

**HAZARD ANALYSIS CRITICAL CONTROL
POINT (HACCP), MICROBIAL SAFETY,
AND SHELF LIFE OF SMOKED
BLUE CATFISH (*ICTALURUS FURCATUS*)**

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of

Master of Science

in

The Department of Food Science

by
Ligia Virginia Antonia da Silva
B.S., Tashkent State University, 1992
August, 2002

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere appreciation to Dr. Witoon Prinyawiwatkul and Dr. Joan M. King, Department of Food Science, for their support and guidance throughout my study. I also want to thank Dr. Michael W. Moody, Head of the Department of Food Science for his advice, instruction, and support and for serving on the examining committee. My appreciation is extended to Dr. Alan Biel, Department of Biological Science, for his advice and serving on the examining committee. A special thanks to Dr. David Bankston for his help and instruction with the data logger and for driving me to New Orleans.

I would like to deeply thank Dr. Donald Day, Audubon Sugar Institute and Dr. Kenneth W. McMillin, Department of Animal Science for their unconditional support, advice, and assistance and for allowing the use of their labs for the microbial and chemical analyses of this research.

My appreciations go to Dr. J. Samuel Godber for his advice. Furthermore, I would like to thank Dr. Henry Njapau for his support, advice and guidance. My thanks are also extended to Dr. Changho Chung, Duwoon Kim, Na Hua, and Alfredo Buanga for their help and friendship. I would like to thank USAID for funding and The Africa-America Institute especially Robin Caldwell for their advice and guidance.

Finally, I would like to thank my daughter Eveline Svetlana Benedita Silva Mendes Pereira, my mother Geralda Sanches Vaz, my father Agostinho Antonio da Silva, my brothers Cicero, Germano and Octoviano, my sisters Ernestina, Eloisa, Elisete, Alameda, Diomena, and Gravelina, my nephews, nieces, and in special my cousins Filomena Mendes and Luisa Sanches Vaz, for their support and encouragement.

TABLE OF CONTENTS

ACKNOWLEDGMENTS.....	ii
LIST OF TABLES	v
LIST OF FIGURES.....	vi
ABSTRACT	viii
INTRODUCTION.....	1
LITERATURE REVIEW.....	4
Catfish	4
Microbial Profile of Catfish	5
Natural Microflora.....	5
Spoilage Bacteria.....	5
Pathogen Bacteria.....	6
Bacteria in Smoked Fish	8
Antimicrobial Preservatives	13
Sodium Lactate.....	13
Sorbic Acid.....	15
Sodium Chloride	20
Antioxidants: Rosemary Extract and Ascorbic Acid	24
Hazard Analysis Critical Control Point (HACCP).....	25
MATERIAL AND METHODS	28
Samples Treatment.....	28
Smoking of Catfish	28
Microbial Analysis.....	29
Physico-Chemical Analysis.....	31
pH Determination.....	31
Water Activity Determination.....	31
Color Determination.....	32
Thiobarbituric Acid Reactive Substances (TBARS) Determination.....	32
Peroxide Value Determination	33
Proximate Analysis	34
Fat Determination.....	34
Protein Determination	34
Moisture Determination	35
Ash Determination	36
Statistical Analysis	36
RESULTS AND DISCUSSION	37
Microbial Analysis.....	37
Physico-Chemical Analysis.....	48

PH.....	48
Water Activity	49
Color	50
Thiobarbituric Acid Reactive Substances (TBARS).....	59
Peroxide Value	62
Proximate Analysis	67
Fat Content.....	67
Protein Content.....	67
Moisture Content.....	68
Ash Content.....	74
Temperature Profile.....	74
Processing Flow Chart and HACCP Plan	75
Smoking Process	80
 SUMMARY AND CONCLUSION.....	 83
 REFERENCES	 86
 APPENDIX 1: MICROBIAL COUNTS IN FRESH CATFISH STEAKS	 93
 APPENDIX 2: pH OF FRESH AND SMOKED CATFISH STEAKS	 94
 APPENDIX 3: WATER ACTIVITY OF SMOKED CATFISH STEAKS	 95
 APPENDIX 4: PROXIMATE ANALYSIS OF FRESH AND SMOKED CATFISH STEAKS.....	 96
 APPENDIX 5: THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) AND PEROXIDE VALUE (PV) OF SMOKED CATFISH STEAKS	 99
 VITA.....	 100

LIST OF TABLES

Table 1: <i>E. coli</i> , <i>Staphylococcus</i> and yeast and mold counts (Mean \pm standard deviation of log CFU/g) in smoked catfish stored for 0, 2, 4, and 6 weeks at room temperature.....	44
Table 2: Internal color changes in smoked catfish steaks during 6-week storage	55
Table 3: External color changes in smoked catfish steaks during 6-week storage.	57
Table 4: Mean and standard deviation of TBA values in raw catfish steaks.	64
Table 5: Mean and standard deviation of peroxide values in raw catfish steaks.....	64
Table 6: HACCP plan	81

LIST OF FIGURES

Figure 1: Total Plate counts (TPC), coliform and yeast and mold counts in fresh catfish steaks.	40
Figure 2: <i>E. coli</i> , <i>Staphylococcus</i> , and <i>Salmonella</i> counts in fresh catfish steaks	41
Figure 3: Total plate counts of smoked catfish steaks stored for 0, 2, 4, and 6 weeks at room temperature.....	42
Figure 4: Coliform counts of smoked catfish steaks stored for 0, 2, 4, and 6 weeks at room temperature.....	43
Figure 5: pH of fresh catfish steaks.....	52
Figure 6: Changes in pH of smoked catfish stored for 0, 2, 4, and 6 weeks at room temperature.....	53
Figure 7: Water activity of smoked catfish stored for 0, 2, 4, and 6 weeks at room temperature.....	54
Figure 8: The TBARS Standard curve	60
Figure 9: Changes in TBARS of smoked catfish during 6 weeks of storage.....	65
Figure 10: Changes in PV values of smoked catfish during 6 weeks of storage	66
Figure 11: Proximate composition of fresh catfish steaks	69
Figure 12: Changes in the fat content of smoked catfish steaks during 6 weeks of storage at room temperature.....	70
Figure 13: Changes in protein content of smoked catfish during 6 weeks of storage at room temperature.....	71
Figure 14: Changes in moisture content of smoked catfish during 6 weeks of storage....	72
Figure 15: Changes in ash content of smoked catfish during 6 weeks of storage at room temperature.....	73
Figure 16: The temperature profile in oven #1 used for cooking.....	77
Figure 17 A: The temperature profile in oven #2 used for smoking and the internal fish temperature (Ft) during smoking (Batch 1).....	78

Figure 17 B: The temperature profile in oven #2 used for smoking and the internal fish temperature during smoking (Batch2).....79

Figure 18: Smoking processing flow chart. 80

ABSTRACT

Quality deterioration of smoked catfish is caused by lipid oxidation and microbial spoilage. Smoked catfish can be cross-contaminated during processing and may harbor several pathogens, e.g., *Listeria monocytogenes*, and *Salmonella spp.* Lipid oxidation causes unpleasant flavor, thus making smoked fish unacceptable. Hydroperoxides and free radicals, formed during oxidation, may directly react with fish tissues to promote complex reactions. Through such complex reactions, physicochemical properties of smoked catfish may be adversely affected. A combination of smoking and treatments with antimicrobial agents and antioxidants would retard microbial spoilage, extend shelf life, and enhance safety of smoked catfish.

The objective of this study was to assess microbial and physicochemical quality of smoked catfish (*Ictalurus furcatus*) treated with antimicrobial agents and antioxidants during 6-week storage at room temperature.

Raw catfish steaks were subjected to the following treatments for 30 minutes prior to smoking: 25% NaCl and 1% ascorbic acid; 3% sodium lactate; 3% sodium lactate and 5% rosemary extract; and/ or 5% sorbic acid. The non-treated catfish served as control. Smoked catfish samples were drawn after 0, 2, 4, and 6 weeks for microbial, pH, water activity, proximate, color, thiobarbituric acid reactive substances (TBARS), and peroxide value (PV) analysis.

