FATTY ACID ALTERATIONS DUE TO BIOCHEMICAL CHANGES DURING THE LIFE CYCLE OF GULF OF MEXICO FINFISH

Julia S. Lytle and Thomas F. Lytle
Environmental and Analytical Chemistry Sections
Gulf Coast Research Laboratory
P.O. Box 7000, Ocean Springs, MS 39564

Recent studies in our laboratory have shown that fatty acid concentrations and distributions in muscle tissue of Gulf finishes vary not only with species but with size, sex, sexual maturation and age (6,7). In some species, the variations are significant, while in others, the changes are very small. Fatty acid modifications which occur as a result of biological change may be due to one or more of the following: changes in diet, changes in hormone levels, changes in sensitivity to hormone actions with age, changes in food availability, changes in health and changes in environment (8).

Nutritional information for fatty acids as well as the saturated, monounsaturated and polyunsaturated fats cannot be adequately evaluated without knowing the extent of these variations. Our laboratory has examined extensively 45 species of warm water finfish species from the Gulf of Mexico to determine the extent of these variables on fatty acid concentration and distribution of muscle tissue.

Eleven species of Gulf finishes will be presented to illustrate fatty acid changes with observed biological change.

MATERIALS AND METHODS

Sample design
A collection scheme was devised that would permit a measure of the fatty acid variability associated with biological changes in fish species (1,2,3,4,5,9,10). Each species was collected during four seasons in sufficient quantities to generate information on seasonal variability of fatty acid concentration and distribution for each of the species as well as biological variations such as age, size, sex and sexual maturity.

Sample preparation
Immediately after delivery of fresh fish to the laboratory, the fish were measured, weighed, sexed and state of health recorded. The fish were filleted and given a unique identification number, placed in plastic containers, flushed with N2 to remove O2 and then frozen at -40°C. At the end of each season, the entire collection of each species of individual fishes were grouped into a minimum of three pools of unique characteristics (e.g. size, sex, sexual maturity, age). Identical quantities of tissue from each of the fish in the subgroups was used in the composites so that no bias was placed upon size of fish. Tissues were taken from the entire longitudinal cross section of fillet so that the sample was representative of all of the edible tissue of each fish. All tissue from individual fish not used for compositing was archived.
Analytical procedure

All solvents used in analysis were HPLC grade or analytical reagent grade. Standards were purchased from NuCheck Prep, Inc. (Elysian, MN). Fillets were homogenized using a Waring blender and 0.5 g aliquots weighed into screw-capped (Teflon-lined) centrifuge tubes (30 ml) and saponified at ambient temperature with ethanolic KOH under N₂ using a magnetic stirrer for one hour. Care was exercised in the volumes of saponifying mixtures used to keep the water level, derived from tissue, sufficiently high to prevent trans-esterification. Solvent ratios were those suggested by Nelson (7). After dilution with distilled water, the neutral fraction was extracted with hexane. The remaining alkaline solution was acidified with 6N HCl, and free fatty acids were extracted with benzene. Benzene aliquots were combined and concentrated using a rotary evaporator. All evaporations were closely monitored to ensure that distillation temperatures did not exceed 25°C. Fatty acids were converted to methyl esters using 7% BF₃-MeOH by the method of Metcalfe et al. (6) modified to use ambient temperatures and a one-hour reaction period.

Identification of fatty acid methyl esters (FAME) was obtained by capillary gas chromatography (GC) using a Perkin-Elmer model Sigma 2000 gas chromatograph equipped with flame ionization detector and fitted with a 30 m x 0.25 mm I.D. fused silica capillary column coated with a 0.25 μm film thickness of Dura Bond WAX (J & W Scientific) and operated with a split ratio of 100:1. The carrier gas was He, maintained at 20 psi. Oven temperature was programmed at 90-250°C at a linear rate of 4°C/min. Data was processed using a Perkin-Elmer Sigma 10 data system with quantification of all compounds based on individual peak area response by GC compared to the internal standard methyl tricosanoate. Quantitative data were corrected for differences in detector responses that were determined through analysis of authentic standards of each reported fatty acid. FAME were tentatively identified by comparison with retention times with those of authentic standards. Verification of identification on select samples was accomplished through gas chromatography mass spectrometry analysis conducted by Charleston Laboratory, National Marine Fisheries Service.

Sample protection

Several precautions were taken to ensure that no degradation or other alteration of lipids occurred during extraction and saponification. All analytical steps were conducted at ambient temperatures, and samples were constantly flushed with N₂ to prevent oxidation. Further, as many steps as possible were conducted in a single extraction tube to reduce loss and degradation that occurs with sample transfer. All solvents were flushed with N₂ immediately before use to remove dissolved O₂ and to prevent oxidative degradation. Likewise, samples requiring storage were placed in sample bags which were then flushed with N₂ before being placed in freezers. In addition, the antioxidant BHT was added in a concentration of 0.005% (w/v) to extraction solvents to prevent oxidative degradation of unsaturated lipids.

Data analysis

One way analysis of variance (ANOVA) with post facto 95% confidence level range test (11) was used to compare fatty acids as well as certain parameters derived from fatty acid data. Those parameters that indicated statistical difference within comparison groups were identified (p<0.05).
RESULTS AND DISCUSSION

Male vs Female  Fatty acid and lipid differences were observed in males and females of the same species of similar size. These differences, however, were significant only during certain seasons of the year. Male and female spot (Lelostomus xanthurus) caught in January show little variation in relative or absolute concentrations of classes of fatty acids or lipids. Figure 1 is representative of the format used for all figures. The top division provides concentrations expressed as relative percent of the total fatty acids; the middle division, concentrations are expressed as absolute amounts in μg/g; the lower division affords results of measured parameter ratios of nutritional significance including percent lipid. As shown in Figure 1, there is no significant difference between male and female spot caught in January from various near shore areas along the Mississippi Coast. Figure 2 depicts fatty acid in male and female spot caught in October from the same areas. In these samples, fatty acids found in males and females are significantly different whether expressed as relative or absolute concentrations. The exception is absolute concentrations of total fatty acids. Even though there are large differences between mean values for males and females, the individual variations were so large as to show no significance when statistics were applied to this parameter.

