QUALITY CHANGES OF AQUACULTURED HYBRID STRIPED BASS FILLET MEAT RESULTING FROM REDUCTION OF POST-HARVEST METABOLISM

by

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(ABSTRACT)

Hybrid striped bass (Sunshine Bass) were raised in an indoor recirculating aquaculture system incorporating concrete culture tanks, sump tanks, rotating biological contactors and liquid oxygen injection and underground U-tubes for aeration. Fish were anesthetized by two methods after harvest to reduce metabolic activity. Hybrid striped bass were either held in cooled water or water with elevated CO_2 levels prior to sacrificing and filleting. Control fish were filleted immediately or three hours after harvest. The objectives of this study were to determine the effects of these treatments on fillet quality and to develop indicators of quality and shelf life. All fillets were stored at 1-4°C and tested over a 14-day period. Analyses included aerobic plate count, pH determination, texture measurements (Instron), color measurements (L* a* b* scale) and sensory panel evaluations of cooked portions for appearance, taste,

odor and texture. Fillets of the cooled water treatment group had the highest pH and were significantly less firm (Instron). Log phase growth and the time for spoilage levels of microorganisms to grow were delayed one day in the CO_2 fillets. In addition, the CO_2 treated fillets were generally rated higher in sensory attributes than the other fillets, especially late in the test period.

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I. INTRODUCTION

Per capita consumption of edible fishery products in the United States increased approximately 24% since 1980 to a record 15.9 lbs. in 1989 (USDC, 1990). More than 12 billion pounds of edible fish, shellfish and molluscs were consumed in the U.S. in 1989 and nearly half was imported from other countries. These trends are expected to continue through the next decade. Aquaculture may be able to satisfy the growing demand for high quality finfish and shellfish.

Furthermore, intensive aquaculture techniques can ensure a consistent high quality product because of strict controls on growing conditions. A possible problem with processing aquacultured finfish is that at harvest they are so fresh that harvest, handling and processing procedures need to be modified to develop products of the highest quality.

The hybrid striped bass is an increasingly important aquacultured food fish, especially in the mid-Atlantic and California. This fish is suitable for intensive culture because of good biological traits, rapid growth, schooling behavior, hardiness and high market value (Van Olst and Carlberg, 1990).

The primary objective of this study was to show the effects on fillet meat quality and shelf life of different post-harvest treatments to reduce metabolism or physical

activity of hybrid striped bass raised by a recirculating aquaculture system. Stress and high metabolic activity associated with harvesting and transportation of live fish may affect final product quality and shelf life.

Post-mortem changes in fish flesh including bacterial and enzymatic degradation, need to be controlled to optimize product quality. Of all the flesh foods, fish are the most susceptible to autolysis, oxidation and hydrolysis of fats and microbial spoilage (Frazier and Westhoff, 1978). In this study, post-harvest metabolism was reduced by placing fish into cooled water or water with an elevated level of carbon dioxide.

Another study objective was to develop consistent or reproducible indicators of chemical, microbiological and sensory quality and/or shelf life. Many previous studies that have reported indices of quality and shelf life in fish have had widely varying or contradictory results. For example, Johnson et al. (1980) reported that variations between texture measurements in fish fillets of the same species are largely due to geographical, seasonal and feeding factors of live fish, post-mortem biochemical factors and the fillet process itself. Since the hybrid striped bass (female white bass x male striped bass) used in this project were raised under controlled conditions, many of the variables (e.g. diet variations or seasonal water

temperatures and photoperiods) that can affect quality indices were not factors.

II. LITERATURE REVIEW

A. Hybrid Striped Bass

The hybrid striped bass is a cross of the striped bass (Morone saxatilis) and the white bass (Morone chrysops). This fish has become an increasingly popular species of aquacultured food fish in the United States along with catfish, trout, salmon and tilapia.

The commercial culture of striped bass and hybrid striped bass began in the mid-1970's (Hodson et al., 1987). They are important recreational fishing species and have been stocked into reservoirs in most States (Smith et al., 1985). Commercial catches of striped bass declined from over 15 million pounds in 1973 to less than one million pounds in 1986 due to overfishing, pollution and fishing regulations (Harvey et al., 1990; Hodson et al., 1987). Hybrid striped bass are primarily raised in ponds, but also, in cages, net pens, raceways and tanks since they can tolerate fresh, brackish and marine waters.

Smith et al. (1985) compared production characteristics of F_1 and F_2 crossed hybrids reared in an intensive culture tank system. They concluded that the original cross F_1 (female striped bass x male white bass) was highly desirable to raise by intensive aquaculture techniques. This cross showed rapid growth, a high survival rate, resistance to diseases and handling stress, a high production level,

ability to grow in fresh or brackish water, and good utilization of commercial feeds. The reciprocal cross hybrids (female white bass x male striped bass) were also proven to be superior to F_2 (F_1 original x F_1 original) hybrids. Other desirable traits of hybrid striped bass are that they can survive in a wider range of temperatures than striped bass and have an increased body depth which gives more edible flesh per fish and less waste (Harvey et al., 1990).

Currently, the marketing of hybrids may be restricted because law enforcement agencies may not easily distinguish between farm raised or aquacultured hybrid striped bass and wild caught striped bass which are protected. In some states, laws may prohibit the sale of striped bass and hybrid striped bass because they are considered game fish (Hodson et al., 1987).

B. Intensive Aquaculture

Aquacultured finfish and shellfish comprise an increasing share of all edible fishery products which is now approximately 12% of the worldwide supply. Additionally, U.S. aquaculture production has increased 290% between 1980 and 1989 (Harvey et al., 1990).

The rapid growth of aquaculture in recent years is due to several factors including a continued decline of capture

fishery production, increases in seafood consumption and concerns over contaminated finfish and shellfish natural environments (Harvey et al., 1990).

Currently, most aquaculture efforts utilize extensive methods since intensive culture systems are expensive due to high overhead costs and difficulties with strict water quality management. Water quality parameters that must be continuously monitored include pH, alkalinity, hardness, dissolved oxygen, nitrite and ammonia. As the aquaculture industry expands, more operations will turn to intensive culture techniques. While they require close monitoring of all growing conditions, there is much less water usage and land needed than in extensive culture methods. Also, intensive systems are more useful for research since experimental conditions can be more easily replicated (Stickney, 1979a). Other advantages of intensive culture systems are that they are generally easy to harvest from, and predator losses are minimized (Stickney, 1979b).

A recirculating aquaculture system is one type of intensive aquaculture system, and is generally considered to be semi-closed. This system usually requires the constant addition of small amounts of water to replace that lost to leakage, evaporation or splash out (Stickney, 1979a). Also, the density of fish is very high in the culture tank. The water is continuously pumped and filtered to remove solid

wastes and reduce ammonia levels.

C. Factors Affecting Quality and Shelf Life

Spoilage of seafood leads to a loss of quality and decreased shelf life due to a variety of causes including microbiological, such as deterioration of organoleptic properties; chemical, such as rancidity; and physical, including freezer burn or dehydration (Curiale, 1991).

Seafood spoils quicker than mammalian muscle due to its higher water content, higher free amino acid content, less connective tissue, higher enzyme activity and higher ulitmate pH (Pedrosa-Menabrito and Regenstein, 1988).

1. Stress and Pre-harvest Condition of Fish

The physiological state of fish prior to capture or harvest has a profound effect on the ultimate quality. For example, starving fish can enter rigor mortis almost immediately after death, and spawning fish are in their worst condition with very soft, watery flesh (Love, 1988). Also, Love (1988) reported that the post-mortem pH of Atlantic cod will be low in well-fed fish, and near neutral for starving or spawning cod.

Digestive enzyme activity is accelerated in the gut of a struggling or stressed fish. The effect is even greater when the fish has been feeding prior to capture. Autolysis

can proceed into the edible flesh after the viscera (Pigott and Tucker, 1990; Venugopal, 1990).

Fish cannot be hauled live to a processing plant under the influence of an anesthetic and, therefore, may become stressed during transport (Stickney, 1979b). This stress leads to corresponding changes in the time and onset of rigor, and numerous biochemical and physical effects.

Carbon dioxide anesthesia prior to slaughter has been used in the poultry and swine processing industries to reduce ante-mortem stress (Ring and Schlager, 1988; Zeller et al., 1988). Mitsuda, et al. (1980) successfully used CO_2 to anesthetize carp. They found that the period of sedation could be controlled by water pH through control of the dissolved carbon dioxide concentration. Sedation by CO_2 produced similar swimming patterns to drug anesthesia, and was considered to be safer and less expensive.

Overcrowding of fish can cause an increased spoilage rate in finfish. When fish are crowded together in a culture tank or harvest net their vigorous movements lead to scale losses and greater entry or penetration of spoilage organisms into the skin and flesh.

2. Metabolic Activity at Slaughter

After capture, a stressed fish expends energy swimming or struggling with a reduction in muscle glycogen.

The breakdown of muscle glycogen to lactate occurs during anaerobic glycolysis and proceeds through the intermediate pyruvate. Black et al. (1962) demonstrated changes in glycogen, pyruvate and lactate in rainbow trout after various levels of muscular activity. Muscle glycogen was depleted by more than 50% from resting levels after the first two minutes of severe activity, accompanied by a sudden accumulation of pyruvate and lactate. After eight hours, resting levels of pyruvate and lactate returned, but muscle glycogen levels took more than 24 hours to recover. In fish, blood lactate levels can remain elevated for long periods since it is only slowly removed from muscle. In moderate exercise, the rate of depletion of muscle glycogen is low.

3. Rigor mortis

Rigor mortis refers to the post-mortem condition of warm-blooded animals and fishes when the muscles become contracted and rigid, due to the disappearance of adenosine triphosphate (ATP) (Partmann, 1963). Rigor mortis may not develop until the glycogen reserve of the animal has been depleted, until then, the breakdown of ATP and its resynthesis by the glycolytic cycle will take place. In the absence of ATP, permanent actomyosin crosslinks are formed which cause muscle stiffening (Pedrosa-Menabrito and

Regenstein, 1988).