All treated smoked catfish had a water activity (a_w) less than 0.85 compared with 0.94 for the control. Total plate count (TPC) for all dried samples was 1.2- 2.2 log CFU/g at day zero and increased to 1.48-3.0 log CFU/g after 4-week storage. The control was moldy after 6-week storage. No mold was observed on samples treated with sodium

lactate, or sorbic acid even after 4-week storage. No *Salmonella* and *Listeria monocytogenes* were isolated from any smoked samples. Samples treated with 3% sodium lactate had the lowest microbial loads and were shelf-stable up to 6 weeks without refrigeration. The sample treated with rosemary extract was more stable to oxidation than all other treatments. Protein content of smoked sample ranged from 54 to 87%, 2.82 to 5.80% for ash, 13.11 to 22% for fat, and 11 to 22% for moisture. No significant change in color of smoked catfish was observed during storage.

Sodium lactate treatment was most efficient in controlling microbial quality and extending shelf life of smoked catfish.

INTRODUCTION

Fish are widespread over the oceans and rivers of the world. They are a major source of food for humans providing a significant portion of the protein intake in the diets of a large proportion of the people, particularly so in the developing countries. Fish are a cheap source of animal protein with little or no religious rejection of it, which gives it an advantage over pork or beef. Fish are a very perishable commodity, more than cattle, sheep, and poultry, and get spoiled very easily even in temperate climates. So unless it is disposed of quickly after capture, it must be preserved in some way.

World fish production was estimated at 100 million tons in 1989, 15% of which was cured in one or another way. One third of the cured fish was smoked and about 20% of the smoked fish goes into international trade. Smoking of fish and/or meat products is one of the most ancient processing technologies. It has been for centuries used for preservation, and is still widely used for this purpose among several communities in the third world where up to 70% of the catch is smoked for preservation (Ward, 1995). Hard curing by salting and smoking permits lengthy preservation by removing moisture, which is essential for bacteriological and enzymatic spoilage. In industrialized countries, however, smoking of fish is done for enhancement of flavor and texture (Dillon et al., 1994), often producing value added products whose preservation is achieved by other means. Technically, smoking is the process through which volatiles from thermal combustion of wood penetrate meat or fish flesh (Simko, 1991). The quality of smoked product is dependent on several factors including the quality of the fish at the time of smoking, and the nature of wood and type of smoking procedure employed. The effect of curing by smoking with respect to quality and shelf life of the product depends on the

preparation of the raw material, the type of smoking, relative humidity, velocity, temperature, density, and composition of the smoke, and the time of smoking (Doe et al., 1998).

There are principally three types of smoking: cold smoking at a temperature not exceeding 30°C, hot smoking in conditions causing thermal denaturation of the proteins, and hot smoke –drying. In some methods cooking of the fish in brine is included. In hot smoking the temperature may be about 80°C inside the fish or only about 50°C (Doe et al., 1998).

Increasing consumer awareness of the nutritional value of seafood has stimulated a strong demand for seafood and seafood products (Pigott and Tucker, 1990). Consumers are rediscovering the good taste of smoked seafood, including smoked catfish. To satisfy the consumer demand, it is necessary to produce good quality and safe smoked seafood products. Fish is one of the most perishable commodities worldwide. Smoked fish and shellfish products can be a source of microbial hazards including *Listeria monocytogenes*, *Salmonella spp.*, and *Clostridium botulinum* (Heinitz and Johnson, 1998). Delay or prevention of microbial spoilage of fish may be achieved by different preservative methods that include the use of smoking and chemical preservatives. Sodium lactate is a food additive used primarily as an antimicrobial. Sodium lactate can be used in fish products to delay the growth of pathogens such as *Listeria monocytogenes* and *Clostridium botulinum*, and to inhibit toxin production of *Clostridium botulinum*. The use of NaCl in combination with other preservatives can inhibit the growth of *L. monocytogenes*, and prevent the germination of *C. botulinum* endospore (USDA, 1999).

In addition, sorbic acid has been used to prevent mold growth in smoked fish during storage.

The principal chemical reaction contributing to the spoilage of smoked fish is oxidation of the lipids. The use of FDA-permitted antioxidant such as rosemary and ascorbic acid can slow down oxidative deterioration (Doe et al., 1998). Lipid oxidation may influence the color, texture, nutrition, and safety, as well as the flavor of smoked fish. In addition, the digestibility of fish proteins may be adversely affected by dehydration and storage of smoked fish. The impact of the smoking process and different storage times at room temperature on microbial, chemical and physical quality of smoked blue catfish (*Ictalurus furcatus*) has not been reported elsewhere.

The objectives of this study were to determine the microbial, chemical and physical quality changes of smoked blue catfish and to evaluate the effect of NaCl, ascorbic acid, sodium lactate, rosemary extract, and sorbic acid, either alone or in combination, on maintaining the quality of smoked catfish during 6-week storage at room temperature.

LITERATURE REVIEW

Catfish

Catfish live in both freshwater and salt water; however, the species cultured by farmers are raised in freshwater. About 1,250 species of catfish exist, but less than 50 species are located in the North America. The most common species of catfish are: channel catfish (*Ictalurus punctatus*), blue catfish (*Ictalurus furcatus*), white catfish (*Ictalurus catus*), flathead (*Pylodictis olivaris*), speckled bullhead (*Ictalurus nebulosus marmoratus*), brown bullhead (*Ictalurus nebulosus*), black bullhead (*Ictalurus melas*), and yellow bullhead (*Ictalurus natalis*). The common species of catfish are usually differentiated by color and arrangement of the external features. The most prominent feature is the fins (Lee, 1991).

Presently, the areas of the United States with the greatest concentration of catfish production are primarily in Alabama, Arkansas, Louisiana, and Mississippi. However, catfish farming is reaching the east and west and a little to the north (Lee, 1991). In 1997, the total production of food size farm-raised catfish exceeded 170 million kg live weight. Of that amount, 72% was sold frozen and 28% fresh. A 14% increase in the production of catfish during the last decade has created a need to develop new products (USDA, 1997). Catfish are a lean and highly nutritious fish that contain high amounts of vitamins, proteins, minerals, and little or no saturated fat, and is low in carbohydrates. Farm- raised channel catfish are also versatile fish that may be prepared in various ways, such as broiled, fried, baked, blackened, grilled, stir fried, and sautéed (Lee, 1991). Smoked catfish may have some appeal as a special catfish product. In developing countries, up to 70% of the total fish catch is preserved by smoking (Ward, 1995).

Smoking prolongs the shelf life of food products because of the combined effects of heat (cooking) and reduced water activity. These effects cause microbial destruction, thereby minimizing spoilage (Hintlian and Hothckiss, 1986).

Microbial Profile of Catfish

Natural Microflora

The number and type of microorganisms found on freshly caught catfish are influenced by the geographical location of the catch, season, methods of harvest, and environment (Nickelson and Finne, 1992). The microflora in the alimentary tracts of freshwater fish contain primarily species of *Aeromonas* and *Plesimonas*, and representatives of the family *Enterobacteriaceae*. These bacteria are distributed widely in freshwater environments, and are believed to survive and multiply under the selective condition of the alimentary tract of fish. *Acinetobacter*, *Bacillus*, *Flavobacterium*, *Moraxella* and bacteria species are also considered to be derived from diet and water, whereas the microflora in the intestines are reported to consist predominantly of fermentative bacteria, including *Aeromonas* and *Enterobacteriaceae* representatives. The dominant bacteria associated with the skin and gills are considered to be *Acinetobacter*, *Flavobacterium*, *Moraxella* and *Pseudomonas*. In addition, *Vibrio sp.* is common aquatic bacteria and typically is isolated as endogenous catfish flora (Austin and Austin, 1989; Davies, 1997).

Spoilage Bacteria

Fish spoilage is a complex process involving both nonmicrobiological and microbiological processes. Nonmicrobiological deterioration is caused by endogenous proteolytic enzymes, which are concentrated in the head and viscera and attack these

organs and surrounding tissues after death. Enzymatic spoilage is followed by the growth of microorganisms, which invade the fish flesh, causing breakdown of tissues and a general deterioration of the product. During processing of catfish (e.g., deheading, eviscerating, cutting), the microorganisms present in the surface slime layer, the gills and the gut can be spread onto the processing equipment, the workers and the flesh of the fillet. Hence, the normal sterile flesh can be contaminated with millions of bacteria (Banwart, 1989; Bonnell, 1994; Garthwaite, 1997; English et al., 1993).

