 a	7.57 c	10.50 d	16.84 e
18:3 *n*-6	0.00 a	0.08 b	0.13 c	0.13 c	0.00 a	0.13 c	0.19 d	0.26 e
20:3 *n*-6	0.02 a	0.33 c	0.55 e	0.64 f	0.10 b	0.47 d	0.67 f	0.94 g
20:4 *n*-6	0.04 a	0.21 b	0.30 c	0.36 d	0.06 a	0.20 b	0.33 cd	0.46 e
sum *n*-3	0.21 a	0.93 ab	1.52 bc	1.72 c	0.35 a	1.51 bc	2.21 c	4.02 d
18:3 *n*-3	0.09 a	0.38 b	0.66 cd	0.77 d	0.14 a	0.65 c	0.93 e	1.33 f
20:5 *n*-3	0.02 a	0.15 b	0.23 c	0.23 c	0.04 a	0.22 c	0.30 d	0.41 e
22:5 *n*-3	0.00 a	0.05 ab	0.10 ab	0.12 bc	0.00 a	0.08 ab	0.22 d	0.19 cd
22:6 *n*-3	0.10 a	0.34 a	0.54 a	0.60 a	0.18 a	0.56 a	0.76 a	2.08 b

[a] Values in a row followed by the same letter are not significantly different ($P > 0.05$).

rescent pigments found in LSU samples compared to AQUA samples. Inhibition of lipid oxidation by phospholipase activity has been demonstrated in model systems (Mazeaud and Bilinski, 1976; Shewfelt et al., 1981) and in fish muscle (Castell and Bishop, 1969) and has been hypothesized to function by reducing the interactions of membrane PUFA which would promote propagation (Shewfelt and Hultin, 1983).

Losses of PL FAME were not consistent with the quantities of FFA generated over similar time periods. Lipase activity would therefore have to account for the bulk of the FFA produced. Furthermore, since LSU had significantly greater quantities of FFA produced than AQUA, LSU in turn would be presumed to have had higher lipase activities than AQUA. FFA generated from lipolysis of TG, being more susceptible to oxidation than TG (Labuza, 1971), could thus be responsible for the volatiles and TBARS detected in samples stored for 3 or 6 months.

Throughout frozen storage, both α- and γ-tocopherols were found in channel catfish muscle tissue. By considering γ-tocopherol to be 45% as effective as α-tocopherol in tissue (Fukuzawa et al., 1982), α-tocopherol equivalents were initially similar in the two samples (Table I). In the first 3 months of frozen storage, tocopherol degradation was not detected. By 9 months of frozen storage, however, <10% of the initial tocopherol levels remained. Loss of tocopherol during storage was consistent with tocopherol's proposed role as a scavenger of oxygen- or carbon-centered fatty acyl radicals. In particular, accelerated degradation of tocopherol after 6 months in conjunction with the increased production of oxidative products suggested that a critical concentration of tocopherol had been reached for both samples. At this critical concentration, the tocopherol would no longer be able to compete effectively for the peroxyl radicals and prevent the propagation step of oxidation. The inability of tocopherol to protect the highly unsaturated PL after 6 months of storage would point to the PL fraction serving as the primary source of generated oxidative products. This shift in effective concentration of tocopherol would also have marked a shift in the relative concentrations of volatiles produced. Alterations in the product distribution by tocopherol have been shown previously (Karahadian and Lindsay, 1989; Frankel and Gardner, 1989), and in this study, changes in the relative concentrations of propanal and acetaldehyde were most evident (Table II). The difference noted between samples in the levels of tocopherol present at the start of the propagation phase (44 and 35 nmol/g dwb for AQUA and LSU, respectively) suggests that different mechanisms of oxidation may be operating in the two samples. Different temporal relationships have previously been found between loss of α-tocopherol and observed lipid peroxidation when oxidant stresses were varied (Palozza et al., 1992). In the present case, since the prooxidant metals, iron and copper, were similar in both samples in LSU compared to AQUA (Table V), the larger levels of both manganese and zinc in LSU compared to AQUA may be responsible for LSU's reduced critical tocopherol concentration. Both manganese and zinc have been shown to exhibit antioxidant properties through their ability to compete with iron for membrane sites (Girotti et al., 1985; Tampo and Yonaha, 1992) and thus could have reduced the concentration of tocopherol needed to protect the membrane PUFA. Alternatively, LSU's high phospholipase activity may have lowered the effective tocopherol concentration.

Table V. Initial Metal Composition (Micrograms per Gram Dry Weight Basis) in Channel Catfish Muscle Tissue

	AQUA	LSU	P[a]
copper	0.8	1.0	NS
iron	10.0	13.3	NS
manganese	0.4	0.7	0.005
zinc	18.6	24.6	0.05

[a] Level of significance for statistical difference between strains using Student's t-test. NS, not significant.

