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Ability of chemical measurements to differentiate oxidative stabilities of frozen minced muscle tissue from farm-raised striped bass and hybrid striped bass

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Minced muscle tissue from farm-raised striped bass (*Morone saxatilis*) and hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) were subjected to fluctuating frozen temperatures (−18° to −6°C) for 0, 3, 6 and 9 months. Oxidative stabilities of the bass samples could be differentiated during the induction phase by monitoring the production of thiobarbituric acid-reactive substances and several headspace volatiles, as well as degradation of α-tocopherol equivalents. Chemical measurements which were successful at differentiating the oxidative susceptibilities of the bass samples during propagation included conjugated dienes, organic fluorescent pigments, total headspace volatiles and loss of α- tocopherol equivalents. Hybrid striped bass was less stable in early phases of storage because of lower concentrations of antioxidants. Higher rates of oxidation observed during propagation in striped bass samples could be due to the greater susceptibility of its phospholipid fraction to oxidize and/or its higher concentration of iron.

INTRODUCTION

Improved adaptation of hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) to aquaculture conditions compared with striped bass (*M. saxatilis*) has led to the development of a farm-raised hybrid striped bass industry. Expansion of this industry, while still in its infancy will be facilitated by freezing the muscle tissue. Frozen storage stability studies are therefore needed to characterize this product further.

Chemical measurements used for lipid oxidation are generally applied to samples with the intent of estimating the oxidative status of that product. However, they may also be applied to stored samples to estimate the product's resistance or stability to oxidation. Since lipid oxidation is a complex process, a systematic study of lipid oxidation, involving a variety of chemical measurements, will be advantageous in monitoring oxidative stability. Unfortunately, since the most popular detection methods are based upon the measurement of oxidative products, they are often of limited value in differentiating oxidative stabilities of samples in the early phases of lipid oxidation. These measurements instead tend to differentiate the tissue lipid's oxidative potential later in the oxidative process when protection is no longer a significant factor. In contrast, antioxidant (tocopherol, ascorbic acid) degradation has been monitored on a limited basis (Deng *et al.*, 1978; Phillippy, 1984; Murphy & Kehrer, 1989) and may be detected during early stages of oxidation. It was the intent of this study, therefore, to provide a comprehensive comparison of the traditional methods of measuring oxidation (conjugated dienes, hydroperoxides, thiobarbituric acid-reactive substances (TBARS), headspace volatiles, fluorescent pigments) with the nontraditional methods (losses in tocopherol, ascorbic acid) for their ability to differentiate muscle tissue oxidative stabilities as well as the tissue lipid's sensitivity to oxidation in farm-raised hybrid striped bass and striped bass. The stabilities and susceptibilities of tissue lipids to oxidize, in addition, will be discussed in relation to the tissue's composition.

MATERIALS AND METHODS

Sample preparation and storage

Striped bass (*Morone saxatilis*) and hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) were obtained

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from the University of Georgia Cohutta Fisheries Research facility. Both groups of fish were approximately 2·5 years old and had been fed a commercial diet (Zeigler 40% protein, 3/18 in (42 mm)) at a daily rate of 3% of body weight. Striped bass (SB) had been stocked in a 0·70-acre pond at 1000 fish/acre while hybrid striped bass (HB) were stocked in a 0·38-acre pond at 1500 fish/acre (1 acre = 4047 m^2). The higher stocking densities encountered by HB compared with SB are due to the better adaptation by HB to stressful culture conditions (Lareau, 1987).

Fish were placed in concrete tanks and starved for three days before harvest. Short-term starvation is typically applied to freshwater farm-raised fish to minimize water quality deterioration during haulage of fish to processing plants. Fish were sacrificed by cutting the gills, then, following bleeding, they were weighed, filleted and minced.

Minced samples were subdivided into four portions with each portion being packaged in polyethylene bags (product no. Curlon 550; Cryovac Co., South Carolina, USA) 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 at least in duplicate on the samples. At the time of analysis, samples were removed from the −100°C freezer and thawed in cold running water.

Compositional measurements

Moisture content was determined on 2·0 g minced-tissue samples by microwave drying. Samples were dried at 100% power using an automatic Volatility computer, model AVC-80 (CEM Corporation; Indian Trail, NC, USA) until constant weight was achieved.

Tocopherols were extracted from saponified tissue samples as described by Erickson (1992). Separation of tocopherol analogs by reverse-phase high performance liquid chromatography (HPLC) was conducted as described by Vatassery & Smith (1987). The HPLC system consisted of a Micromeritics 752 gradient programmer, Micromeritics 750 solvent delivery system, and a Brinkmann 656 electrochemical detector. Conversion of γ- and δ-tocopherols to α-tocopherol equivalents was accomplished by applying the conversion factors 0·45 and 0·14, respectively (Fukuzawa *et al.*, 1982).