The number and types of microorganisms in catfish fillets are determined by the natural microflora of catfish and the manner in which the fish was handled during harvesting, processing and storage (Nickelson and Finne, 1992). The primary spoilage organisms of fresh fish stored aerobically at refrigeration temperature are *Aeromonas* sp., *Pseudomonas* sp., and *S. putrefaciens* (Davies, 1997). These bacteria are psychotropic, they multiply at refrigeration temperature, and they attack various substances in fish tissue to produce off-flavors and off-odor compounds such methyl mercaptan, dimethyl disulfide, dimethyl trisulfide, 3-methyl-1-butanol, trimethylamine, and ethyl esters of acetate, butyrate and hexanoate (Nickelson and Finne, 1992).

Pathogen Bacteria

Seafood has been implicated as one of the leading foods in the transmission of foodborne outbreaks for the period of 1977-1984 in the U.S. with an incident rate of 24.8% (Bryan, 1988). Human infections may be caused by bacteria endogenous to fish. Bacterial pathogens, which may be transferred from fish to human beings include: *A. hydrophila* (septicemia, diarrhea), *Campylobacter jejuni* (gastroenteritis), *Clostridium botulinum* type E (botulism), *Edwardsiella tarda* (diarrhea), *Erysipelothrix rhusiopathiae*

(fish rose), *Leptospira interrogans* (leptospirosis), *Mycobacterium fortuitom/ marinum* (mycobacteriosis), *Plesiomonas shigelloides* (gastroenteritis), *Pseudomonas aeruginosa* (wound infections), *P. fluorescens* (wound infections), *Salmonella sp.*(food poisoning), and *vibrio parahaemolyticus* (food poisoning) (Austin and Austin, 1989). The main pathogens associated with contaminated fish are *Clostridium botulinum*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella sp.*, and *Vibrio sp.* (Faber, 1991; Jay, 1992). However, there have been no reported illnesses of foodborne disease linked to the consumption of catfish (Bryan, 1988).

Bacteria isolated and identified from whole catfish and catfish fillets, included the following pathogenic microorganisms that are known to cause foodborne illness: *Aeromonas sp.*, *C. freundii*, *E. coli*, *H. alvei*, *K. pneumoniae*, *Listeria sp.*, *P. shigelloides*, *Proteus sp.*, *S. aureus*, and *Vibrio sp.* High aerobic plate count (APC) (8.4×10^6 CFU/g) and *E. coli* (7.4×10^3 CFU/g) counts found in whole catfish (WC) indicated that catfish are highly contaminated from several potential sources, including the aquatic environment, holding tanks, and processing. Similarly, APC (2.6×10^7 CFU/g) and *E. coli* (3.2×10^3 CFU/g) counts for catfish fillets (CF) indicated that fillets are cross-contaminated during processing of catfish (e.g., deheading, skinning, eviscerating). The coliform counts and the presence of *E. coli* may indicate contamination from fecal water, or from the evisceration process. The high number of microorganisms in WC and CF along with the presence of psychrotrophic bacteria equate to the possibility of a high rate of spoilage, and thus a shorter shelf life of chilled catfish products (Ramos, 1999). The presence of pathogens in fresh aquacultured channel catfish fillets does raise the question of their safety. According to the ICMFS criterion, the presence of pathogens such as

Salmonella is considered a case 10 hazard, and cooking would reduce the degree of hazard posed by the pathogens isolated in aquacultured channel catfish fillets (Fernandez et al., 1997).

Bacteria in Smoked Fish

Smoked fish and shellfish products can be a source of microbial hazards including *Listeria monocytogenes*, *Salmonella* species, and *Clostridium botulinum*. *L. monocytogenes* has been identified in several foodborne outbreaks, in which pasteurized milk, coleslaw, and soft cheese were implicated (Fleming et al., 1985). These organisms have also been isolated from a variety of fish and shellfish products (Ben Embarek, 1994). *L. monocytogenes* is ubiquitous in nature and able to grow at low temperatures and in high salt concentration up to 10%. Studies have shown that *L. monocytogenes* can significantly increase in numbers on smoked salmon during storage at 4°C (Guyer and Jemmi, 1991). Regulatory agencies in the United States have adopted a zero-tolerance policy toward this organism in ready-to-eat food products.

L. monocytogenes is a nonsporeforming, psychrotrophic bacterium that causes the disease, listeriosis. In humans, the primary manifestations of listeriosis are meningitis, abortion and pre-natal septicemia. Immuno-compromised individuals, pregnant women and infants are most at risk. The estimated annual incidence of foodborne listeriosis in the United States is 1,850 cases and 425 deaths. Sporadic cases and outbreaks of listeriosis associated with seafood products have been reported: a 1980 outbreak (29 cases, 9 deaths) in New Zealand associated with fish or molluscan shellfish; an outbreak (9 cases) in Connecticut caused by contaminated shrimp; a case in which fish was implicated; a case in which smoked cod roe was implicated, 3 case in Tasmania caused

by smoked mussels, and 9 cases of listeriosis in Sweden suspected to have been caused by gravid cold-smoked rainbow trout. FDA surveys of domestic and imported cooked; ready-to-eat seafood products found *L. monocytogenes* in crabmeat samples and smoked fish samples (Gram, 2001). Over 17 % of cold-smoked products were positive for the organism (Gram, 2001). *Listeria monocytogenes* has been detected in a wide range of sea products (Dillon et al., 1994). *L. monocytogenes* was found in 9% of the samples of cold smoked salmon (Rorvik et al., 1991). Guyer and Jemmi (1991) examined the behavior of *L. monocytogenes* during the manufacture and storage of cold-smoked salmon. In two of the three trials, a significant increase in the level of *L. monocytogenes* occurred during storage at 4 or 10°C, which implied an increasing risk of infection for the consumer by storing cold-smoked fish for a long period of time. Although there are no reports of listeriosis caused by the consumption of this foodstuff, there have been cases which other seafoods have been implicated (Dillon et al., 1994). Goktepe and Moody (1998) determined that the initial count of *Listeria spp.* in raw catfish fillets was high (4.37 log CFU/g). The count slightly decreases (3.24 log CFU/g) after the brining process. However, after hot smoking, the count was 0 log CFU/g. The combination of heat and smoke was responsible for the elimination of this pathogen. Catfish fillets were exposed to a temperature of 82.2 °C for 3 h in order to reach an internal temperature of 67.39 °C. This temperature was high enough to destroy *Listeria spp.* In addition, smoked components such as formaldehyde and phenols have antibacterial activity that aids in the further elimination of *Listeria* (Goktepe and Moody, 1998). There may be a bactericidal effect of smoke on *L. monocytogenes* that remains on the surface of the product (Gram, 2001).

The inhibitory effect of smoke still needs to be characterized and the best method for the application of smoke needs to be identified. The inhibitory effects of salt, nitrite, and sodium lactate on microbial growth have been investigated. While these preservatives have little effect when used alone, there is some evidence that when used in combination, there is an inhibitory effect on low levels of *L. monocytogenes*. Other factors that may provide inhibitory effects include pH control, water activity, and competitive microorganisms. In hot-smoked products, *L. monocytogenes* is usually assumed to be a result of postprocessing contamination. There is some evidence that *L. monocytogenes* can survive on the surface of salmon fillets processed to an internal temperature of 83°C (181°F) without application of smoke. Other factors that may affect the survival of *L. monocytogenes* include the formation of a “pellicle,” where the surface dries before the application of smoke, which decreases the inhibitory effect of smoke (Gram, 2001). Smoked fish, especially those with lower water activity (a_w) show inhibited growth of most bacteria. However, molds can grow on its surface (Ray, 1996).

Foods commonly involved in transmission of *Salmonella* spp. include eggs, meat and meat products, and milk. *Salmonella* food poisoning is only occasionally associated with fish other than shellfish (Varnam, 1991). Processed seafood products are usually considered to present a lower risk for infections attributed to *Salmonella* species, but outbreaks have occurred. *Salmonella's* paratyphi B infections were associated with consumption of smoked halibut in Germany (Kuhn, 1994).