Male and female harvest fish (Pepilus burti) caught in spring show very little significant fatty acid variations (Figure 3). Again, the mean percent fat in females is higher than males though statistically not significant. Male and female pigfish (Orthopristis chrysoptera) exhibit significant differences in percent lipid and absolute amounts of n-3 fatty acids (Figure 4). When comparing differences between other male and female species, there is a trend for males to have less fat than females and for males to have a higher relative amounts of omega-3 (denoted by n-3) fatty acids than females.

Age  Cobia (Rachycentron canadum) was the only species in which ages were ascertained. Ages were determined by Mr. Jim Franks, Gulf Coast Research Laboratory (GCRL) fisheries biologist, by counting the number of rings in their otoliths using the method of image analysis. Figure 5 represents female cobia caught in late summer of 1988. The older 4-7 year females in these fish are lower in fat with higher relative n-3 fatty acid concentrations as compared to the younger 2-3 year females. On the other hand, the older 4-6 year females caught in May 1988 (Figure 6) are higher in fat and lower in relative n-3 concentrations indicating that cobia may exhibit greater variation among themselves than other species. Relative differences in cobia of different ages are small or insignificant while absolute differences are much greater.

Size  Red snapper (Lutjanus campechanus) caught in the fall of 1989 were pooled into three size groups ranging from 800 to 1500 g. All were caught from one geographical area and each group contained both males and females. No significant differences in fatty acid parameters are observed for the three sizes as indicated in Figure 7, however, the range in the three weight groups was small. Blackfin tuna (Thunnus atlanticus) caught in June of 1990 ranged in weights from 13 to 23.5 lbs. and were pooled into three size groups (Figure 8). There is wider variation in fatty acids among the three size groups with relative concentrations of saturates, n-6 and n-3 showing no significant differences.

Juvenile and adult maturation  Atlantic croaker (Microcogonias undulatus) have a much wider fatty acid range of variations than the majority of species examined which may be clue in part to their feeding habits. Even though the range is wide, there are no significant differences in relative concentrations nor for measured ratios of fatty acid parameters. Saturates, polyunsaturates, n-3 and n-6 are significantly different when measured as absolute concentrations (Figure 9). Southern kingfish (Menticirrhus americanus) (Figure 10) and hybrid striped bass (Morone saxatilis x Morone chrysops) show similar trends
Figure 1. Fatty acids in male and female spot (winter).

Figure 2. Fatty acids in male and female spot (fall).
Figure 3. Fatty acids in male and female harvestfish.

Figure 4. Fatty acids in male and female pigfish.
Figure 5. Fatty acids in female cobia of various ages (summer).

Figure 6. Fatty acids in female cobia of various ages (spring).
Figure 7. Fatty acids of red snapper of varying sizes.

Figure 8. Fatty acids of blackfin tuna of varying sizes.
Figure 9. Fatty acids of adult and juvenile Atlantic croaker.

Figure 10. Fatty acids of adult and juvenile southern kingfish.
between juvenile and adult species (Figure 11). That is, juveniles have less fat but higher relative amounts of n-3 fatty acids. Juveniles of all species have higher total n-3/total n-6 ratio. Some fatty acid differences between adult and juvenile striped bass may also be due to differences in diet. Juvenile hybrid striped bass were reared in 500 gallon indoor tanks fed trout grower 1/8" by Zeigler from week 15 until sampled at approximately 5 months. Adult fish were reared in outdoor ponds for 6 months before sampling where they most likely fed on the epifauna and flora of the pond in addition to the same commercial feeds that fish reared in indoor tanks were fed.

**Maturation development** Red porgy (*Pagrus pagrus*) males caught in March, 1990 from the same geographical area but in developmental stages 2 and 4 show no significant differences except relative differences in total fatty acids (Figure 12). On the other hand, female ladyfish (*Elops saurus*) caught during the summer months from the Mississippi Sound in early developmental stage 1 and in gravid development, show significant differences whether expressed as relative concentrations or absolute concentrations (Figure 13). Only lipid and the ratio unsaturated/saturated are not significantly different. Greatest differences in fatty acids are seen when comparisons are made on species having the greatest differences in developmental stages. Southern kingfish (*Menticirrhus americanus*), for example, caught on June 20, 1989 from the Mississippi Sound were separated into developing females stage 3 and gravid females stage 5, and results indicated no significant differences (Figure 14).

**CONCLUSIONS**

Variations in fatty acids are observed in males and females during certain seasons of the year. These differences are most likely due to hormonal changes during maturation/spawning which may also change eating habits. Males are usually leaner and enriched in n-3 compared to females. Likewise, variations in size of species produce variations in the fatty acid patterns. Most likely, these changes are due to wide differences in fat content. Younger fish of all species examined are enriched in relative percent n-3 fatty acids compared to older fish. Juvenile species are enriched in n-3 and have higher n-3/n-6 ratios than do adult species. Species within families (pinfish and red porgy, little tunny and blackfin tuna) show similar trends. However, diet most likely is the over-riding factor in biological variability.

**ACKNOWLEDGMENTS**

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Figure 11. Fatty acids of adult and juvenile hybrid striped bass.

Figure 12. Fatty acids of red porgy of varying development state.
Figure 13. Fatty acids of ladyfish of varying development state.

Figure 14. Fatty acids of southern kingfish of varying development state.
REFERENCES


FLAVOR AND TEXTURE COMPARISON OF HYBRID STRIPED BASS
(Morone saxatilis/chrysops)
AND RED SNAPPER (Lutjanus campechanus)

L.S. Andrews, R.M. Grodner, J.A. Liuzzo
Department of Food Science
L. Dellenbarger and A. Schupp
Department of Agricultural Economics
Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center
Baton Rouge, LA  70803

The increasing consumption of seafood in the United States (1) has encouraged the development of new seafood products by the aquaculture industry. One of the newer aquaculture species, hybrid striped bass, is the object of much attention by aquaculture promoters (5). Information regarding consumer acceptance of hybrid striped bass could be beneficial when developing marketing strategies for this new aquaculture species.

