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BACTERIOLOGICAL PROFILE OF SELECTED AREAS OF WHITE AMUR (*CTENOPHARYNGODON IDELLA*) AND ITS FLESH

A Thesis

Presented for the

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Degree

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ABSTRACT

A bacteriological profile was established for selected areas on fresh white amur (*Ctenopharyngodon idella*) and its flesh. The numbers and types of bacteria occurring on white amur fed two different diets (animal or plant) were studied. Significant differences ($P<0.05$) between the diets were found with APC and coliform counts of the flesh which were most likely due to cross-contamination. The types of bacteria isolated from white amur were *Acinetobacter*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Micrococcus*, *Moraxella*, *Pseudomonas*, *Staphylococcus* and *Streptococcus*.

Several storage studies on white amur flesh were conducted. A 200 ppm chlorine dip for 2 minutes reduced the coliform population from an initial log 3.5 to log 0.7 at day 2. The initial APC was also reduced, though not as dramatically. Freezing for 8 weeks at -18°C caused reductions in APC, psychrotrophic, and coliform counts. Storage at 4°C was more effective than 7°C in keeping the coliform population stabilized, but had little effect on APC. The use of CO_2 was significantly ($P<0.05$) effective in reducing the aerobic bacterial load compared to air. In contrast, coliforms showed a small increase in CO_2 atmosphere. Results indicated that bacterial growth on white amur flesh could be controlled using the storage conditions studied.

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CHAPTER I

INTRODUCTION

The white amur (*Ctenopharyngodon idella*) is one of the largest members of the minnow family, Cyprinidae. It has been reported to reach lengths of 4 feet and weights of up to 100 pounds in its natural range (Plieger, 1975). It is native to the Amur River system in Northern China and its natural range extends to Southern China and into parts of Thailand (Riemer, 1984). The white amur, also known as the grass carp, has been introduced by man throughout most of Asia, Europe, and into North America (Riemer, 1984). Except for the United States, its introduction into most countries has been primarily as a food fish. It was introduced into the United States in 1963 by the Federal government at Stuttgart, Arkansas, and by Auburn University, in Alabama, primarily for aquatic vegetation control (Sutton, 1977). Since the white amur's diet consists mainly of aquatic vegetation, its ability to control a wide variety of submerged and floating plants is beyond question (Cross, 1969).

White amur have been shown to be an acceptable human food source in several sensory evaluation studies. In one, 64% of a 28 member taste panel gave scores indicating they liked the taste of the fish (Wilson and Cottrell, 1979).

Another study showed that the white amur ranked ahead of channel catfish and other fish species in several taste tests performed (Avault, 1986). Bakir (1989) reported that the flavor of grass carp fed alfalfa pellets scored as high as that of catfish.

Another reason for considering the white amur as a food source is that per capita consumption of fish has risen dramatically in the past few years (Anonymous, 1987). A large part of the increase is due to increased demand by the food service industry. Interrelated is the fact that consumers have become more health and nutrition conscious. Therefore, the white amur as a cultured fish has potential to become another item alongside the varieties now available.

Since fish flesh is perishable, the way it is handled may greatly influence the quality and value of the final product. The single most important consideration in fish processing is microbial contamination. A major problem concerning the microbiological integrity of fish flesh is the cross-contamination that may occur during the processing procedures. For all practical purposes, it can be said the flesh of unprocessed fish is sterile (Hackney and Garrett, 1985). Thus, contamination of the flesh usually occurs during the cleaning process. Large numbers of spoilage bacteria are found in the skin and mucous, gut cavity (intestines), and mouth and gills. During cleaning,

evisceration, and skinning, bacteria are transferred from these areas of the fish onto all work surfaces, implements, worker's hands, and exposed surfaces of the processing area. These bacteria, in turn, are transferred to the freshly exposed flesh during preparation of the fish for market. It is known that high levels of bacteria may shorten shelflife, cause off-odors or off-flavors, or produce a public health hazard (Byrd, 1973; Castell and Greennough, 1957; Shewan, 1976). Since the white amur has potential to be utilized as a cultured food source, it is pertinent that information be available concerning bacteriological contamination and spoilage of its flesh.

The objective of this research was to compile a bacteriological profile of the white amur flesh in three stages of processing/storage: (1) fresh, (2) spoiled, and (3) frozen. Contaminating bacteria were identified to genera. Several storage conditions for white amur flesh were studied. Potential cross-contamination from areas of the fish such as gills, intestines, and mucous was examined to determine if a correlation existed between the microflora of these areas and the microflora of the flesh due to processing.

CHAPTER II

REVIEW OF THE LITERATURE

A. Microbiology of Fish

Humans consume a broad range of fish and seafood products from the more than 3000 different commercial species of seafoods available globally and more than 250 in the United States (Martin et al., 1978; Otwell, 1986; Ward and Baj, 1988). With such diversity, it would seem that a similar diversity of bacterial organisms might be associated with fish and seafood products.

It is generally accepted that the bacteria associated with fresh fish and fishery products are species of *Achromobacter*, *Aeromonas*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Flavobacterium*, *Micrococcus*, and *Pseudomonas* (Byrd, 1973; Cox and Lovell, 1970; Shewan, 1971). Other workers add to this list *Alteromonas* types and *Moraxella-Acinetobacter* types (Hobbs, 1983). Reported, as well, are such pathogenic types as: *Shigella*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus* (Saddik et al., 1985). Several species of *Listeria* have recently been isolated from samples of frozen seafood products (Weagant et al., 1988). Other researchers have found *Aeromonas hydrophila* to occur on fish flesh and cause its subsequent spoilage (Alur et al., 1988; Alur et al., 1989; Ingham and Potter, 1988a;b).

Several researchers believe the specific types of bacteria found on fish are influenced by harvesting environment. Fish from clean cold waters generally have fewer organisms than fish taken from polluted or tropical waters (Hackney and Garrett, 1985). The reason for this is that cool temperatures of the sea bed (<10°C) make bacterial populations of fish from temperate waters mostly psychrotrophic (Shewan, 1976). Most marine bacteria isolated from the cool waters in the Northern hemisphere have been found to be able to grow at ice (0°C) temperature, even down to -10°C, although their generation time is lengthened by these temperatures (Hess, 1950). Shewan (1976) suggested these psychrotrophic bacteria have comparatively short generation times at temperatures between 0-5°C, and easily outgrow other bacterial types present. Gram-negative rod-shaped bacteria predominate in this psychrotrophic population. Pseudomonads, *Moraxella-Acinetobacter*, *Alteromonas*, *Cytophaga*, *Flavobacterium*, and *Vibrio* species are the primary organisms isolated (Hackney and Garrett, 1985). In contrast, bacteria on fish from tropical areas are thought to be primarily mesophilic, gram-positive microflora with micrococci, bacilli, and coryneforms prevailing (Hackney and Garrett, 1985; Hobbs, 1950; Ward and Baj, 1988).

It is generally recognized that the internal flesh of healthy, live fish is sterile, or normally free of bacteria

(Hackney and Garrett, 1985; Hess, 1950). Therefore, bacterial contamination must come from other sources. Bacteria that exist on fresh fish are generally found in the outer protective mucous covering, the gills, or the intestines (Hackney and Garrett, 1985; Hess, 1950). Other potential bacterial sources are the contaminated contact surfaces on the deck and in the holds of fishing vessels, and in processing plants (Hess, 1950). It is the cross-contamination from these areas that is the important consideration. The microbiology of market fish and seafood is affected by several factors including method of catch and handling, type of processing, methods of preservation and packaging.

B. Fresh Marine Fish

It is believed the microflora of marine fish is halophilic, with microorganisms that are able to grow over a wide range of salt concentrations from <0.5-10% (Hackney and Garrett, 1985). The APHA (1976) reported that most slightly halophilic bacteria contributing to spoilage of fresh marine fish and shellfish originate from marine environments and include the genera *Pseudomonas*, *Moraxella*, *Acinetobacter*, and *Flavobacterium*. For example, Molin and Stenstrom (1984) found the initial flora of herring fillets was dominated by *Alteromonas putrefaciens* and pseudomonads. After spoilage in air, these organisms constituted 62-95% of the flora.

Hobbs (1983) studied the microflora of skin for four different fish and found the following organisms to occur most commonly: *Pseudomonas-Alteromonas* types, 32-60% and *Moraxella-Acinetobacter* types, 18-37%.

Bacteria found on fresh fish muscle are invariably introduced during handling. Cross-contamination from the highly contaminated areas of the fish (mucous, gills, intestines) is of great importance. APHA (1976) reported that fresh-caught fish typically carry populations of 100-1000 bacteria/cm^2 of skin surface or per gram of gill tissue. Bacterial numbers in the alimentary canal may range from very few to $>10^7$ bacteria/g of gut contents. Gram et al. (1989) found the total surface bacterial load on Nile perch (*Lates niloticus*), immediately after catch, was 5 x 10^3-5 x 10^5 CFU/cm^2. Ravesi et al. (1985) found that whole spiny dogfish (*Squalus acanthias*) had the lowest microbial counts compared to gutted or headed and gutted fish. They determined that microbial numbers on filleted fish depended on the level of handling and processing prior to the filleting procedure.

The growth of microorganisms on fresh marine fish is affected by several factors: (1) initial bacterial load, (2) type of bacteria present, and (3) storage conditions such as temperature, humidity, and atmosphere (Alur et al., 1989; Hess, 1950). Effects of microbial growth on fish include changes in color, flavor, odor, and texture resulting in a

deterioration of quality and concurrent reduction in shelflife. A study by Alur et al. (1989) illustrated the effects of spoilage bacteria on stored fish. They first isolated *Pseudomonas marinoglutinosa* and *Aeromonas hydrophila* from spoiling Indian mackerel (*Rastrelliger kanagurta*). These microorganisms were then inoculated in sterile mackerel homogenate and a sarcoplasmic protein fraction. These authors reported that an experienced sensory panel (Miyauchi et al., 1964) reduced odor scores for both fractions from 9 to 3 within 15 days of incubation at 0-2°C.

Schoebitz et al. (1985) studied the microbiological quality of *Trachurus murphyi*, a retail fish species sold in Valdivia, Chile. At the time of the study, fish were piled into wooden boxes, without ice, and taken to market by truck. The fish market was located in the open, next to a river. Fish were held at ambient temperature for up to 2-3 days. Thus, rapid spoilage occurred because of high storage temperature and length of storage before sale. The mean aerobic plate count (APC) of the fish at the time of purchase was found to be 1.91×10^6/g with a range of 3.90×10^4/g-3.12×10^8/g.

Len (1987) investigated the spoilage of fish subjected to inadequate icing and ambient temperature storage. Fish handling procedures used were typical of tropical countries. Fish were first held 4 days at 10°C (partially iced) to

simulate a boat hold. They were then held at 29°C (ambient temperature) prior to sensory analysis to simulate post-catch market conditions. Fish were rejected by odor panelists after 13 hours at 29°C . At rejection, the total bacterial count was 10^8-10^9/g, compared to the initial count (start of 29°C incubation) of 10^3-10^5/g.

Adequate icing or low temperature storage does not prevent spoilage problems. Chen and Hu (1989) discovered that common dolphin (*Coryphaena hippurus*) meat became discolored by green pigment when inoculated with various strains of bacteria isolated from dolphins on board fishing vessels. Discoloration occurred after 6 days at 4°C. Gram et al. (1989) found that the iced storage life of Nile perch inoculated with psychrotrophic spoilage bacteria was reduced by 2 weeks when compared with uninoculated fish. Much of the natural bacterial flora of uninoculated fish was also capable of growth at 5°C, which led the authors to believe the difference in storage was not simply due to a difference in initial bacterial loads. They concluded that the 2-week extended shelflife was due to a reduced growth rate of the natural psychrotrophic flora in addition to low numbers.

In addition to spoilage microorganisms, pathogenic bacteria have been reported to be associated with marine fish and seafood products (Alur et al., 1988; Barrow, 1973; Connell, 1980; Hackney and Garrett, 1985; Saddik et al.,

1985). Saddik et al. (1985) collected samples of raw and cooked fish and other seafoods from hotels, restaurants, markets, street vendors, and small-cook shops in Egypt, and analyzed them for common foodborne pathogens. *Shigella* was isolated from one sample of raw fish and two samples of raw shrimp. *Vibrio parahaemolyticus* was isolated from three fish samples and one raw shrimp sample. Forty-eight percent of raw fish samples contained *Staphylococcus aureus*. It was also found in 30% of raw shrimp and one sample of raw mussels and crab. According to Saddik et al. (1985) and ICMSF (1980), isolation of *Shigella* from raw fish is a rare occurrence. The staphylococci, however, may contaminate fish during capture through contact with on-board materials or during marketing (Saddik et al., 1985). Cooked fish products were found to be free of shigellae and *V. parahaemolyticus* but approximately one-third contained *S. aureus*. Saddik et al. (1985) suggested contamination after cooking as a reason for the presence of *S. aureus*.

Hackney and Garrett (1985) stated that pathogenic marine vibrios such as *V. parahaemolyticus* and *V. vulnificus* have been implicated in illness associated with the consumption of raw or cooked seafood products. Garrett (1988) reported that nearly 90% of all reported foodborne outbreaks of illness associated with seafoods are attributable to shellfish and a few species of fish (i.e., those associated with ciguatoxin and scrombrotoxin).

C. Processed Marine Fish

Processing of fish and seafood products implies subjecting them to particular methods, systems, or handling techniques to effect a desired result. These processes may range from simply filleting (Nickerson and Goldblith, 1964) to complex operations such as surimi production (Elliot, 1986; Lee, 1984). Other processing methods involve special techniques such as freezing, pasteurization, cooking, smoking, or fermentation. Many of these processing methods have been studied to determine their effect on the microbial quality of seafood products.

The microbiological quality of processed seafood may initially be effected by the microflora of the raw product. For example, Pace et al. (1988) found that the microflora of raw oysters (*Crassostrea virginica*) was composed mainly of gram-negative types, with *Moraxella* and *Vibrio* species dominating. Other organisms isolated were *Pseudomonas*, coliform types, *Bacillus*, *Alcaligenes*, *Acinetobacter*, *Flavobacterium* and *Proteus*, respectively.