Heintz and Johnson (1998) analyzed the frequency of occurrence of *Listeria* spp., *Salmonella* spp., and *Clostridium botulinum* in samples of smoked finfish and smoked shellfish, and they isolated *Listeria monocytogenes* from 14% of 1,080 samples. The

incidence of *L. monocytogenes* was higher in cold-smoked than hot-smoked products (51 of 240 cold-smoked compared of 19 of 215 hot-smoked products). *Listeria* species other than *L. monocytogenes* were also detected in 7.2% of cold-smoked and 3.8% of hot-smoked products. *Listeria innocua* was the most common species isolated, followed by *Listeria seeligeri* (Heintz and Johnson, 1998).

Efiuvwevwere and Ajiboye (1996) evaluated the microbial characteristic of smoked catfish subjected to different concentrations of sodium benzoate or potassium sorbate and stored at tropical ambient temperature. They found that unsmoked fish samples showed diverse microflora (*Enterobacter*, *Escherichia*, *Serratia*, *Bacillus*, *Staphylococcus*, *Streptococcus*, *Penicillium*, *Aspergillus*, and *Achlya* genera), while smoked samples were dominated by Gram-positive bacterial flora (*Bacillus*, *Staphylococcus* and *Streptococcus*) and spoilage molds (*Penicillium verrucosum*, *Aspergillus flavus* and *Achlya* spp.). Smoking reduced the total viable count significantly in all samples, but sample treated with 0.4% (w/v) potassium sorbate showed the greatest microbial reduction especially on day 0. Abrupt and significant increase of the total bacteria counts occurred in all samples within the first 4 days of storage and remained significantly high until the end of storage. A similar trend occurred in *staphylococci* population, but control and samples treated with sodium benzoate showed the maximum load at the end of storage. A marked decrease was observed in coliform counts following smoking, but there was a less apparent (26%) reduction on day 0 in control samples, compared to 56.6% and 57.8% reduction in 0.2% and 0.4% (w/v) potassium sorbate treated samples, respectively. The samples treated with 0.4% potassium sorbate showed the minimum fungal load throughout storage (Efiuvwevwere and Ajiboye, 1996).

Hot-smoked fish receives a cook, for example, 62.8°C (145°F) for 30 min that should inactivate vegetative pathogens. The issue for *L. monocytogenes* in hot-smoked fish is the need to prevent recontamination after the cook through plant sanitation and other methods (Gram, 2001). Recommended salt levels and heat treatments used in cold and hot-smoked fish are intended to control *Clostridium botulinum* type E. In vacuum-packed products, 3.5% water phase salt is needed, and 2.5% for air packed products. *L. monocytogenes*, however, is relatively tolerant of salt, so concentrations adequate to control *C. botulinum* type E have relatively little effect. *C. botulinum* can grow fairly well in cold-smoked fish with 6% water phase salt at refrigerated temperatures. During hot smoking, products are normally heated to an internal temperature of 62.8°C (145°F) for 30 min to inactivate vegetative pathogens (Gram, 2001).

It should be noted that, although pathogens may be present, an effective HACCP program that incorporates sanitation standard operating procedures (SSOPs) and good manufacturing practices (GMPs) would result in products having a low microbial food safety risk. Furthermore, temperature-abused products containing pathogenic microorganisms may not result in food poisoning or intoxication since either the natural or adventitious flora present in the catfish product would result in spoilage prior to toxin formation. Additionally, the heat treatment during preparation would destroy or inactivate most of the pathogens (Fernandez, 1997a).

Fernandez et al. (1997b) observed a significant difference ($P \leq 0.05$) in aerobic, psychotropic, and total coliform, *E. coli* and *S. aureus* counts due to temperature effects during production and variations in processing protocols. *E. coli* and *S. aureus* counts were significantly different during the four seasons. *E. coli* and *S. aureus* counts were

high during summer and low during winter weather. There was a significant difference ($P \leq 0.05$) in aerobic, psychrotrophic, and total coliform counts among the three processors during warm weather; however, these differences were significantly ($P \leq 0.05$) reduced in cold weather.

Antimicrobial Preservatives

Sodium Lactate

Antimicrobial preservatives, either present naturally or formed during processing or legally, intentionally added as ingredients, are capable of killing microorganisms or controlling their growth in catfish. Some antimicrobial preservatives used to prolong the shelf-life of catfish are: acetic acid, lactic acid, lactic cultures, polyphosphates, potassium sorbate, propionic acid, sodium acetate, sodium ascorbate, sodium chloride and sodium lactate. Fernandez et al. (1998) stated that antimicrobial properties vary with quality and quantity of preservatives, and time of exposure.

Generally, lactic acid and lactates are used by the food industry for one of the following properties: (1) the acidification potential of lactic acid, (2) pH regulation property of sodium and potassium lactates, (3) reduction of water activity by sodium lactate, (4) synergism with common antioxidants such as ascorbic acid, and (5) antimicrobial activity. Experiments have been conducted with sodium lactate added to raw and salted salmon. Growth of *L. monocytogenes* was completely inhibited by 2% lactate at 5°C (41°F), where 3% lactate was required to inhibit growth at 10°C (50°F) (Pelroy et al., 1994). While lactate is used as a flavor enhancer in some products, it is not known what the sensory effect of lactate would be on smoked fish. Also, it may be difficult to adsorb 2% lactate into the water phase of fish. Lactate is inhibitory to

psychotropic *C. botulinum*. Meng and Genigeorgis (1993) found that the lag phase of 10^4 spores/sample of turkey roll was prolonged from 8 h to 28h at 8°C (46°F) when 2% of lactate was added. The effect of lactate was more pronounced with the concurrent addition of NaCl; adding 2% NaCl to 2% lactate increased the lag phase to 58 days.

Lactate is considered to be an effective additional hurdle against the growth of contamination flora and pathogens such as *Salmonella*, *Listeria*, *E. coli O157:H7* and *Clostridium*. In addition, lactate is acknowledged to extend product shelf life, control pathogens and enhance flavor without adversely affecting other product characteristics (Purac, 1997). Lactate acts as a bacteriostat by increasing the lag phase or dormant phase of microorganisms. Studies on the specific action of lactates indicate they stimulate mechanisms that interfere with the metabolism of the bacteria, such as intercellular acidification, interference with proton transfer across the cell membrane and feedback inhibition. Lactate also lowers water activity. This antimicrobial action suppresses growth for extended periods of time, assuring a longer shelf life and increased product safety. Since lactate does not kill bacteria, it cannot be used to mask poor sanitation practices (Purac, 1999).

A study at the University of Wageningen in the Netherlands determined the minimal inhibitory concentrations (MIC) of sodium lactate and sodium citrate for three microorganisms: *Listeria monocytogenes*, *Salmonella* and *E. coli O157:H7*. The addition of 2.5, 2, and 1.5% of sodium lactate was needed to fully stop the growth of *Listeria monocytogenes*, *Salmonella* and *E. coli*, respectively. However, were needed 7.0, 12.0, and 9.0% of citrate to inhibit the growth of these pathogens (Purac, 1999).

The effectiveness of lactate varies with its concentration. Both spoilage and pathogenic organisms found in meat are relatively sensitive to lactate, even those that are more salt tolerant. At a 3.3% use level, the shelf life of a further processed meat product may be extended by 30 to 100% (Purac 1999). Lactate is used in the further processed fish industry (fish cakes, smoked salmon, injected fillets, marinated fish). Lactate in combination with salt is the only proven ingredient that can control *Listeria monocytogenes*. The use of 3% lactate in the end product will increase the shelf life by 30 to 60%. For whole muscle products, lactate can be either injected or tumbled into the product. For the further processed fish products 2.5 to 3.0% sodium lactate (60% commercial available solution), based on the weight of the final product, can be added to the brine as the final ingredient (Purac, 1997).

Williams et al. (1995) studied the effectiveness of sodium lactate for maintaining stability of fresh catfish fillets during storage under simulated retail conditions, and they found that shelf-life of fillets treated with 2% sodium lactate was extended from 4 to 7 days. Aerobic plate counts and TBA values were lower ($P < 0.05$) for fillets treated with 2% sodium lactate, compared to controls. But, total and fecal coliforms, as well as psychrotrophs were not affected by sodium lactate.