Hybrid striped bass is a cross between striped bass and white bass. This new species has gained much attention recently and most sea grant universities have it under study with production occurring predominantly in the Middle Atlantic region of the United States. "At least two farms, one in California and one in North Carolina, are now harvesting product" (5). Hybrid striped bass is fairly cold tolerant and has many of the characteristics of saltwater finfish.

Market acceptability of new aquaculture species depends on the acceptability of flavor and texture attributes associated with what consumers consider good fish flavors and tenderness. Obviously, consumer acceptability of new products has a direct impact on the economic outcome of any food industry. The primary objective of this study was to establish flavor and texture descriptors for the hybrid striped bass, and secondly, to compare these descriptors to those of another popular saltwater finfish, red snapper.

MATERIALS AND METHODS

Volunteer sensory panelists were selected from undergraduate students, graduate students, faculty and staff in the College of Agriculture, Louisiana State University. Eight panel members were selected from potential panelists screened for their ability to identify and distinguish intensities of various control flavors and textures and for their frequent finfish consumption. Previous sensory and/or consumer panel studies involving seafood were reviewed (2)(4)(6) to provide input into planning and conducting training sessions for the eight-member panel and to establish the evaluation criteria for the actual evaluation sessions. Following established procedures, the panel was trained using red snapper as the control fish. Procedures outlined by Meilgaard, et al. (3) were followed, during three training sessions, to assist the panel members in identifying the kinds and intensities of flavors and textures present in the red snapper. Training in recognizing good fish flavor and texture in the red snapper was used in rating the overall acceptability of the unknown samples.
Three formal sensory panel sessions were held, with each panel member evaluating unidentified duplicate samples of both hybrid striped bass and red snapper finfish using a randomized complete block design. The sensory room was lighted with red light to reduce the influence of flesh color on the flavor evaluation. Each panel booth was equipped with evaluation forms, cooked samples, expectorant cups, water and scissors (for opening plastic bags). The identities of all the fish samples were unknown to the panelists.

The hybrid striped bass used in the evaluation was pond reared by the Fisheries Department at the Louisiana State University. Red snapper was purchased from a local seafood specialty store. All products were filleted, wrapped, frozen and stored in a -20°C freezer until use. Just prior to testing, the fillets were removed from the freezer (cut while in frozen state) into approximately 3 cm squares and sealed individually in 4" x 4" polyethylene bags.

All fish fillet samples (sealed in polyethylene bags to retain moisture and flavor) were cooked in a boiling water bath (100°C) for 8-9 min, optimum cooking times for texture was determined for both species prior to actual panel sessions. Cooking times longer than 8-9 min for these two species resulted in a drier and tougher meat protein structure. Each of the samples was clearly labeled with random numbers and presented in random order to the panelists. Each panelist rated all samples for flavor, juiciness, texture, and overall acceptability using a 0-9 hedonic scale. The hedonic acceptability scale ranged from 0 - highly unacceptable to 9 - highly acceptable. In addition, the panelists were asked to record specific flavor and texture attributes identified in each sample. The panel results were used to determine mean values for the panel as a whole and also with the panel divided into two groups based on their preference and consumption of fresh or salt water finfish.

Freshly thawed hybrid striped bass fillets and red snapper were also breaded in a "Cajun" style fish fry breading and pan fried in pure vegetable oil (175°C) for 3-5 min until golden brown. Samples of breaded and fried hybrid striped bass and red snapper were rated for overall acceptability by the panel following the final session of evaluating boil-in-bag samples.

RESULTS AND DISCUSSION

Flavor descriptors identified by the panel for the red snapper and hybrid striped bass are presented in Table 1. All of the flavor and texture descriptors identified by the panel during the training sessions for the red snapper were also identified in the blind samples of red snapper and hybrid striped bass with the addition of pleasant, grainy, and buttery/oily identified in the hybrid striped bass only. The majority of the descriptors (mild fishy, sweet, moist, firm, etc.) were considered "good fish flavors" expected from a marine finfish such as red snapper. A few variations among panelists occurred in determining the degree of desirable fishy flavor in the boiled fish fillets. The "fishy" flavors described by the panel members, particularly those who consumed marine finfish on a regular basis, were considered good and expected flavors for fresh saltwater fish.

Overall acceptability ratings of the boiled fish samples are presented in Table 2. The overall mean acceptability scores for red snapper and the hybrid striped bass were very similar with ratings of 3.85(acceptable) and 3.18 (somewhat acceptable) respectively. When the panel scores were split into two groups based on the panelists normal consumption of fresh or salt water fish, the acceptability of both fish species approximately doubled to around 7.00 (very acceptable) for the salt water fish group specifically.
Table 1. Flavor and texture descriptors identified in red snapper and hybrid striped bass.

<table>
<thead>
<tr>
<th>Flavor related</th>
<th>Texture related</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>weak fishy</td>
<td>flaky</td>
<td>nutty</td>
</tr>
<tr>
<td>good fishy</td>
<td>moist</td>
<td>creamed corn</td>
</tr>
<tr>
<td>mild fishy</td>
<td>firm</td>
<td>cardboard</td>
</tr>
<tr>
<td>bland</td>
<td></td>
<td></td>
</tr>
<tr>
<td>strong fishy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>buttery/oily*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sweet light</td>
<td>grainy*</td>
<td></td>
</tr>
<tr>
<td>typical fishy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pleasant*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Descriptors identified in hybrid striped bass only.

Table 2. Acceptability* Rating.

