Dietary Protein Level Has Minimal Effect on Flesh Quality of Frozen Stored Sunshine Bass, *Morone chrysops* × *M. saxatilis*

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ABSTRACT. The storage stability of frozen muscle from sunshine bass, *Morone chrysops* × *M. saxatilis*, fed diets containing various levels of protein (29, 36, 42, and 45%) was determined by measuring thiobarbituric acid reactive substances (TBARS), an indicator of lipid oxidation, and changes in shear stress and tensile strength of the muscle during storage. Fish fed a 45% protein diet had a higher ($P < 0.05$) dressed yield than fish fed diets containing 29 and 36% protein. Percentages of moisture, protein, fat, and ash of fresh fillet were not different ($P > 0.05$) among all dietary treatments. The n-3 fatty

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acids composed one-third of the total fatty acids in muscle, and the n-3/n-6 fatty acid ratio was lower in muscle of fish fed from the 29% protein diet (1.89) than those fed from the higher protein diets (2.70-3.40). Other fatty acids, however, were similar among all muscle samples. Storage at −20°C for 6 months did not cause significant ($P > 0.05$) increases in lipid oxidation for skin-on fillets, but from month 4 to month 6, skinless fillets exhibited marked increases ($P < 0.05$) in TBARS. Changes in TBARS were generally not affected by dietary protein levels. Shear stress and tensile stress of muscle tissue showed inconsistent changes during storage and no differences ($P > 0.05$) due to dietary regimen were observed. The results indicate that physical and chemical characteristics of hybrid striped bass muscle after 6 months of frozen storage were minimally influenced by dietary protein level. *[Article copies available from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: getinfo@haworth.com]*

INTRODUCTION

Striped bass, *Morone saxatilis*, and its hybrids have recently received considerable attention in the United States, particularly in the southeast region, as a commercial aquacultured species. The potential for expansion of the species appears to be great since it adapts well to inland ponds in most temperate regions of the United States. The mild flavor characteristics and firm, white flesh of striped bass and hybrid striped bass make the fish well accepted by consumers.

Currently, an increasing number of reports are available on the nutritional requirements of striped bass and its hybrids. Tuncer et al. (1990) reported that palmetto bass, *M. saxatilis* × *M. chrysops*, exhibited superior growth and survival compared to striped bass. There have been reports on nutritional requirements of larval and juvenile striped bass (Webster and Lovell 1990; Griffin et al. 1992; Keembiyehetty and Gatlin 1992; Nematipour et al. 1992; Griffin et al. 1994). The information has allowed producers to feed economical, nutritious diets to hybrid striped bass. However, little research has been conducted on fillet quality of cultured hybrid striped bass.

In order to expand the hybrid striped bass production to a large commercial scale, an improved understanding of the fish storage stability is essential. Boyd et al. (1992) showed that chillpack (−2°C) effectively prolonged shelflife (flavor and texture, judged by a tasting panel) of unfrozen hybrid striped bass to 21 days, compared to only 8 days for refrigerated samples. Freezing and storage at sub-freezing temperatures is an excellent method to minimize chemical and microbiological deterioration

of fish muscle. However, mechanical damage of muscle tissue due to ice formation, as well as resulting alterations in muscle ultra-structure and toughening in texture, have been observed in certain fish species (Jarenback and Liljemark 1975a; Shenouda 1980). Although frozen hybrid striped bass muscle has a long shelflife compared to refrigerated muscle, when temperature fluctuations occur, the beneficial effect of frozen storage could diminish (Erickson 1993). Information concerning chemical and textural stability of hybrid striped bass fillets during frozen storage is lacking in the literature, and a quantitative assessment of physical and chemical changes in frozen hybrid striped bass muscle is needed.

The objectives of the present study were to measure carcass and fatty acid compositions in hybrid striped bass fed diets containing various levels of protein and to quantitate storage quality of frozen (−20°C) muscle fillets by monitoring lipid oxidation (TBARS), along with muscle shear and tensile stresses.

MATERIALS AND METHODS

Fish Production and Processing

Four experimental diets with various protein levels (30, 36, 42, and 48%) were formulated from practical ingredients (Table 1). Peruvian anchovy meal and soybean meal served as protein sources. Menhaden fish oil was used as the lipid source for energy and to supply essential n-3 polyunsaturated fatty acids, PUFA (Webster and Lovell 1990). The fatty acid composition of the diets is shown in Table 2. Diets were extruded into floating pellets by a commercial feed mill (Integral Fish Foods, Inc., Grand Junction, Colorado[1]). Diets were stored at −40°C in plastic-lined bags until fed.