Sorbic Acid

Sorbic acid is a natural component of the mountain tree berries (*Sorbus aucuparia*, L. *Rocaceae*). The crude sorbic acid produced by decomposition of the ketene-crotonaldehyde reaction polymer is purified and stabilized for storage, distribution, and use as an antimicrobial agent. Sorbic acid is generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA). According to The Code of

Federal Regulations, when a food preservative is used in food products, its common name should be listed on the label and its function should be indicated by an explanatory description (e.g., “to maintain freshness,” “to extend shelf-life, or “as a preservative”). Sorbic acid is active against yeasts and molds, as well as against many bacteria. Extensive research during the 1950s demonstrated the antimicrobial effectiveness of sorbates against yeasts and molds, and led to the use of the compounds as fungistatic agents in many foods. Effective antimicrobial concentrations of sorbic acid in most foods are in the range of 0.02-0.30% (Sofos & Busta, 1993).

Inhibition of yeasts, first demonstrated in the 1950s in fermented vegetables products, includes species of the genera *Brettanomyces*, *Candida*, *Cryptococcus*, *Debaryomyces*, *Endomycopsis*, *Hansenula*, *Kloeckera*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Sporobolomyces*, *Torulaspora*, *Torulopsis* and *Zygosaccharomyces*. In addition to its effectiveness in fermented vegetables, sorbic acid inhibits yeast in fruit juices, wines, cottage cheese, dried fruits, meat and fish products. Use of sorbates for inhibition of yeasts is especially important in low-pH and/or intermediate water activity products (Sofos and Busta, 1993). Sorbate also inhibits molds in butter, sausages, fruits and juices, cakes, grains, breads and smoked fish. The antimicrobial activity of sorbic acid against molds includes species of the genera *Alternaria*, *Ascochyta*, *Aspergillus*, *Botrytis*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Cunnibghamella*, *Curvularia*, *Fusarium*, *Sporotrichum*, *Trichoderma*, and others (Sofos, 1989). Sorbates have been reported to inhibit bacterial species belonging to the genera *Bacillus*, *Campylobacter*, *Clostridium*, *Enterobacter*, and *Escherichia*, including *E. coli* O157:H7, *Klebsiella*, *Lactobacillus*, *Listeria*, *Micrococcus*, *Moraxella*, *Salmonella*,

Serratia, *Staphylococcus*, *Vibrio*, *Yersinia*, and others. Depending on pH and concentration, sorbate inhibited or inactivated *Listeria monocytogenes* in a broth and in a cold-pack cheese food (Ryser and Marth, 1988). It was concluded that combinations of sorbate with propionate or lactate, which inhibited growth, could extend shelf life and increase safety. Overall, sorbates can inhibit Gram-positive and Gram-negative, catalase-positive and catalase-negative, aerobic and anaerobic, and mesophilic and psychrotrophic microorganisms, as well as spoilage and pathogenic bacteria. Inhibition of bacteria by sorbate appears to affect an extension of the lag phase, with a lesser influence on rate and extent of growth and/or vegetative cell division (Sofos and Busta, 1993).

The antimicrobial activity of sorbic acid is influenced by compositional, processing and environmental factors, such as ingredients, pH, concentration, water activity, temperature, gas atmosphere, packaging, microbial flora, and additives. The antimicrobial activity increases as the pH of the substrate decreases and approaches the dissociation constant ($pK_a = 4.76$), but sorbic acid shows effectiveness at pH values as high as 6.5 while certain studies have indicated small antimicrobial activity by sorbate at pH values as high as 7.0 (Sofos, 2000). Increased amounts of fat in a product reduce the concentration of sorbate in the water phase, where it is needed for microbial control.

Other food ingredients such as salt and sugar also reduce the concentration of sorbate in the aqueous phase; however, they act synergistically to enhance the antimicrobial activity of sorbate. In general, solutes should increase the inhibitory activity of sorbate by reducing the water activity of substrate. Sucrose, glucose, and sodium chloride, however have reduced the synergistic effect of sorbate and heat on

thermal inactivation of microorganisms, and sodium chloride (1.25 and 2.5%) reduce the inhibition of *C. botulinum* by sorbate in a nutrient broth (Sofos and Busta, 1993).

Presence of sorbic acid in food formulations may affect the rate and extent of microbial destruction during heating, as well as dormancy and recovery of heated microorganisms (Sofos, 2000). Sorbate may enhance heat activation and destruction of spores and may inhibit repair and growth of thermally injured cells, but its influence on thermal inactivation and recovery of injured microorganisms is variable among species and strains. Reduced storage temperature enhances inhibition of microbial growth by sorbic acid, which indicates that the compounds should be more useful as a preservative in refrigerated foods. However, when combined with acidification, treatment with sorbic acid may enhance the storage stability of fruits juices even at temperatures higher than refrigeration. Although food acids may reduce the water solubility of sorbate, they can enhance its antimicrobial activity by increasing the concentration of undissociated sorbic acid. In addition, the specific anion itself may contribute to antimicrobial activity (Sofos and Busta, 1993). Sorbic acid combinations with antioxidants have shown increased antimicrobial activity compared to individual components. Although results varied with type of microorganisms, antioxidants and substrates, these combinations offer the advantage of simultaneous inhibition of microbial growth and rancidity (Sofos, 2000).

Application of sorbates to foods includes direct addition in the formulation, spraying or immersing the food material in a solution, dusting with a powder, or addition in coating or packaging material. Selection of the most appropriate method depends on processing procedures, type of foods, objective to be accomplished, equipment available, and convenience. Concentrations of sorbic acid used in food preservation are in the

range of 0.02-0.3% while higher levels may cause undesirable changes in taste. In general, amounts of 0.1-0.3% are tolerated, but levels as low as 0.1% may be detectable in some foods. Commonly, the amount of sorbate used in smoked and salted fish is 0.05 to 0.3%. Sorbic acid can be applied to smoked and salted fish by immersion or spraying methods, using 5% solution (Sofos and Busta, 1993).

Sorbates are used to inhibit molds on dried and smoked fish (e.g., dried cod), while in Asian countries they are commonly used in combination with other preservatives to preserve fish and meat sausages, as well as fermented plant foods (Sofos, 2000). El-Shenawy and Marth (1988) reported that 0.05% sorbate at pH 5.6 caused a marked reduction in the growth rate of *L. monocytogenes* at 4°C (39°F). In control samples growth increased from 10³ to 10⁸ cells in 24 days, whereas a count of 10⁶ was reached after 45 days when sorbate was added. In different types of meat sausage products (which include salt and nitrite), addition of potassium sorbate delays or inhibits growth of the organism (Hu and Shelef, 1996). In some studies, sorbate was reported to inhibit spore germination, whereas others have found no effect of 1% sorbate (at pH 6.7) on germination. Vegetative cell growth is inhibited by sorbate, particularly at low pH. For instance, sorbic acid is most effective at pH < 6.0-6.5 (Kim and Foegeding, 1993).

Overall, sorbic acid appears to be one of the safest food preservatives available. In addition to being considered less toxic, sorbic acid is also more effective than benzoate or propionate in preserving foods such as cheese, fish and bakery products. The usefulness of sorbates as preservatives relies on their ability to inhibit the growth of numerous yeasts, molds, and bacteria. Inhibition of microorganisms by sorbates, however, is variable with microbial types, species, strains, food, and environmental

conditions. Some microbial strains are resistant to inhibition by sorbate or even metabolize the compound under certain conditions. In general, however, sorbates are considered effective food preservatives when used under sanitary conditions and in products processed following good manufacturing practices (Sofos, 2000). As with lactate, the application of sorbate may be difficult from a technology point of view.

Sodium Chloride

Sodium chloride, commonly known as salt, table salt, or rock salt, is a vital part of human life. Salt enhances the flavor of foods and plays a functional role in food processing. For instance, salt controls microbial growth and controls yeast activity; it enhances the texture, ripening and shelf life extension in cheese; it lowers water activity, strengthens gel structure and enhances color in processed meats (Ravishankar and Juneja, 2000).

The antimicrobial activity of sodium chloride may be either direct or indirect depending upon the purpose it serves and the amount added in a food product. In the case of dried and smoked meats, a large amount of sodium chloride is added, which makes these products shelf stable. These products depend solely on sodium chloride for their preservation, and hence the effect could be referred to as “direct”. In recent years sodium chloride is added in a minimal amount and is combined with other preservatives or hurdles to prevent microbial growth. The amount of sodium chloride that needs to be added in foods required to prevent microbial growth is large (16.54% salt solution to bring the water activity to 0.9), and will cause an unacceptable taste and hence salt is usually combined with other preservation techniques. In certain instances, sodium chloride is added mainly as a flavoring and functional ingredient and hence in these cases

the effect could be “indirect.” Another reason that the antimicrobial effect of sodium chloride may be called indirect is that it reduces the water activity in many foods and thereby indirectly prevents microbial growth (Ravishankar and Juneja, 2000).