Rigor mortis is especially important in fish preservation because it can retard post-mortem autolysis and bacterial decomposition. Shelf life can be prolonged by procedures to lengthen rigor mortis such as lowering of muscle activity, careful handling and lowering of holding temperatures. The time and onset of rigor mortis is affected by the degree of struggling on capture and is hastened by exhaustion prior to death (Korhonen et al., 1990). Crawford et al. (1970) found that exercised tuna went into rigor within half an hour, while rested fish had weak signs of rigor up until the time of the canning process. Rigor mortis is also hastened by a lack of oxygen and warm temperatures and delayed by low pH and cooling (Frazier and Westhoff, 1978; Mayer and Ward, 1991).

4. Post-mortem Microbial Changes

Fresh fish muscle provides an excellent substrate for the growth of spoilage microorganisms because of high water activity, near neutral pH and a high level of soluble nutrients. The greater exposed surface area of cut fillets can further enhance the rate of microbial spoilage (Liston, 1980).

Freshly caught fish may harbor 10^2 - 10^6 bacteria per cm^2 of skin or per gram of gill tissue, but muscle tissue is

relatively sterile. Microbial levels in the alimentary canal depend on the quantity of food present, and can range from 10^1 to 10^7 cfu/gram if fish have been feeding recently (Liston and Matches, 1976; Shewan, 1977).

As bacterial numbers increase during cold storage to 10^7 cfu/gm or higher, psychrotrophic Gram negative organisms of the genera Psuedomonas and Alteromonas dominate the spoilage microflora (Venugopal, 1990; Kraft and Rey, 1979). These organisms can grow near temperatures of 0°C and can attack the amino acids methionine and cysteine with the production of hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulfide ($(CH_3)_2S$). When bacterial levels exceed 10^6/gm, significant amounts of these volatile sulfur compounds are produced and spoilage becomes organoleptically evident (Shewan, 1977; Liston, 1982). Other commonly found spoilage organisms include Acenitobacter - Moraxella, Flavobacterium and Vibrio spp. (Liston and Matches, 1976).

Other metabolites of post-mortem microbial action can yield objectionable odor and flavor compounds. These include the volatile bases such as diamines, cadaverine, histamine and ammonia from the microbial degradation of amino acids (Jacober and Rand, 1982). Additionally, bacterial action leads to the development of acetic acid, CO_2 and H_2O from carbohydrates and lactate. Also, glycine,

leucine and serine can be converted to esters of acetic, propionic, butyric and hexanoic acids and production of fruity odors (Shewan, 1977).

Gram et al., (1987) isolated 309 strains of bacteria from spoiled fish and found that Gram negative, non-fermentative, motile rods of Alteromonas sp. were the major spoilage (H_2S-producing) organisms after 0°C storage. After storage at 20°C, the predominate organisms were Gram negative, fermentative, motile rods belonging to the family Vibrionaceae and Alteromonas.

The ability to reduce trimethylamine oxide (TMAO) to trimethylamine (TMA) or to produce H_2S are regarded as prominent characteristics of fish spoilage bacteria (Gram et al., 1987). But, not all psychrotrophic bacteria can produce TMA from TMAO, therefore evaluation of spoilage by TMA formation may not be valid (Kraft and Rey, 1979).

Higher levels of microorganisms are found on fish from tropical and subtropical waters, but psychrotrophic organisms, which are the predominant spoilage microflora, are more abundant in fish from colder, temperate waters (Shewan, 1977). Therefore, the shelf life of fish from temperate waters is generally considered to be shorter (8-12 days) than fish from tropical or subtropical waters (2-3 weeks).

5. Post-mortem Chemical and Physical Changes

Post-mortem changes in fish flesh include the lowering of the pH from near neutral to approximately 6.2-6.5 due to the anaerobic degradation of glycogen to pyruvic and lactic acid, and the enzymatic degradation of ATP (Barrett et al., 1965; Bendall, 1973; Korhonen et al., 1990; Sikorski et al., 1990). Lactic acid accumulation is the principal factor in determining post-mortem muscle acidity (Tarr, 1966).

If fish struggle or swim excessively prior to death, then glycogen reserves will be depleted, and post-mortem pH will be higher than in rested fish where lactic acid may accumulate through glycolysis. For example, Botta et al. (1987) showed that the muscle pH of cod caught by gillnet was significantly higher than that of cod caught by longline or handline.

The quantity of muscle glycogen at death does not always relate to the ultimate post-mortem pH due to incomplete conversion to lactic acid. Variations occur in the quantity of glycogen converted hydrolytically to glucose by amylases, which does not affect pH (Love, 1979; Tarr, 1966).

Flesh texture in fish is toughest during rigor and when pH is low (Dunajski, 1979). Even though a relatively low pH can provide a desirable firm flesh texture, an excessively low pH toughens flesh by increasing the resistance of the

myofibrils to rupture. Furthermore, cold (frozen) storage of fish will further toughen flesh texture due to maintenance of greater integrity of whole cells, and will likely make the product unacceptably tough (Love, 1979 and 1988). Microbial and enzymatic spoilage leads to a softer flesh texture due to weakening of connective tissue and fragmentation of myofibrils. In advanced spoilage, endogenous bacterial proteinases degrage myofibrilar proteins (Sikorski et al., 1990).

Flesh color can also undergo post-mortem changes. As with red meats, the muscle heme pigment myoglobin can become oxidized to oxymyoglobin and further oxidized to metmyoglobin. The color of myoglobin is best retained during 0°C storage. Also, Brown et al. (1967) reported that a higher pH was associated with better overall color in canned tuna. Higher pH is associated with a slower rate of oxidation of oxymyoglobin and oxyhemoglobin to the less desirable metmyoglobin and methemoglobin.

In fish after death, decomposition of adenosine triphosphate (ATP) proceeds through adenosine diphosphate (ADP), adenosine 5'-phosphate (AMP), inosine 5'-phosphate (IMP), inosine (HxR), hypoxanthine (Hx), xanthine (x) and finally, uric acid. In a live fish, ADP is produced in muscle contraction and can be rephosphorylated either by respiratory action or by phosphocreatine; therefore the AMP

level remains low. After death IMP accumulates via dephosphorylation and deamination of ATP (Surette et al., 1988).

IMP is produced at death and early post-mortem, and then, degraded relatively slowly to HxR and bitter tasting Hx. The degradation process is primarily autolytic and catalyzed by the enzymes IMP phosphohydrolase and inosine ribohydrolase (Hiltz et al., 1971). The gradual loss of IMP during storage is largely responsible for the loss of typically desirable fish flavor (Karube et al., 1984; Sikorski et al., 1990; Surette et al., 1988).

Surette, et al (1988) showed that bacterial enzymes contribute to nucleotide catabolism in cod fillets stored at 3°C. Changes in IMP, HxR, and Hx concentrations were measured in sterile and non-sterile tissue over 14 days. They found that the rates of IMP degradation were similar, possibly because levels of spoilage bacteria are low and biochemical changes during the first few days of storage are primarily due to autolysis. Beyond day four of the study, the disappearance of inosine and accumulation of hypoxanthine was accelerated in the non-sterile samples. Also, they suggested that nucleotide degradation is affected by mechanical handling of the fish. They found an accelerated rate of decomposition in filleted fish versus gutted and whole fish. Mechanical damage to the flesh can

lead to enzyme decompartmentalization and a rapid onset of autolysis.

Enzymatic spoilage can increase in muscle stored at temperatures between -1°C and -5°C since enzymes can concentrate as the water in the fish freezes. Also, a slow freezing procedure leads to the formation of ice crystals which can break cell membranes and lead to greater enzymatic decay and loss of texture. Storage of fish below -5°C slows chemical activity greatly in muscle, while storage below -30°C can prevent enzymatic spoilage (Jacoby, 1987).

Muscle proteins denature during cold storage because of ice crystal damage, dehydration, increased salt concentration, and a change in pH due to the removal of water in ice formation (LeBlanc et al., 1988). During frozen storage, muscle proteins interact gradually to form insoluble complexes and thaw drip increases (Awad et al., 1969).

Proteolysis is negligible initially post-mortem, but the non-protein nitrogen compounds in muscle, especially TMAO and amino acids, can be attacked with the production of TMA, dimethylamine (DMA), NH_3 and volatile acids. Deamination of amino acids is the primary pathway leading to ammonia and volatile fatty acids accumulation in muscle tissue. Proteolysis becomes more important in later stages of spoilage when most of the free amino acids have been

depleted (Liston, 1982).

Trimethylamine oxide (TMAO) is believed to be involved in the osmoregulation of marine fish, as well as being a part of the body's buffer system. Saltwater fish generally lower their concentration of TMAO to a level that counteracts the osmotic pressure of their environment. The breakdown of TMAO to trimethylamine (TMA), dimethylamine and formaldehyde has been implicated in fish spoilage. TMA is volatile and has a very low odor threshold. Formaldehyde is believed to react with fish proteins to accelerate undesirable textural changes (Regenstein et al., 1982).

6. Post-mortem Oxidative Changes

Lipids can undergo deterioration during storage and produce off-flavors (Awad et al., 1969). Low-fat (1-2%) fish have a very mild flavor if fresh, since minimal rancidity of fat takes place. High-fat (>6%) fish can be more flavorful when fresh, but fats can oxidize rapidly to produce off-flavors (Gorga and Ronsivalli, 1988a). For example, Regenstein and Santos (1990) reported that lipid oxidation limited the shelf life of frozen stored fatty fish (mackerel), but the use of vacuum packaging or the application of the antioxidant erythrobic acid prolonged shelf life.