Diets were analyzed for crude protein, fat, fiber, ash, and moisture using AOAC methods (AOAC 1990). Crude protein was determined using the Kjeldahl method; crude fat was determined using the acid-hydrolysis method; ash was determined by placing 10 g of sample in a muffle furnace (600°C) for 6 hours; crude fiber was determined using the fritted glass crucible method; and moisture was determined by placing a 10-g sample in a drying oven (95°C) until a constant weight was achieved. Analyzed protein levels of the diets differed from calculated values using feed ingredient compositions (NRC 1983); the actual protein contents were 29, 36, 42, and 45%, respectively. These values will be used throughout the paper.

1. Use of trade or manufacturer's name does not imply endorsement.

TABLE 1. Composition of diets fed to juvenile bass containing various percentages of protein.

	Diet (% protein)			
Ingredient	29	36	42	45
Anchovy meal	25.0	30.0	35.0	40.0
Soybean meal	17.0	26.5	35.0	43.0
Wheat flour	13.0	0.0	0.0	0.0
Corn grain	32.2	31.4	18.4	8.9
Menhaden oil	11.0	10.5	10.0	6.5
Dicalcium phosphate	0.6	0.4	0.4	0.4
Vitamin mix[1]	0.5	0.5	0.5	0.5
Mineral mix[2]	0.6	0.6	0.6	0.6
Ascorbic acid	0.1	0.1	0.1	0.1
Chemical analysis				
Moisture (%)	10.75	10.88	9.59	8.05
Protein (%)[3]	29.06	36.33	42.32	45.43
Lipid (%)[3]	15.92	17.06	15.25	13.34
Energy[4]	4.44	4.46	4.30	4.17
P:E[5]	65.5	81.5	98.4	108.9

[1]Vitamin mix supplied the following vitamins (IU or mg/kg of diet): vitamin A, 6000 IU; vitamin D, 2200 IU; vitamin E, 150 IU; vitamin K, 10 mg; niacin, 200 mg; pantothenic acid, 60 mg; thiamin, 30 mg; riboflavin, 20 mg; pyridoxine, 20 mg; folic acid, 5 mg; B_{12}, 0.01 mg; biotin, 2 mg.
[2]Mineral mix supplied the following (mg/kg of diet): manganese, 180 mg; copper, 8 mg; cobalt, 1.5mg; iron, 66 mg; zinc, 150 mg; iodine, 6 mg; selenium, 0.3 mg.
[3]Moisture-free basis.
[4]Available energy in kcal/g of diet.
[5]P:E = protein to energy ratio in mg protein/kcal.

Juvenile sunshine bass, *Morone chrysops* × *M. saxatilis*, with an average weight of 125 ± 30 g, were obtained from a commercial producer (Nature's Catch, Jamestown, Kentucky) and stocked into 12 cages (1.2 × 1.2 × 2.4 m; H × W × L) on 17 May 1993 at a rate of 200 per cage. Fish in each cage were randomly selected to be fed one of the four experimental diets, with each diet being fed to three cages. Fish were fed twice daily (0800 and 1630) all they could consume in 30 minutes.

Temperature and dissolved oxygen were monitored three times daily (0800, 1630, and 2030) outside the cages, at a depth of 0.75 m using a YSI

TABLE 2. Selected fatty acids of diets fed to juvenile hybrid striped bass containing various percentages of protein. Values are means of two replicates.

	Diet (% protein)			
Fatty acid	29	36	42	45
18:2 (n-6)	8.94	7.78	7.32	8.14
18:3 (n-3)	1.18	1.16	1.20	1.29
18:4 (n-3)	1.74	1.80	1.86	1.66
20:4 (n-6)	1.12	1.16	1.22	1.20
20:5 (n-3)	10.54	10.91	11.18	10.83
22:5 (n-3)	2.28	2.36	2.37	2.28
22:6 (n-3)	6.75	7.01	7.42	7.50
Others	67.45	67.82	67.43	67.10

Model 58 oxygen meter (YSI, Inc., Yellow Springs, Ohio). If the dissolved oxygen was predicted to decline below 4.0 mg/L, aeration was provided using a 10-hp electric paddlewheel aerator.