<table>
<thead>
<tr>
<th>Whole Panel</th>
<th>Ocean Fish Panel</th>
<th>Whole Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unseasoned/boiled</td>
<td>Unseasoned/boiled</td>
<td>Seasoned/Fried</td>
</tr>
<tr>
<td>HSBass</td>
<td>3.18</td>
<td>7.12</td>
</tr>
<tr>
<td>Snapper</td>
<td>3.85</td>
<td>6.93</td>
</tr>
</tbody>
</table>

*Acceptability scores were measured on a 9 point hedonic rating scale. (9 = highly acceptable; 0 = highly unacceptable).

Acceptability ratings, by the entire panel, for the red snapper and hybrid striped bass prepared in a seasoned batter and fried were markedly higher than unseasoned samples with scores of 7.15 for red snapper and 7.89 for hybrid striped bass (Table 2).

CONCLUSION

Flavor and texture descriptors identified with hybrid striped bass were very similar to those described for red snapper. The overall flavor and texture acceptability scores rated by this sensory panel was similar for the two species. Consequently, it would appear that the farm raised hybrid striped bass could favorably compete with a popular marine species such as red snapper. The investigators recognize the limited scope of this experiment and recommend test marketing of hybrid striped bass also be conducted in other regions of the United States.
REFERENCES


QUALITY CHANGES OF AQUACULTURED HYBRID STRIPED BASS
FILLET MEAT RESULTING FROM POST-HARVEST
COOLING OR CO₂ TREATMENTS

Joseph D. Eifert, Cameron R. Hackney, George S. Libey
and George J. Flick, Jr.
Department of Food Science and Technology,
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061
and
**Department of Fisheries and Wildlife Sciences
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

Aquacultured finfish and shellfish comprise an increasing share of all edible fishery
products which is now approximately 12% of the worldwide supply. Additionally,
aquaculture production increased 290% between 1980 and 1989 in the United States (3).

The hybrid striped bass has become an increasingly popular aquacultured food fish
in the United States along with catfish, trout, salmon and tilapia. This fish is suitable for
intensive culture because of good biological traits, rapid growth, schooling behavior,
hardiness and high market value (10).

The primary objective of this study was to examine ways to increase the quality or
shelf life of hybrid striped bass fillets from fish raised in an indoor recirculating aquaculture
system. In this study, the post-harvest metabolism of hybrid striped bass was reduced with
either a carbon dioxide gas treatment or a cooled water treatment.

Stress and high metabolic activity associated with harvesting and transportation of live
fish may affect final product quality and shelf life. Of all the flesh foods, fish are the most
susceptible to autolysis, oxidation and hydrolysis of fats and microbial spoilage (2). Rigor
mortis is especially important in fish preservation because it can retard post-mortem
autolysis and bacterial decomposition. Rigor mortis is hastened by struggling, lack of
oxygen and warm temperatures and delayed by low pH and cooling (2,6). Shelf life can
be prolonged by procedures to lengthen rigor mortis which include lowering muscle activity,
careful handling and lowering of holding temperatures.

Another study objective was to develop quality indices for this species which have
been lacking. Many previous studies that have reported biochemical, microbiological and
sensory indices of quality and shelf life in other fish have widely varying or contradictory
results. Since the hybrid striped bass used in this project were raised under controlled
conditions, many of the variables (i.e. diet variations or seasonal water temperatures and
photoperiods) that can affect quality indices were not factors.
MATERIALS AND METHODS

Source of Fish

The reciprocal cross hybrid striped bass used in this study were a cross between the female white bass *Morone chrysops* and the male striped bass *Morone saxatilis*. This cross is readily available from commercial sources and is the most common cultured hybrid striped bass. The accepted common name of this cross is the Sunshine Bass (4). All fish were obtained from the Virginia Polytechnic Institute & State University Aquaculture Research Facility (ARF). Fingerlings were purchased from Keo Fish Farms (Lonoake, Arkansas) and stocked at 1800 per culture tank (approximately 4 fish per cubic ft.).

Recirculating Aquaculture System

The Aquaculture Research Facility contains nine, indoor independent recirculating aquaculture systems. Each system consists of five major components: an 8,330 liter rectangular culture tank, a 1,970 liter sump tank with a multi-tube clarifier for the removal of suspended solids, a 1/4 hp pump (50 gpm), a 1,990 liter biofilter tank housing a three stage rotating biological contact filter, and a U-tube aeration system incorporating pure oxygen injection. Rotating biological contactors aid to maintain appropriate ammonia, nitrate and nitrite levels. Oxygen concentration is maintained by surface aerators, injection of liquid oxygen and the use of U-tube aerators.

Air and water temperatures in the facility are controlled by four propane heaters suspended in each corner of the building. Lighting in the building is kept to a minimum to reduce fish stress and algal growth. Lighting simulated an approximately 14 hour light and 10 hour dark cycle.

Water quality parameters were consistently measured. These parameters included ammonia, nitrate, nitrite, dissolved oxygen, pH, alkalinity, hardness, and temperature. Culture tank water temperatures at time of fish harvest were 24.4 - 25.1 ºC.

The fish were fed a high protein floating diet (Biosponge Aquaculture Products, Sheridan, Wyoming) formulated for hybrid striped bass. The diet was composed of 44% crude protein, 8% fat, 3% crude fiber and 13% moisture. Feed was administered once or twice daily depending on water quality and feeding activity. Fish were not fed within 15 hours of harvest time.

Treatments

For each treatment or control group, 25-40 fish were harvested from a single tank by grading and dip net. Fish were transported to the Department of Food Science & Technology (FST) for filleting.

Fish were sacrificed on day zero. All analyses were started the following day (day one). Four test groups were used as follows in each of three experiments.

Control group fish were harvested, placed in waxed cardboard boxes, transported to FST and filleted.