Singh et al. (1987) examined the microbiological quality of raw and cooked frozen shrimp from fresh water and marine sources. Samples were analyzed for aerobic plate count (APC) at 35°C, coliforms, *Escherichia coli* and *Staphylococcus*. Analysis was also done for foodborne pathogens such as *Salmonella*, *Shigella* and *Vibrio cholerae*. In general, the bacteriological quality of the raw frozen

marine shrimp was better than the raw frozen fresh water shrimp, with the former having lower APC, coliform and *E. coli* counts. The researchers suggested this was due to the fecal contamination of the fresh water source. They stated that marine shrimp are usually harvested from coastal areas and the deep sea which are less likely to be subjected to fecal contamination. The cooked shrimp were of better bacteriological quality than the raw shrimp with much lower APC, coliform and *E. coli* counts. The researchers concluded that the cooking process had resulted in a lower bacterial load on the shrimp. *Shigella* and *V. cholerae* were not detected in any sample, while *Salmonella* was detected in three (0.5%) samples: one raw fresh water sample, one raw marine sample and one marine cooked sample.

The bacterial composition of 11 samples of domestically produced Alaska pollock surimi was determined by Elliot (1986). Of 220 isolates examined, about 90% stained gram-negative and fewer than 0.5% were yeasts. *Pseudomonas*, *Acinetobacter* and *Moraxella* were present in highest numbers, while only one or two isolates of *Lactobacillus* and *Escherichia* were found.

During processing of fish or seafood products, a variety of bacteria may be introduced. Cleanliness, hygiene and sanitation at all stages of processing are important (Connell, 1980). Staphylococci contaminate fish after primary processing, and in virtually all instances the cause

of contamination is human handling (Korkeala and Pakkala, 1988). For example, staphylococci found on 75% of cooked fish samples from small cook-shops, hotels, restaurants and street vendors in Egypt most likely resulted from contamination by persons who handled the fish after cooking (Saddik et al., 1985). Hackney and Garrett (1985) stated that organisms such as coliforms, staphylococci and nonhalophilic spoilage organisms may be introduced by contamination from boxes, human hands and clothing.

Most evidence indicates that sanitary conditions in the seafood processing plant or operation correlates well with the microbial quality of the finished product (Duran et al., 1983; Phillips and Peeler, 1972; Surkiewicz et al., 1967; Ward and Baj, 1988; Wentz et al., 1985). Cleaning and sanitation significantly contribute to the prevention of product contamination and spoilage (Comar, 1988). Nickerson and Goldblith (1964), in a study of haddock fillets, found that most microbial contamination occurred during filleting and subsequent handling prior to packaging. They established that total microbial counts could increase in the plant over the work day. For example, total counts for one processor increased from log 5.6 in the morning to log 5.7 at noon and log 5.9 in the evening. Ravesi et al. (1985) found that the numbers of microorganisms on fillets of fresh spiny dogfish were related to the amount of prior handling and processing.

The number of bacteria on fish has been shown to be

significantly reduced by certain processing techniques. Pace et al. (1988) demonstrated that pasteurization of freshly-shucked oysters for 8 minutes at 72°C reduced APC from 3.7×10^5 to 5.2×10^2/g. A somewhat larger reduction was achieved (1.1×10^5 to 9.0×10^1) with pasteurization for 8 minutes at 74°C. Saddik et al. (1985) found that cooked fish and shrimp from food service establishments and street vendors in Egypt were free from *Salmonella*, *Shigella* and *V. parahaemolyticus*. Cooking (frying or roasting) was effective in destroying these pathogens. Hackney and Garrett (1985) stated that most spoilage bacteria are heat-sensitive and are killed by cooking.

Growth of bacteria after processing treatments may be affected by several factors. Comparing microbiological changes in smoked and charred Baltic herrings during storage at different temperatures, Korkeala and Pakkala (1988) found coliforms and fecal streptococci to be present in higher numbers at 20°C than at 4°C on both types. High *Staphylococcus aureus* counts were found in two samples of each at 20°C. The researchers concluded that careless human handling after processing and temperature abuse during storage and sale may allow staphylococci and other potentially harmful organisms to become a health risk to consumers.

Resistance characteristics of initial microflora before processing may also affect bacterial growth after treatment.

Pace et al. (1988) found that pasteurization for 8 minutes at 72°C decreased the microbiological level of predominately gram-negative organisms found in fresh oysters. However, during subsequent refrigerated storage for 5 months at 0.5°C all surviving organisms were found to be gram-positive. Genera surviving after pasteurization and cold storage were *Bacillus*, *Clostridium*, *Corynebacterium*, *Listeria*, *Peptostreptococcus* and *Staphylococcus*. Ward et al. (1977) analyzed crab meat following pasteurization and isolated species of *Bacillus*, *Corynebacterium*, *Lactobacillus*, *Flavobacterium*, *Acinetobacter*, *Pseudomonas* and *Brevibacterium*. Bacteria isolated from smoked *Clarias lazera* bought from a Nsukka, Nigeria market were *Micrococcus*, *Lactobacillus*, *Acinetobacter*, *Bacillus*, *Staphylococcus*, *Proteus* and *Escherichia* (Okafor and Nzeako, 1985).

Zuberi et al. (1988) studied the bacterial flora of fermented fish sauce made from sardines. Samples of fish sauce were prepared from sardines layered with salt and fermented in barrels for 3 months. A further 3-month fermentation in the presence of 10% tartaric acid was performed. Samples from this two-stage fermentation process were examined for residual aerobic bacterial flora. Results indicated bacilli predominated, followed by micrococci.

D. Fresh Water Fish

Much information is available on the microbiology of marine fishery products. Yet very few data have been published on the microflora of fish reared in fresh water (Acuff et al., 1984). Lima dos Santos (1981) attributed this to the situation that fresh water fish from cold or temperate areas are generally less commercially important than marine fish and have not been so extensively studied. However, information does exist on several tropical fresh water species.

Okafor and Nzeako (1985) compared the microflora of fresh water *Clarias lazera* before and after smoking. Fresh fish were caught from the Ogurugu River in Nigeria and smoked at 38-43°C and 65-78°C. Smoked fish bought on the open market were also analyzed. Bacteria isolated from freshly-caught fish were *Micrococcus*, *Lactobacillus*, *Klebsiella*, *Acinetobacter*, *Aeromonas*, *Pseudomonas*, *Enterobacter*, *Flavobacterium*, *Moraxella*, and coryneforms. *Micrococcus*, *Lactobacillus* and *Acinetobacter* were isolated after smoking at 38-43°C. At the higher smoking temperature (65-78°C) only *Micrococcus* was found. For fish bought on the open market, *Micrococcus*, *Lactobacillus*, *Acinetobacter*, *Bacillus*, *Staphylococcus*, *Proteus*, and *Escherichia* were all detected. The absence of several genera on smoked fish was attributed to the antibacterial effects of both smoke and temperature. The presence of *Bacillus*, *Staphylococcus*,

Proteus, and *Escherichia* on the purchased smoked fish was attributed to poor handling and storage after smoking.

Gram et al. (1989) studied the storage life of Nile perch from Lake Victoria in relation to bacterial load and storage temperature. Freshly-caught whole fish and fillets were held at ambient temperature (20-30°C) and at chill temperature (0-5°C). Immediately after catch, the total surface bacterial load on the fish was 5×10^3-5×10^5 CFU/cm^2. Bacteria on the fish held at ambient temperature increased rapidly to 5×10^6-10^8 CFU/cm^2 after 12-17 hours. Fillets were declared spoiled by sensory, chemical and microbiological analysis at 13 hours. Whole fish had a shelflife of 11-14 hours. In contrast, the iced whole fish had a shelflife of approximately 4 weeks. Iced fillets, which were handled observing strict hygiene, spoiled faster than the iced whole fish although the initial bacterial count was significantly lower. The researchers thought this could be explained by the protection against bacteria offered by the tough skin of *Lates niloticus*, but a similar protection was not seen when fish were deliberately contaminated. The reason for the rapid spoilage of the fillets was not obvious to the researchers. However, they concluded that icing proved an efficient way of preserving the fish.

Other researchers have studied fresh water fish stored on ice. Obanu and Ajayi (1985) examined the quality

deterioration of whole and gutted *Tilapia nilotica* during storage in refrigerated brine at 4°C. The shelflife of whole fish was 16 days and that of gutted, 28 days. Perigreen et al. (1987) reported on the iced storage shelflife of common fresh water murrel (*Channa striatus*). Total bacterial counts decreased rapidly after initial storage on ice (0°C), then decreased gradually upon further storage on ice. The fish remained in acceptable condition for 8-9 days on ice according to chemical, microbiological and organoleptic methods.

E. Cultured Fish

Aquaculture production is important on a global basis. Future demands for aquatic food products must be met through aquaculture because traditional (wild) fishery and seafood stocks are being harvested close to their maximum sustainable yields (Acuff et al., 1984; Redmayne, 1989). However, farmed fishery production presents special problems, especially in the area of microbiological quality of products. Microbiological problems generally stem from production practices which may lead to contamination by pathogens and increased antibiotic resistance among these pathogens (Ward, 1989). For example, in some countries the culture of fish in ponds enriched with human and animal excreta has long been a tradition. Fong and Brooks (1989) also noted that aquaculturists rely upon the use of approved

antibiotics to overcome problems such as diseases and stress related traumas. It is because of these conditions that the bacteriological quality of cultured fishery products may be more influenced by their environment than other fishery systems.

The relationship of the rearing water and the bacterial flora of tilapia was studied by Acuff et al. (1984). The researchers found the distribution of microorganisms in water and tilapia differed. The microbial flora of the pond water from which the fish were harvested contained approximately equal percentages of gram-negative and gram-positive types with *Streptococcus*, coryneforms, *Moraxella-Acinetobacter*, and *Flavobacterium* predominating. Immediately after harvesting, the fish had a predominance of gram-positive organisms including *Micrococcus*, *Moraxella-Acinetobacter*, *Flavobacterium*, *Pseudomonas* and *Vibrio* . At harvest, the mean aerobic mesophilic bacterial count of the pond-reared fish was $7.3 \times 10^2/cm^2$ while the pond water contained 2.2×10^4/ml. In studies with fish cultured in wastewater (Phelps, 1985; Guttman-Bass et al., 1986), traditional fecal indicator organisms and pathogens (*Salmonella*) were present, yet the levels of bacteria encountered were within the range encountered in commercially-sold fish (Ward, 1989).

In intensive aquaculture, water quality is most important. Polluted or highly-contaminated systems may

reflect on the quality of the fishery products in them. Filter-feeding bivalve mollusks such as oysters, mussels and clams can concentrate toxic chemicals and pathogenic microorganisms from the water in which they are grown (Thrower, 1989). Water quality and crowded conditions may interact to influence bacterial populations. Fong and Brooks (1989) reported that hundreds of thousands and sometimes millions of animals may be confined in a single pond or rearing tank. Studies by Buras et al. (1980) have shown that fish grown in ponds containing wastewater accumulate fecal bacteria and that, above a threshold concentration of about 10^4 bacteria/ml, detectable levels of bacteria penetrate into the muscle tissue (Ward, 1989). Such conditions produce physiological stress on fish, make transmission of diseases easier, and suggest the potential for human health risks. The U.S. Food and Drug Administration (FDA) reported that *Salmonella* (<5% of samples tested), *Edwardsiella* (0.3% of samples tested) and high fecal coliform counts were detected in domestically-raised retail catfish (Andrews et al., 1977). The current condition of these pathogens occurring in commercially-grown catfish is uncertain. However, Ward (1989) felt that aquaculture practices have improved and for microorganisms to cause foodborne illness, abusive conditions and/or cross-contamination would have to occur.

Another microbiological problem associated with

aquaculture is the formation of off-odors and off-flavors in fish caused by certain microorganisms. This is the most significant production problem of the fresh water catfish industry (Johnsen, 1989). Geosmin (GSM) and 2-methylisoborneol (MIB) are earthy/musty smelling metabolites produced by microorganisms and are among the most potent olfactory stimuli known to man (Johnsen, 1989). Johnsen and Kuan (1987) demonstrated that pure cultures of streptomycetes and algae can produce either GSM or MIB or both.

Studies conducted on processed cultured fish have dealt with several issues. One was how to make fish available to the consumer in a fresh, rather than frozen, state. To accomplish this, alternate processing and packaging methods were examined. These were designed to extend shelflife and maintain a quality product.

It has been recently reported that modified atmospheric packaging (MAP) can significantly extend the fresh life of fishery products (Brown et al., 1980; Lannelongue and Finne, 1986; Scott et al., 1984). Przybylski et al. (1989) investigated the extension of shelflife of iced fresh channel catfish using modified atmospheric packaging and low dose irradiation. It was found that irradiation treatments with or without elevated carbon dioxide (CO_2) atmosphere packaging significantly reduced the bacterial load and extended shelflife from 5-7 days to 20-30 days. A study by

the National Marine Fisheries Service (Barnett et al., 1987) involved the combination of an antimicrobial agent, special packaging, temperature and modified atmosphere. Boned trout, treated or not treated with a 2.3 % potassium sorbate dip, were packaged in laminated high/low density polyethylene bags with a CO_2-enriched modified atmosphere (MA). All samples were held at 35°F and analyzed at intervals. Results showed microbial growth was controlled and chemical and organoleptic changes were minimized by the use of the polyethylene bags and MA. Pretreatment with sorbate increased control of microbial growth but did not increase shelflife beyond what was obtained by the packaging and MA alone.

Quality changes and microbial characteristics of aquacultured fish stored on ice have also been examined. Studies have shown that low temperature (i.e., ice) storage can decrease initial bacterial counts. Acuff et al. (1984) attributed this decrease to the disappearance of mesophilic organisms (not including coliform types) that could not adapt to the cold environment. Perigreen et al. (1987) noted a rapid decrease in coliform bacteria on common murrel due to cold shock by ice (0°C) storage.

New food products from fish was an issue examined by Gelman and Benjamin (1989). The characteristics of mince from pond-bred silver carp (*Hypophtalmichthys molitrix*) were studied. Its application in an all-fish sausage was

investigated. Deboned halves of silver carp were minced and portions stored for 1 week at 5-6°C to determine microbial stability. Aerobic plate counts increased from 7.9×10^4 to 1.1×10^5 after 6 days at 5-6°C. Coliform and psychrotroph counts increased from 1.0×10^1 and 7.8×10^3 to 5.4×10^2 and 1.3×10^4, respectively. No spoilage was detected in any of the fish sausage products during storage for 7 days at 5-6°C.

F. Storage Studies

Fish is an extremely perishable food. Maintaining freshness and quality is paramount. Fish tissues provide an ideal nitrogenous substrate for the growth of proteolytic and putrefactive organisms (Tarr, 1954). Special storage methods and processing treatments have been developed with the primary purpose of extending shelflife by reducing microbial load and/or inhibiting growth. Extension of shelflife may involve special treatment such as low temperature storage or freezing, use of microbial decontaminating chemical dips or preservatives or modified atmosphere packaging (MAP). Many of these methods, individually or in combination, have been studied to determine their effects on the shelflife of fishery products.