Studies on the effect of sodium chloride on various organisms have indicated that sodium chloride could have a role in interfering with substrate utilization in these organisms. In *Staphylococcus aureus* sodium chloride was found to inhibit respiration, glucose utilization, phospho- β -galactosidase induction, and staphylococcal enterotoxin-A synthesis as well as hydrolysis of O-nitrophenyl- β -galactoside (ONPG), thereby inhibiting substrate transport into the bacterial cell (Ravishankar and Juneja, 2000).

Sodium chloride has been found to inhibit toxin production in *Clostridium botulinum*. Greenberg et al. (1959) studied the inhibitory effect of sodium chloride on growth and toxin production in *C. botulinum* types A and B in cured meat. With less than 6.25% sodium chloride there was no inhibition of toxin production as well as putrefactive changes. Between 6.25 and 9% there was no inhibition of toxin production, but the putrefactive changes did not occur, and above 9% the growth was inhibited. A concentration of 5.0% sodium chloride at pH lower than 5.03 was needed to inhibit growth of *C. botulinum* type E (Segner et al., 1966).

The effect of sodium chloride in combination with other factors on *Escherichia coli* has been investigated. A concentration of 8% or more of sodium chloride completely inhibited growth of enteropathogenic *E. coli* at different temperatures and pH levels, while a concentration of 4% in combination with pH 5.6 and 200 ppm of nitrite did not. *E. coli* O157:H7 was inhibited by 8.5% or more sodium chloride in TSB. A concentration of 2.5% did not have any inhibiting effect, while at 4.5% the generation

time was longer and at 6.5% the lag time was very long (36 h) which could have been attributed to the presence of a salt tolerant population of the organism (Ravishankar and Juneja, 2000).

Another organism studied with regard to its sensitivity to sodium chloride is *Listeria monocytogenes*. The influence of sodium chloride, temperature and pH on the growth of *L. monocytogenes* in cabbage juice was investigated. A 2% and higher concentration of sodium chloride in juice inhibited the organism. With 5% sodium chloride the survival of one strain declined 90% over 70 days at 5°C. Sodium chloride inhibits the repair of thermal injury in *L. monocytogenes* cells (Ravishankar and Juneja, 2000). Jujena and Eblen (1999) studied the interactive effect of temperature, pH, sodium chloride and sodium pyrophosphate on the heat inactivation of *L. monocytogenes* in beef gravy. In their findings, sodium chloride protected the organism against the lethal effects of heat.

Staphylococcus aureus grew in 5% sodium chloride at 12°C and *L. monocytogenes* was able to grow in 5 and 9% sodium chloride at 5 and 12°C, respectively. Sodium chloride (9%) at -2°C was either bacteriostatic or bacteriocidal depending on the organism. *L. monocytogenes* was able to survive 20% sodium chloride at -12°C for 30 days.

Li et al. (1997) tested the efficacy of sodium chloride spray to reduce *Salmonella typhimurium* in prechilled chicken carcasses. Prechilled chicken carcasses inoculated with *S. typhimurium* were sprayed with tap water, 0.85% sodium chloride solution and other antimicrobial sprays. Compared to tap water, sodium chloride did not significantly

reduce *S. typhimurium* population and hence did not prove as effective as antimicrobial spray.

In many food formulations salt is added for flavoring, and functional as well as preservative effects. Some of these food products include butter, cheese, fermented vegetables and fish, cured meats, and bread. In fish, salting has been one of the oldest methods of preservation. Fish is one of the commodities to which a large amount of salt is sometimes added and if less salt is added, it is usually combined with other methods of preservation. Dipping fish in sodium chloride solution preserves the texture and color combined with modified atmospheric packaging (MAP) and storage (Mitsuda et al., 1980). Hake slices were dipped in sodium chloride (5 min in 5% brine) and MAP stored and these were compared with MAP stored slices of hake without sodium chloride dipping (Pastoriza et al., 1998). In sodium chloride dipped slices, biochemical, microbial and sensory deterioration changes were inhibited, shelf life was extended and the total volatile bases and total viable microbial counts were significantly lower than those of non-dipped slices. The postmortem changes (rigor mortis) of Atlantic salmon influenced the salt uptake of the fish muscle (Wang et al., 1988). The equilibrium salt concentration of pre-rigor fillet was much lower (0.53 g/g salt-free solids) than that of in-rigor (0.66 g/g salt-free solids) and post-rigor mortis (0.75 g/g salt-free solids) salmon fillets in 20% (w/v) sodium chloride solution at 10°C.

In summary, salt enhances the flavor of foods and plays a preservative as well as functional role in food processing. The microbial activity of salt can be both direct and indirect depending on the amount added and the purpose it serves. Since the amount of sodium chloride needed to be added to foods to prevent microbial growth is large and

will cause an unacceptable taste, it is usually added in combination with other hurdles. The mechanism of inhibition of microorganisms by sodium chloride is mainly by lowering the water activity of the substrate. Studies have also indicated that sodium chloride could have a role in interfering with substrate utilization in microorganisms.

Antioxidants: Rosemary Extract and Ascorbic Acid

One of the principal causes of food deterioration is the oxidation of fats and oils. When foods are exposed to air, oxidation reactions start to produce undesirable flavors, rancid odors, discoloration and other forms of quality deterioration. During storage of prepared or dehydrated food, lipid autoxidative degradation products, e.g., hydroperoxides, malonaldehydes, aldehydes, ketones, and hydroxy fatty acids may occur if the food is not properly protected by antioxidants. These products not only result in unpleasant flavors but may also pose health risk. Lipid oxidation is an autocatalyzed radical chain reaction induced by free radicals. The function of antioxidants in the food matrices is to act as radical scavengers. Polyphenolic antioxidants possess a relatively reactive phenolic hydrogen atom, which functions as a donor, allowing the formation of the antioxidant phenoxy radical (Shahidi, 1997). To prevent or retard the oxidative deterioration of foods, antioxidants such as ascorbic acid and rosemary extract have been used widely as additives in fats, oils, and in food processing (Shahidi, 1997). The interest of the food industry in the phenolic antioxidants, including vitamin C and antioxidant from spice such as rosemary, is related primarily to their antioxidant activity resulting in increased shelf-life of food products. Rosemary extract has been shown to act as an effective antioxidant in several food systems, especially in animal fats and vegetable oils. The advantage of rosemary as a natural source of antioxidant is the ready availability of

the raw material and its high antioxidant potential. In practical use rosemary retarded oxidation in plain lard, piecrusts, mayonnaise, and French dressing (Shahidi, 1997). With the Rancimat test, in which fats and oils are oxidized under elevated temperatures and accelerated aeration, the induction time of lard was longer in the presence of rosemary extract or its most active components, carnosol and carnosol acid, and had stronger antioxidant activities than butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Shahidi, 1997).

Ascorbic acid (vitamin C) is a well-known biological antioxidant and exists in rather high concentration in plants, especially chloroplasts. Ascorbic acid, a water-soluble antioxidant, provided protection against oxidation of lipids and low-density lipoprotein (LDL) in plasma (Shahidi, 1997). Several researchers have proposed that vitamin C acts as an antioxidant-synergist with the lipid-soluble vitamin E and that both vitamins can work together as a powerful antioxidative system in cells. Ascorbate can reduce $O_2^{\cdot -}$ and may react with 1O_2 . Vitamin C inhibits the peroxide radical-initiated oxidation of methyl linoleate in methanol/t-butanol at 37°C. Vitamin C also acts as a chain-breaking scavenger for peroxy radicals and acts synergistically with vitamin E.