Initially, LSU contained nearly 1.4 times the level of ascorbic acid than AQUA (Table I). Degradation was similar for both LSU and AQUA during the first 3 months of storage, but while ascorbic acid degradation continued in the LSU samples during the next 3 months of storage, AQUA samples showed no further losses. By 9 months of storage, neither strain contained detectable amounts of ascorbic acid. Ascorbic acid degradation in both strains exhibited a similar pattern to that shown by Phillippy (1984), with large losses occurring in the initial phases of storage. Since levels of ascorbic acid in LSU were initially

Table VI. Pearson Correlation Coefficients between Levels of Oxidative Products and Fatty Acid or Antioxidant Composition[a]

	compositional parameter									
			PL				FFA			
			wt %		mg/g dwb		wt %		mg/g dwb	
oxidative product	TocEq	Asc	18:2	22:6	18:2	22:6	18:2	22:6	18:2	22:6
hydroperoxides	−0.91**	−0.62	−0.76*	−0.56	−0.62	−0.51	−0.59	−0.15	0.53	0.41
conjugated dienes	−0.94**	−0.55	−0.84**	−0.56	−0.64	−0.51	−0.72*	0.08	0.59	0.54
TBARS	−0.95***	−0.76*	−0.86**	−0.71	−0.72*	−0.63	−0.63	−0.12	0.71	0.59
organic fluorescent pigment	−0.85**	−0.62	−0.68	−0.53	−0.56	−0.48	−0.46	−0.22	0.46	0.30
aqueous fluorescent pigment	−0.95***	−0.82*	−0.85**	−0.77*	−0.78*	−0.71*	−0.59	−0.16	0.74*	0.59
total volatiles	−0.97***	−0.55	−0.95***	−0.70	−0.75*	−0.62	−0.79*	0.05	0.78	0.77*
acetaldehyde	−0.66	−0.90**	−0.68	−0.89**	−0.83*	−0.85**	−0.37	−0.38	0.84**	0.68
propanal	−0.98***	−0.74*	−0.95***	−0.74*	−0.78*	−0.67	−0.79*	−0.06	0.80*	0.77*
ethyl acetate	−0.97***	−0.78*	−0.96***	−0.82*	−0.85**	−0.76*	−0.78*	0.05	0.88**	0.84**
pentanal	−0.85**	−0.65	−0.94***	−0.69	−0.71*	−0.59	−0.83*	−0.34	0.86**	0.95
hexanal	−0.98***	−0.76*	−0.92**	−0.74*	−0.79*	−0.68	−0.72*	−0.03	0.77*	0.70
heptanal	−0.91**	−0.72*	−0.95***	−0.84**	−0.92**	−0.82*	−0.84**	−0.01	0.92**	0.87**
tocopherol (TocEq)		−0.75*	0.96***	0.73*	0.79*	0.66	0.80*	−0.02	−0.79*	−0.72*
ascorbic acid (Asc)	−0.75*		0.74*	0.90*	0.82*	0.82*	0.38	0.27	−0.83*	−0.65

[a] *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

much higher than levels in AQUA and LSU's degradation of tocopherol during the first 6 months was much greater than AQUA's, ascorbic acid's main role may not have been in regenerating tocopherol. Kunert and Ederer (1985) found that protection against peroxidation was enhanced when the ratio of ascorbic acid:tocopherol was 10:1 to 15:1, whereas peroxidative damage was evident when the ratio was 1:1 or lower. Consequently, efficient regeneration of tocopherol may only be possible when ascorbic acid levels are in excess of tocopherol levels, which was not the case for samples in the present study. Regeneration of tocopherol by ascorbic acid may also have been minimized by mincing the tissue which would have increased the exposure of metal ions to ascorbic acid. Alternatively, the larger pool of nonmembrane FFA requiring protection in LSU samples compared to AQUA samples at 3 months may explain the observed ascorbic acid losses. Preferential utilization of ascorbic acid by this pool of lipids, in turn, would have minimized the potential regeneration of tocopherol and thus account for the greater tocopherol degradation observed in LSU samples compared to AQUA samples between 3 and 6 months.

Comparison of the fatty acid or antioxidant compositions to levels of oxidative products exposed some potential associations (Table VI). Very good correlation coefficients that were greater than 0.6 (Burgard and Kuznicki, 1990) were demonstrated between levels of α-tocopherol equivalents and levels of oxidative products. Oxidative products also exhibited a higher degree of association with 18:2 n–6 or 22:6 n–3 when present in the PL fraction than if present in the FFA fraction. On the basis of the correlation coefficients, the fatty acid's weight percentage would also have to be considered to be more instrumental in dictating oxidative susceptibilities than absolute amounts of these fatty acids.

Measurement of expressible moisture was conducted to determine whether oxidative reactions occurring during storage affected this physical property. Initially, the expressible moisture of AQUA was greater than that of LSU (Table I). After 9 months of frozen storage, levels in both AQUA and LSU had increased by factors of 1.2 and 1.3, respectively. These increases were explained to a greater extent by the changes in FFA levels ($r = 0.60$) than by changes in oxidative products ($r \leq 0.54$). Lipid hydrolysis, rather than oxidative reactions, was therefore considered to be more instrumental in modifying moisture retention properties during storage.

In summary, this study has provided insights into the degree to which compositional factors affect lipid oxidation. During initial stages of oxidation, tocopherol appeared to play a vital role in protecting membrane lipids. As tocopherol levels decreased to a critical nonprotecting concentration, propagation commenced with fatty acid composition of the phospholipids appearing to affect rates of lipid oxidation. In addition, oxidative stabilities could also have been affected by phospholipases, lipases, and zinc and manganese ions.

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