Low temperature storage is an effective way to retard microbial growth and extend shelflife. Acuff et al. (1984)

found that the total bacterial count of pond-reared tilapia (*Tilapia aurea*) decreased during the first 3 days of storage on ice and remained stable for the next 6 days. Manthey et al. (1988) studied the quality changes of European catfish (*Silurus glanis*) during storage in melting ice held in containers in a chill room at 0-2°C. Samples for total aerobic plate count were taken from the skin and the muscle at 3 day intervals. They found no significant increase of aerobic bacteria occurring on the skin surface during the first week of storage on ice. Bacterial numbers in the muscle remained constant (<log 1.5) up to day 16 of storage.

Other researchers have found similar results. Whole and gutted *Tilapia nilotica* stored in refrigerated brine at 4°C, were found by taste panel assessment to be of acceptable quality for 16 days and 28 days, respectively (Obanu and Ajayai, 1985). Gram et al. (1989) reported that whole Nile perch remained fresh for 27-30 days on ice, while fillets remained fresh for 23 days. Perigreen et al. (1987) found bacterial counts of common murrel decreased from 1.1×10^6 to 1.4×10^4 after 3 days storage on ice.

Freezing is another effective method to extend the shelflife of fishery products. Effects of freezing on microorganisms varies with type (Jay, 1978), although eventual mortality depends on the freezing process itself (Simmonds and Lamprecht, 1985). However, Jay (1978) clearly pointed out that freezing should not be regarded as a means

of destroying foodborne microorganisms. With fish, enzyme activity and chemical reactions such as oxidation of fats and denaturation of proteins far outweigh the effects of bacterial action (Simmonds and Lamprecht, 1985). It is reported that fish must be frozen rapidly and held at as low a temperature as possible to maintain best quality (Jay, 1978; Simmonds and Lamprecht, 1985). Equally important on the quality of frozen fish products are handling and packaging. Josephson et al. (1985) suggested that ice-glazing and packaging of frozen whitefish (*Coregonus clupeaformis*) in vacuum-sealed pouches of low oxygen permeability provided oxidation protection and quality retention.

Microbial decontamination by chemicals has also been investigated as a means to extend shelflife. Kosak and Toledo (1981) used a combination of chlorine pretreatment with various packaging techniques to delay microbial growth. A 3.5-minute dip in a 1000 mg/ml free chlorine solution with either a vacuum pack or polyethylene bag yielded a 12-day extension of fresh fish quality. Pace et al. (1988) showed that chlorination at 150 ppm for 30 minutes reduced the total aerobic plate count (APC) of freshly-shucked oysters from 4.5×10^5 to 5.8×10^4/g. In contrast, Surendran et al. (1985) found EDTA dips were not effective in extending the shelflife of fatty fish such as oil sardine (*Sardinella longiceps*) or mackerel (*Rastrelliger kanagurta*). The EDTA

treatment controlled bacterial growth but no increase in shelflife was realized due to deterioration of fat in the fishes.

It has been known for years that carbon dioxide (CO_2) may inhibit bacterial growth and extend the shelflife of perishable foods. The principal effect of CO_2 on fish spoilage bacteria is an extension in the lag phase and a reduced growth rate in the logarithmic phase (Ward and Baj, 1988; Gray et al., 1983). Carbon dioxide is also known to be bactericidal at high levels (Barnett et al., 1987; Parkin et al., 1981).

Increased lag phase by use of CO_2 has been demonstrated in fresh water crayfish (*Pacifastacus leniusculus*) by Wang and Brown (1983) and in fresh retail fish from the Midwest U.S.A. (Richter and Banwart, 1983). Both groups of researchers use an 80% CO_2:20% air compared to air storage at low temperature (1-4°C). They reported a 0-7 day lag phase in bacterial growth in CO_2. In other studies, CO_2 was effective in retarding the growth of microorganisms on various fish. Banks et al. (1980) demonstrated this on packaged finfish from the Gulf of Mexico. They found at least a log difference in bacterial counts up to six days between fish stored in CO_2 as compared to control fish in air. Brown et al. (1980) found similar results on rockfish (*Sebastes miniatus*) fillets and silver salmon (*Oncorhynchus kisutch*) steaks held in atmospheres of 20% or 40% CO_2.

Lannelongue and Finne (1986) found carbon dioxide caused a decrease in growth rates of microorganisms isolated from black drum. They reported this inhibitory effect was greatly enhanced by low temperature (4°C). Przybylski et al. (1989) found lower microbial levels on fresh channel catfish fillets stored in 80% CO_2:20 % air or 100% CO_2. In contrast, they were not significantly different ($P<0.05$) from controls flushed with air. Scott et al. (1984) found CO_2 most effective at reducing growth of sulphide-producing bacteria on snapper fillets. Parkin et al. (1981) demonstrated the bactericidal effects of high levels of CO_2 on the initial flora of rock fish fillets.

Carbon dioxide may be used in combination with other treatments, packaging, or storage conditions. Barnett et al. (1987) investigated the refrigerated shelflife of boned rainbow trout (*Salmo gairdneri*) in a CO_2-enriched environment. Aluminum trays (about 7-8 fish/tray) with perforated lids were placed in laminated high/low density polyethylene bags and back-flushed once with a premix gas of 80% CO_2 and 20% N_2. Control trays were packaged in 1.5-mil polyethylene bags. All samples were stored at 2.8°C and analyzed at selected intervals for microbial and chemical changes. Aerobic plate count (APC) for the controls increased slightly from the initial counts during the first 6 days, but increased sharply thereafter. The APC of the specially packaged CO_2 samples decreased during the same

period and remained only slightly higher than initial counts for 18 days. This led the researchers to conclude that the combination of packaging, refrigeration, and modified atmosphere was effective in doubling the shelflife of the trout product.

Modified atmosphere packaging (MAP) using CO_2 has some limitations. Strenstom (1985) reported that packaging of fish in high concentrations of CO_2 has some technical disadvantages: (1) CO_2 dissolves into the fish liquids, causing deformation of the package, and (2) discoloration of highly pigmented fish occurs (Ward and Baj, 1988).

There also is concern whether pathogenic bacteria such as *Clostridium botulinum* may grow to substantial levels in CO_2 prior to spoilage. Carbon dioxide may inhibit the normal spoilage flora sufficiently to allow growth of *C. botulinum* during the extended shelflife of the product (Statham, 1984). The concern is whether toxin may be formed by *C. botulinum* during refrigerated storage or temperature abuse situations before spoilage is detected. Eyles (1986) indicated that some types of *C. botulinum* may produce toxin at refrigerated temperatures, however growth may be so slow that spoilage will occur before a detectable amount of toxin is produced. Several studies have revealed the effect of temperature abuse on toxin formation by *C. botulinum* in fish fillets stored under CO_2. Post et al. (1985) used inoculated cod, whiting and flounder fillets. They found

spoilage to occur before toxin detection at 8-12°C, but detected toxin before spoilage at 26°C. Stier et al. (1981), using inoculated salmon steaks, observed spoilage prior to toxin detection at temperatures from 4.4-12°C but after toxin detection at 22.2-26°C.

CHAPTER III

MATERIALS AND METHODS

A. Collection and Preparation of Fish

The white amur used for this project were obtained from several sources. Those used in the first phase of the experiment, isolation and identification of bacteria occurring on the flesh, were obtained from the Tennessee Wildlife Resources Agency (TWRA) Eagle Bend Hatchery in Clinton, Tennessee. The mean weight of the fish was 1.36 kg (3.00 lb). Fish used in the storage/shelflife studies were obtained from two sources. The first was a commercial aquaculture farm, Hill's Farms Distributors, Inc., Lonoke, Arkansas. White amur at this establishment are sold for weed eradication in ponds and shipped to the Western United States to be used as a human food source (Hill, 1987). The live fish used in the present study were shipped by air freight in plastic bags with water and an oxygen gas headspace. The mean weight of these fish was not ascertained. The second source of white amur used for the storage/shelf-life studies were obtained from the pond of Dr. D.J. Krahwinkel, Jr., of The University of Tennessee Veterinary Teaching Hospital, Knoxville, Tennessee. The mean weight of these fish was 3.7 kg (8 lbs 2 oz).

All fish used in this study were housed at the

University of Tennessee Fisheries Laboratory and maintained in 122 cm diameter circular tanks equipped with venturi-type drains, having a flow-through water supply (ca. 2.8-5.4 L/min) from a municipal source (Bakir-Al-Hakkak, 1985).

Fish used for isolation and identification of bacteria for fresh, spoiled, and frozen aspects of the study were divided into two groups with the three fish in each group representing triplicate samples. One group was fed a diet of bermuda grass pellets (plant protein diet). The other group was maintained on a diet of trout chow pellets (animal protein diet). On the day of processing, dissolved oxygen, chlorine, pH, and ammonia concentrations were determined for water in the tank using a portable testing kit (Hach Model DR-EL/1, Hach Chemical Co., Ames, IA).

B. Preparation of Sampling Materials

All work surfaces, utensils, and materials used in processing were sanitized and aseptic methods were utilized to prevent cross-contamination among the samples. Work table surfaces were sanitized with a 400 ppm quaternary ammonium compound solution (Industrial Colloids and Chemicals, Inc., Knoxville, Tennessee). Utensils such as forceps, scissors, knives and scalpels were initially wrapped in brown kraft paper and sterilized for 25 minutes at 121°C. Utensils used more than once during processing were placed in 95% ethanol and flamed prior to use.

C. Sample Processing and Storage

1. Isolation and Identification Analyses

Three fish from each group were processed in the meat laboratory of the Department of Food Technology and Science, The University of Tennessee, Knoxville. Each fish was processed and samples taken of gills, intestines, flesh and body mucous according to the following methods. Fish were stunned with a blow to the head by a hammer. A 2 x 12.5 cm^2 area on the fish was swabbed using a sterile precut aluminum foil template and a sterile dacron swab (American Scientific Products, Inc., McGaw Park, IL)., dipped in 0.1 % peptone buffer solution. The swab was aseptically broken into a sterile screwcap culture tube containing a premeasured amount of 0.1 % peptone buffer solution. The fish was then measured and weighed. A portion of the gill was removed using sterile forceps and scissors. The fish was carefully eviscerated and a section of the intestine was removed. Flesh was removed from the body. All solid samples were placed into sterile stomacher bags (Seward Laboratory, England), placed on ice and transported to the microbiology lab of the Department of Food Technology and Science, The University of Tennessee, Knoxville.

Three samples from the flesh of each fish were taken. One flesh sample was placed in a -18°C freezer and held for 6 months. Following frozen storage, the sample was analyzed to determine microbial flora. A second flesh sample was

refrigerated at 7°C and held until spoilage was detected by visual and olfactory changes. When spoilage was detected, this sample was also analyzed for microbial flora. The third flesh sample, gills, intestines, mucous samples, and tank water were immediately analyzed for microbial count and types.

2. Microbial Enumeration

Enumeration of microorganisms in samples consisted of aerobic plate count (APC) of mesophilic organisms, psychrotrophic plate count (PPC) and a coliform count (AOAC, 1984; APHA, 1985). For all phases of microbial enumeration, 25g portions of each solid sample were homogenized with 225 ml of 0.1% peptone diluent in a Stomacher Lab Blender (Model 400; Seward Laboratory, England). Serial dilutions were prepared for each sample in 0.1% peptone. Aliquots of each dilution were mixed with Standard Methods Agar (SMA; BBL, Cockysville, MD) for APC and PPC, or Violet Red Bile Agar (VRB;BBL) for coliform count using the pour-plate technique. The APC plates were incubated for 48 hours at 32°C prior to enumeration. The PPC plates were incubated for 7 days at 7°C. Coliform plates were incubated for 24 hours at 32°C.

3. Bacterial Isolation and Identification

The Harrison Disc Method (Harrigan and McCance, 1976) was used for random selection of bacterial colonies. A

maximum of 20 colonies per plate were selected and individually transferred to Trypticase Soy Broth (TSB;BBL) and incubated for 48 hours at 32°C. A loopful of broth from each tube was streaked for isolation onto Trypticase Soy Agar (TSA;BBL) plates and incubated for 48 hours at 32°C. This method was repeated to ensure culture purity. Isolates were maintained in TSB and on TSA until biochemical testing was conducted. They were transferred routinely to maintain viability. Isolates were initially characterized as to morphology and gram-stain reaction. A protocol for biochemical testing was modified from existing techniques (Breed et al., 1957; Hugh and Leifson, 1953; Jay, 1976; Kiss, 1984; Post, 1983) and used for identification of isolates to genus level (Figures 1, 2, and 3).

D. Storage Studies

Microbial log counts over time were established for fresh white amur flesh using four different storage conditions or treatments. These involved use of a chemical sanitizing solution, frozen storage, a comparison of two low storage temperatures, and modified atmosphere packaging. For each treatment, a large portion of fresh flesh was subdivided into 25g samples.