7. Harvesting, Handling and Processing

Freshly caught fish is both delicate and highly perishable and must be handled and processed carefully to assure optimum quality. Various harvesting and processing procedures can have a significant effect on final product quality and shelf life. For example, fish that have been caught by trawl may carry 10-100 times the bacterial load of line caught fish due to dragging the catch along the sea bottom where bacterial levels are high. In addition, when fish are hoisted onto a trawler the gut contents of many fish will be expressed onto other fish of the catch (Shewan and Hobbs, 1967). Botta et al. (1987) demonstrated that the method of catching raw Atlantic cod had important effects on sensory quality. Color grades were lower when these fish were caught by a gillnet as a result of the fish struggling extensively prior to being brought onboard ship.

Freshly caught fish that are iced immediately and held in ice can remain of high quality for eight to nine days and of edible quality for approximately two weeks. Unfortunately, fish are not often held at a constant temperature throughout distribution channels. Ideally, the temperature should be held near 0°C as the fish passes from the fishing vessel to the processing plant, warehouse or distribution center, retail outlet and finally the home or a foodservice establishment. Holding temperatures of 42°F

(5.6°C) can reduce shelf life to six days, and a lowered storage temperature of 29°F (-1.7°C) can extend shelf life to three to four weeks (Ronsivalli, 1982).

The fillet process is a chief source of post-harvest contamination of fresh seafood. Often fish are held in water with high bacterial levels from previously cleaned fish or are piled on top of each other prior to filleting. Fillet boards and knives can be the largest source of post-harvest contamination if they are not rinsed and sanitized properly or frequently (Shewan and Hobbs, 1967).

Spoilage in fish is mostly a surface phenomenon since most enzymes and bacteria require oxygen. Therefore, fillets and smaller fish with a high surface-to-volume ratio will spoil at a faster rate (Gorga and Ronsivalli, 1988c). In some cases, filleting and bleeding of fish can delay spoilage. Fillets are less susceptible to autolytic spoilage by digestive tract enzymes than either whole or headed and gutted fish. Also, bleeding of fish prior to processing removes heme compounds which accelerate oxidative rancidity (Jacoby, 1987).

8. Preservation

Many preservation techniques are available to the seafood industry, such as drying, salting, smoking, fermenting, pickling and canning. Other techniques include,

the use of chemical preservatives, modified atmosphere packaging, and, in some countries, irradiation (Shewan and Hobbs, 1967). Nevertheless, lowering storage temperatures through chilling, refrigeration and freezing remain as the most important methods to preserve the quality of fish and fish products. After processing, spoilage is accelerated by a lack of chilling and poor storage conditions, or deficiencies in the distribution and marketing of the final product (Pedrosa-Menabrito and Regenstein, 1988).

Temperature is the most important factor limiting spoilage rate and microbial growth. Temperatures generally must be held below -10°C before bacteria cease to grow and multiply (Jacoby, 1987). Psychrotrophic gram-negative organsims of the genera Pseudomonas and Alteromonas predominate during cold storage of fish (Venugopal, 1990; Kraft and Rey, 1979). Psychrotrophic bacteria grow well at low temperatures even though their optimum growth may be at 10-30°C. While refrigeration can lower their growth rate, freezing can maintain low bacterial numbers (Kraft and Ray (1979). A temperature of -18°C is considered the proper storage temperature for frozen fish (Awad et al., 1969).

Many studies have reported the effects of storage temperature on shelf life of fish. Lee and Toledo (1984) reported that the shelf life of mullet increased from seven to ten days when the storage temperature was lowered from

0°C to -2°C. Storage of fresh yellow perch fillets at 42°F (6°C) reduced storage life by at least 50% from fillets held at 33°F (1°C) (Emerson et al., 1966). Gorga and Ronsivalli (1988c) stated that the shelf life of fresh fillets held at 5.6°C (42°F) is six days, but at 0°C increases to two weeks, and then to three to four weeks at -1.7°C (29°F).

Physical and chemical changes can occur in frozen muscle even though microbial changes are inhibited (Awad et al., 1969). For example, Regenstein et al., 1982 reported that freezing and frozen storage of fish can cause textural changes which often decrease the water retention of muscle proteins, particularly in fish of the Gadidae family. This process is probably due to the enzymatic breakdown of TMAO to dimethylamine and formaldehyde, which is believed to react with the fish proteins to accelerate undesirable textural changes.

In the United States, there is still a common belief among consumers that fresh fish is superior than frozen, and therefore, is of higher quality and should command a higher price (Ronsivalli, 1982). The consumer demand for fresh over frozen fish and requirements for longer shelf life prompts researchers to consider alternative preservation methods to freezing.

Modified and controlled atmosphere systems are increasingly used in the seafood industry. These systems

may use combinations of gases including carbon dioxide (CO_2), nitrogen (N_2), oxygen (O_2), carbon monoxide (CO) and sulfur dioxide (SO_2) (Pedrosa-Menabrito and Regenstein, 1990a). The use of these gases in controlled or modified atmosphere packaging (MAP) must consider the optimum concentrations to produce an inhibition of bacterial growth, and the potential for the development of Clostridium botulinum toxin under anaerobic conditions. Also, MAP without oxygen can retard the oxidation of myoglobin to metmyoglobin in fresh and frozen fish (Pedrosa-Menabrito and Regenstein, 1988).

The effect of carbon dioxide on microorganisms in foods is to increase the lag phase and generation time of spoilage microorganisms, but the mechanism for this bacteriostatic effect is not clear (Daniels, 1985). Among the proposed mechanisms of action are 1) displacement of oxygen which slows the growth rate of aerobes, 2) lowering of internal cell pH and effects on metabolic processes since CO_2 easily penetrates the cell wall after solubilization to carbonic acid (H_2CO_3) and 3) disruption of cell enzyme systems. Jensen et al. (1980) and Senstrom (1985) demonstrated an extension of shelf life of cod and cod fillets held at 2°C under elevated CO_2 atmospheres.

Daniels et al. (1986) demonstrated that cod fillets dipped in carbonic acid (pH 4.6) achieved a moderate

extension of shelf life due to inhibition of surface spoilage microorganisms. This treatment compared favorably with fillets stored in a 98% CO_2 atmosphere during 21 days of storage at 2°C. They concluded that the dip could enhance bacteriostatic effects early in the storage period compared to the slow solubilization of carbon dioxide to carbonic acid in MAP.

The National Marine Fisheries Service has developed a CO_2-saturated refrigerated sea water system. They demonstrated that this medium was effective in extending the shelf life of whole halibut, yellowtail rockfish, pink shrimp and dressed chum salmon when compared with refrigerated sea water or ice storage (Nelson and Barnett, 1971).

The use of carbon dioxide in modified atmosphere packaging has the potential to allow the growth of anaerobic pathogens under high CO_2 and low O_2 concentrations (Daniels et al., 1985). Other drawbacks to the use of carbon dioxide in MAP are that CO_2 dissolves into the fish liquids and can cause deformation of the package, and that highly pigmented fish can become discolored (Senstrom, 1985).

Irradiation may become an important method of fish preservation in the future. Emerson et al. (1966) found that the storage life of unirradiated yellow perch fillets held at 1°C was approximately 10 days, whereas irradiation

with 0.3 or 0.6 megarad of cobalt-60 gamma radiation increased the storage life four or five-fold as measured by a sensory evaluation panel. Similar effects were found by Przybylski et al. (1989) for iced catfish fillets with and without CO_2 modified atmospheric packaging. But, in this study, sterilizing doses of radiation reduced sensory quality.

D. Indicators of Quality and Shelf Life

The intrinsic quality of fish depends on several factors including the species (i.e. percent body fat), size, sex, condition or composition (i.e. spawning), environmental conditions (i.e. toxic elements, pollution), parasites, disease or physical damage (Pedrosa-Menabrito and Regenstein, 1990b).

Many methods are available to assess the quality and shelf life of fish including subjective or organoloeptic evaluations and objective or instrumental methods. Objective tests usually only gain acceptance when they correlate well with sensory or organoleptic analyses. On the other hand, many researchers consider some subjective tests to be unreliable (Gorga and Ronsivalli, 1988b).

1. Organoleptic Measurements

The appearance, odor, flavor and texture of seafood are

all accepted criteria for quality judgments (Gorga and Ronsivalli, 1988a). Sensory analysis is popular in the food industry because it can be inexpensive, effective and samples can be quickly judged of acceptable or non-acceptable quality (Gorga and Ronsivalli, 1988b; IFT, 1981). Typically, analyses involove the presentation of several samples of a product to an experienced or trained panel of judges who are asked to rate or rank various attributes of the product or to indicate their preference for a sample. For example, Reed et al. (1983) used an experienced taste panel to rate the odor and flavor of chilled catfish. The panel rated samples stored for seven days at -2.2°C significantly higher than those stored in ice.

Several compounds contribute to overall fish flavor which can be judged by subjective measures. Fresh fish flavors primarily result from glycine, glucose (glycogen breakdown), and pleasant tasting IMP. During storage, IMP is catabolized to tasteless inosine and then to bitter hypoxanthine. The gradual loss of IMP is largely responsible for the loss of typically desirable fish flavor (Karube et al., 1984; Sikorski et al., 1990). Also, during post-mortem spoilage, fatty acids with distinctinve flavors are liberated from relatively tasteless triglycerides.

Izutsu and Wani (1985) suggest that the most important sensory property is texture in foods, such as fish, which

have a relatively low flavor intensity. For example, in high quality fish, the raw tissue is resilient after depression, and resumes its original shape when pressure is removed. Or, in cooked samples, sensory panel judges may rate the chewiness or moistness of masticated samples.

2. Objective Measurements

Instrumental methods to determine fish freshness have the advantages of speed, accuracy, non-destructivity and objectivity.