Ascorbic acid was extracted from minced tissue (4·0 g) and quantified by HPLC as described previously (Thed & Erickson, 1992). Reported levels are expressed as nmol ascorbic acid/g dry weight.

Selected metals (copper, iron, manganese and zinc) were determined on unstored samples. Following exposure of the tissue to acid digestion (McDaniel, 1991), the diluted digest was aspirated into a Thermo Jarrell Ash inductively coupled plasma atomic emission spectrometer.

Lipid extraction was performed on muscle tissue (1·00 ± 0·02 g) using a chloroform methanol (2 : 1) solvent system (Erickson, 1992). A silica Sep-Pak cartridge was used to fractionate triacylglycerols from phospholipids (Juaneda & Rocquelin, 1985). Esterification of fractions and gas chromatographic separation of esterified fatty acids was conducted as described by Erickson (1992).

Measurements of oxidative products

Conjugated dienes were measured spectrophotometrically (232 nm) on an aliquot of a lipid extract which had been previously washed with 0·88% KCl, evaporated and reconstituted in iso-octane. Peroxides were also analyzed on a washed lipid extract by the method of Buege & Aust (1978).

TBARS (thiobarbituric acid-reactive substances) were quantified according to the extraction procedure of Vyncke (1970). Results were expressed as nmol malonaldehyde/g dry weight.

Fluorescent pigments were determined on 10·0 ml of a lipid extract which had been washed with 2·50 ml of 0·88% KCl. Diluted samples of both the aqueous and organic layers were measured in a Turner fluorometer, Model 112, using a quinine sulfate standard (1×10^{-8} M) set equal to 100 fluorescence units.

Headspace volatiles were collected with a gas-tight syringe from the headspace of tissue (1·5 g) which had been heated in a 5 ml vial at 90°C for 15 min. Chromatographic conditions as described by Young & Hovis (1990) were used for separation of headspace volatiles. Purified standards (Sigma, St Louis, MO, USA) were used for tentative identification of sample peaks while quantification of volatiles was based on the response of a known quantity of the internal standard, 4-heptanone.

Statistical analysis

A StatGraphics software package (Rockville, MD, USA) was used for statistical analysis of the data. Oxidative data were subjected to analysis of variance and, where statistical differences were noted for a measurement, differences among samples were determined using the least significant difference test. Statistical differences in lipid and metal compositions were determined using Student's *t*-test. The level of significance was set at p = 0·05 for all tests unless otherwise noted.

RESULTS AND DISCUSSION

During the first 3 months of frozen storage, the primary products of lipid oxidation, peroxides, remained at constant levels for both bass samples (Table 1). By 6 months of storage levels increased by a factor of 40 for SB only, whereas at 9 months both bass samples had comparable elevated levels of peroxides. In contrast, a

Table 1. Oxidative measurements of frozen, minced bass tissue[a]

Oxidative measurement	Hybrid striped bass				Striped bass			
	Storage (months)							
	0	3	6	9	0	3	6	9
Peroxides (nmol/g dry weight)	31·6a	0·0a	31·9a	631·3c	2·9a	0·0a	117·6b	656·1c
Conjugated dienes (A_{232})	1·78ab	1·76ab	1·65a	2·26c	1·68a	1·85b	1·83b	2·45d
TBARS (nmol/g dry weight)	0·4a	5·2b	33·0e	95·3g	0·0a	8·8c	15·0d	80·4f
Total volatiles (μg/g dry weight)	34·2a	59·2b	104·1d	160·2f	53·0b	82·7c	121·1e	236·6g
Aqueous fluorescent pigments (units/g dry weight)	6 808d	6 281d	6 326d	8 738e	3 594b	3 736b	2 993a	5 701c
Organic fluorescent pigments (units/g dry weight)	453bc	242a	565d	792e	479bcd	383b	550cd	1 213f
α-Tocopherol equivalents (nmol/g dry weight)	53·1d	46·0c	45·3c	13·1a	84·3e	85·3e	89·6e	35·7b
Ascorbic acid (nmol/g dry weight)	337·9c	nd[b]	0·0a	0·0a	413·3d	48·0b	56·3b	0·0a

[a] Values in a row followed by the same letter are not significantly different ($P > 0{\cdot}05$).
[b] Not determined.

slight increase in conjugated dienes for SB was noted between O and 3 months, but by 9 months significantly larger levels were found in SB tissue than in HB tissue.