Fish were harvested on 8 October 1993 and were not fed 16 hours prior to harvest. Total number and weight of fish in each cage were determined at harvest. Fish were killed by decapitation, eviscerated, and the fillet was removed from each side of the scaleless carcass. Carcasses and waste (head and viscera) of three fish were selected from each cage, homogenized separately in a blender, and analyzed for protein, fat, ash, and moisture as previously described for the diets, except that fat was analyzed by ether extraction (AOAC 1990).

Storage Quality Evaluation

Sample preparation. Three fish were randomly selected from each cage to determine storage stability. Immediately after dressing, fillets from one side of the carcass were skinned by hand, while fillets from the other side of the carcass were not skinned. Fillets were placed separately in plastic freezer bags and frozen (−20°C) within 1 hour postmortem. Prior to freezing, all fillets were kept in a −10°C commercial freezer. At the end of 1, 2, 3, 4, and 6 months, three skinned fillets and three non-skinned fillets from each cage were removed from the freezer and thawed for 2 hours in 10°C tap water while still in plastic bags.

Lipid oxidation. The development of oxidative rancidity in frozen stored fillets was estimated with 2-thiobarbituric acid (TBA) reagent by extracting 10 g of ground fish with 100 mL of 7.5% trichloroacetic acid (Witte et al. 1970). Malonaldehyde tetraethoxypropane was used as standard. Absorbance was read against a blank at 538 nm. Results were expressed as mg malonaldehyde/kg of muscle.

Textural evaluation. From each fillet, two strips of muscle samples, each measuring close to 30 × 20 × 8 mm (L × W × H), were prepared along the vertebral bone by using a sharp-edged knife. The actual dimension of each muscle strip was re-measured after sampling, and the cross-sectional area (W × H) was calculated. One strip was used for tensile stress measurement and the other for shearing test. For tensile test, one end of the muscle strip was fastened to a load cell of a Model 4301 Instron (Instron Corp., Canton, Massachusetts), and the other end was secured to the stationary platform using rubber clamps, allowing about 15 mm length of the strip freely suspended between the two cribs. The muscle strip was pulled apart at 20 mm/minutes until it ruptured. Tensile stress was defined as the rupture force (N) divided by the cross-sectional area (i.e., W × H; in m^2) (Xiong et al. 1993).

Shear tests were performed using a Warner-Bratzler shear device mounted to the Instron. The muscle strip was placed in a triangular hole of a 1-mm-thick blade. When the blade was allowed to move through a 1.5-mm slot at 20 mm/min, the muscle sample was cut. Shear stress was expressed as the maximum shear force divided by the cross-sectional area (W × H) of the sample.

Lipid Analysis

Lipid was extracted from diets with chloroform-methanol by the method of Bligh and Dyer (1959) as modified by Kates (1986). Fatty acid methyl esters were obtained according to the method of AOAC (1990) and analyzed using a Hewlett-Packard 5890 II gas chromatograph equipped with a DB-225 fused silica capillary column (30 m × 0.25 mm ID; J&W Scientific, Folsom, California) and a flame-ionization detector (FID). The carrier gas was helium. Oven temperature was programmed from 160 to 220°C at a rate of 2°C/minute, and then from 220 to 270°C at a rate of 10°C/minute. Detector response was recorded and quantitated with an electronic integrator-recorder. Fatty acid methyl esters were identified with those of authentic standards (Nu-Chek Prep, Elysian, Minnesota).

Muscle samples were subjected to direct transesterification using acetyl chloride in methanol (Lepage and Roy 1986), and the fatty acid methyl esters were analyzed by a Perkin-Elmer auto GC (Perkin-Elmer, Norwalk,

Connecticut) using a DB-225 fused silica capillary column (30 m × 0.25 mm ID, phase thickness 0.25 um). The GC analysis was temperature-programmed from 190°C to 220°C at a rate of 3°C/minute and held at 190°C for 8 minutes and at 220°C for 20 minutes. Other parameters were: split injection, helium (carrier gas) at a flow rate of 0.77 kg/cm^2; injection port with temperature at 250°C; detector (FID) temperature at 250°C. TURBOCHROM software (PE-NELSON, Cupertino, California) was used for data analysis. The individual fatty acids were identified by comparison with the retention time of a reference standard (Nu-Chek Prep, Elysian, Minnesota).