Aerobically stored and refrigerated meat spoils when bacterial counts exceed 10^7 CFU per cm^2 or per gram. Gram negative psychrotrophs predominate in advanced spoilage even though their initial numbers may be low. One method of determining the level of these spoilage organisms is with the aerobic plate count or psychrotrophic plate count (Liston and Matches, 1976). For example, El Marrakchi et al. (1990) reported that the shelf life of sardines stored in ice for 18 days was considered to be nine days since the aerobic plate count exceeded 10^7 cfu/gm.

Another method to assess bacterial counts in meat is to analyze for aminopeptidase activity. Gram negative bacteria convert L-alanine-p-nitroanilide to p-nitroaniline which can be detected spectrophotometrically. Perez de Castro et al. (1988) developed this method to quickly measure levels of

10^7 cfu/cm^2 in beef and pork muscle.

Pivarnik et al. (1990) used a Torrymeter to assess the freshness of six species of finfish. The Torrymeter measures changes in dielectric properties which occur during fish muscle degradation. They found a significant linear correlation between the sensory panel evaluation of appearance and odor of whole fish and Torrymeter values through eight days of storage at 1-2°C.

The amino acid content of fresh fish is very low, but increases rapidly in spoiled fish. The microbial decarboxylation products of amino acids such as histamine from histidine and tyramine from tyrosine can be used as spoilage indicators. Veciana-Nogues et al. (1990) found a high correlation between these two spoilage indicators and TMA, Hx and pH in anchovies stored at 4-6°C and at 18-22°C.

The Instron Universal Testing Instrument has been used extensively to determine various textural qualities in foods. Often, a Warner-Bratzler Shear attachment or Kramer Shear/Compression cell are utilized with this instrument to measure the force necessary to shear or compress fish muscle and to determine the corresponding firmness (Dunajski, 1979; Manthey et al., 1988; Prusa et al., 1982).

Le Blanc et al. (1988) used an Instron Universal Testing Instrument and Kramer Shear/Compression Cell to measure the peak force of compression of raw and cooked,

previously frozen, cod fillets. The researchers felt that measurements of peak force for compression on raw muscle gave a rapid indication of frozen fillet quality. They found that the peak force was negatively correlated with water content. Thus, as the sample texture deteriorated, the amount of free water increased. Buttkus and Tarr (1962) stated that mechanical measures of fish texture should be performed on cooked samples or related between raw and cooked samples. But, in the previous study, measurements on cooked samples were not reliable, possibly due to variances in cooking procedures such as final product thickness, internal temperature, heat transfer or oven temperature.

For example, Buttkus and Tarr (1962) used a Mangold sclerometer to measure texture in frozen stored fish. The hardness of muscle was measured by its resistance to a deforming force or plunger falling at a constant force and time interval. "Tenderness" was proportional to percent penetration of the plunger.

Concentrations of inosine monophosphate (IMP) and inosine were positively correlated with overall desirability and hypoxanthine (Hx) was negatively correlated with flavor and desirability in cod and pollock (Greene and Bernatt-Byrne, 1990). Hiltz et al. (1971) and Martin et al. (1978) concluded that analysis for hypoxanthine has the best potential for use as a quality index since Hx concentrations

in most species increase progressively with a change in quality from pre-spoilage to definite spoilage. Jacober and Rand (1982) concluded that unlike IMP, hypoxanthine increases as a result of autolytic changes and may be useful as an index of freshness.

Karube et al. (1984) developed an enzyme sensor system to measure concentrations of ATP and its catabolites. An index of fish freshness was developed and designated as K_1:

$$K_1 = \frac{[HxR] + [Hx]}{[HxR] + [Hx] + [IMP]} \times 100$$

This index primarily measures the level of degradation of IMP to inosine (HxR) and hypoxanthine (Hx). Also, they felt that the K value should be considered as a freshness index since ATP, ADP and AMP remain in some species of fish even after two weeks.

$$K = \frac{[HxR] + [Hx]}{[ATP] + [ADP] + [AMP] + [IMP] + [HxR] + [Hx]} \times 100$$

Many other objective measures of fish quality and degree of spoilage are available including analysis of volatiles by gas chromatography (Dupuy et al., 1978) and mass spectrometry, determination of pH, and quantitative color measurements.

3. Problems in Quality Assessment

Determination of post-harvest quality and shelf life can be difficult due to differences in composition both within and between species of fish and shellfish. Diverse or contradictory results of analytical tests for quality and shelf life of the same species have been reported (Flick et al., 1986). Furthermore, the methods of capture, area of the catch and handling and processing techniques make quality assessment difficult (Martin et al., 1978).

Numerous problems exist with the determination of quality of freshwater fish. Studies on the bacterial spoilage of freshwater fish are rare compared to studies on marine fish. Various freshwater species can be found in a wide variety of water tempertures such as arctic lakes or tropical rivers. The fat content and composition of fish can vary greatly with location of catch which can affect processing techniques and spoilage patterns. Other environmental factors can complicate the developement of quality indices such as fish parasites that can lessen appearance and earthy odors and flavors caused by geosmin produced by actinomycetes (Bligh, 1971).

Trimethylamine, a reduction product of trimethylamine oxide, is a volatile amine produced by bacteria growing on the surface of marine fish. Trimethylamine oxide (TMAO) is widely accepted as an indicatior of freshness but this

compound is generally lacking in freshwater fish (Bligh, 1971). In freshwater fish, TMAO either is absent, or if present, is not reduced to TMA. Therefore TMA is not useful for determining quality deterioration in these fish. Furthermore, as a bacterial process, production of TMA would not be useful in determining quality in fish stored frozen (Martin et al., 1978).

No single index of quality can be applied to marine or freshwater fish. Therefore, procedures to determine the end of shelf life may range from organoleptic tests to bacterial counts to quantitation of catabolites such as TMA to combinations of the above.

Ward and Baj (1988) point out that there are many contradictions in the scientific literature concerning the relationship of microbiological techniques to measurements of the quality or shelf life of seafood. Standard procedures among researchers are lacking. Different control methodologies are common, including variations in product storage temperatures from -20°C to 20°C and variations in incubation temperatures (20°C to 35°C) for microbial media. Bacterial processes used for quality indices may not start until shortly before organoleptic spoilage is reached. Tests for TMA, TVB, volatile acids and oxidized lipids (TBA) are limited since they measure compounds indicative of advanced deterioration and spoilage (Jacober and Rand,

1982).

Pedrosa-Menabrito and Regenstein (1990b) outlined other problems with the use of microbiological methods to determine quality. These include that not all species identified or enumerated can cause spoilage, testing may require two or three days to complete, and as new methods of preserving fish are implemented, the levels of bacteria that would indicate potential problems might change. Furthermore, it is possible that spoilage of fresh fish may no longer be due to microbiological processes, primarily, if spoilage organisms are controlled during processing.

Objective measurements of quality or freshness have various advantages and disadvantages over subjective or sensory assessments. A taste panel can be time-consuming and expensive since difficulties selecting and training judges often arise (Buttkus and Tarr, 1962; Dupuy et al., 1976). Also, panelists may fatigue during the testing and test results are less reproducible (Gorga and Ronsivalli, 1988b). But, the use of objective methods such as a Torrymeter to measure quality or the use of hypoxanthine as an index of freshness only measure one property of the seafood (Jacober and Rand, 1982). Another example is the analysis for 2-thiobarbituric acid to measure the developement of oxidative rancidity. Martin et al. (1978) states that this test is unreliable as an index of

freshness, since a wide variation exists between and within species and many factors affect the development of rancidity.

Surette et al. (1988) concluded that no single nucleotide catabolite can be considered as a reliable index of quality since many factors are involved in their decomposition and the rates of the breakdown of intermediates differs between species. Therefore, the use of K and K_1 values to measure the freshness of fish has some drawbacks. Both measure the extent to which IMP is degraded in muscle and do not take into account if inosine (HxR) or hypoxanthine (Hx) is the dominant catabolite. HxR has a positive impact, and Hx a negative impact on overall flavor. Even though the K value includes the added parameters of ADP and AMP, these products are usually converted to IMP within 24 hours post-mortem (Greene and Bernatt-Byrne, 1990).

PART ONE

Use of a Recirculating Aquaculture System and Pre-sacrifice Treatments on Hybrid Striped Bass to Improve Product Quality

III. MATERIALS AND METHODS

A. 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 (Hodson, 1989). 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.).

B. 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 with 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. Toxic nitrogenous wastes are converted to nitrite and then non-toxic nitrate by bacteria living on the rotating biological contactors (Stickney, 1979a). Dissolved 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°C.

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. All fish were fed within 15 hours of harvest time.

C. 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). Two treatment and two control 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 ($NaHCO_3$, 200 g) and calcium chloride ($CaCl_2 \cdot 2H_2O$, 215 g) were added to increase alkalinity and hardness. Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$, 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.

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 (CO_2) was harvested and placed in a holding tank at ARF for approximately 30 minutes. Water (approx. 200 gallons) for the holding tank was obtained from the culture tank and aerated only prior to addition of fish. Carbon dioxide (CO_2) was injected at 10-20 cfh until most fish remained on the bottom of the tank. 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.

D. 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.

E. Analyses

1. Aerobic plate count

Microbial levels of thawed fillets were determined with an aerobic plate count. For each group, a composite sample of 20-25 g from three fillets was tested. The composite was mixed with 0.1% peptone (Difco Laboratories, Detroit, MI) to acheive 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 (Liston and Matches, 1976).

2. 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).

3. 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 (Dunajski, 1979; Segars et al., 1981). 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 used to give a total of 24 tested pieces 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.

4. 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 (Stone and Sidel, 1985). 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.

5. Statistical analyses

Analysis of variance was performed with the General Linear Models procecure (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.

IV. RESULTS AND DISCUSSION

A. 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). Fillets were skinless, boneless, and without belly flap. The fillet operation yielded an average of 29.2% fillets per fish. 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.