The effect of a chemical sanitizing agent on the microflora of the flesh during storage was studied. Portions of flesh were dipped into a 200 ppm sodium

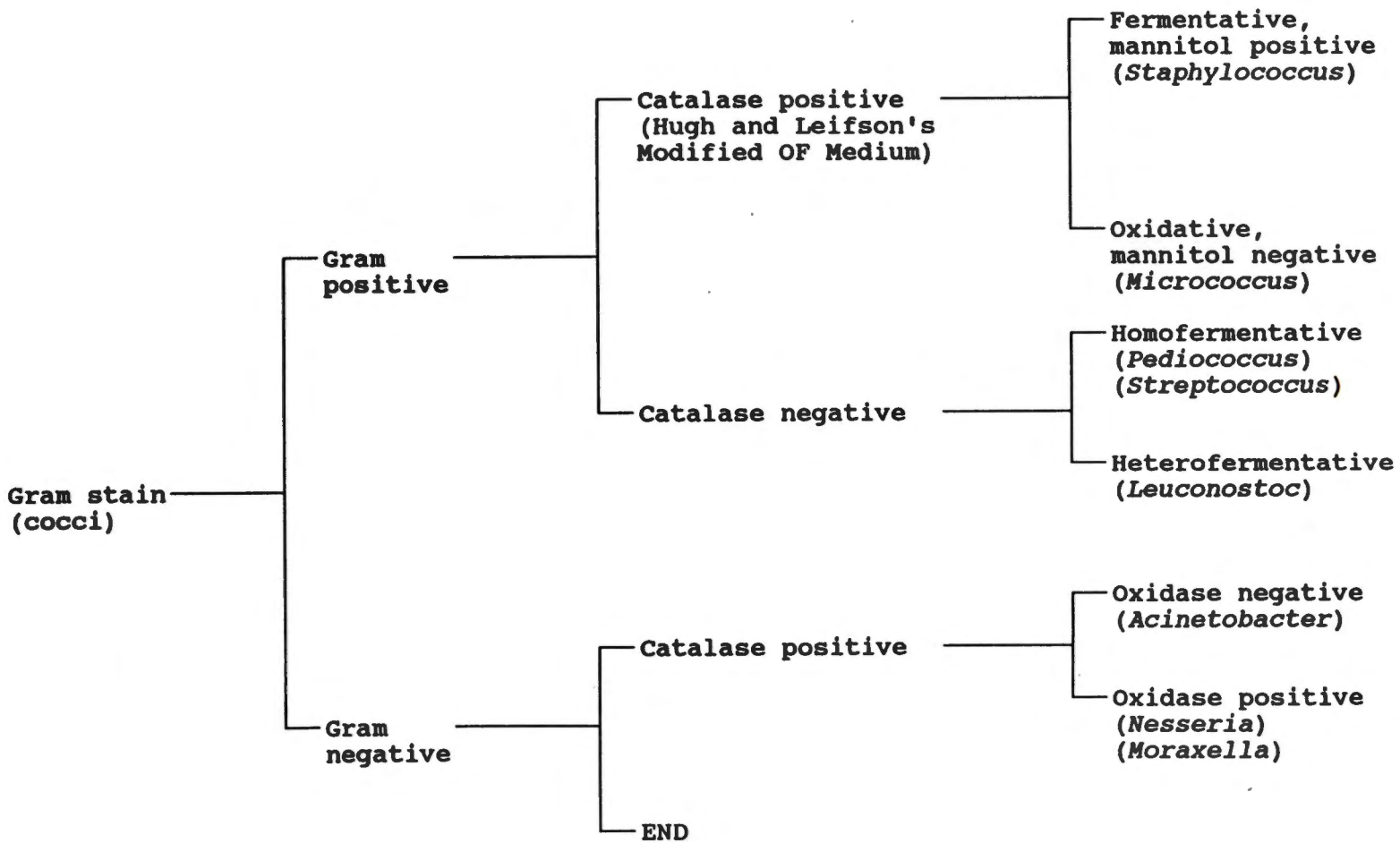

Figure 1. Key for the identification of the heterotrophic cocci.

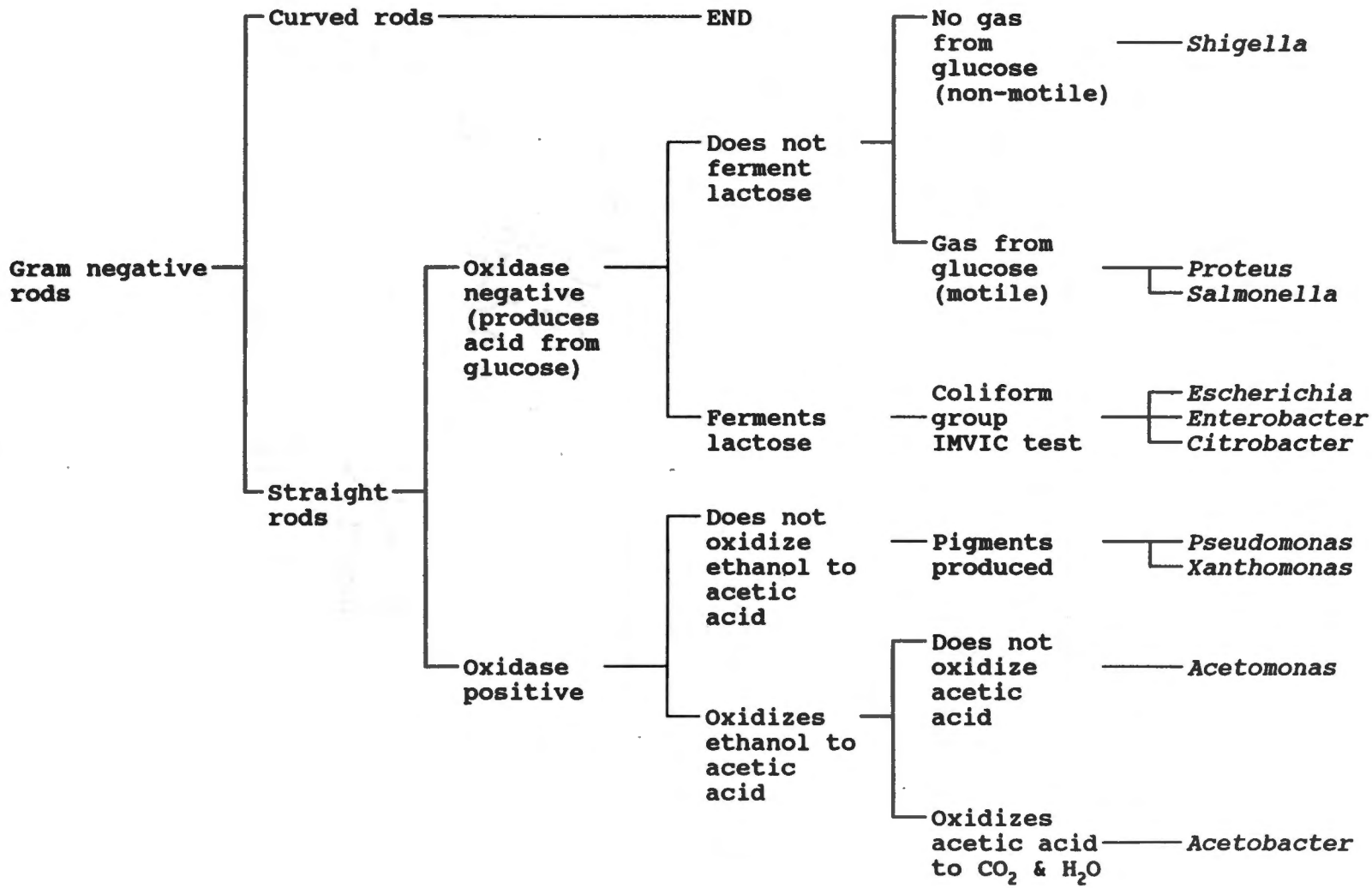

Figure 2. Key for the identification of the Gram-negative rods.

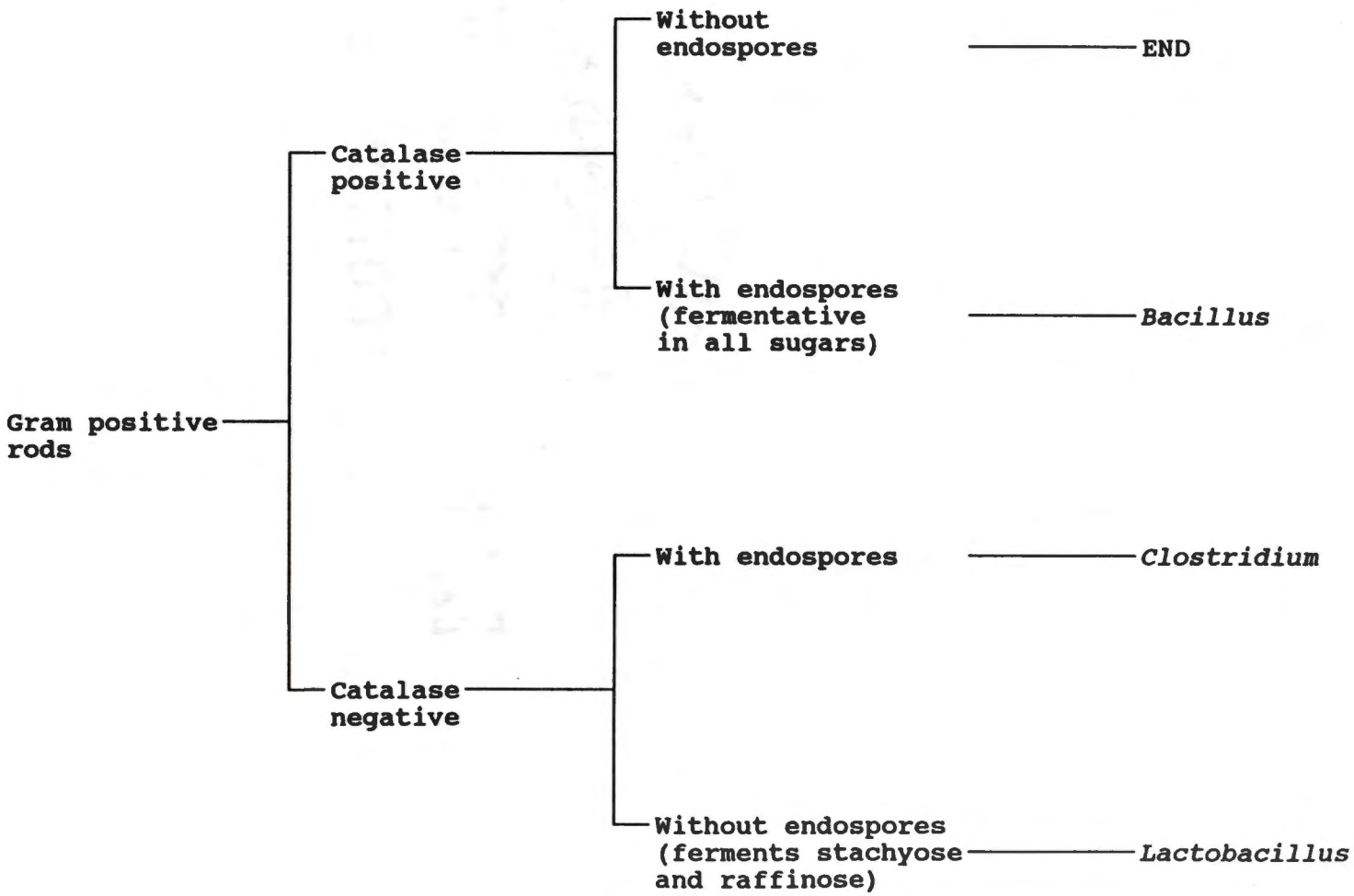

Figure 3. Key for the identification of the Gram-positive rods.

hypochlorite solution for 2 minutes and placed in styrofoam trays overwrapped with an air-permeable plastic film. These samples were stored at 7°C. Microbial enumeration was carried out on the treated samples at 2 day intervals for 14 days.

Microbial log counts over time were also established for frozen white amur flesh. Flesh was frozen at -18°C in sterile stomacher bags for a period of 2 months. Initial and semimonthly samples were enumerated.

A comparison of two refrigerator storage temperatures was conducted. Samples were placed in styrofoam trays overwrapped with an air-permeable plastic film to simulate commercial packaging conditions. Half of the samples were stored at 4°C, the other half at 7°C. Microbial enumeration was conducted at 2 day intervals for a period of 14 days.

The effects of modified atmosphere environment on storage of flesh were studied. White amur flesh was packaged under an atmosphere of 100% carbon dioxide gas using a gas-flush vacuum packaging machine (Model AG4, Cryovac, Inc.). Samples were stored at 7°C and were enumerated at 2 day intervals for 14 days. The effects on overall growth were determined.

E. Statistical Analyses

Analysis of variance (ANOVA) was used to compare diets and storage treatments. When significant differences

were detected by ANOVA at $p < 0.05$, Duncan's New Multiple Range Test was used to separate means. Two replications were performed on storage treatments.

CHAPTER IV

RESULTS AND DISCUSSION

A. Enumeration of Bacteria on White Amur

One objective of this experiment was to determine the number of aerobic and coliform bacteria occurring on white amur. Various areas of the fresh fish were analyzed, as well as flesh following spoilage (14 d at 7°C) and freezing (6 month at -18°C).

1. Fresh White Amur

The surface (skin/mucous area) of fresh white amur had the lowest aerobic plate count (APC), while the gill area and intestine were highest (Table 1). The reason for the lower numbers on the surface area of the fish can easily be explained. One purpose of the mucous on the skin of fish is that it acts as a means of protection (Pflieger, 1975). Bacteria and other agents that collect on the mucous are periodically sloughed off (Lagler et al., 1962; Pflieger, 1975). The gill and intestine areas, moreover, are known to harbor high numbers of bacteria and are often implicated as areas from which cross-contamination of the flesh occurs. The intestine of animals contain high numbers of a variety of microorganisms (APHA, 1976). These can be aerobic or facultative anaerobic bacteria.

Table 1. Mean aerobic (APC) plate counts from fresh white amur samples fed animal or plant food diets.

Sample	Surface	Gill	Intestine	Flesh
	Log CFU/25cm^2		Log CFU/g	
Animal	3.6^x	6.0^x	8.0^x	4.0^x
Plant	3.7^x	5.7^y	8.1^x	3.6^y

^{x},^{y}Means within columns with different superscripts are significantly different ($P<0.05$).

A significant difference ($P<0.05$) existed between the two diets in the numbers of bacteria occurring on the gill area and the flesh (Table 1). No difference between diets was found for the surface (skin/mucous) area or the intestine.

The coliform enumeration for this study was done by procedures described by AOAC (1980) and APHA (1985) and utilized Violet Red Bile (VRB) Agar as the selective medium. Mean coliform counts among white amur fed different diets had a similar pattern to that for the aerobic plate count (Table 2). The surface (skin/mucous) area had lowest numbers, while gill and intestinal areas had highest. A significant difference ($P<0.05$) in the number of coliforms on the flesh was found between diets (Table 2). More than likely, the presence of coliforms on the flesh was due to the cross-contamination during processing. The diet had no effect on the numbers of coliforms found on the other areas of the fish. Comparing the mean log counts among the different areas of the fish from Tables 1 and 2, it can be seen that a range of 2 to 12.5% of the bacteria found on the fresh fish in this study were coliforms.

2. Spoiled White Amur Flesh

The mean mesophilic (APC) and psychrotrophic (PPC) plate counts from spoiled flesh of white amur fed animal or plant food diets is shown in Table 3. There was no

Table 2. Mean coliform counts from fresh white amur samples fed animal or plant food diets.

Sample	Surface	Gill	Intestine	Flesh
	Log CFU/25cm^2		Log CFU/g	
Animal	2.3^x	4.4^x	7.4^x	3.2^x
Plant	2.7^x	4.5^x	7.4^x	2.5^y

^{x,y}Means within columns with different superscripts are significantly different ($P<0.05$).

Table 3. Mean psychrotrophic (PPC) and aerobic (APC) plate counts from spoiled (14 d at 7°C) flesh of white amur samples on animal or plant food diets.