Statistical Analysis

Statistical analysis of data was performed using Statistix 3.5 software for personal computers (Analytical Software, St. Paul, Minnesota). When a treatment effect was found to be significant ($P < 0.05$) by the analysis of variance (ANOVA), the general linear model (GLM) procedure was followed to identify significant differences among treatment means using Tukey's pairwise comparisons (Zar 1984).

RESULTS AND DISCUSSION

Increases in the dietary protein level tended to increase dressed carcass yield, with the 45% protein dietary treatment producing the highest yield ($P < 0.05$). Trends also indicated that increased dietary protein resulted in a higher percentage of muscle protein and moisture and a lower percentage of fat. Muscle composition of fish fed diets containing 29 and 36% protein had higher percentages of fat compared to fish fed diets containing 42 and 45% protein, although not significantly different ($P > 0.05$) (Table 3). The level of protein in the diet was positively correlated with carcass yield ($P < 0.01$), muscle protein ($P < 0.01$), and moisture ($P < 0.05$) and was negatively correlated with muscle lipid content ($P < 0.05$) (Table 4). Percent muscle protein and moisture were highly correlated ($P < 0.01$) with each other, and as expected, both were negatively correlated ($P < 0.01$) with percent muscle fat.

The energy level of digestible materials in a diet affects the amount of food consumed by fish, and the protein:energy (P:E) ratio of the diet will influence conversion efficiency of the diet. A low ratio may increase fat deposition while a high ratio will cause protein to be used as an energy source. Diets used in the present study had different P:E ratios. The diet containing 29% protein had the lowest P:E ratio, while the diet containing 45% protein had the highest value.

TABLE 3. Dressed carcass yield and composition of muscle fillet from hybrid striped bass fed diets containing different levels of protein. Fillets were with skin on; dressed carcass yield was expressed as the weight percentage of dressed carcass over the whole fish weight. Muscle composition was calculated on a wet-weight basis. Values are means of three replications. Means with different letters were significantly different ($P < 0.05$).

Diet (% protein)	Carcass yield (%)	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
29	56.93b	67.09a	19.23a	10.56a	2.88a
36	57.14b	67.51a	19.26a	10.24a	2.83a
42	57.92ab	67.98a	19.88a	9.25a	2.83a
45	59.56a	68.65a	20.16a	8.63a	2.69a

TABLE 4. Correlation coefficients between dietary protein level, carcass yield, and muscle chemical composition of hybrid striped bass fed diets containing different levels of protein.

	Diet	Yield (%)	Ash (%)	Fat (%)	Moisture (%)
Yield (%)	0.74**				
Ash (%)	−0.07	0.00			
Fat (%)	−0.66*	−0.33	−0.26		
Moisture (%)	0.61*	0.16	−0.11	−0.88**	
Protein (%)	0.74**	0.42	0.14	−0.95**	0.92**

*$P < 0.05$.
**$P < 0.01$.

Fatty acid composition of muscle samples from the four dietary treatments were largely similar; however, there were some exceptions. The n-3 fatty acids composed approximately one-third of the total fatty acids in muscle for all dietary treatments (Table 5). Total polyunsaturated fatty acids (PUFA) were slightly higher in muscle from fish fed the 29% protein diet than from those fed higher protein diets. The percentage of muscle n-3 fatty acids was higher than that reported by Fair et al. (1993) for hybrid striped bass, probably due to differences in the feed formulation.

In fish, highly unsaturated fatty acids (HUFA), such as eicosapentaenoic acid (EPA), 20:5(n-3), and docosahexaenoic acid (DHA), 22:6 (n-3), are important to maintain membrane fluidity. Lee et al. (1967) stated that EPA is selectively incorporated into the phospholipid fraction of the lipid pool. In the present study, levels of linoleic acid, 18:2 (n-6), linolenic acid, 18:3 (n-3), octadecatetraenoic acid, 18:4 (n-3), and arachidonic acid, 20:4 (n-6) were similar in diets and in muscle tissue of hybrid striped bass. However, percentages of EPA and DHA were higher in muscle than in the diets (Tables 2 and 5). The percentages of DHA in muscle of hybrid striped bass were almost twice as much as in the diet. This is consistent with the essential fatty acid requirement of juvenile hybrid striped bass (Nematipour and Gatlin 1993). Webster and Lovell (1990) stated that striped bass larvae did not appear able to elongate and desaturate linolenic acid into EPA and DHA. Thus, striped bass may selectively incorporate these two fatty acids in tissues. Muscle n-3/n-6 fatty acid ratios for the 29, 36, 42, and 45% protein diets were 1.89, 3.07, 3.40, and 2.70, respectively.