Hazard Analysis Critical Control Point (HACCP)

Organisms of primary concern are *L. monocytogenes* and *C. botulinum*. In addition, the extensive handling provides opportunities for other foodborne pathogens (that is *Salmonella spp.*, *Shigella spp.*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio parahaemolyticus*, and *Vibrio cholerae*) to contaminate products if insufficient attention is given to Good Manufacturing Practices (GMPs), Sanitation Standard Operating Procedures (SSOPs), and hygienic practices of plant employees. The

HACCP concept developed for controlling foodborne diseases is based on a simple yet fundamental premise: “identify and control.” The United States policy has a “zero tolerance” (nondetectable, by the current methods) for *L. monocytogenes*. Experiments with naturally contaminated hot-smoked fish produced from fish with high levels of *C. botulinum* show that toxin may be formed under conditions of temperature abuse. Based on a range of model studies in broth and inoculation studies with hot- or cold-smoked fish, it can be concluded that a combination of 3.5% NaCl (as water phase salt) and chill storage at 4.4 °C, (40°F), allowing for short time periods of elevated temperature up to 10°C (50°F), will prevent toxin formation in reduced oxygen packaging cold-smoked fish for several weeks beyond its sensory shelf life. For air-packaged products, levels of NaCl can, theoretically, be reduced; however, scientific data that support this argument do not exist and are needed before any reduction is recommended. Even when not packed under vacuum or modified atmosphere, pockets of anaerobic conditions may be created where slices of fish overlap or where aerobic spoilage bacteria consume the oxygen present (Ward, 2001).

The FDA regulations mandated (starting December 1997) the application of the Hazard Analysis and Critical Control Point (HACCP) principles to seafood processing. The four most prominent driving forces for use of HACCP are: (1) HACCP is focused on food safety, (2) is science based, (3) relies on preventive controls rather than retrospective end-product testing, and (4) focuses control on those food safety hazards that are reasonably likely to occur. Although HACCP holds great promise for minimizing the risk of foodborne disease, application of HACCP principles to a few foods and food processes is challenging because no useful strategies are available to

control some identified food safety hazards. Smoked fish and the process generally used for this product are examples of foods and processes that pose such challenges (Ward, 2001).

MATERIAL AND METHODS

Samples Treatment

Fresh blue catfish (*Ictalurus furcatus*), cut into steaks, were obtained in 3 separate batches from a processing plant in New Orleans, LA. Catfish steaks were randomly chosen and divided into six groups of 16 steaks each (14 lb per treatment): 1) control (untreated samples); 2) treated with 25% NaCl and 1% ascorbic acid for 1 hour; 3) treated with 25% NaCl and 1% ascorbic acid for 30 min; 4) treated with 3% sodium lactate for 30 min; 5) treated with 3% sodium lactate and 5% rosemary extract for 30 min; and 6) treated with 5% sorbic acid for 30 min. The temperature of all treatment solutions including brine was continuously monitored. The solutions temperature was adjusted to 10°C. Fresh samples were separated from each treatment for analysis. The fresh samples were placed in sterile plastic bags, placed in the icebox covered with ice, and then transported to the Food Science department at Louisiana State University in Baton Rouge. The samples were then stored at -20°C over night. The rest of the samples were smoked at 365°F for 31-36 hour, and also transported to the Food Science department and stored at room temperature (26-33°C) in plastic bags and kept in cardboard waxed paper boxes for the shelf life study. The smoked samples separated for day 0 analysis were stored at -20°C for 5 hour before analyzed.

Smoking of Catfish

The samples were processed in three different batches of 6 treatments. Treated samples of fresh catfish steaks were loaded into the first oven (oven #1), set at 325°F and cooked for 5 hours. The initial and final oven and internal temperatures of catfish steaks were recorded. After cooking, the samples were flipped over and transferred to the

second oven (oven # 2), then smoked with firewood. During the smoking process the temperatures were continuously monitored by thermocouples placed inside of oven #1 and oven #2, and also in the center of the largest fish samples in oven two.

After smoking, the catfish samples were cooled and packed in bulk and separated by treatment in plastic bags and kept in cardboard waxed paper boxes. The samples were stored at room temperature (26-33°C) in different boxes. This was done to mimic commercial practices. The samples were drawn after two, four and six weeks of storage; then subjected to analysis.

Microbial Analysis

Both raw and smoked samples were analyzed for the presence of pathogens after 0, 2, 4, and 6 weeks of storage. A 10g representative sample was obtained from the loin muscle of the fish (the thickest part of muscle) to prepare serial dilutions (10^{-1} – 10^{-3}) using sterile water as a diluent. The samples were homogenized for 60 seconds using a Seward Stomacher Lab Blender 400C (Weber Science, Hamilton, NJ). Total plate count, coliforms, *E. coli*, *Staphylococcus*, *Salmonella*, yeast and mold counts were determined by the Grid-Membrane Filtration method (GMFM) (Peterkin et al., 1998). Homogenized sample (10ml) was passed through a 0.45 mm grid membrane filter. After that, the filter was placed on a plate with media and incubated. *Listeria spp.* was determined by a qualitative method. The enrichment step was done with 10 g of the sample added to 100 ml of Demi Fraser broth, and the solution was incubated at 30°C for 24h, followed by plating (0.1ml) in selective and differential media ALOA (Agar Listeria Ottaviani & Agosti) at 37°C for 24h. ALOA is a prepared selective and differential medium for the isolation of *Listeria spp.* from foods for presumptive identification of *L. monocytogenes*.

The selectivity of the medium is due to lithium chloride and the addition of an antimicrobial mixture. The differential activity is due to the presence in the medium of the chromogenic compound X-glucoside as a substrate for the detection of beta-glucosidase enzyme, common to all *Listeria* species. The specificity is obtained by detecting the metabolism of a substrate by an enzyme (phospholipase) that is only present in the *L. monocytogenes* species. The combination of both substrates allows the differentiation of *Listeria spp.* “non monocytogenes”, which develops blue colonies, from *Listeria monocytogenes*, which develops blue colonies surrounded by an opaque halo. ALOA allows differentiation *L. monocytogenes* even in the presence of competitive flora (Microbiology International Co., 2000). Presumptive *Listeria* colonies from ALOA agar were identified using a Gram staining technique followed by API Listeria test (bioMerieux Industry, Hazelwood, MO). API Listeria is a system for the identification of *Listeria*. It uses standardized and miniature tests with a specially adapted database. The API Listeria strip consists of 10 microtubes containing dehydrated substrates, which enable the performance of enzymatic tests or sugar fermentation. The API test was done using the kit containing 10 API Listeria strips, 20 ampoules of suspension medium, 10 incubation boxes and 10 results sheets. The inoculum was prepared by suspending few well-isolated colonies in 2ml suspension medium and the strips were placed in the incubation boxes with 3 ml of distilled water. After that, 50µl of bacterial suspension was distributed into the tubes and the incubation box was closed and incubated 35°C for 24 hours. The result was read and identification was obtained in the list of profile or with identification software (bioMerieux Industry, Hazelwood, MO).

Isolation, characterization and identification of *E. coli* and coliforms were done by using m-ColiBlue24 broth (Hach Co., Loveland, CO), and for yeast and mold m-Green YM broth was used (Gelman Co., Ann Arbor, MI). For enumeration of coliforms were counted all blue and red colonies, and for *E. coli* was counted only the blue colonies. For total plate count, *Staphylococcus* and *Salmonella* testes, m-plate count broth, m-Staphylococcus broth and MacConkey broth, respectively, were used (Diffco Lab, Detroit, MI). The Total Aerobic Plate Count (APC) plates were incubated at 37°C for 48h, and the plates with *Staphylococcus* and *Salmonella* cultures were incubated at 35°C for 48h. The yeast and molds cultures were incubated for 48h at room temperature. Following incubation, colonies were counted and data reported as log CFU/g (colony forming units). Triplicate experiments were conducted, and each dilution was plated in duplicate.

Physico-Chemical Analysis

pH Determination

pH determination was performed using a digital pH/mV/temperature meter (Model IQ 150, IQ Scientific Instruments, Inc., San Diego, California). pH of each sample was measured after pre-treatment and after smoking for day 0, and weeks two, four, and six, in duplicate. Ten grams of each sample was blended with 30ml of deionized water for 1 min, and the sample homogenate was allowed to stay for 5 min before taking a reading.

Water Activity Determination

Water activity of smoked catfish samples for all three batches and for stored samples (0, 2, 4, and 6 weeks) was determined in quadruplicate using two different water

activity meters a Novasina Thermoconstanter TH200 (Novasina, Tempe, AZ) and an AwQuick (Rotronic Instrument Corp., Huntington, NY). The sample was taken from the thickest part of the muscle loin of the fish.