Generation of TBARS and total headspace volatiles was noted throughout storage for both bass samples (Table 1). Between 3 and 6 months, however, the rate of formation of TBARS increased to a much greater extent for HB than for SB, whereas TBARS production rates between 6 and 9 months were similar. In contrast, comparable rates of generation of total headspace volatiles by HB and SB during the first 6 months of storage, and a much larger increase by SB than by HB during the last 3 months of storage, were noted. Consequently, while TBARS levels were 1·2 times higher in HB than in SB tissue samples after 9 months of storage, total volatile levels were 1·5 times higher in SB than in HB tissue samples. These different trends may be attributed to the different secondary products participating in each measurement. In the case of the TBARS measurement, although a number of products react with thiobarbituric acid, the primary oxidative product is malonaldehyde (Hoyland & Taylor, 1991). The major changes in headspace volatiles, however, are accounted for by the peaks corresponding to the saturated aldehydes (hexanal and propanal) (Table 2).

Differentiation of individual headspace volatiles also revealed different profiles with storage time (Table 2). By 9 months of storage, noted decreases were evident in two of the eleven headspace volatile peaks from initial (0 month) levels. Seven of the remaining peaks showed increases over this same time period with SB levels 1·2–1·5 times higher than HB levels. In contrast to total headspace volatiles, production rates between 3 and 6 months for peaks eluting at 1·91, 2·94 and 5·10 min were higher for HB than for SB by factors ranging from 2·1 to 4·8.

Stored samples were also monitored for fluorescent pigments, tertiary products of lipid oxidation (Table 1). Carbonyls linked through a conjugated Schiff base to phospholipids or amino acids/peptides may be measured in the organic and aqueous layers, respectively, of a chloroform/methanol (2 : 1) lipid extract washed with a KCl solution. Compared with levels in 0-month samples, aqueous fluorescent pigments in both bass samples increased to similar degrees after 9 months of frozen storage while the change in organic fluorescent pigment levels was twice as great in SB as in HB.

Table 2. Headspace volatiles (μg/g dry wt) in bass muscle tissue subjected to varying periods of frozen storage[a]

Retention time (min)	Volatile	Hybrid striped bass				Striped bass			
		Storage (months)							
		0	3	6	9	0	3	6	9
1·03	?	25·1a	48·0b	67·7c	78·6d	43·2b	68·9c	96·7e	118·4f
1·29	?	3·1a	5·2ab	8·7cd	10·2d	4·4a	7·1bc	8·7cd	14·6e
1·91	Propanal/acetone	4·2a	4·4a	19·6c	49·8d	4·4a	5·2a	11·1b	72·6e
2·61	Butanal	0·0a	0·7b	1·2cd	1·9e	0·2a	1·1bc	1·6de	2·5f
2·82	?	1·9c	0·0a	0·0a	0·0a	1·6c	1·1b	0·8b	0·0a
2·94	?	0·0a	0·0a	1·9b	5·4c	0·0a	0·0a	0·4a	6·9d
3·24	?	5·5ab	6·0ab	15·6c	7·3ab	7·9ab	8·5b	1·6a	2·4ab
3·70	?	1·0a	3·4a	6·8a	7·0a	5·4a	3·6a	1·3a	3·5a
3·87	?	1·5c	0·0a	5·6d	0·0a	0·8b	1·4bc	0·0a	0·0a
4·02	Pentanal	0·0a	0·0a	2·5b	9·9c	0·3a	0·0a	2·3b	15·1d
5·10	Hexanal	1·8ab	1·6a	8·1c	21·6d	1·0a	1·5a	4·6b	31·0e

[a] Values in a row followed by the same letter are not significantly different ($P > 0{\cdot}05$).

During the first 6 months of storage, no significant changes were noted in HB samples for aqueous fluorescent pigments, while a decrease was noted for SB samples. The lack of sensitivity in measuring oxidative changes with aqueous fluorescent pigments in early stages of oxidation is not surprising, given that secondary products must first be formed. The contributions of other endogenous aqueous compounds to the background fluorescence may also limit the sensitivity of the aqueous fluorescent pigment measurement. The fluorescence background of SB, in particular, was nearly two-fold higher than the HB background.

During the first 6 months of storage, SB tocopherol levels (as expressed in α-tocopherol equivalents) were stable (Table 1). In contrast, HB lost approximately 13% of its initial tocopherol levels in the first 3 months of storage. By 9 months, however, the amount of tocopherol degraded in SB samples exceeded that in HB samples.

Degradation of ascorbic acid in both bass samples was observed primarily over the first 6 months of frozen storage (Table 1). Large losses of ascorbic acid in the initial phases of storage have also been reported by Phillippy (1984). While regeneration of tocopherol by ascorbic acid (Lambelet *et al.*, 1985) would have reduced the levels of ascorbic acid, it is contended that losses occurred from reduction of iron (Davies *et al.*, 1991) since exposure of ascorbic acid to metal ions would have been enhanced upon mincing of the tissue.