Erickson (1993) found only small increases in lipid oxidation, as measured by thiobarbituric acid reactive substances (TBARS) in minced hybrid striped bass during frozen storage for 3 months under temperature-abusing conditions, i.e., fluctuation between $-6°C$ and $-18°C$. However, from month 3 to month 6, TBARS content increased markedly. In the present study, the TBARS content in both skin-on and skinless fillets changed very little ($P > 0.05$) during storage in the first 4 months, but from month 4 to month 6, TBARS values increased in all fillet samples, particularly in skinless fillets ($P < 0.05$) (Figure 1; Table 6). When mean TBARS values were pooled, irrespective of storage time, we observed a more pronounced lipid oxidation in the 29% protein group than in the 42% and 45% protein groups. This could be partially attributed to differences in muscle total PUFA and to the higher percentage of lipid in muscle of fish fed the 29% protein diet.

With the exception of the 6-month skinless samples, all other samples exhibited similarly low TBARS, regardless of storage time. This indicates that the storage conditions employed in the present study were effective in preventing lipid oxidation. Erickson (1993) showed that hybrid striped bass had a relative low copper (0.8 μg/g dry muscle) and iron (8.0 μg/g dry muscle) content, and during frozen storage for 6 months, the concentration of α-tocopherol decreased very little. The higher numerical TBARS values of skinless fillets, compared to those of skin-on fillets, after 6-month storage, probably resulted from more extensive disruption of muscle tissue and cells due to skin removal and the loss of an oxygen barrier (skin).

Instron textural analysis revealed no difference in shear stress of the

TABLE 5. Fatty acid composition (%) of total extractable lipids from muscle of hybrid striped bass fed diets containing different levels of protein. Values are means of six replications.

	Dietary protein (%)			
Fatty acid	29	36	42	45
14:0	4.40	2.04	6.02	2.43
15:0	0.44	0.28	0.00	0.00
iso-16:0	0.60	0.47	0.82	0.60
16:0	19.72	18.3	20.30	20.46
16:1(n-7)	6.40	6.73	10.10	5.95
16:1(n-5)	0.69	0.75	0.00	0.00
16:2(n-4)	0.71	0.61	0.00	0.70
16:4(n-1)	0.58	0.34	0.61	0.60
18:0	4.82	3.94	4.87	5.06
18:1(n-9)	16.37	12.84	13.05	14.25
18:1(n-7)	2.33	2.40	2.50	2.42
18:2(n-6)	6.97	6.29	5.83	7.18
18:2(n-4)	0.44	0.00	0.00	0.00
18:3(n-6)	0.43	0.45	1.15	0.42
18:3(n-3)	0.73	0.93	0.98	1.02
18:4(n-3)	0.80	0.88	0.76	0.81
20:1(n-11)	1.71	1.56	1.92	1.73
20:2 NMID*	1.08	0.88	1.79	1.07
20:4(n-6)	3.06	2.49	2.81	2.86
20:4(n-3)	0.76	0.75	0.98	0.74
20:5(n-3)	16.71	14.86	11.45	11.29
22:5(n-6)	1.64	1.33	0.00	1.42
22:5(n-3)	2.75	2.78	4.27	3.17
22:6(n-3)	13.07	12.24	14.79	15.06
Total PUFA	49.73	44.83	45.42	46.34
Total n-3	34.82	32.44	33.32	32.09
Total n-6	18.39	10.56	9.79	11.88
n-3/n-6 ratio	1.89	3.07	3.40	2.70

*NMID = non-methylene interrupted diene.

FIGURE 1. Changes in thiobarbituric acid-reactive substances (TBARS, mg/kg muscle) of hybrid striped bass fillets stored at −20°C for various time periods. Values are means of three replications; for statistical information, see Table 6.