Stressed Control group fish were harvested, and placed in a truck mounted holding tank for transport to FST. Transport tank water was obtained from the fish culture tank. After arrival at FST the fish were transferred by dip net to another holding tank. Approximately 200 gallons of water for the second holding tank was obtained from
municipal supply and aerated by a surface agitator. Sodium bicarbonate (NaHCO₃ 200 g) and calcium chloride (CaCl₂·2H₂O, 215 g) were added to increase alkalinity and hardness. Sodium thiosulfate (Na₂S₂O₃·5H₂O, 20 g) was added to inactivate chlorine.

Fish were held in this tank for approximately three hours and subjected to periodic agitation. Also, these fish were additionally stressed since they were transferred between culture tank, transport tank and holding tank and experienced a change in water quality in the holding tank. After three hours, the fish were removed by dip net, weighed and filleted within three hours.

The cool water treatment group (CW) was harvested and placed in a holding tank at ARF for approximately two hours. Water for the holding tank was obtained from the culture tank and aerated with a surface agitator. Crushed ice was periodically added to the water to lower the temperature to 10-12°C at the end of two hours. Sodium thiosulfate (10 g) was added to approximately 100 gallons of water in the tank to inactivate chlorine from the melting ice. At the end of the holding period the fish were removed by dip net, placed in waxed cardboard boxes with crushed ice and transported to FST for filleting.

The carbon dioxide treatment group (CO₂) was harvested and placed in a holding tank at ARF for approximately 30 minutes. Carbon dioxide (CO₂) was injected at 10-20 cfh until most fish remained on the bottom of the tank. After five to ten minutes, fish were gasping at the water surface. Water (approx. 200 gallons) for the holding tank was obtained from the culture tank and aerated only prior to addition of fish. Dissolved oxygen level dropped from approximately 6.7 ppm to 4 ppm over 30 minutes as measured with a YSI model 58 dissolved oxygen meter (Yellow Springs Instrument Co., Yellow Springs, OH). At the end of the holding period the fish were removed by dip net, placed in waxed cardboard boxes and transported to FST for filleting.

Sampling of fillet meat

Skinless, boneless fillets were removed from all fish within 30 minutes after arrival at FST, except filleting occurred after the three hour holding period for the stressed control group. Fillets were stored in ice covered plastic boxes in a refrigerator maintained at 1-4°C.

For each experiment, 15 fillets from the control group were vacuum packaged in plastic bags. These fillets were stored at -20°C and used as a reference for the sensory evaluation panel.

Analyses

Aerobic Plate Count

Microbial levels of thawed fillets were determined with an aerobic plate count. For each group, a composite sample of 20-25g from three fillets was tested. The composite was mixed with 0.1% peptone (Difco Laboratories, Detroit, MI) to achieve a 10⁻¹ dilution. The dilution was blended in a Stomacher Model 400 (Tekmar Co., Cincinnati, OH) for two minutes. Further dilutions in 0.1% peptone were prepared and plated in duplicate on Plate Count Agar (Difco Laboratories) using a pour plate technique. Plates were incubated at 20°C for four days.
pH

The pH level of a fillet meat composite was determined on the same test days as the aerobic plate count. A composite sample of approximately five grams (from three fillets) was mixed with an equivalent weight of distilled water with a glass rod in a beaker. Determinations of pH level were performed with a Corning Model 240 pH meter (Corning Glass Works, Corning, NY) meter with a combination electrode (Corning #476530).

Texture measurement

Measurements of the texture of raw and cooked fillets were performed with an Instron Model 1011 Universal Testing Instrument (Instron Corp., Canton, MA) equipped with a L.E.E. - Kramer Shear/Compression Cell (1). Approximately 15 g pieces of raw (experiment 2) and cooked (experiments 1 and 2 only) fillets were brought to room temperature prior to testing. Cooked pieces were oven baked at 350°F for eight minutes. Two pieces per fillet, and three fillets per group were tested each test day. Measurements of peak force in units of kilograms force (kgf) to compress the sample to 30% of its original thickness were recorded.

Sensory evaluation panel

An experienced taste panel of 15 graduate students or employees of the Department of Food Science & Technology were used to evaluate the taste, aroma, appearance and texture of cooked fillets. Fillets presented to the panel were prepared by cutting 10-15 gram portions, wrapping in aluminum foil, labelling and baking for eight minutes in a 350°F convection oven. Each panelist was presented five cooked portions of fish and asked to rate the samples with a nine-point scale (1 = inedible, 5 = borderline, 9 = excellent) for appearance, odor, texture and taste (6). Each panelist received one piece each of fish representing each of the four test groups. A fifth fish portion for each panelist was a portion from the vacuum packaged and previously frozen at -20°C fillets, and was coded as "A". Panelists were directed to compare all attributes of the other fillet portions to the reference "A" portion.

Statistical analysis

Analysis of variance was performed with the General Linear Models procedure (Version 6.06, SAS Institute, Inc., Cary, NC). Additional comparisons were made with Duncan’s Multiple Range Test, Tukey’s Studentized Range Test and Dunnett’s T test. The results of three experiments are combined and reported.

RESULTS AND DISCUSSION

Hybrid striped bass measurements

The average weight of the 369 fish filleted was 337 g (11.9 oz.). The average fillet weight obtained from each fish was 98.8 g (49.4 g per fillet). The fillet operation yielded an average of 29.2% fillets per fish. All fillets were skinless, boneless, J-cut and without belly flap. Fillets were studied because they are a popular market form and are less susceptible to autolytic spoilage by digestive tract enzymes than either whole or headed and gutted fish (9). The average flesh temperatures of the fish groups prior to filleting were 9.6°C for the CW fish group and approximately 23°C for the other three groups.
Aerobic plate count

The aerobic plate count of all test groups exceeded finfish spoilage levels of \(10^7\) cfu/gm (5) by day 11 (Figure 1). The cool water treatment fillets reached this level by day 9 and the two control groups near day 10. The fillets from the carbon dioxide treated fish did not achieve log 7 growth of microorganisms until eleven days of storage. Also, log phase growth in the CO\(_2\) treatment fillets did not occur until between day 7 and 8 (Figure 1). A one log increase in counts occurred at least one day earlier for the other three test groups of fillets. Differences in levels of aerobic organisms were not significant \((p<0.05)\) between test groups.

pH

The pH of the refrigerated fillets in all groups increased from an average of approximately 6.24 initially to 6.54 by day 14 and 6.86 by day 18. Throughout the test period the pH of the cool water treatment fillets were highest overall and were significantly higher \((p<0.05)\) than that of the control fillets. On day 1 the pH of the CO\(_2\) fillets (6.13) was lowest. A significant correlation \((p<0.001, r = 0.57)\) existed between pH level and aerobic plate count \((\log \text{cfu/gm})\) over time for all test groups combined.