In this study, hybrid striped bass were not fed within 15 hours prior to capture, therefore high levels of digestive enzymes that could lead to rapid autolytic spoilage were not present (Thrower, 1988). Fillets were studied because they are less susceptible to autolytic spoilage by digestive tract enzymes than either whole or headed and gutted fish, and they are a popular market form (Thrower, 1988).

B. Aerobic Plate Count

The aerobic plate count of all test groups exceeded finfish spoilage levels of 10^7 cfu/gm (ICMSF, 1986) by day 11 (Figure 1). The cool water treatment fillets reached this level by day 9 and the two control groups about day 10. The fillets from the carbon dioxide treated fish did not

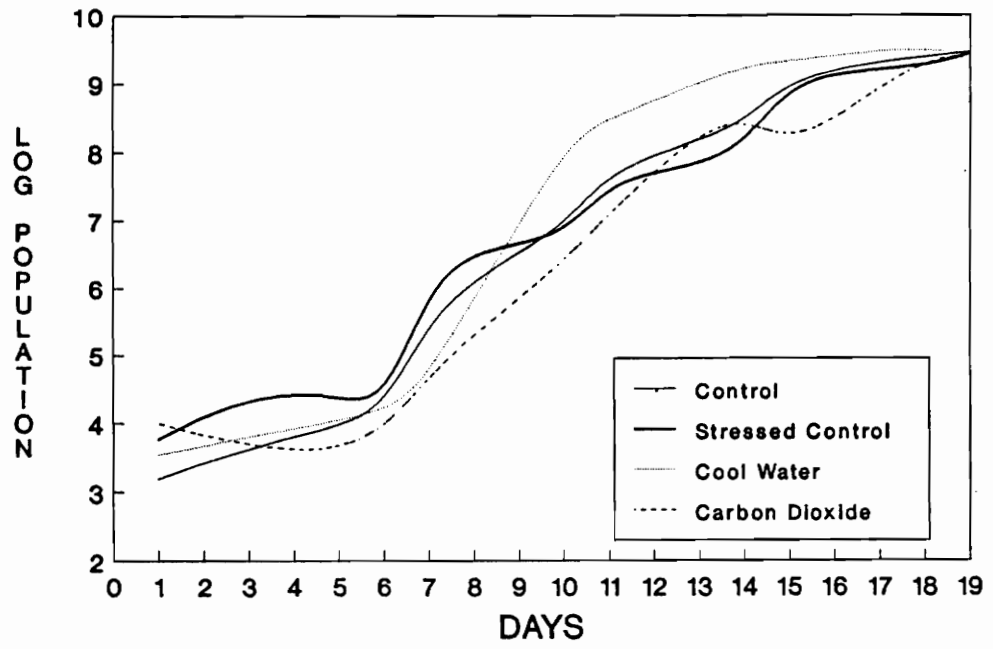


Figure 1 - Mean aerobic plate count of fillets stored at 1-4°C.

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 ($p \leq 0.05$) between test groups.

C. 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 (Figure 2). 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 cfu/gm) over time for all test groups combined.

D. 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

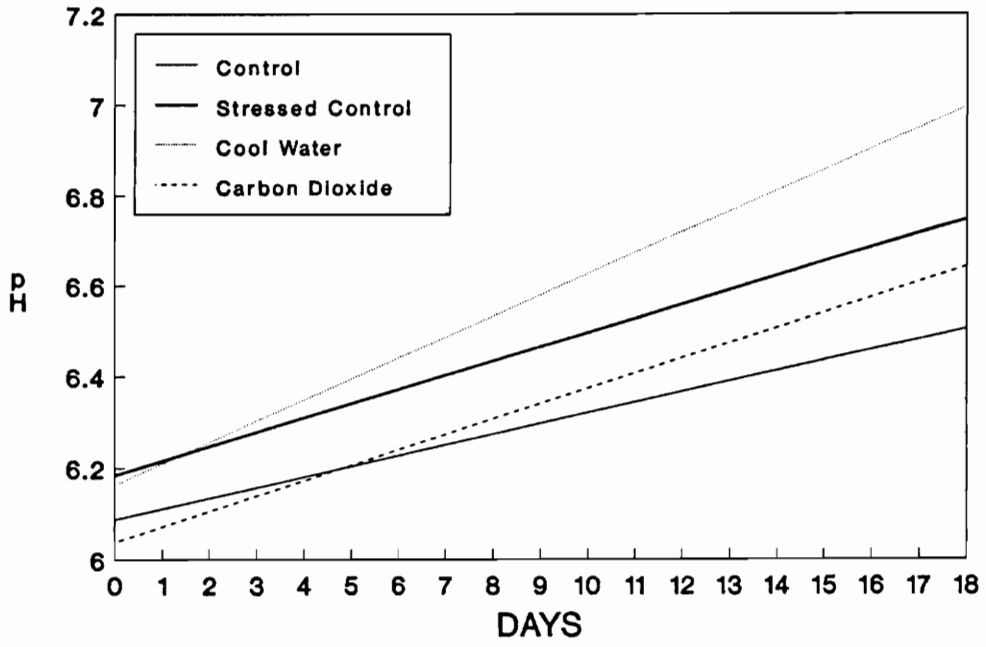


Figure 2 - Mean pH of fillets.

(Figure 3) 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 26.2 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.

E. 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 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

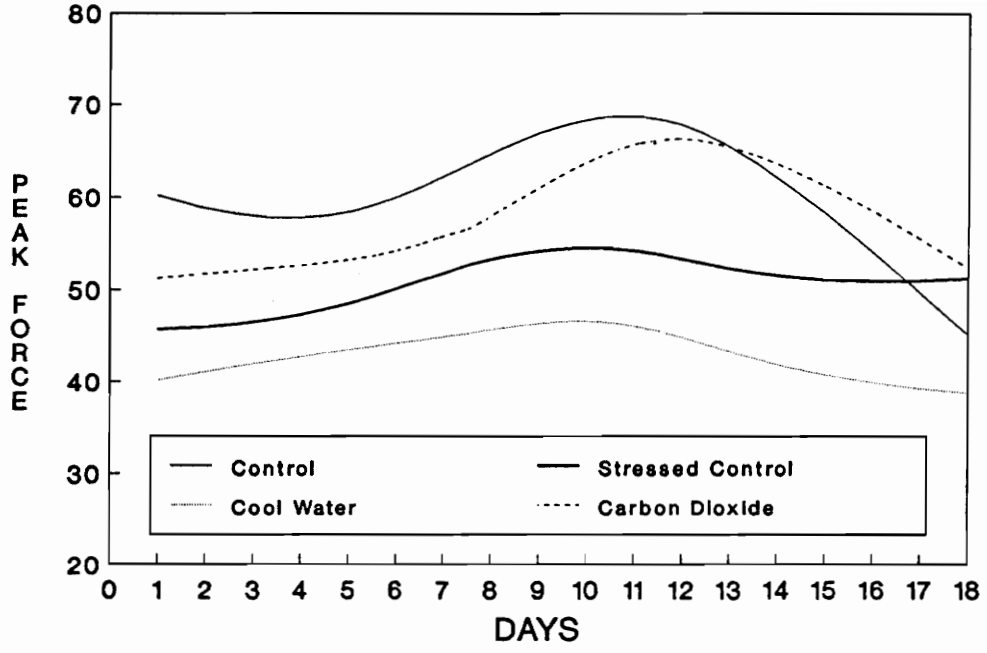
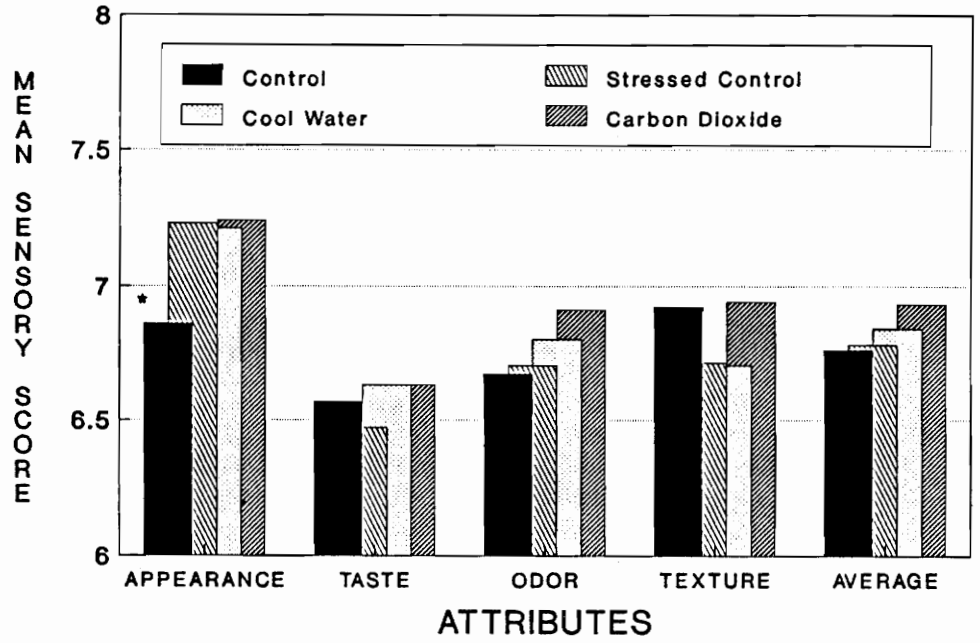


Figure 3 - Mean peak force energy (kgf) for compression of cooked fish portions.

rated lower in appearance than the other three groups throughout the test period. In Figure 4 the sensory scores for all test days 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.

In Figure 5 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).



5=Borderline 6=Fair 7=Good 8=Very Good

Figure 4 - Mean sensory evaluation panel ratings for each test group per attribute. * = significance, $p<0.05$.