The results from these oxidative measurements demonstrated differences in the ability to differentiate oxidative stabilities of HB and SB. No differences in generation of peroxides, conjugated dienes, fluorescent pigments or total headspace volatiles were seen for the bass samples during the induction phase of oxidation (less than 6 months of storage). The similar ascorbic acid losses over this same time period also indicated that the bass samples exhibited similar oxidative stabilities. α-Tocopherol equivalent losses, conversely, pointed to a greater instability of HB tissue to oxidation than SB tissue. These results were supported by the TBARS data and the data for several individual headspace volatiles (RT 1·91, 2·94, 5·10). Only when monitoring tocopherol levels, however, could the HB oxidative instability be distinguished by 3 months of frozen storage. Measurement of tocopherol, therefore proved more sensitive in detecting oxidative stabilities compared with other, more traditional, oxidative measurements.

The ability to differentiate in tissue the sensitivity of the lipids to oxidize during propagation, referred to in this study as oxidative susceptibility, was not attained through measurements of peroxides, TBARS, aqueous fluorescent pigments or loss of ascorbic acid. The measurements which successfully differentiated oxidative susceptibility in bass samples (conjugated dienes, organic fluorescent pigments, total headspace volatiles, loss of tocopherol) were consistent in ranking the oxidation rate for SB greater than that for HB. These results implied that SB lipids were more susceptible to oxidation than the lipids of HB.

Table 3. Initial lipid and metal composition of minced bass muscle tissue

	Hybrid striped bass	Striped bass	P^a
Lipids[b]			
Phospholipids			
PUFA (mg/g dry weight)	7·3	8·3	0·005
$22:6n-3$ (mg/g dry weight)	1·3	2·0	0·01
Peroxidizability index[c]	25·8	32·1	0·005
Triacylglycerols			
PUFA (mg/g dry weight)	32·8	27·6	0·005
$22:6n-3$ (mg/g dry weight)	2·0	2·3	NS
Peroxidizability index	68·0	65·1	0·01
Metals (μg/g dry weight)			
Copper	0·8	1·0	NS
Iron	8·0	15·2	0·05
Manganese	0·4	0·5	0·025
Zinc	15·1	17·3	0·01

[a] Level of significance for statistical difference between strains using Student's *t*-test.
NS = not significant.
[b] A more detailed profile of the lipid composition of these samples was presented in Erickson (1992).
[c] Peroxidizability index = [(sum mg monoenes × 0·025) + (sum mg dienes × 1) + (sum mg trienes × 2) + (sum mg tetraenes × 4) + (sum mg pentaenes × 6) + (sum mg hexaenes × 8)]/g dry weight (Murata & Yamauchi, 1990).

Oxidative susceptibilities as well as oxidative stabilities indicated by these chemical measurements were compared with the tissue's initial lipid and metal composition (Table 3) and initial tocopherol and ascorbic acid compositions (Table 1). The peroxidizability index of the phospholipids, which factored in the tissue weight of each fatty acid as well as susceptibility of each fatty acid to oxidize, was greater in SB than in HB, whereas for triacylglycerols the peroxidizability index was greater in HB than in SB. The greater rate of oxidation for SB than for HB during propagation may be attributed to a greater contribution to oxidation by phospholipids than by triacylglycerols, and/or to the presence of higher levels of the pro-oxidant iron (Castell & Spears, 1968) in SB tissue compared with HB tissue. In contrast, the lower levels of antioxidants present in the HB tissue during the induction phase of oxidation may account for the oxidative instability noted in the HB samples. Besides the antioxidant tocopherol, lower levels of ascorbic acid, manganese and zinc were found in HB compared with SB. Both metals have been reported to exhibit antioxidant activity (Girotti *et al.*, 1985; Coassin *et al.*, 1992; Tampo & Yonaha, 1992). It appeared, therefore, that during early phases of oxidation, the greater susceptibility of SB phospholipids to oxidize was offset by the higher concentrations of antioxidants found in SB tissue compared with HB tissue. It may also be presumed that since tocopherol resides primarily in membranes and protects the membrane phospholipids, the absence of tocopherol degradation in SB tissue during the first 6 months of storage implied the absence of phospholipid degradation. Thus, the most likely source for the

oxidative products detected in SB tissue during this time period would appear to have been the triacylglycerol fraction.

CONCLUSION

Oxidative chemical measurements varied in their ability to differentiate oxidative stabilities and oxidative susceptibilities of the two bass samples. Monitoring the degradation of α-tocopherol equivalents was the most sensitive measure for distinguishing stability of samples, while the conjugated dienes, organic fluorescent pigments and total headspace volatiles, in addition to loss of tocopherol, succeeded in differentiating oxidative susceptibilities of the samples. The greater instability of HB tissue, noted during the induction phase of oxidation, could be explained by its lower concentration of antioxidants (tocopherol, ascorbic acid, manganese, zinc) compared with SB tissue. During propagation, when oxidative susceptibilities could be distinguished, the iron content and the phospholipid fraction could have accounted for the higher oxidative rates observed in SB than in HB.

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