Texture measurement

Treatment differences and time differences due to aging of fillets were highly significant \((p<0.001)\) for cooked fillet pieces for peak force energy measurements. Initial peak force measurements on cooked fillets (Figure 2) ranged from an average of 40.1 kilograms force (kgf) for the CW fillets to 60.2 kgf for the control group fillets. Cool water fillet peak force measurements were significantly lower \((p<0.05)\) throughout the test period than the control and CO\(_2\) fillets.

Differences in peak force energy measurements on raw fish portions (one experiment only) were not significant over time (18 days) or between test groups. Average initial (day 1) peak force was 25.2 kgf for raw fish portions and 49.4 kgf for cooked portions. Average final peak force was 22.7 kgf for raw fish portions and 46.8 kgf for cooked portions.

Sensory evaluation panel

Significant differences \((p<0.05)\) over time (day 1 - 14) exist for all four sensory attributes. The average sensory attribute scores across all test groups declined over 14 days as follows: appearance score from 7.51 to 6.58, taste score from 7.06 to 6.00, odor score from 7.44 to 6.43, and texture score from 7.08 to 6.40. Texture scores and Instron measurements of fillet texture were not correlated. Texture scores and microbial levels \((\log \text{cfu/g})\) were not correlated \((r=-0.31, p=0.053)\).

The only significant \((p<0.05)\) treatment difference for a sensory attribute was that the control group fillets were rated lower in appearance than the other three groups throughout the test period. In Figure 3 the sensory scores at each time have been combined and averaged to show differences in sensory qualities of fillets of each test group by attribute. Overall, the two treatments were rated higher in sensory quality than both control groups when all times are combined.

In Figure 4 the four attribute scores have been combined and averaged at each time to show the changes in sensory qualities of each test group over time. The control group fillets were rated lowest initially (day 1-3), and the CO\(_2\) group fillets were rated highest late in the test period (days 10-14).
Figure 1. Mean aerobic plate count of fillets stored at 1.4 °C.

Figure 2. Mean peak force energy (kgf) for compression of cooked fish portions.
5: Borderline 6: Fair 7: Good 8: Very Good

Figure 3. Mean sensory evaluation panel ratings for each test group per attribute. * = significance, p<0.05.

Figure 4. Mean sensory evaluation panel ratings for each test group over time. Appearance, odor, texture and taste scores are combined.
CONCLUSION

The CO₂ treatment increased the shelf life of fillets by at least one day over the other treatments used since log phase growth and the time to reach a seven log microbial level was delayed. In addition, sensory attributes of fillets from the carbon dioxide treated hybrid striped bass were judged superior during extended storage of fillets. Also, the firmness of cooked fish portions as measured by a Kramer Shear Compression Cell (peak force) was significantly higher for these fillets than the cool water treatment fillets.

Procedures to reduce fish stress during handling and processing are critical to increased productivity of intensive aquaculture (7). The use of a post-harvest CO₂ treatment to increase the quality and shelf life of fish obtained from a recirculating aquaculture facility can lead to several economic benefits. First, an increase in consumer confidence in the high quality and consistency of hybrid striped bass will lead to a greater market demand and increases in price or sales. Increased shelf life can lead to distribution over greater distances and a product less sensitive to storage temperature abuse. Also, processing plants can operate year-round since a recirculating aquaculture system can produce a predictable supply of raw material leading to planned production schedules (9). Finally, post-harvest use of carbon dioxide gas on fish awaiting processing is less costly than the use of ice or refrigeration to slow the metabolism of live fish.

The carbon dioxide treatment described here may be useful for extensively aquacultured fish after harvest and during or after transport to a processing or packaging facility. The results of this research may be applicable to other food fish species that are raised in recirculating aquaculture systems.

REFERENCES


STORAGE STABILITY OF TILAPIA
IN RELATION TO LIPID AND TOCOPHEROL COMPOSITION

Marilyn C. Erickson and Swee Tee Thed
Department of Food Science and Technology
Food Safety and Quality Enhancement Laboratory
University of Georgia Agricultural Experiment Station
Griffin, GA 30223

Displaying an efficient conversion of low protein diets, an ability to breed easily, a resistance to handling stress and disease, and a high palatability, tilapia are considered to be one of the most important aquacultured food fish in the world. In the United States, culture of tilapia has been restricted to the warmer states. Despite this limitation, production of these fish within this country could increase dramatically necessitating in turn that a greater percentage of the product be marketed as frozen. The storage stability of two varieties of tilapia was therefore examined to explore the potential of tilapia to be marketed in this form. The lipid and tocopherol composition of these tilapia was also examined in an attempt to understand the degree to which these components dictate the tilapia’s susceptibility to oxidation.

MATERIALS AND METHODS

Sample preparation
Two varieties of tilapia were obtained from the Owens and Williams Fish Farm in Hawkinsville, Georgia. One variety, *Tilapia nilotica*, exhibited the traditional black/blue coloration of skin and was designated as "Blue" tilapia (BT). The other variety designated "Red" tilapia (RT) was a hybrid, having inherited a red body coloration from a cross of *Tilapia nilotica* and *Tilapia mossambica* 10 generations earlier. Both fish had been spawned in April, stocked the following month in a 0.38 acre pond at 12,000 fish/acre, and harvested in October of the same year. Transported on ice to Griffin, seven fish from each variety were weighed, filleted, pooled to form a homogeneous sample, and minced.