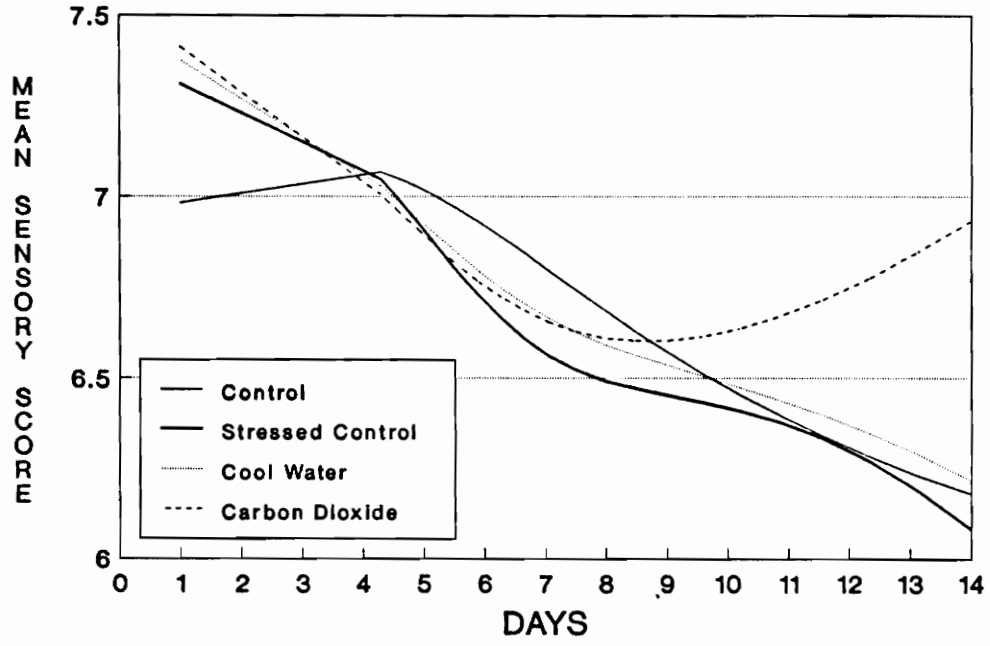


Figure 5 - Mean sensory evaluation panel ratings for each test group over time. Appearance, odor, texture and taste scores are combined.

PART TWO

Quality Changes of Aquacultured Hybrid Striped Bass Fillet Meat Resulting from Post-Harvest Cooling or CO_2 Treatments

V. MATERIALS AND METHODS

A. Source of Fish

The hybrid striped bass used in this study were a reciprocal cross between female white bass Morone chrysops and male striped bass Morone saxatilis. The accepted common name of this cross is the Sunshine Bass. Fingerlings were purchased from Keo Fish Farms (Lonoake, Arkansas) and stocked at 1800 per culture tank (approximately 4 fish per cubic ft.). The fish were fed a high protein floating diet (Biosponge Aquaculture Products, Sheridan, Wyoming) formulated for hybrid striped bass and maintained at the Virginia Polytechnic Institute & State University Aquaculture Research Facility (ARF).

This facility contains nine independent recirculating aquaculture systems. Rotating biological contactors aid to maintain appropriate ammonia, nitrate and nitrate levels. Dissolved oxygen concentration is maintained by surface aerators, injection of liquid oxygen and the use of U-tube aerators that use hydrostatic pressure to supersaturate water with oxygen. Air and water temperatures and lighting in the facility are all controlled. Culture tank water

temperatures at time of fish harvest were 24.4-25.1°C.

B. 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. After arrival at FST all fish except for the stressed control group were sacrificed, weighed and filleted within 30 minutes. All fish survived treatments and transport. The day of sacrifice is considered day zero for all experiments. All analyses began the following day (day one).

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

Stressed control fish were harvested, and placed in a truck mounted holding tank for transport to FST (10 minute trip). 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 and 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. After three hours, the fish were removed by dip net, weighed and filleted.

The cool water treatment group (CW) fish were 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. 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.

The carbon dioxide treatment group (CO_2) fish were harvested and placed in a holding tank at ARF for approximately 30 minutes. Water (approx. 200 gallons) for the holding tank was obtained from the culture tank and aerated only prior to addition of fish. Carbon dioxide was injected at 10-20 cfh until most fish remained on the bottom of the tank. The 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.

C. Sampling of Fillet Meat

Skinless, boneless fillets were removed from all fish within 30 minutes after arrival at FST, except filleting occurred after a three hour holding period for the stressed

control group. Approximately twenty fillets each were placed into separate sanitized plastic boxes and placed into ice chests. Boxes were covered with ice and placed in a refrigerator maintained at 1-4°C.

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

D. Analyses

1. Aerobic plate count

Microbial levels of thawed fillet meats were determined with an aerobic plate count. Each test group was usually sampled on days 1, 4, 7, 11, 14 and 18.

For each group, a composite sample of 20-25 g from three fillets were tested. The composite was mixed with 0.1% peptone (Difco Laboratories, Detroit, MI) to acheive 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 (Liston and Matches, 1976).

2. 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) (Manthey et al., 1988).

3. 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 (Dunajski, 1979; Manthey et al., 1988). Approximately 15 g pieces of raw (experiment 2) or cooked (experiments 1 and 2 only) fillets were tested. Fillets were brought to room temperature prior to testing and cooked pieces were oven baked at 350°F for eight minutes. Two pieces per fillet, and three fillets per group (4) were tested.

Flesh pieces were oriented with the grain perpendicular to the grating of the Shear/Compression Cell. Measurements of peak force in kilograms force (kgf) and total energy in kilograms force meters (kgf x m) to compress the sample to

30% of its original thickness were recorded (Borderias et al., 1983). Instron operating parameters were 100 kg load range for a 500 kg load beam and 200 mm/min head speed.

4. Color measurement

Color was measured on raw fillets prior to cooking for the sensory evaluation panel and total color difference from a standard white plate was calculated. For each experiment, three fillets per group were sampled each test day. Three sets of measurements were taken on each of the three fillets per group. The color of fillet meats was measured with a Minolta Chroma Meter Model CR-200 (Minolta Corp., Ramsey, NJ). The reflective color of the surface of each tested fillet was measured at three points (8 mm diameter area). Color measurements were recorded in the L* a* b* coordinates of the Hunter Lab system (Anonymous, 1990; Berrang et al., 1990). The Minolta Chroma Meter was calibrated with a white plate provided with the meter (L* 97.91, a* -0.68, b* 2.45), and the total color difference of the samples from calibration values was calculated.

5. Sensory evaluation panel

An experienced taste panel of 15 graduate students or employees of the Department of Food Science & Technology was used to evaluate the taste, odor, appearance and texture of

cooked fillets. Fillets were prepared by cutting 10-15 gram portions, wrapping in aluminum foil, and baking for eight minutes in a 350°F convection oven. Each panelist received one piece each of fish representing each of the four test groups. All of these samples were coded with a three-digit random number. A fifth fish portion for each panelist was a portion from the vacuum packaged and previously frozen (-20°C) fillets, and was coded as "A". The panelists were asked to rate the samples with a nine-point scale (1 = inedible, 5 = borderline, 9 = excellent) for appearance, odor, texture and taste (Larmond, 1977; IFT, 1981). Panelists were directed to compare all attributes of the other fillet portions to the reference "A" portion. Sensory evaluation panels were conducted on days 1, 4, 7, 11, and 14.

6. Statistical analyses

Analysis of variance was performed with the General Linear Models procecure (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.

VI. RESULTS AND DISCUSSION

A. Aerobic Plate Count

The aerobic plate count of all test groups exceeded spoilage levels for finfish of 10^7 cfu/gm (ICMSF, 1986) by day 11. (Figure 1). Jahncke et al. (1988) found this level in the aerobic plate count by day 13 in iced hybrid striped bass fillets, and day 16 in iced, headed and gutted hybrid striped bass. The cool water treatment fillets reached this level by day 9 and the two control groups at day 10. The fillets from the carbon dioxide treated fish did not achieve log seven growth of microorganisms until eleven days of storage. Also, log phase growth in the CO_2 treatment fillets was not observed 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 when the differences were combined among the three trials. Differences in levels of aerobic organisms were not significant ($p>0.05$) between test groups.

B. 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 was highest overall and was significantly higher ($p<0.05$) than

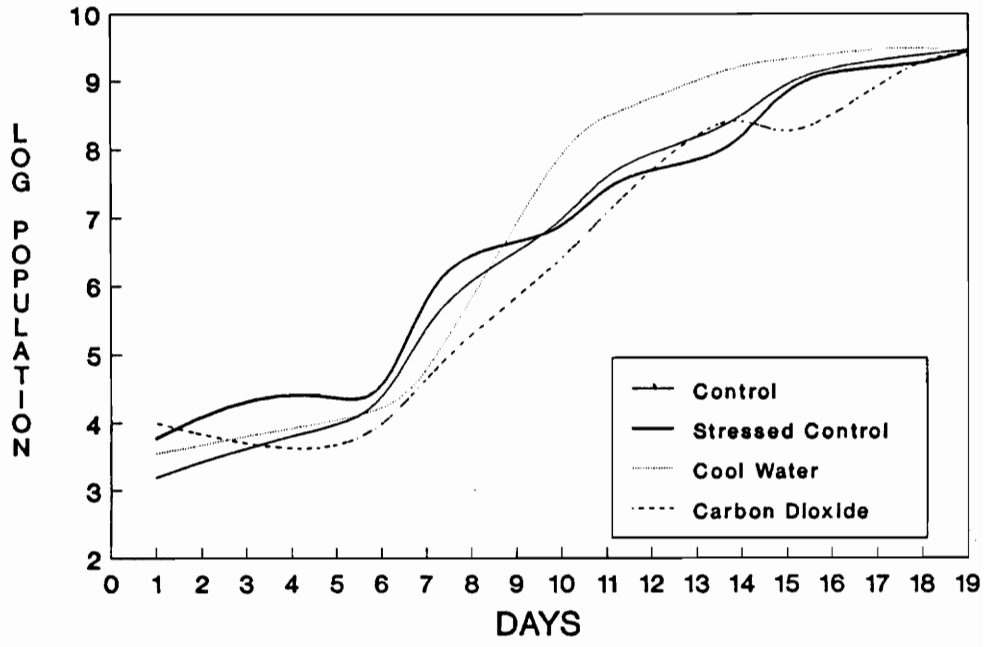


Figure 1 - Mean aerobic plate count of fillets stored at 1-4°C;