	PPC[a] (Log CFU/g)	APC[b] (Log CFU/g)
Sample	Spoiled Flesh	Spoiled Flesh
Animal	8.3[x]	7.8[x]
Plant	8.9[x]	8.0[x]

[a]PPC=Microorganisms detected on Standard Methods Agar (SMA) incubated aerobically for 7 days at 7°C.

[b]APC=Microorganisms detected on Standard Methods Agar (SMA) incubated aerobically for 48 hours at 32°C.

[x]Means within columns with the same superscripts are not significantly different ($P<0.05$).

significant difference ($P<0.05$) between diets for either bacterial type. However, more psychrotrophic bacteria were detected than mesophilic bacteria for both diet-types. The temperature (7°C) at which the flesh was stored while spoilage occurred apparently favored the development of psychrotrophic spoilage bacteria over the mesophilic types.

3. Frozen White Amur Flesh

The type of diet fed had no significant effect ($P<0.05$) on the numbers of psychrotrophic (PPC), mesophilic (APC), or coliform bacteria occurring on the frozen flesh of white amur stored 6 months at -18°C (Table 4). However, freezing of white amur flesh caused a decline in all microbial numbers over time. Higher numbers of mesophilic bacteria survived freezing conditions than did psychrotrophic or coliform types. The coliform population decreased >3 logs by the second week of storage. This agrees with several previous studies in which coliforms were found to be very susceptible to frozen storage (Perigreen et al., 1987; Sulzbacher, 1950).

B. Identification of Bacteria on White Amur

A second objective of this study was to identify the types of bacteria occurring on white amur flesh in a fresh, spoiled (14 d at 7°C), and frozen (6 months at -18°C) state.

Table 4. Mean bacterial counts on frozen (6 months at -18°C) flesh of white amur samples on animal or plant food diets.

	Frozen Flesh (Log CFU/g)		
Sample	PPC[a]	APC[b]	Coliforms[c]
Animal	0.8[x]	1.0[x]	<1.0[x]
Plant	0.9[x]	1.0[x]	<1.0[x]

[a]PPC=Microorganisms detected on Standard Methods Agar (SMA) incubated aerobically for 7 days at 7°C.

[b]APC=Microorganisms detected on Standard Methods Agar (SMA) incubated aerobically for 48 hours at 32°C.

[c]Coliforms=Microorganisms detected on Violet Red Bile (VRB) Agar incubated for 24 hours at 32°C.

[x]Means within columns with the same superscripts are not significantly different ($P<0.05$).

1. Fresh White Amur Flesh

It is generally accepted that the bacteria associated with fresh fish and fishery products include species of *Achromobacter*, *Acinetobacter*, *Aeromonas*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Flavobacterium*, *Micrococcus*, and *Pseudomonas*, (Acuff et al., 1984; Byrd, 1973; Cox and Lovell, 1970; Okafor and Nzeako, 1985; Shewan, 1971). *Pseudomonas* was the most abundant aerobic bacterium found on different areas of fresh white amur, and the only aerobic bacterium isolated from the intestine (Table 5). *Pseudomonas* is known to occur widely throughout nature in soils, water, on plants, and in the intestine of man and other animals (Jay, 1970).

All areas of fresh white amur analyzed contained coliforms, with the highest numbers occurring in the intestine (Table 6). *Escherichia* was the dominant genus, comprising 54% of coliforms identified. *Escherichia* is a common bacterial group found on fish and seafood products. It has been noted, however, that fish and other free-swimming marine animals do not usually carry those organisms generally considered to be typical of the mammalian microflora, including *E. coli*, the fecal coliforms, and enterococci (Roberts et al., 1986). These authors consider the presence of human enteric organisms on marine food products clear evidence of contamination from a terrigenous source.

Table 5. Aerobic bacteria[a] of fresh white amur.

Bacteria	No. of Occurrences (%)			
	Fresh Flesh	Gills	Intestines	Skin/Mucous
Acinetobacter	5 (18)	4 (12)	0	4 (14)
Micrococcus	6 (22)	9 (27)	0	2 (7)
Moraxella	0	1 (3)	0	1 (4)
Pseudomonas	4 (14)	8 (24)	5 (63)	9 (32)
Staphylococcus	9 (32)	4 (12)	0	6 (21)
Streptococcus	2 (7)	0	0	3 (11)
Unknown	2 (7)	7 (22)	3 (37)	3 (11)
Total	28	33	8	28

[a]Bacteria detected on Standard Methods Agar (SMA) incubated for 48 hours at 32°C.

Table 6. Coliform[a] bacteria of fresh white amur.

	No. of Occurrences (%)			
Coliform	Fresh Flesh	Gills	Intestines	Skin/Mucous
Citrobacter	2 (5)	4 (14)	4 (8)	0
Enterobacter	14 (37)	14 (48)	10 (22)	11 (41)
Escherichia	16 (42)	6 (21)	32 (70)	14 (52)
Unknown	6 (16)	5 (17)	0	2 (7)
Total	38	29	46	27

[a] Bacteria detected on Violet Red Bile (VRB) Agar incubated for 24 hours at 32°C.

2. Spoiled White Amur Flesh

Pseudomonas and *Acinetobacter* were the only types of bacteria identified on spoiled white amur flesh held for 14 days at 7°C (Table 7). Lerke et al. (1967) found the bacteria of spoiling fish to consist almost entirely of pseudomonads and *Acinetobacter-Moraxella* types. Byrd (1973), in a study on pond-raised catfish found these organisms along with *Aeromonas* and *Flavobacterium*. The data from the present study also is supported by other workers using fish or fishery products (Castell and Greenough, 1956; Cox and Lovell, 1970; Shewan, 1971). The reason for the emergence of the pseudomonads as the primary spoilage organism is probably due to their growth characteristics. Pseudomonads are usually classified as low temperature microorganisms. According to Shewan (1976), many of them are psychrotrophic, capable of growth down to temperatures of -5°C to -10°C, and have comparatively short generation times at temperatures between 0-5°C, so that they easily outgrow other bacterial types present. Ingraham and Stokes (1959) defined psychrophiles as true cold-loving microorganisms that grow well at 0°C within 2 weeks. However, Ayres et al. (1980) stated that true psychrophiles are rare both in nature and in foods. The latter felt that a great variety of low temperature mesophiles mimic psychrophiles in low temperature tolerance. Organisms that grow well at low temperatures but do not require low

Table 7. Bacteria of spoiled (14 d at 7°C) flesh of white amur.

Bacteria	No. of Occurrences (%)	
	PPC[a]	APC[b]
Acinetobacter	4 (8)	5 (12)
Pseudomonas	48 (92)	36 (86)
Unknown	0	1 (2)
Total	52	42

[a]PPC=Bacteria detected on Standard Methods Agar (SMA) incubated for 7 days at 7°C.

[b]APC=Bacteria detected on Standard Methods Agar (SMA) incubated for 48 hours at 32°C.

temperature for growth are called "psychrotrophs".

Two incubation temperatures (7°C and 32°C) were used to enumerate the bacteria of the spoiled flesh. Both bacterial types identified were recovered equally well at either temperature (Table 7). The storage temperature (7°C) and time (14 d) used in this study to reach spoilage apparently allowed for psychrotrophic forms of *Pseudomonas* and *Acinetobacter* to become the dominant spoilage organisms. This study shows that these organisms were capable of growth and survival at low temperatures and were able to be recovered over a wide range of incubation temperatures.

3. Frozen White Amur Flesh

Bacteria identified on white amur flesh stored 6 months at -18°C were primarily mesophilic (Table 8). These data are supported by the enumeration data shown in Table 4. It is generally agreed that some types of microorganisms are more resistant to freezing than others. Shewan (1971), Jay (1978), and Simmonds and Lamprecht (1985), maintain that gram-negative bacteria are more sensitive to freezing than gram-positive ones, while the cocci (spherical) bacteria are the most resistant. *Staphylococcus*, *Micrococcus*, and *Streptococcus* (all gram-positive cocci) were detected on flesh that had been frozen.

It is interesting to note the association between the type of bacteria recovered and the incubation temperature.

Table 8. Bacteria of frozen (6 months at -18°C) flesh of white amur.

Bacteria	No. of Occurrences (%)	
	PPC[a]	APC[b]
Acinetobacter	16 (55)	6 (15)
Micrococcus	0	7 (18)
Moraxella	2 (7)	0
Pseudomonas	8 (28)	8 (20)
Staphylococcus	0	15 (38)
Streptococcus	0	3 (7)
Unknown	3 (10)	1 (2)
Total	29	40

[a]PPC=Bacteria detected on Standard Methods Agar (SMA) incubated for 7 days at 7°C.

[b]APC=Bacteria detected on Standard Methods Agar (SMA) incubated for 48 hours at 32°C.

Gram-positive cocci were all recovered at 32°C. Surviving gram-negative organisms, *Acinetobacter* and *Pseudomonas* were recovered equally well at both temperatures from the frozen flesh as was demonstrated with spoiled flesh (Table 7). This demonstrates that the latter organisms were capable of growth and survival at a wide range of temperatures.

C. Storage Studies of White Amur Flesh

The third objective of this study was to examine various storage methods for extending the shelflife of white amur flesh. Special storage methods and processing treatments were studied to determine their effectiveness in reducing bacterial load or inhibiting growth.

1. Effect of Chemical Sanitizing Agent

This storage study was conducted to determine the effect that a chlorine sanitizing solution had on the microbial load of white amur flesh.

There was a significant difference ($P<0.05$) in the coliform population of white amur flesh between the sanitized and non-sanitized samples (Figure 4). The chlorine dip was effective in decreasing the number of coliforms from an initial log 3.5 to log 0.7 at day 2. After day 2 the remaining coliform population increased dramatically from day 4 to day 8. However, the chlorine dip was effective in maintaining the coliform population at

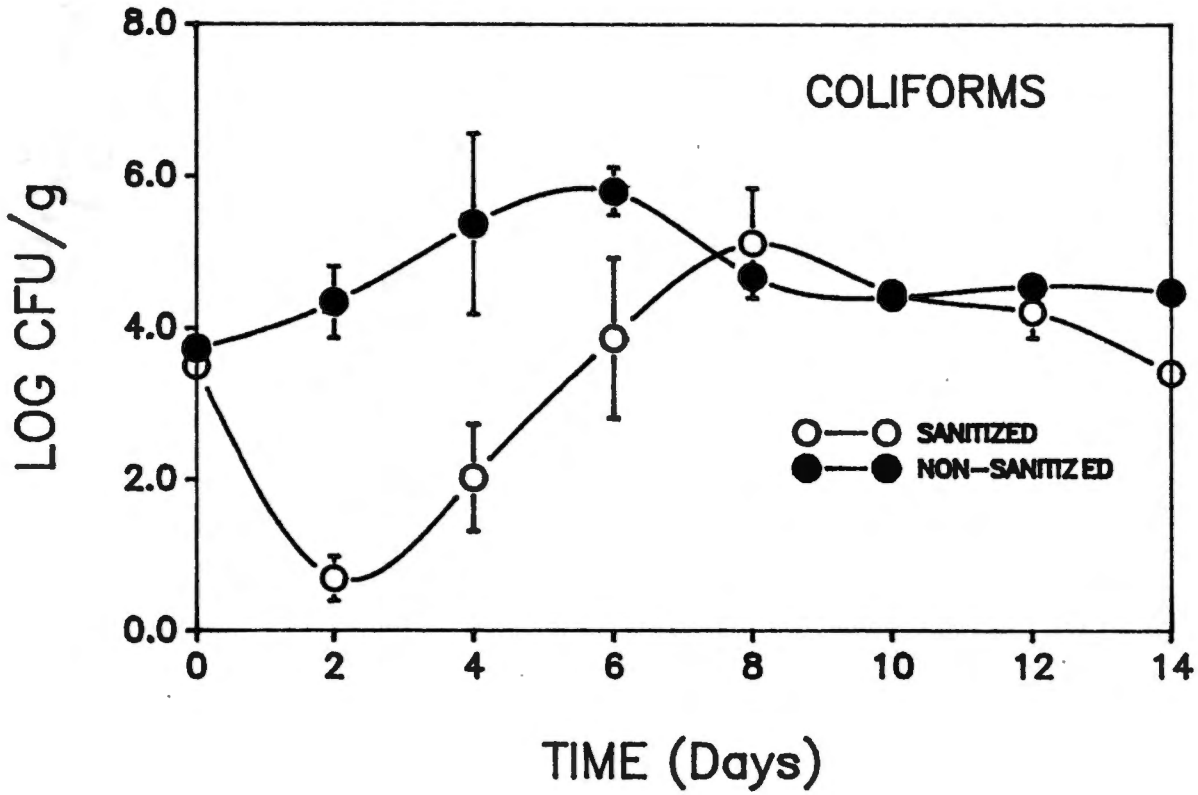

Figure 4. Effect of a 200 ppm sanitizing solution on the coliform population of white amur flesh.

least equal to or lower than the initial number for approximately 6 days.

The effect of the chlorine dip on the aerobic bacterial population is shown in Figure 5. The aerobic plate count of the non-sanitized sample tended to increase slowly over the 14 day holding period. In contrast, there was a reduction in the initial APC of the sample which had been treated with sodium hypochlorite. The APC of the sanitized sample remained equal to or less than the initial count until approximately day 6. However, while the APC was lower for the sanitized sample, there was no significant difference ($P<0.05$) between the two treatments.

The results of the chemical decontamination treatment show that the sodium hypochlorite solution was effective in reducing the initial coliform population by a significant amount. The APC was not as dramatically affected. The subsequent rise in coliform and APC count was probably due to the decrease in residual chlorine over time or to the resistance of the surviving microflora. Pace et al. (1988) reported that chlorination reduced the total aerobic plate count (APC) from 4.5×10^5 to 5.8×10^4/g on oysters. They also stated that a slight rise in APC was probably due to a decrease in residual chlorine effects. Kosak and Toledo (1981) used chlorine dips and various packaging techniques as a means of delaying microbial growth on fresh fish.