Storage
The samples, divided into 4 portions, were subjected to varying periods (0, 3, 6 and 9 months) of fluctuating frozen storage (-6 to -18°C), then stored at -100°C 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
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-60 (CEM Corporation; Indian Trail, NC) until constant weight was achieved.

Lipid Extraction and Characterization
Chloroform:methanol (2:1) was used to extract lipid from 1.00 ± 0.02 g muscle tissue as described by Erickson (3). Polar (phospholipids) and nonpolar lipid (triacylglycerol and cholesterol) fractions were obtained using silica Sep-Pak cartridges (5).
Fatty acid composition of the phospholipid and total lipid fractions was monitored by esterification using 4% H₂SO₄ in methanol and subsequent gas chromatography of the fatty acid methyl esters.

Triacylglycerols were measured by saponifying an aliquot of the nonpolar lipid fraction and quantitating the glycerol moiety (6).

Following saponification of another aliquot of the nonpolar lipid fraction, quantitation of total cholesterol was based on the formation of a chromogen with FeCl₃ (11).

**Tocopherol Analyses**

After saponification in the presence of ascorbic acid and ethanol, tocopherols were extracted from 1.0 g minced muscle tissue with 10% ethyl acetate in hexane (3). The extract evaporated to dryness under nitrogen was reconstituted in 1.0 ml methanol:water (98:2). Reverse-phase high performance liquid chromatographic separation of tocopherols was conducted as described by Vatassery and Smith (7). The chromatographic system consisted of a Micromeritics 752 Gradient Programmer, Micromeritics 750 Solvent Delivery System, and a Brinkmann 656 Electrochemical Detector.

**Oxidative Measurements**

Two five ml aliquots of lipid extract (25 ml total) were dispensed into separate clean tubes and each washed with 1 ml of 0.88% KCl. After removing the upper layer by aspiration, one of the tubes was analyzed for hydroperoxides according to Buege and Aust (1), while the other tube was analyzed for conjugated dienes by the spectrophotometric procedure (A₂₃₅).

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 x 10⁻⁸M) set equal to 100 fluorescence units.

Thiobarbituric acid reactive substances (TBA-RS) were quantified according to the extraction procedure of Vyncke (8). Results were expressed as nmol malonaldehyde/g dry weight.

Following heating of tissue (1.5 g) in a 5 ml conical vial for 15 minutes at 90°C, headspace (1.5 ml) was removed with a gas-tight syringe and subjected to chromatographic conditions described by Young (10). Peaks corresponding to propanal and hexanal were tentatively identified by comparison of their relative retention times to purified standards (Sigma, St. Louis, MO) subjected to the same heating and chromatographic conditions as the samples. Based on the response of the internal standard, 4-heptanone, quantities of propanal and hexanal were calculated.

**RESULTS AND DISCUSSION**

At harvest, BT's fish weights ranged from 375 to 508 g with an average of 414 g while RT's fish weights ranged from 309 to 508 g with an average of 363 g. As shown in Table I, BT was found to have larger quantities of both lipid and moisture than found in RT. By accounting for the moisture differences between the two tilapia varieties, differences in fish weights were largely attributed to the larger quantities of triacylglycerol deposited in the BT. In addition, BT contained slightly higher quantities of phospholipid and cholesterol than RT on a dry weight basis.
Table I. Lipid composition of tilapia.

<table>
<thead>
<tr>
<th></th>
<th>RT</th>
<th>BT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Lipid</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Percent Moisture</td>
<td>79.4</td>
<td>80.2</td>
</tr>
<tr>
<td>mg Triacylglycerol/</td>
<td>58.5</td>
<td>71.8</td>
</tr>
<tr>
<td>g dry wt.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg Phospholipid/</td>
<td>22.0</td>
<td>23.2</td>
</tr>
<tr>
<td>g dry wt.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg Cholesterol/</td>
<td>2.4</td>
<td>2.6</td>
</tr>
<tr>
<td>g dry wt.</td>
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The two most abundant polyunsaturated fatty acids present in both tilapia's triacylglycerol fractions were linoleic acid (18:2) and docosahexaenoic acid (22:6). RT's triacylglycerol fraction, however, was characterized with a greater degree of unsaturation than BT's when expressed as a percentage of the total fatty acids in this fraction (Table II). When all fatty acid percentages and the varying susceptibilities of each fatty acid to oxidize are used to calculate a peroxidizability index (9), RT's triacylglycerol fraction is considered more unstable than BT's fraction. Alternatively, when the peroxidizability index is modified to take into account the quantity of each fatty acid that would be present in one g of tissue, BT's tissue would be considered more susceptible to oxidation than RT if triacylglycerols serve as the primary site of lipid oxidation.

While representing a much smaller percentage of the total lipid, the phospholipid fractions from each tilapia variety were also characterized as to their fatty acid composition (Table II). Again, 18:2 and 22:6 were the most dominant polyunsaturated fatty acids in both fractions and constituted a larger percentage of the total fatty acids and hence higher peroxidizability index in RT's phospholipid fraction than BT's fraction. The tissue peroxidizability index in turn indicated that RT's tissue could be more susceptible to oxidation than BT's tissue if phospholipids alone were the primary rate determining factor.

The tocopherol antioxidants, however, must also be factored into the susceptibility of phospholipids to oxidize. Residing in membranes, the tocopherols act as antioxidants by scavenging oxygen- or carbon-centered fatty acyl radicals via donation of a hydrogen atom from the phenyl hydroxyl of the chromanol ring. In tilapia, both the alpha and gamma isomers of tocopherol were present in greater abundance in BT than RT (Table III). Adjusting for the gamma isomer being approximately 31% as effective an antioxidant as the alpha isomer (2), BT's tocopherol protection was nearly 1.2 times greater than RT. Therefore, in accounting for both the phospholipid and tocopherol composition (peroxidizability index/nmol alpha-tocopherol equivalents), RT's minced muscle sample with a ratio of 2.2 could be expected to oxidize sooner than the BT sample with a ratio of 1.8 provided membrane phospholipid serve as the primary site of lipid oxidation.
Table II. Fatty acid composition and peroxidizability of triacylglycerol and phospholipid fractions.