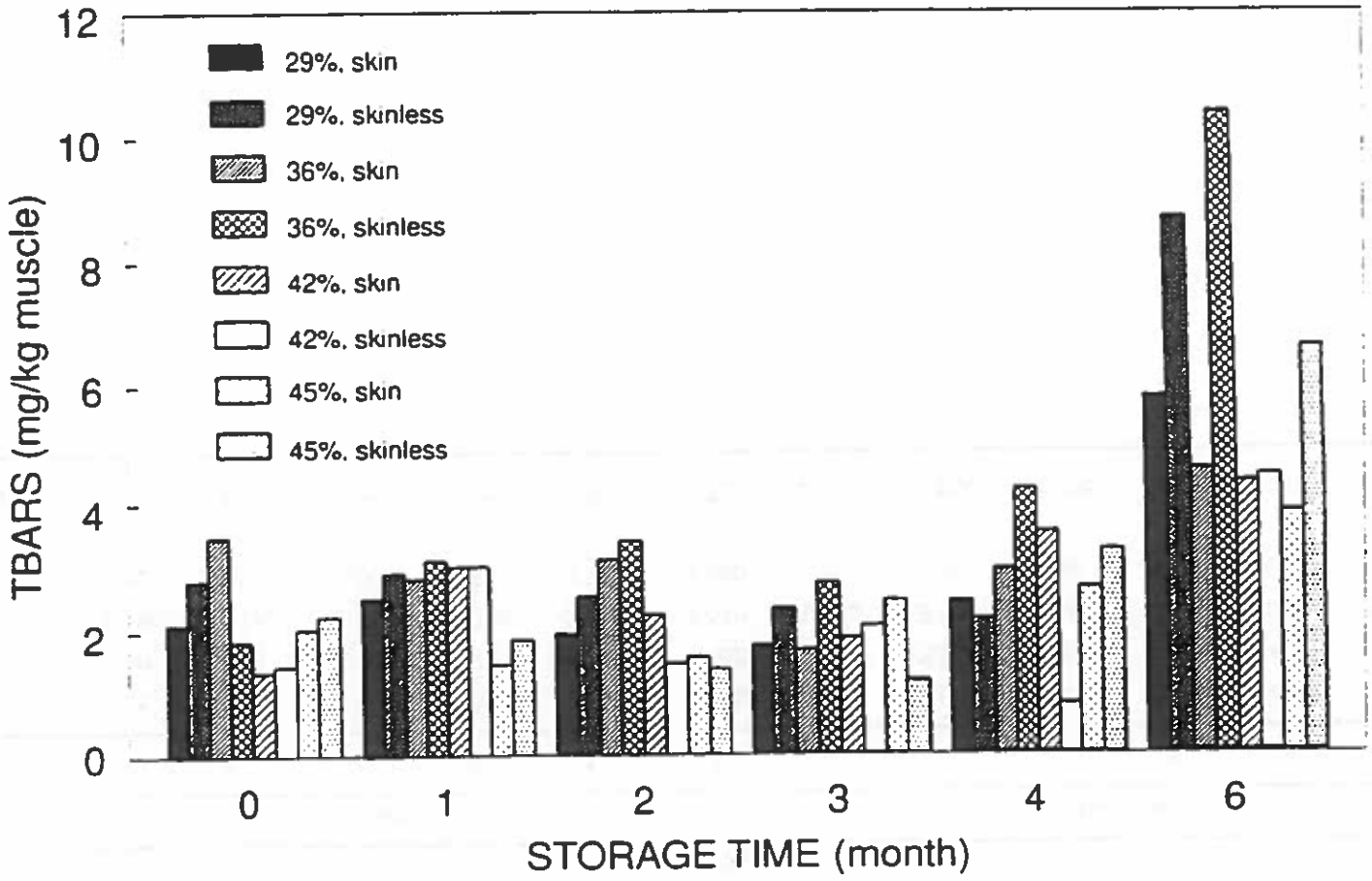

TABLE 6. Changes in thiobarbituric acid-reactive substance (TBARS, mg/kg muscle) of hybrid striped bass fillet stored at −20°C for various time periods. Values are means of three replications. Means in the same row with different letters are significantly different ($P < 0.05$). No significant difference ($P > 0.05$) was observed between means within the same column.