Shelflife extension provided by the sodium hypochlorite

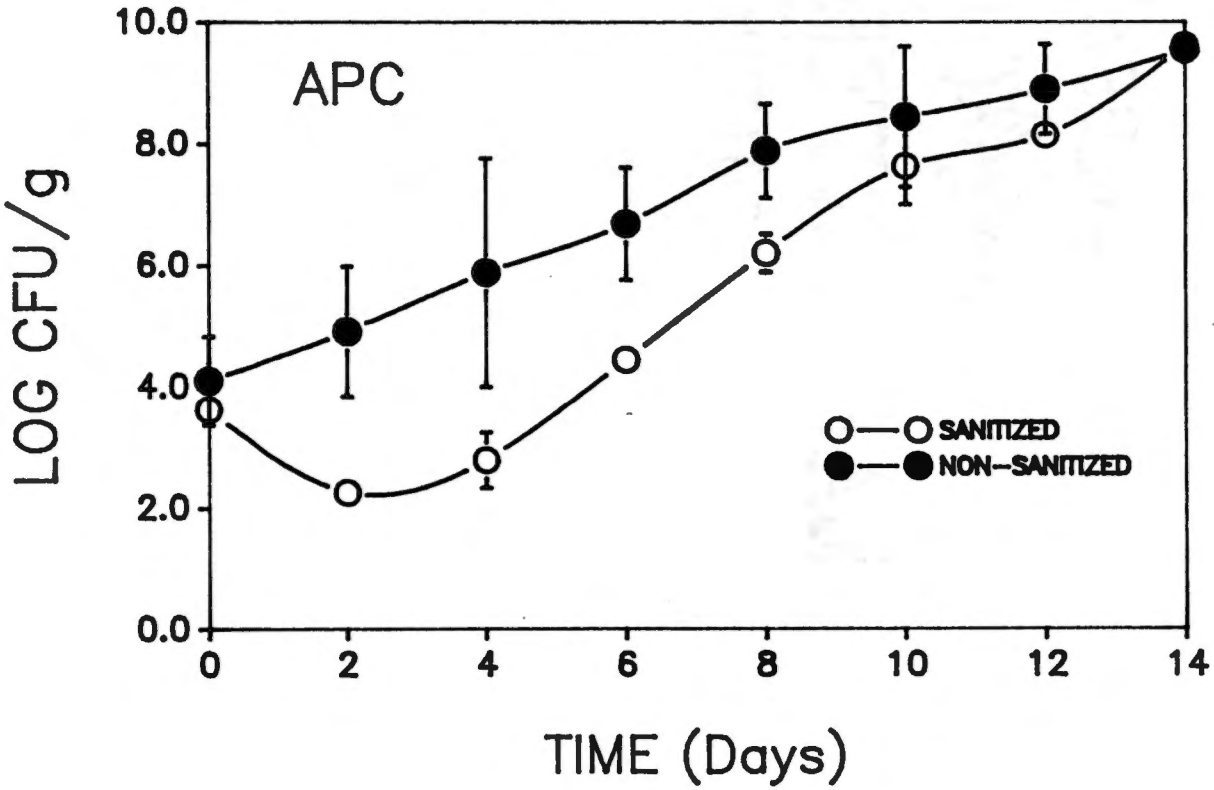

Figure 5. Effect of a 200 ppm sanitizing solution on the aerobic bacterial population of white amur flesh.

pretreatment was considered to be 6 days, based on the theory of Kosak and Toledo (1981). They quantified shelflife extension of fresh fish after chlorine dips based on microbial counts of the fish prior to treatment. Shelflife extension was considered to be the time at which the number of bacteria on the treated fish increased during storage to reach the number of bacteria on the fish prior to treatment. Both coliform and aerobic populations in this study were stabilized for 6 days.

2. Effect of Freezing

The effects of frozen storage (8 weeks at -18°C) on the bacterial population of white amur flesh is shown in Figure 6. Freezing was detrimental to all three bacterial groups examined. Psychrotrophic and aerobic plate counts exhibited a similar pattern of gradual decline. The coliform group showed the most dramatic decrease in number. After 2 weeks of storage, the coliform population was reduced by over 2 logs. There were no coliform survivors detected at the end of the study period. Coliforms are considered very susceptible to frozen storage. Jay (1978) stated that gram-negative organisms are generally more sensitive to freezing than gram-positive ones, with the *Enterobacteriaceae* among the most sensitive types. According to Jay (1978), however, coliforms can sometimes survive long periods at freezing temperatures.

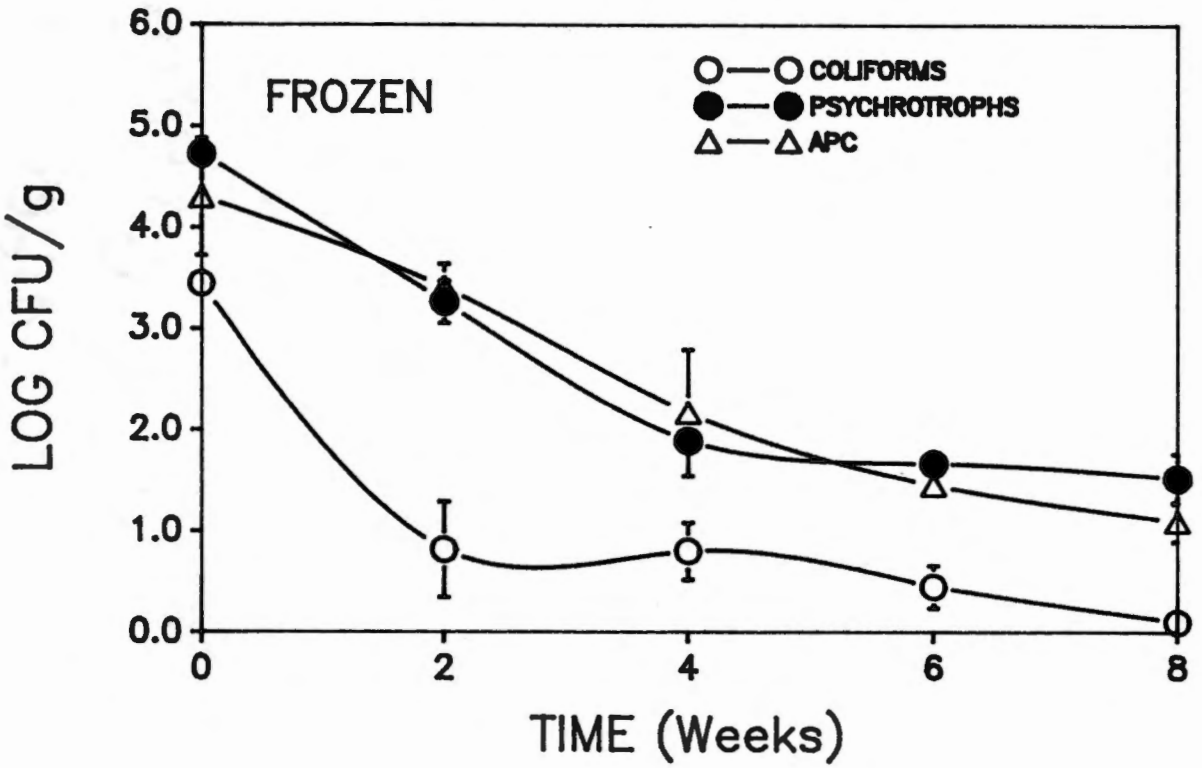

Figure 6. Effect of frozen (8 weeks at -18°C) storage on the bacterial population of white amur flesh.

It is generally agreed that the effect of freezing on the bacterial population is dependent upon several factors. Effects of freezing on microorganisms varies with type (Jay, 1978), although eventual mortality depends on the freezing process itself (Simmonds and Lamprecht, 1985). These authors agree that there is a sudden mortality immediately on freezing, with a gradual die-off over the period of time in a frozen state. The data presented in the present storage study display these characteristics (Figure 6). Evaluated on a microbial basis, these data show that freezing can be an effective method to extend the shelflife of white amur flesh.

3. Comparison of Storage at 4°C and 7°C

The effect of 4°C and 7°C storage on the coliform population of white amur flesh is shown in Figure 7. These data indicate that there was no significant difference in coliform growth on white amur flesh stored for eight days between the two storage temperatures. By day 10, however, the coliform population at 7°C was significantly ($P<0.05$) larger than that on the white amur stored at 4°C. This population had increased to >log 5 by this time. Coliforms at 4°C remained equal to or less than the initial number (<log 3.5) throughout the time period. Therefore, it was concluded that 4°C was effective in keeping the coliform population stabilized.

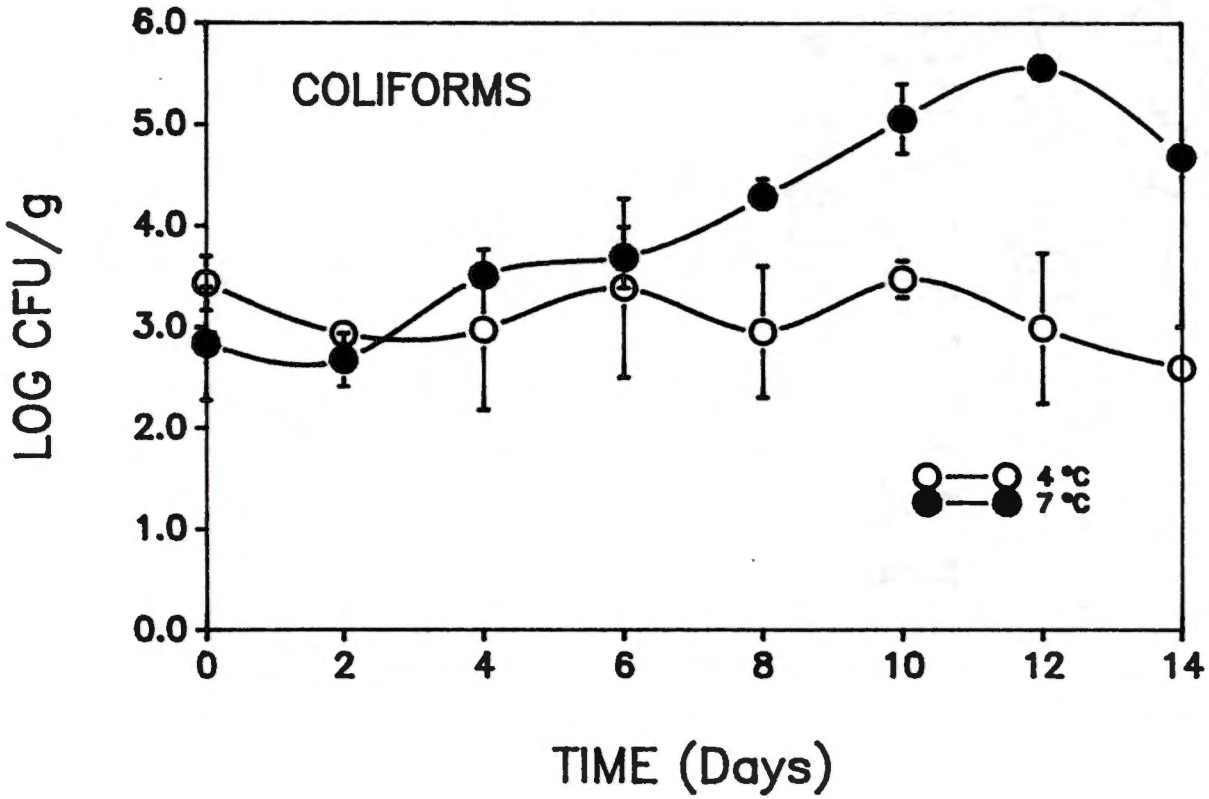

Figure 7. Effect of 4°C and 7°C storage on the coliform population of white amur flesh.

This effect was not evident with the APC (Figure 8). The aerobic bacteria increased gradually at both temperatures for 10 days. The storage temperature had no significant effect on aerobic bacteria growth until day 14, at which time spoilage had already occurred.

These data indicate that 4°C storage would provide a marginal benefit for reducing the growth of coliforms on white amur flesh. Since coliforms are mesophiles, their growth and development would expect to be retarded at lower temperatures. Perigreen et al. (1987) noted that cold shock due to low temperature storage caused a large decrease in the coliform population of fish studied. In contrast, 4°C did not have a significant advantage against aerobic bacteria. This could be due to the types of bacteria found on fish flesh. A large portion of aerobic bacteria identified on the flesh in this study were psychrotrophs (Table 7). Psychrotrophs are capable of growth at lower temperatures, and over time become the dominant flora of spoiling fish.

4. Effect of 100% CO_2 Atmoshpere

A 100% CO_2 environment had no significant effect on the growth of coliforms in comparison to the control (air) storage (Figure 9). The initial population of coliforms was approximately log 3.0 and showed a small increase in both atmospheres over the storage period. Coliforms are

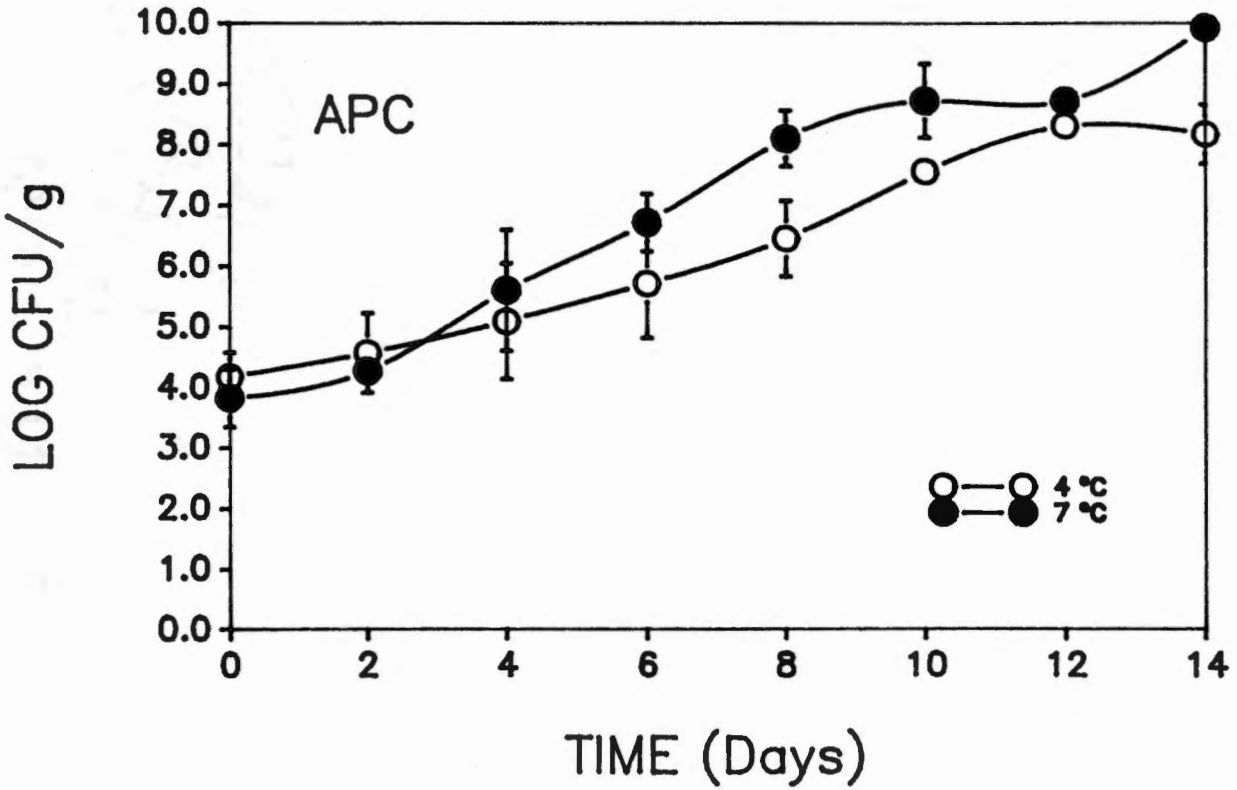

Figure 8. Effect of 4°C and 7°C storage on the aerobic population of white amur flesh.