Table 1

MEAN pH OF FILLETS

Test Day	Test Group			
	CONTROL	STR CONTROL	COOL WATER	CO_2
1	6.29 ±0.20 [b]	6.27 ±0.23 [a]	6.26 ±0.10 [a]	6.13 ±0.10 [a]
4	6.08 ±0.04 [a]	6.38 ±0.11 [a]	6.47 ±0.27 [a]	6.21 ±0.19 [ab]
7	6.19 ±0.06 [b]	6.30 ±0.00 [a]	6.30 ±0.09 [ab]	6.29 ±0.35 [ab]
11	6.22 ±0.04 [b]	6.39 ±0.41 [a]	6.54 ±0.06 [ab]	6.24 ±0.21 [ab]
14	6.38 ±0.18 [b]	6.52 ±0.37 [a]	6.91 ±0.10 [b]	6.37 ±0.02 [ab]
18	6.64 ±0.38 [b]	6.91 ±0.18 [a]	7.04 ±0.13 [b]	6.83 ±0.38 [b]

Means with the same letter in each column are not significantly different over time for each test group ($p<0.05$).

that of the control fillets (Table 1). A significant correlation ($p<0.001$, $r=0.57$) existed between pH level and aerobic plate count (log cfu/gm) over time for all test groups combined.

C. Texture Measurement

Treatment differences and time differences due to aging of fillets were highly significant ($p<0.001$) for cooked fillet pieces for both total compression energy and peak force energy measurements. A significant correlation ($r=0.87$) was observed between these two Instron measures throughout the test period.

Initial total compression energy on cooked fillets (Figure 2) ranged from an average of 351 kilograms (kgf x m) for the cool water fillets to 420.7 (kgf x m) for the control group fillets. Fillets from the CW treatment fish had significantly lower ($p<0.05$) total energy measures throughout the 18 day test period than the control group and CO_2 group fillets.

Initial peak force measurements on cooked fillet meats (Figure 3) 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 throughout the test period than the control and CO_2 fillets.

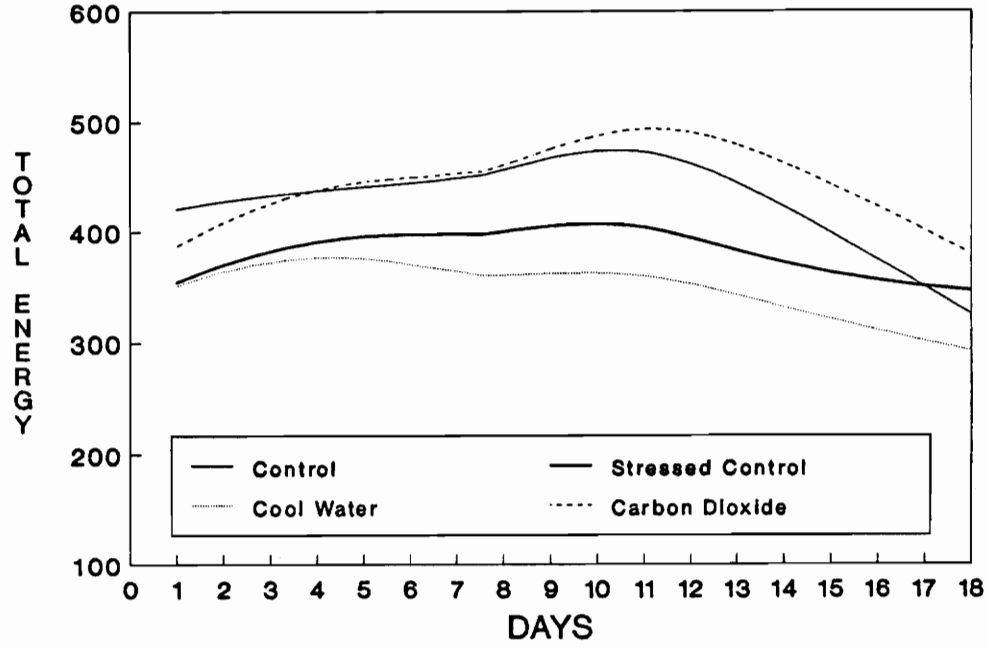


Figure 2 - Mean total compression energy (kgf x m) of cooked fish portions as measured with an Instron and Kramer Shear/Compression Cell.

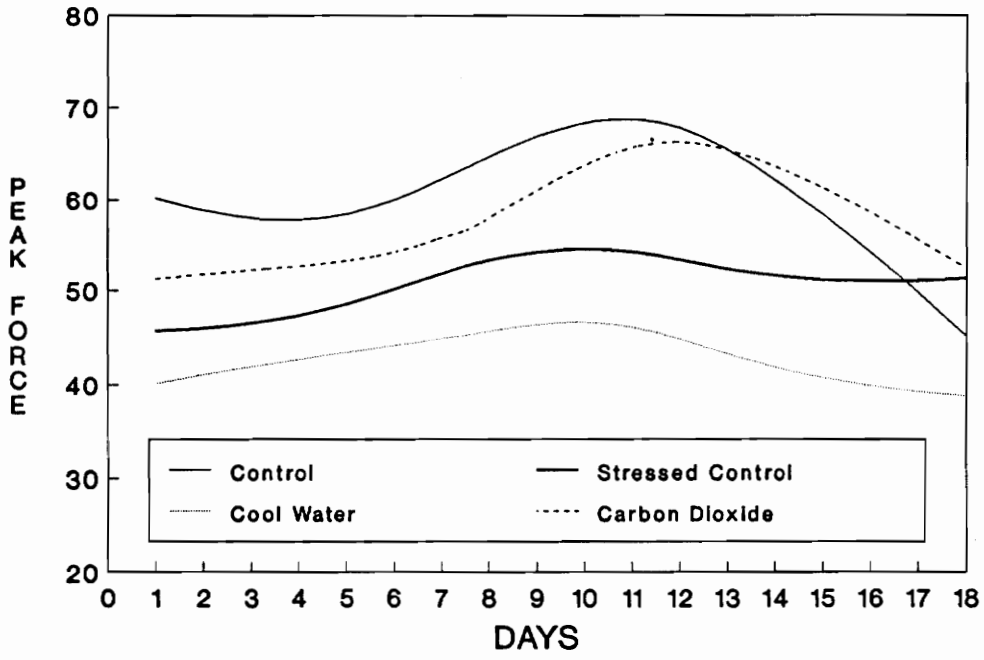


Figure 3 - Mean peak force energy (kgf) of cooked fish portions as measured with an Instron and Kramer/ Shear Compression Cell.

The post-mortem pH is the most important factor affecting flesh texture (Love, 1988). An inverse relationship existed between pH and these two instrumental measures of texture. For example, Figure 4 shows that for the two control groups a higher total compression energy corresponds to lower pH values. Also, decreases in total energy late in the test period occur with an increase in pH value. Similarly, the CW fillets had lower total compression energy scores and higher pH than the CO_2 fillets, and late in the test period pH increases while compression force decreases (Figure 5). Comparisons of peak force measurements (kgf) and pH give similar results due to the high correlation between peak force and total compression energy. The correlation between the relationship of peak force ($r=-0.28$) or total compresssion energy ($r=-0.28$) and pH is significant at $0.05< p <0.06$ in each case. Other researchers have reported high correlation between shear/compression force and pH in finfish (Botta et al., 1987; Kramer and Peters, 1981). Dunajski (1979) found that a decrease of pH by one unit caused a 2.5X increase in toughness scores in cooked fish.

Differences in total compression energy and peak force energy measurements on raw fish portions (one experiment only) were not significant ($p>0.05$) over time (18 days) or between test groups. Average initial (day 1) total

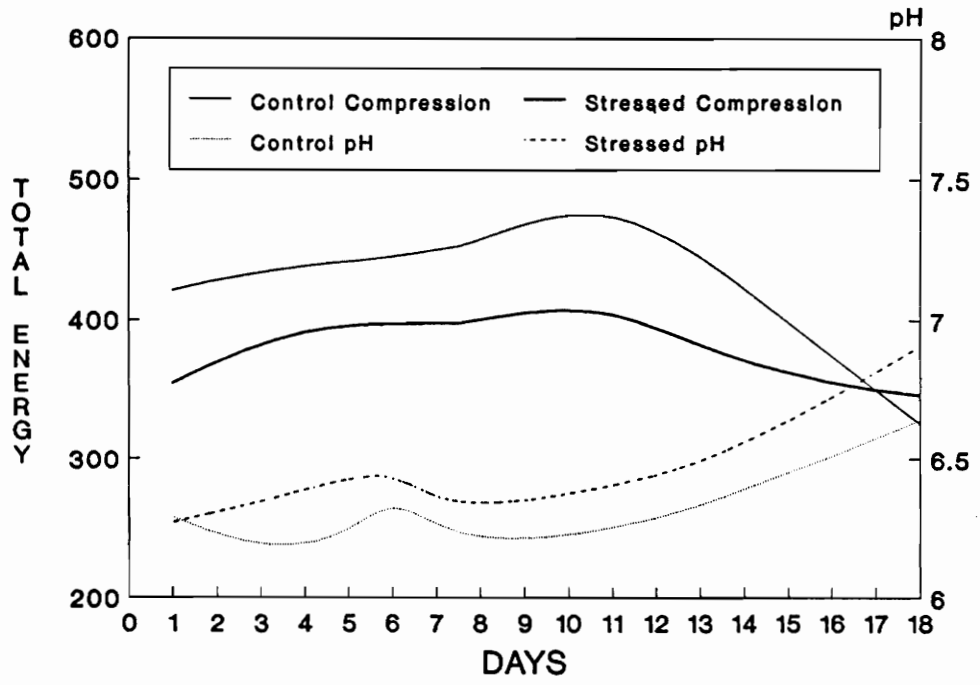


Figure 4 - Mean total compression energy (kgf x m) and pH of control and stressed control group fish.