Diet	Storage time (months)											
	With skin						Without skin					
	0	1	2	3	4	6	0	1	2	3	4	6
29	2.2b	2.6b	2.0b	1.8b	2.5b	5.8ab	2.9ab	3.0ab	2.6b	2.4b	2.2b	8.7a
36	3.6b	2.9b	3.2b	1.7b	3.0b	4.6ab	1.9b	3.2b	3.5b	2.8b	4.3b	10.4a
42	1.4ab	3.1ab	2.3ab	1.9ab	3.6ab	4.4ab	1.5ab	3.1ab	1.5ab	2.1ab	0.8b	4.5a
45	2.1b	1.5b	1.6b	2.5b	2.7b	3.9ab	2.3b	1.9b	1.4b	1.2b	3.3b	6.6a
Pooled mean	2.3bc	2.5bc	2.3bc	2.0c	3.0bc	4.7b	2.1bc	2.8bc	3.0bc	2.1bc	2.6bc	7.5a

muscle tissue obtained from different dietary regimens and from different storage periods, whether or not the skin was removed prior to freezing (Table 7). This was similar for tensile stress, although tensile stress measurement conspicuously resulted in more variable data (Table 8) than did shear stress measurement. Previous studies have shown that fish muscle tissue undergoes toughening during frozen storage due to protein denaturation and cross-linkages between peptides, which are associated, in part, with lipid oxidation (Jarenback and Liljemark 1975b). Many lipid degradation products, such as malonaldehyde (the main TBA-reactive compound), are capable of crosslinking adjacent polypeptides, thereby increasing meat toughness (Buttkus 1967). The lack of production and accumulation of a large quantity of lipid oxidation products (e.g., TBARS) during frozen storage might explain why texture of the striped bass fillets did not change appreciably during storage. Furthermore, increased meat toughness and sponginess are common problems associated with freezing and frozen storage of gadoid fish muscle tissue, due to protein-protein interactions via formaldehyde derived from its precursor trimethylamine oxide (Shenouda 1980). However, this did not seem to be the case for hybrid striped bass, a non-gadoid fish species.

Overall, frozen storage of the hybrid striped bass fillet up to 6 months under the conditions used in this study did not cause appreciable increases in lipid oxidation as measured by TBA analysis, nor alterations in textural characteristics as determined by shear and tensile stress tests. Therefore, frozen storage at $-20°C$ appears to be a feasible means for preservation of hybrid striped bass meat quality. Since skin-on fillets were less susceptible to lipid oxidation during long-term storage, it is recommended that the skin be left on the fillet before the freezing process.

TABLE 7. Tensile stress (kN/m^2) of hybrid striped bass fillet stored at −20°C for various periods. Values are means of three replications. Pooled means shown in the bottom row with different letters are significantly different ($P < 0.05$). No significant differences ($P > 0.05$) were observed between means in all other rows and columns.

Diet	Storage time (months)											
	With skin						Without skin					
	0	1	2	3	4	6	0	1	2	3	4	6
29	18.6	20.5	13.9	23.4	9.8	17.4	16.9	18.4	22.7	31.0	15.2	22.9
36	19.5	21.7	15.8	18.7	13.0	18.7	19.4	21.0	16.6	21.1	13.9	20.1
42	18.0	13.3	19.8	19.1	12.7	17.9	20.1	17.0	18.8	16.4	13.2	23.3
45	14.5	17.5	21.4	21.0	16.5	18.8	16.1	13.7	19.9	20.9	15.2	18.6
Pooled mean	17.7abc	18.3abc	17.7abc	20.6ab	13.0c	18.2abc	18.1abc	17.5abc	19.5abc	22.4a	14.4bc	21.2ab

TABLE 8. Shear stress (kN/m^2) of hybrid striped bass fillet stored at −20°C for various periods. Values are means of three replications. Means in the same row with different letters are significantly different ($P < 0.05$). No significant differences ($P > 0.05$) were observed between means in the same column.

Diet	Storage time (months)											
	With skin						Without skin					
	0	1	2	3	4	6	0	1	2	3	4	6
29	39.0a	55.3a	33.5a	32.0a	37.8a	48.5a	25.1a	52.4a	42.8a	44.9a	53.0a	39.4a
36	36.1a	46.5a	32.1a	32.3a	47.2a	53.2a	29.6a	47.9a	29.5a	29.3a	45.1a	37.0a
42	34.5bc	56.8abc	29.8bc	26.4c	49.9abc	68.3a	24.2c	49.7abc	35.4abc	35.0bc	48.5abc	42.3ab
45	42.5ab	50.6ab	37.9ab	27.1b	62.5a	36.6ab	26.9b	50.8ab	26.2b	27.4b	60.8a	40.5a

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