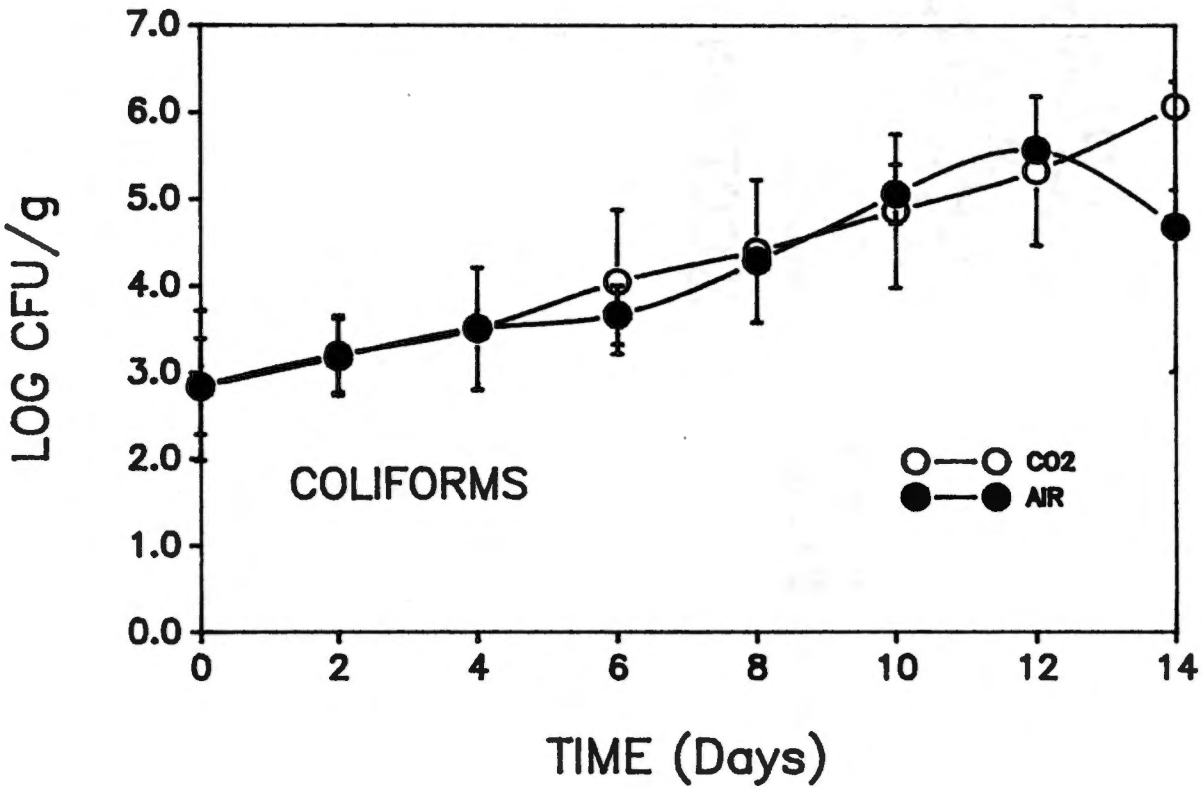

Figure 9. Effect of CO_2 modified atmosphere packaging on the coliform population of white amur flesh.

considered facultative anaerobes that can survive and grow under both aerobic and anaerobic conditions. The 100% CO_2 and air atmospheres apparently provided suitable environments for these organisms to grow.

In comparison to coliforms, CO_2 had a significant effect ($P<0.05$) on the growth of aerobic organisms throughout the study period (Figure 10). There was a sharp decrease in the bacterial population up to day 6 in CO_2. The CO_2 environment apparently was detrimental to the strict aerobic bacteria present. The microbial population in air, however, maintained steady growth from the initial sampling. After day 6, the APC in CO_2 began to increase. However, the growth rate was less than the population in air. This growth could have been due to the presence of the facultative anaerobes or to the loss of CO_2 in the environment. Carbon dioxide is known to be bactericidal at high levels. Parkin et al. (1981) showed the bactericidal effects of high levels of CO_2 on the initial flora of rockfish fillets. Barnett et al. (1987) also demonstrated the bactericidal effects of CO_2 on the bacteria of boned trout. The results of the MAP study showed that the elimination or displacement of oxygen from the package atmosphere disturbed the equilibrium of the microflora. The aerobic bacteria that are normally present and responsible for spoilage were inhibited by the CO_2. This can be considered an advantage and should be viewed as a means of extending the shelflife.

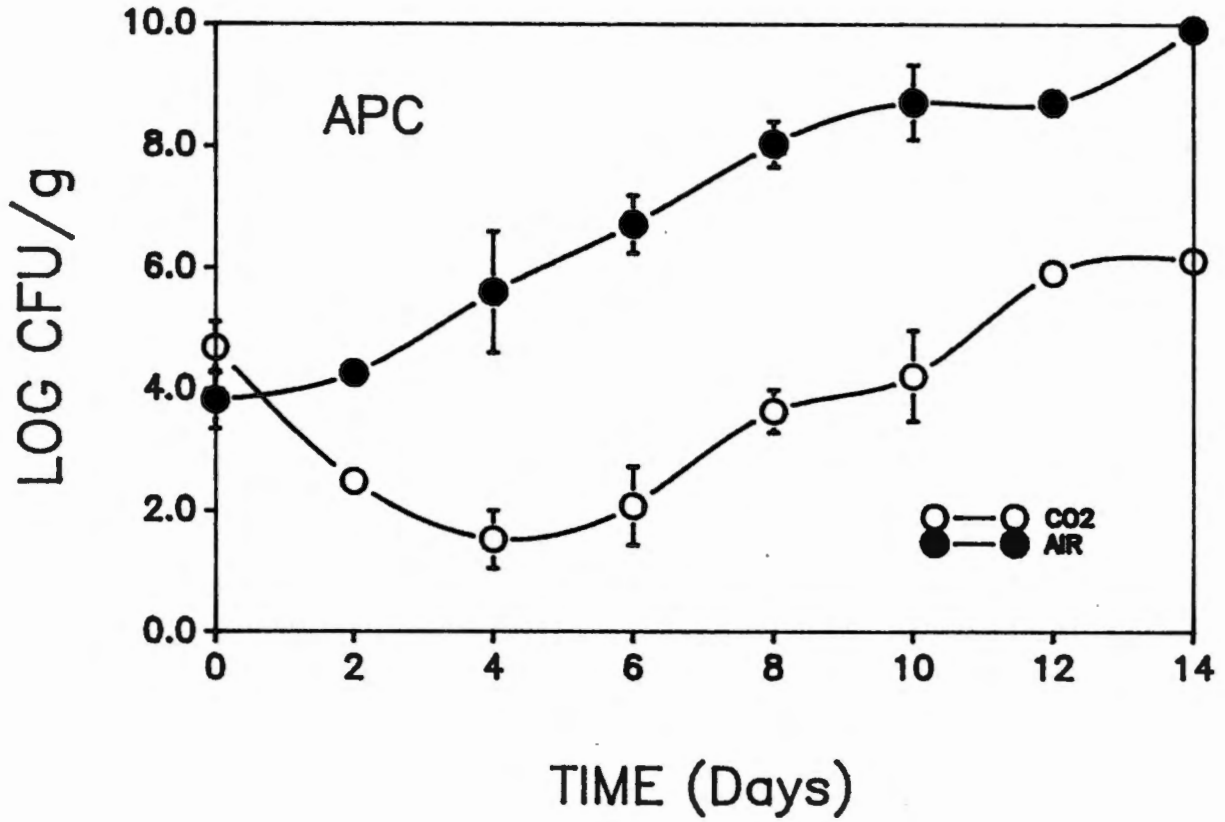

Figure 10. Effect of CO_2 modified atmosphere packaging on the aerobic population of white amur flesh.

Other studies have shown CO_2 to be effective in retarding the growth of microorganisms on fish (Brown et al., 1980; Przybylski et al., 1989; Scott et al., 1984).

A disadvantage of the CO_2 environment is that it favors the growth of some strict anaerobic bacteria which would normally be inhibited in air. There is concern whether pathogenic bacteria can grow to substantial levels prior to spoilage. This study did not address this potential problem.

The use of CO_2 was effective in reducing the initial aerobic bacterial load. Also, the coliform population showed only a small increase in CO_2. However, the growth and development of coliforms was shown to be retarded in air at 4°C in this study (Figure 7). Lannelongue and Finne (1986) also found that the inhibiting effect of CO_2 was greatly enhanced at 4°C. Therefore, the use of CO_2 modified atmospheric packaging and low temperature storage (4°C or less), was demonstrated to be inhibitory to the bacterial populations found on white amur flesh.

CHAPTER V

CONCLUSIONS

The type of diet (animal or plant) had little effect on the number or type of bacteria found on white amur. The only significant differences ($P<0.05$) between diets were the aerobic plate count (APC) of gills and APC and coliform counts of flesh. The differences between flesh samples were likely due to cross-contamination during processing. The chance for cross-contamination is great. For example, *Staphylococcus* found on fresh and frozen flesh could have been from gills, skin/mucous or the hands of the researcher. From this study, it can be recommended that using effective sanitation before and during processing would lessen the chance for contamination.

There was little difference between types of bacteria found on the white amur and those bacteria found on other fresh water fish. The spoilage bacteria consisted of psychrotrophic pseudomonads and *Acinetobacter*. These were found to be capable of growth at low temperatures and were recovered equally well at 7°C and 32°C.

The best results of the storage studies were obtained using carbon dioxide, low temperature storage and a chlorine dip. The 100% carbon dioxide packaging and 200 ppm chlorine dip were quite effective in reducing the initial aerobic

bacterial load. Low temperature (4°C) was shown to retard the growth and development of coliforms, while the chlorine pretreatment had a significant effect on lowering their numbers. These data indicate that these three conditions, separately, would be useful in reducing the bacterial load of white amur flesh during extended storage. The combination of these conditions may provide even better control of the bacterial quality. However, further tests are required before this can be verified.

The white amur has been proven to be a relatively easy fish to culture. Its fast growth rate and minimal feeding requirements are well-documented. Taste tests also have shown that white amur flesh is acceptable as foodfare. However, the presence of intramuscular bones in the flesh is a concern, according to Bakir (1985) and Hill (1988). Processing methods may have to be developed to remove these bones before fresh white amur can become a commercially viable source of food. Alternate methods such as canning, or production of surimi or a comminuted product may be investigated. The research from this study shows that bacteriological quality of white amur flesh can be maintained should it become established as a food item.

LIST OF REFERENCES

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Acuff, G., A.L. Izat, and G. Finne. 1984. Microbial flora of pond-reared tilapia (*Tilapia aurea*) held on ice. J. Food Prot. 47:778.

Alur, M.D., D.P. Nerkar, and V. Venugopal. 1988. Growth and protease secretion by spoilage bacteria: influence of nitrogen fractions of proteinaceous foods on *Aeromonas hydrophila*. J. Food Sci. 53:243.

Alur, M.D., V. Venugopal, and D.P. Nerkpar. 1989. Spoilage potential of some contaminant bacteria isolated from Indian mackerel (*Rastrelliger kanagurta*). J. Food Sci. 54:1111.

APHA. 1976. *Compendium of Methods for the Microbiological Examination of Foods*. M.L. Speck (ed.). American Public Health Association, Washington, D.C.

Andrews, W.H., C.R. Wilson, P.L. Poelma, and A. Romero. 1977. Bacterial survey of the channel catfish (*Ictalurus punctatus*) at the retail level. J. Food Sci. 42:359.

Anonymous. 1987. Fish industry to double in ten years. Aquaculture Magazine. 13:6.

AOAC. 1984. *Officical Methods of Analysis*. 14th ed. Assoc. of Official Analytical Chemists, Washington, DC.

Avault, Jr., J.W. 1986. Personal communication. School of Forestry, Wildlife and Fisheries. Louisiana State University Agricultural Center. Baton Rouge, LA.

Ayres, J.C., J.O. Mundt, and W.E. Sandine. 1980. *Microbiology of Foods*. W.H. Freeman and Co., San Francisco.

Bakir, H.M. 1985. Growth and organoleptic qualities of grass carp fed expermential diets. M.S. Thesis, The University of Tennessee, Knoxville.

Bakir, H.M. 1989. Sensory characteristics and lipid composition of white amur (*Ctenopharyngodon idella*) fed different diets. Ph.D. Dissertation, The University of Tennessee, Knoxville.

Banks, H., R. Nickelson, II, and G. Finne. 1980. Shelf-life studies on carbon dioxide packaged finfish from the Gulf of Mexico. J. Food Sci. 45:157.

Barnett, H.J., J.W. Conrad, and R.W. Nelson. 1987. Use of laminated high and low density polyethylene flexible packaging to store trout (*Salmo gairdneri*) in a modified atmosphere. J. Food Prot. 50:645.

Barrow, G.I. 1973. *The Microbiological Safety of Food.* B.C. Hobbs and J.H.B. Christian (Ed.). Academic Press, London.

Breed, R.S., E.G.D. Murray, N.R. Smith. 1957. *Bergey's Manual of Determinative Bacteriology.* The Williams & Wilkins Co., Baltimore.

Brown, W.D., M. Albright, D.A. Watts, B. Heyer, B. Spruce, and R.J. Price. 1980. Modified atmosphere storage of rockfish (*Sebastes miniatus*) and silver salmon (*Oncorhynchus kisutch*). J. Food Sci. 45:93.

Buras, N., H. Balfour, and E. Sandbank. 1980. Public aspects of fish culture in wastewater. Annual Progress Report. Technion Research and Development Foundation, Ltd. Fish and Aquaculture Research Station, Dor, Israel.

Byrd, L.A. 1973. The microbiology of pond raised catfish. M.S. Thesis. Auburn University, Auburn.