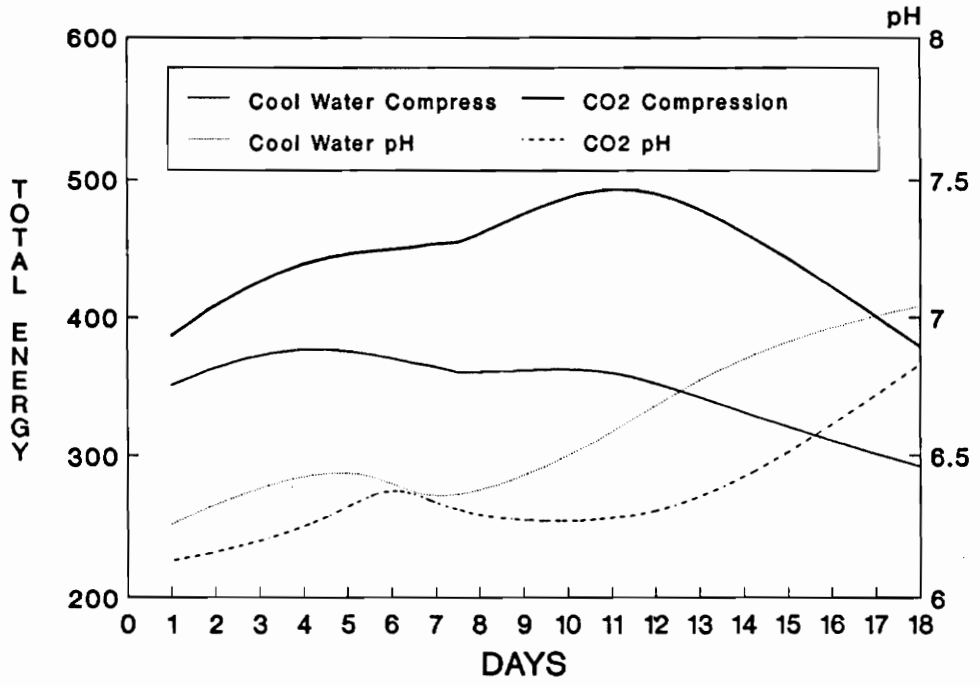


Figure 5 - Mean total compression energy (kgf x m) and pH of cool water and carbon dioxide group fish.

compression energy was 287.8 kgf x m for all raw fish portions and 378.7 kgf x m for cooked portions. Average initial peak force was 26.2 kgf for raw fish portions and 49.4 kgf for all cooked portions. Average final (day 18) total compression energy was 270.6 kgf x m for raw fish portions and 335.7 kgf x m for cooked portions. Average final peak force was 22.7 kgf for raw fish portions and 46.8 kgf for cooked portions.

D. Color Measurements

Treatment differences and time differences due to aging of fillets were highly significant ($p<0.01$) for all four color measurements on raw fillet portions. Significant differences between test groups, though, may be hard to observe visually.

Average L* values for the groups ranged from 53.3 for CW fillets to 59.1 for the stressed control fillets. These CW fillets were significantly darker than both control fillet groups.

Average a* values for the test groups ranged from 4.5 for the stressed control fillets to 7.0 for the control fillets. The stressed control and CO_2 treatment fillets were significantly less red (more green) than the control and cool water fillets.

Average b* values for the test groups ranged from 11.4

for the CW fillets to 13.3 for all three other test groups. The cool water fillets were significantly less yellow (more blue) than the other three groups.

Average color difference values ranged from 42.3 for the stressed control fillet meats (lowest total color difference from white calibration plate) to 46.9 for the CW fillets. Only these two groups were significantly different from each other.

E. Sensory Evaluation Panel

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. The average ratings for each attribute by test group and time are listed in Table 2.

Significant differences ($p<0.05$) over time (day 1-14) exist for all four sensory attributes. Generally, all attribute scores decline over time, but the decline in texture scores is less than that of the other attributes. The largest decreases in attribute scores occurred between days four and seven when mean scores dropped by 0.93, 0.71, 0.59 and 0.22 for taste, odor, appearance and texture, respectively. Generally, the cooked fish was rated acceptable (mean scores >6.0 or fair) in quality for 14 days for each group. Heaton et al. (1972) showed that

Table 2

MEAN SENSORY ATTRIBUTE SCORES

A. APPEARANCE

Test Day	Test Group			
	CONT	S CON	CW	CO2
1	7.17 ^{a}	7.66 ^{a}	7.48 ^{ab}	7.72 ^{a}
4	7.20 ^{a}	7.63 ^{a}	7.63 ^{a}	7.43 ^{ab}
7	6.96 ^{a}	6.88 ^{b}	7.04 ^{ab}	6.67 ^{c}
11	6.68 ^{a}	6.93 ^{b}	6.79 ^{b}	7.14 ^{abc}
14	5.73 ^{b}	6.73 ^{b}	6.87 ^{ab}	7.00 ^{bc}

B. TASTE

Test Day	Test Group			
	CONT	S CON	CW	CO2
1	6.86 ^{a}	6.97 ^{ab}	7.41 ^{a}	7.00 ^{ab}
4	7.13 ^{a}	7.23 ^{a}	6.87 ^{ab}	7.10 ^{a}
7	6.54 ^{ab}	5.75 ^{c}	6.13 ^{bc}	6.17 ^{ab}
11	6.04 ^{b}	6.25 ^{bc}	6.43 ^{abc}	6.11 ^{b}
14	5.93 ^{b}	5.53 ^{c}	5.80 ^{c}	6.73 ^{ab}

C. ODOR

Test Day	Test Group			
	CONT	S CON	CW	CO2
1	7.07 ^{ab}	7.45 ^{a}	7.59 ^{a}	7.69 ^{a}
4	7.30 ^{a}	7.00 ^{ab}	7.20 ^{a}	7.03 ^{ab}
7	6.50 ^{bc}	6.42 ^{bc}	6.33 ^{b}	6.42 ^{b}
11	5.89 ^{c}	6.18 ^{c}	6.25 ^{b}	6.36 ^{b}
14	6.33 ^{bc}	6.07 ^{c}	6.27 ^{b}	7.07 ^{ab}

D. TEXTURE

Test Day	Test Group			
	CONT	S CON	CW	CO2
1	6.83 ^{a}	7.17 ^{a}	7.03 ^{a}	7.28 ^{a}
4	7.07 ^{a}	7.10 ^{a}	6.80 ^{ab}	6.83 ^{a}
7	7.17 ^{aA}	6.29 ^{bB}	6.71 ^{abAB}	6.75 ^{aAB}
11	6.75 ^{a}	6.57 ^{ab}	6.42 ^{ab}	6.86 ^{a}
14	6.73 ^{a}	6.00 ^{b}	5.93 ^{b}	6.93 ^{a}

Scale: 5=borderline, 6=fair, 7=good, 8=very good

Means with the same lower case letter are not significantly different over time for each test group and attribute.

Means with the same upper case letter are not signficantly different between test groups ($p<0.05$).

refrigerated dressed catfish were considered acceptable by a sensory panel for 12, but not 16 days. However, Jahncke (1989) showed that frozen, skinless fillets of hybrid striped bass can retain good flavor characteristics after four to six months storage at -20°C.

A significant correlation did not exist between the texture evaluations by sensory evaluation panel and Kramer Shear/Compression cell measurements. Borderias et al. (1983) also found no significant correlation between indices from instrumental analyses and sensory tests in fillet meats from other fish. Texture scores and aerobic plate counts (log cfu/g) were not correlated ($r=-0.31$, $p=0.053$).

VII. SUMMARY AND CONCLUSIONS

The use of carbon dioxide gas to reduce post-harvest metabolism of aquacultured fish has several advantages over the use of ice or refrigeration to cool live fish. The CO_2 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 (11-14 days) of fillets. Cooked fillets were generally considered to be of acceptable quality after 14 days of refrigeration. Also, Instron texture measurements of peak force and total compression energy were significantly lower for cooked fillets from the cool water treated fish than the CO_2 treated fish. Therefore, these latter fish fillets can be considered to have a firmer and more desirable texture.

Procedures to reduce fish stress during handling and processing are critical to increased productivity of intensive aquaculture (McCraren, 1984). The use of a post-harvest CO_2 treatment to increase the quality and shelf life of fillets 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 (Thrower, 1988). Finally, post-harvest use of carbon dioxide gas on fish awaiting processing is less costly than the use of ice or refrigeration to cool live fish.

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

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IX. VITA

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Mr. Eifert has been employed by Loyola Marymount University as a Chemistry Teaching Assistant (1979-1980). Also, he worked as a Research Assistant for the Veterans Administration Medical Center, Brentwood, California and the UCLA School of Medicine, Los Angeles, California (1980-1983). His recent work experience was as a Chemist for the U.S. Food and Drug Administration in Los Angeles (1983-1989) and as a Graduate Teaching Assistant in the Department of Food Science and Technology at Virginia Polytechnic Institute and State University (1989-1991).