<table>
<thead>
<tr>
<th></th>
<th>TRIACYLGlycEROL</th>
<th>PHOSPHOLIPID</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
<td>BT</td>
</tr>
<tr>
<td>Percent 18:2</td>
<td>11.6</td>
<td>11.2</td>
</tr>
<tr>
<td>Percent 22:6</td>
<td>2.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Peroxidizability Index&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.2</td>
<td>50.6</td>
</tr>
<tr>
<td>µmol 18:2/g dry wt</td>
<td>23.0</td>
<td>27.2</td>
</tr>
<tr>
<td>µmol 22:6/g dry wt</td>
<td>5.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Tissue Peroxidizability Index&lt;sup&gt;b&lt;/sup&gt;</td>
<td>107.4</td>
<td>123.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Peroxidizability Index = (sum % monoenes × 0.025) + (sum % dienes × 1) + (sum % trienes × 2) + (sum % tetraenes × 4) + (sum % pentaenes × 6) + (sum % hexaenes × 8)

<sup>b</sup> Tissue 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)

Table III. Tocopherol content of tilapia muscle tissue.

<table>
<thead>
<tr>
<th></th>
<th>NMOL TOCOPHEROL/G DRY WT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
</tr>
<tr>
<td>Alpha-Tocopherol</td>
<td>58.8</td>
</tr>
<tr>
<td>Gamma-Tocopherol</td>
<td>15.7</td>
</tr>
<tr>
<td>Alpha-Tocopherol Equivalents</td>
<td>63.7</td>
</tr>
</tbody>
</table>

During the first 3 months of frozen storage, no change was noted in the alpha-tocopherol equivalents for either BT or RT (Fig 1). Between 3 and 6 months, degradation of tocopherol had occurred in both tilapia varieties but at a similar rate. After 6 months, a dramatic drop in tocopherol levels was seen for both tilapia varieties increased rate of tocopherol degradation which occurred after 6 months implied that a critical concentration had been reached at which point the tocopherol was no longer able to compete effectively for the peroxyl radicals and prevent the propagation step of oxidation. Previously, this critical tocopherol concentration was found to be dependent on the effectiveness of the oxidative stress (4). Consequently, it may be surmised that the higher apparent critical concentration exhibited by BT arose from a greater oxidative stress in BT than RT muscle tissue.
Fig. 1. Tocopherol content of tilapia muscle tissue following frozen storage.

Measurement of the oxidative products, hydroperoxides, also demonstrated the presence of a lag phase for both RT and BT (Fig. 2). In the case of this data, though, increased rates of formation occurred prior to 6 months for BT muscle. The larger hydroperoxide levels found in BT muscle compared to RT muscle supported the tocopherol data in indicating that BT was more susceptible to oxidation than RT. The conjugated diene data, however, did not follow the same trend as the hydroperoxide data (Fig. 2). Higher levels of conjugated dienes were found in BT at 0, 3 and 6 months, yet at 9 months, levels of conjugated dienes in RT surpassed those in BT.

For the TBA test which measures some of the breakdown products of hydroperoxides, the lag phase was evident for both RT and BT only during the first 3 months of storage (Fig. 3). Notable differences in rate of formation of these oxidative products between the two varieties was just evident during the last 3 months of storage. Similarly, dramatic differences for propanal and hexanal were only noted between varieties in muscle tissue which had been stored 9 months (Fig. 4). The larger production of propanal compared to hexanal which occurred is likely attributed to the relative susceptibility of the fatty acids from which they originate, propanal being an endproduct of n-3 fatty acid oxidation (i.e. 22:6) and hexanal being an endproduct of n-6 fatty acid oxidation (i.e. 18:2).
Fig. 2. Hydroperoxide and conjugated diene content of tilapia muscle tissue following frozen storage.

Fig. 3. TBA-RS content of tilapia muscle tissue following frozen storage.
Fig. 4. Propanal and hexanal content of tilapia muscle tissue following frozen storage.

Fluorescent pigments, another type of secondary oxidative product, was also monitored on the stored samples (Fig. 5). Consisting of carbonyls that have formed a Schiff base to the amine group of a phospholipid, organic fluorescent pigments were produced at similar rates in the two varieties. On the other hand, aqueous fluorescent pigments, or those carbonyls that have formed Schiff bases to a primary amino acid or peptide, formed to a greater extent in the BT than RT muscle tissue. In contrast to the TBA data, the lag phase extended through 6 months of storage for both types of fluorescent pigments. Such a delay, however, is expected given that the fluorescent pigment precursor, carbonyls, must first be produced.

Fig. 5. Fluorescent pigment content of tilapia muscle tissue following frozen storage.
CONCLUSION

Based on the majority of oxidative measurements conducted in this study BT was considered less stable than RT. From the phospholipid and tocopherol composition data, however, the opposite scenario would have been predicted. Only when the absolute amount of triacylglycerol and the relative oxidative stabilities of this fraction's fatty acid composition were considered was BT predicted to be less stable than RT. These results suggest that the triacylglycerols would be serving as the primary site of lipid oxidation and not the phospholipids in these minced fish. It is further speculated that the triacylglycerol contribution to oxidation is via the accelerated action of lipases released on mincing. Since the contribution of lipases in an intact muscle tissue would be minimized, it is unknown whether similar oxidative susceptibilities would exist for these tilapia varieties with unmixed muscle tissue. Thus, further studies are needed on both minced and unmixed muscle tissue to provide us with additional information on the relative contributions of compositional factors to the oxidative stability of tilapia tissue.

REFERENCES