Castell, C.H., and M.F. Greenough. 1956. The action of *Pseudomonas* on fish muscle: organisms responsible for odours produced during recipient spoilage of chilled fish muscle. J. Fish. Res. Board Can. 14:617.

Chen, H.C., and C.C. Hu. 1989. Microbiological studies of the cause of discoloration of common dolphin (*Coryphaena hippurus*) fillets. J. Food Sci. 54:835.

Comar, D. 1988. Equipment and the manufacturing environment-microbiological quality assurance. Food Technol. in Aust. 40:214.

Connell, J.J. 1980. *Control of Fish Quality.* Fishing News Books Ltd. Surrey, England.

Cox, N.A., and R.T. Lovell. 1970. Identification and characterization of spoilage bacteria in fresh-water crayfish (*Procambarus clarkii* Girard). J. Food Sci. 35:6.

Cross, D.G. 1969. Aquatic weed control using grass carp. J. Fish Biol. 1:72.

Duran, A.P., B.A. Wentz, J.M. Lanier, F.D. McClure, A.H. Schwab, A. Swartzentruber, R.J. Barnard, and R.B. Read, Jr. 1983. Microbiological quality of breaded shrimp during processing. J. Food Prot. 46:974.

Elliot, E.L. 1986. Microbiological quality of Alaska pollock surimi. In *Seafood Quality Determination*. D.E. Kramer and J. Liston (eds.). Elsevier Applied Science Publishers, Amsterdam.

Eyles, M.J. 1986. Microbiological hazards associated with fishery products. CSIRO Food Res. Q. 46:8.

Fong, W.G., and G.M. Brooks. 1989. Regulation of chemicals for aquaculture use. Food Technol. 43(11):88.

Garrett, E.S., III. 1988. Microbiological standards, guidelines, and specifications and inspection of seafood products. Food Technol. 42(3):90.

Gelman, A., and E. Benjamin. 1989. Characteristics of mince from pond-bred silver carp (*Hypophtalmichthys molitrix*) and preliminary experiments on its use in sausages. J. Sci. Food Agric. 47:225.

Gram, L., J.O. Oundo, and J. Bon. 1989. Storage life of Nile perch (*Lates niloticus*) in relation to temperature and bacterial load. Trop. Sci. 29:221.

Gray, J.R.H., D.G. Hoover, and A.M. Muir. 1983. Attenuation of microbial growth of modified atmosphere-packaged fish. J. Food Prot. 46:610.

Guttman-Bass, N., N.M. Noe, and B. Fattal. 1986. Microbiological content and health effect of fishponds enriched with wastewater effluent. Water Sci. Technol. 18:211.

Hackney, C.R., and E.S. Garrett. 1985. Safety and quality of seafood. In *Reciprocal Meat Conference Proceedings*. Amer. Meat Sci. Assoc. 38:135.

Harrigan, W. F., and M. E. McCance. 1976. *Laboratory Methods in Food and Dairy Microbiology*. Academic Press, New York.

Hess, E. 1950. Bacterial fish spoilage and its control. Food Technol. 4(12):477.

Hobbs, G. 1983. Microbial spoilage of fish. In *Food Microbiology: Advances and Prospects*. T.A. Roberts and F.A. Skinner (eds.). Academic Press, London.

Hill, L. 1987. Personal communication. Hill's Farm Dist., Inc. Ronoke, AR.

Hill, T.K. 1988. Personal communication. Dept. Forestry, Wildlife and Fisheries. University of Tennessee, Knoxville, TN

Hugh, R., and E. Leifson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. J. Bacteriol. 66:24.

International Commission on Microbiological Specifications for Foods. 1980. *Microbial ecology of foods. Vol. II. Food commodities*. Academic Press, New York, N.Y.

Ingham, S.C., and N.N. Potter. 1988a. Survival and growth of *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, and *Staphylococcus aureus* on cooked mince and surimi made from Atlantic pollack. J. Food Prot. 51:634.

Ingham, S.C., and N.N. Potter. 1988b. Growth of *Aeromonas hydrophila* and *Pseudomonas fragi* on mince and surimi made from Atlantic pollack and stored under air or modified atmosphere. J. Food Prot. 51:966.

Ingraham, J.L., and J.L. Stokes. 1959. Psychrophilic bacteria. Bacteriol. Rev. 23:97.

Jay, J.M. 1978. *Modern Food Microbiology*. D. Van Nostrand Co., New York, NY.

Johnsen, P.B. 1989. Factors influencing the flavor quality of farm-raised catfish. Food Technol. 43(11):94.

Johnsen, P.B., and J.W. Kaun. 1987. Simplified method to quantify geosmin and 2-methylisoborneol concentrations in water and microbiological cultures. J. Chromatogr. 409:337.

Josephson, D.B., R.C. Lindsay and D.A. Stuiber. 1985. Effect of handling and packaging on the quality of frozen whitefish. J. Food Sci. 50:1.

Kiss, I. 1984. *Testing Methods in Food Microbiology*. Elsevier Publishing Co., New York.

Korkeala, H.J., and P.K. Pakkala. 1988. Microbiological changes in smoked and charred Baltic herrings during storage. J. Food Prot. 51:197.

Kosak, P.H., and R.T. Toledo. 1981. Effects of microbiological decontamination on the storage stability of fresh fish. J. Food Sci. 46:1012.

Lagler, K.F., J.E. Bardach, and R.R. Miller. 1962. *Ichthyology*. John Wiley and Sons, Inc., NY.

Lannelongue, M., and G. Finne. 1986. Effect of carbon dioxide on growth-rates of selected microorganisms isolated from black drum (*Pogonias cromis*). J. Food Prot. 49:806.

Lee, C.M. 1984. Surimi process technology. Food Technol. 38(11):69.

Len, P.P. 1987. Mesophilic spoilage of marine fish: bay trout (*Arripis trutta*), bream (*Acanthopagrus butcheri*) and mullet (*Aldrichetta forsteri*). Food Tech. in Aust. 39:277.

Lerke, P., L. Farber, and R. Adams. 1967. Bacteriology of spoilage of fish muscle. IV. Role of protein. Appl. Microbiol. 15:770.

Lima dos Santos, C.A.M. 1981. The storage of tropical fish in ice - a review. Trop. Sci. 23:97.

Manthey, M., G. Karnop and H. Rehbein. 1988. Quality changes of European catfish (*Silurus glanis*) from warm-water aquaculture during storage on ice. Internat. J. Food Sci. Technol. 23:1.

Martin, R.E., R.J. Gray, and M.D. Pierson. 1978. Quality assessment of fresh fish, and the role of the naturally occurring microflora. Food Technol. 32(5):188.

Miyauchi, D., M.K. Eklund, J. Spinelli, and N. Stoll. 1964. Irradiation preservation of Pacific coast shell fish. I. Storage life of king crab meats at 33 and 42°F. Food Technol. 18:928.

Molin, G., and I.M. Stenstrom. 1984. Effect of temperature on the microbial flora of herring fillets stored in air or carbon dioxide. J. Appl. Bacteriol. 56:275.

Nickerson, J.T.R., and S.A. Goldblith. 1964. A study of the microbiological quality of haddock fillets and shucked, soft-shelled clams. J. Milk Food Technol. 27:7.

Obanu, Z.A. and F.O. Ajayi. 1985. Quality deterioration in tilapia during storage in refrigerated brine. Fish. Technol. 22:24.

Okafor, N. and B.C. Nzeako. 1985. Microbial flora of fresh and smoked fish from Nigerian fresh-water. 1985. Food Microbiol. 2:71.

Otwell, W.S. 1986. Why is seafood quality less regulated than meat or poultry? Page 3. In *Water Log*. C. Jarman and D. Conner (eds.). Mississippi-Alabama Sea Grant Consortium, University, MS.

Pace, J., C.Y. Wu, and T. Chai. 1988. Bacterial flora in pasterized oysters after refrigerated storage. J. Food Sci. 53:325.

Parkin, K., M. Wells, and W. Brown. 1981. Modified atmosphere storage of rockfish fillets. J. Food Sci. 47:181.

Perigreen, P.A., J. Joseph, P.K. Surendran, and K. Gopakumar. 1987. Studies on iced storage of common murrel (*Channa striatus*). Fish. Technol. 24:99.

Pflieger, W.L. 1975. *The Fishes of Missouri*. Missouri Dept. of Conservation, Columbia, MO.

Phelps, R.P. 1985. Bacteria of public health significance associated with fish reared in treated wastewater, EPA/600/S1-85/012. Environmental Protection Agency, Washington, D.C.

Phillips, F.A., and J.T. Peeler. 1972. Bacteriological survey of the blue crab industry. Appl. Microbiol. 24:958.

Post, F.J. 1983. *Laboratory Manual for Food Microbiology*. Star Publishing Co., USA.

Post, L.S., D.A. Lee, M. Solberg, D. Furgang, J. Specchio, and C. Graham. 1985. Development of botulinal toxin and sensory deterioration during storage of vacuum and modified atmosphere packaged fish fillets. J. Food Sci. 50:990.

Przybylski, L.A., M.W. Finerty, R.M. Grodner, and D.L. Gerdes. 1989. Extension of shelf-life of iced fresh channel catfish fillets using modified atmospheric packaging and low dose irradiation. J. Food Sci. 54:269.

Ravesi, E.M., J.J. Licciardello, B.E. Tuhkunen, and R.C. Lundstrom. 1985. The effect of handling or processing treatments on storage characteristics of fresh spiny dogfish, *Squalus acanthias*. Mar. Fish. Rev. 47:48.

Redmayne, P.C. 1989. World aquaculture developments. Food Technol. 43(11):80.

Richter, E.R. and G.J. Banwart. 1983. Microbiological and sensory evaluation of fresh fish packaged in carbon dioxide for retail outlets in the midwest. J. Food Prot. 46:245.

Riemer, D.N. 1984. *Introduction to Freshwater Vegetation*. AVI Publishing Co., Westport, CT.

Roberts, T.A., F.L. Bryan, J.H.B. Christian, D. Kilsby, J.C. Olsen Jr, and J.H. Silliker. (Ed.) 1986. Microorganisms in Foods. The International Commission on Microbiological Specifications for Foods (ICMSF), University of Toronto Press, Toronto.

Saddik, M.F., M.R. El-Sherbeeny, B.M. Mousa, A. El-Akkad, and F.L. Bryan. 1985. Microbiological profiles and storage temperatures of Egyptian fish and other sea foods. J. Food Prot. 48:403.

Schoebitz, R., R. Tamayo, and M. Davis. 1985. Microbiological quality of the fish species *Trachurus murphyi*, sold at the retail level in the town of Valdivia, Chile. Food Microbiol. 2:243.

Scott, D.N., G.C. Fletcher, and G. Summers. 1984. Modified atmosphere and vacuum packing of snapper fillets. Food Technol. in Aust. 36:330.

Shewan, J.M. 1971. The microbiology of fish and fishery products - a progress report. J. Appl. Bacteriol. 34:299.

Shewan, J.M. 1976. The biochemistry and microbiology of low temperature spoilage. Food Technol. in Aust. 28:409.

Simmonds, C.K., and E.C. Lamprecht. 1985. Microbiology of Frozen Fish and Related Products. In *Microbiology of Frozen Foods*. R.K. Robinson. (Ed.). Elsevier Applied Science Publishers, London.

Singh, D., M. Chan, H.H. Ng, and M.O. Yong. 1987. Microbiological quality of frozen raw and cooked shrimps. Food Microbiol. 4:221.

Statham, J.A. 1984. Modified atmosphere storage of fisheries products: the state of the art. Food Technol. in Aust. 35:233.

Stenstrom, I. 1985. Microbial flora of cod fillets (*Gadus morhau*) stored at 2°C in different mixtures of carbon dioxide and nitrogen/oxygen. J. Food Prot. 48:585.

Stier, R.F., L. Bell, K.A. Ito, B.D. Shafer, L.A. Brown, M.L. Seeger, B.H. Allen, M.N. Porcuna and P.A. Lerke. 1981. Effect of modified atmosphere storage on *C. botulinum* toxigenesis and the spoilage microflora of salmon fillets. J. Food Sci. 46:1639.

Sulzbacher, W.L. 1950. Survival of microorganisms in frozen meat. Food Technol. 4(10):386.

Surendran, P.K., K.M. Iyer, and K. Gopakumar. 1985. Effectiveness of EDTA dips on the shelf-life of oil sardine (*Sardinella longiceps*), mackerel (*Rastrelliger kanagurta*) and prawn (*Metapenaeus dobsoni*) in iced storage. Fish. Technol. 22:28.

Surkiewicz, B.F., J.B. Hyndman, and M.V. Yancy. 1967. Bacteriological survey of the frozen prepared food industry. II. Frozen breaded raw shrimp. Appl. Microbiol. 15:1.

Sutton, D.L. 1977. Grass carp (*Ctenopharyngodon idella* Val.) in North America. Aquat. Bot. 3:157.

Tarr, H.L.A. 1954. Microbiological deterioration of fish post mortem, its detection and control. Bacteriol. Rev. 18:1.

Thrower, S.J. 1988. Aquaculture products; sanitation and quality assurance. Food Technol. in Aust. 40:314.

Wang, M.Y., and D. Brown. 1983. Effects of elevated CO_2 atmosphere on storage of freshwater crayfish (*Pacifastacus leniusculus*). J. Food Sci. 48:158.

Ward, D.R. 1989. Microbiology of aquaculture products. Food Technol. 43(11):82.

Ward, D.R., and N.J. Baj. 1988. Factors affecting microbiological quality of seafoods. Food Technol. 42(3):85.

Ward, D.R., M.D. Pierson, and K.R. Van Tassell. 1977. The microflora of unpasteurized crabmeat. J. Food Sci. 42:579.

Weagant, S.D., P.N. Sado, K.G. Colburn, J.D. Torkelson, F.A. Stanley, M.H. Krane, S.C. Shields, and C.F. Thayer. 1988. The incidence of *Listeria* species in frozen seafood products. J. Food Prot. 51:655.

Wentz, B.A., A.P. Duran, A. Swartzentruber, A.H. Schwab, F.D. McClure, D. Archer, and R.B. Read, Jr. 1985. Microbiological quality of crab meat during processing. J. Food Prot. 48:44.

Wilson, J.L., and K.D. Cottrell. 1979. Catchability and organoleptic evaluation of grass carp in East Tennessee ponds. Trans. Am. Fish. Soc. 108:97.

Zuberi, R., S.I. Shamshad, and R.B. Qadri. 1988. Bacterial flora of fermented fish sauce prepared from sardines in Pakistan. Trop. Sci. 28:239.

VITA

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