Color Determination

The color measurements of smoked catfish were determined for all three batches and stored samples (0, 2, 4, and 6 weeks) in five replicates, using a Minolta Spectrophotometer (Model CM-508d, Minolta Camera Co., Ltd., Osaka, Japan). The instrument was calibrated with zero and white calibrations to compensate for the effects of stray light and to eliminate variations in measured values due to changes in ambient temperature and the internal temperature of the Spectrophotometer. The Spectrophotometer was set to obtain color values based on 10° Standard Observer and D65 illuminants. Results were expressed in terms of L*, a*, and b* values. Psychometric color terms involving hue angle $[(\tan^{-1} (b^*/a^*))]$ and chroma $[(a^{*2} + b^{*2})^{1/2}]$ were calculated for all six treatments of smoked catfish. The L* value measures lightness (0=black and 100=white); a positive a* value represents redness and a negative a* represents greenness; a positive b* value represents yellow and a negative b* value represents blue. The hue angle represents an actual color, and chroma evaluates purity or intensity of the color. The ΔE (total color difference) was also determined.

Thiobarbituric Acid Reactive Substance (TBARS) Determination

Oxidative stability of smoked catfish was measured by Thiobarbituric acid-reactive substance (TBA-RS) following the procedure Cd19-90 (AOCS, 1996). Results were expressed as mg malonaldehyde /Kg dry weight. A 10g sample of fat from the belly flap of the fish was combined with distilled water and thoroughly mixed. After

addition of 2.5 ml of 4 N HCl, Dow antifoam A, and boiling beads, the sample was distilled and the first 50 ml collected. An aliquot of the distillate (5 ml) was combined with 5 ml of TBA reagent and heated in a boiling water bath for 35 min. Absorbance of the solution was determined at 530 nm, using a slope of TEP (1,1,3,3-tetraethoxypropene, Sigma Chem. Co., St. Louis, MO) standard curve, which was derived to correlate with the expected range of values. Absorbance readings were calculated as μ moles TBARS/kg and then converted to milligrams of malonaldehyde (or TBARS) per kilogram of sample. The TBARS was calculated by the following formula:

$$\text{TBARS} = (T * V1 * 1000) / (V2 * W)$$

Where: TBARS value in μ moles/kg fish

T = μ moles malonaldehyde (TEP) equivalent to absorbance of sample as determined from the standard curve;

V1= volume (ml) of distillate collected, usually 50ml;

V2= volume (ml) of distillate aliquot withdrawn for analysis, usually 5ml;

W= weight of fish added to distill, usually 10g.

Peroxide Value (PV) Determination

Oxidative stability of smoked catfish was also measured using titrimetric determination of the amount of peroxide or hydroperoxide groups, the initial product of lipid oxidation. To 5g of sample (fat from belly flap of the fish) 30 ml glacial acetic acid-chloroform solution (3:2 v/v) was added and swirled. Excess potassium iodide (0.5ml of KI solution) was added to react with the peroxides and iodine was liberated. After 1 min., 30 ml of H₂O was added. Then the solution was titrated with 0.1 N sodium thiosulfate (Na₂S₂O₃). 0.5ml of 1% starch indicator was added and the titration was continued until blue color disappeared. The peroxide value was calculated by multiplying ml of Na₂S₂O₃

by normality and by 1000 then divided by grams of catfish sample (AOAC 965.33, 1990).

Proximate Analysis

The fat, protein, moisture, and ash contents of the raw and smoked catfish were determined by using the following AOAC methods for fat (AOAC991.36, 1995), protein (AOAC 981.10, 1995), moisture (AOAC 950.46B, 1995), and ash determination (AOAC 942.05, 1995).

Fat Determination

The fat content of raw and smoked catfish was determined by a solvent extraction (Submersion) method for fat (Crude) in Meat and Meat Products (AOAC991.36, 1995). Soluble material was extracted from dried samples by a 2-step treatment with petroleum ether solvent. Solvent was recovered by condensation, leaving extracted soluble material, which was determined by weight after drying. Three grams of homogenized fish samples were mixed with sand and dried 1h in an oven at 125°C. Samples were extracted with 40ml of petroleum ether at boiling temperature for 25 min, and rinsed for 30 min. The fat deposited from the sample was recovered with the extraction cups. The cup and contents were dried for 30 min at 125°C, then cooled and weighed. Percent of fat in the sample was calculated by subtracting the weight of the extraction cup after drying from the weight of the extraction cup before extraction times 100, then divided by the sample weight (AOAC 991.36, 1995).

Protein Determination

The protein content was determined by a Block Digestion method (AOAC 981.10, 1995). Approximately 2g of well-ground and mixed catfish samples were

weighed and transferred to a 250 ml digestion tube. H₂SO₄ (15 ml) was added to each tube, and 3ml of 30-35% H₂O₂ was slowly added. After the reaction subsided, the tubes were placed in a block digester, and the mixture was digested at 400°C until it became clear (for 45 min.). The tubes were removed and cooled for 10 min. Carefully 50-75 ml H₂O was added. The NaOH-Na₂S₂O₃ solution was placed in an alkali tank of the steam distillation unit. A distillation tube containing diluted digest was attached to the distillation unit. A receiving flask, containing 25 ml H₃BO₃ solution with mixed indicator, was placed on the receiving platform. The mixture was steam distilled and 100-125 ml were collected (absorbing solution turns green from liberated NH₃). Digestion tubes and receiving flasks from the unit were removed. The absorbing solution and reagent blank were titrated with 0.2N HCl to a neutral gray end point. The protein content was calculated using the following formula:

% Protein = $(V_A - V_B) \times 1.4007 \times N / \text{g sample}$, where V_A and V_B = volume standard acid required for sample and blank respectively; 1.4007 = milliequivalent weight N × 100 (%); N = normality of standard acid.

Moisture Determination

The moisture content was determined by a mechanical convection oven drying method (AOAC 950.46B, 1995). With lids removed, samples containing ca. 2g dry material was dried to constant weight (2-4h) in a mechanical convection oven at 125°C. The samples were covered with an Al dish ≥50 mm diameter and ≤40 mm deep, cooled in a desiccator and weighed. Loss in weight was reported as moisture.

Ash Determination

Ash content was determined by measuring the mass of a dried sample before and after it had been heated in a muffle furnace. Approximately 2g of catfish sample was weighed into crucibles and placed in a temperature-controlled furnace preheated to 600°C. The crucible was held at this temperature for 2h; then it was directly transferred to a desiccator, cooled, and weighed. The percent of ash was reported to the first decimal place (AOAC 942.05, 1995).

Statistical Analysis

Analysis of variance (ANOVA) was performed to determine differences in physico-chemical properties among raw and smoked catfish samples. The procedure General Linear Model (GLM) was used in data analysis. Tukey's studentized range test was performed for post-hoc multiple comparison (SAS 8.1, 2000).

RESULTS AND DISCUSSION

Microbial Analysis

A study for the absence and presence of the target foodborne pathogens such as *Listeria*, *Salmonella*, *Staphylococcus*, and *E. coli* is required to evaluate microbial safety of smoked catfish (*Ictalurus furcatus*). The data obtained from microbial analysis will be used in the development of generic HACCP plans for smoked catfish processing.

Pathogens can enter the process through raw materials. They can also be introduced into foods during processing from the air, unclean hands, unsanitary utensils and equipment, unsafe water, and sewage, and through cross contamination between raw and cooked product (FDA, 2001. Fish and Fishery Products Hazards & Controls Guidance manual).

The range of specified microbiological limits recommended by ICMSF (1986) for fish and fishery products is as follows: for the TPC, the maximum recommended bacterial counts for good quality products (m) is 5×10^5 ($5.7 \log_{10}$ CFU/g) and the maximum recommended bacterial counts for marginally acceptable quality products (M) is 10^7 ($7 \log_{10}$ CFU/g). For *E. coli*, the m value is 11 ($1.0 \log_{10}$ CFU/g) and the M value is 500 ($2.7 \log_{10}$ CFU/g), and for *Staphylococcus*, m value is 10^3 ($3 \log_{10}$ CFU/g) (IMSF, 1986). The FDA and EPA safety levels relating to safety attributes of fish and fishery products published in regulations and guidance are the following: for ready-to-eat fishery products (minimal cooking by consumer), the Enterotoxigenic *Escherichia coli* (ETEC) level is 1×10^3 ETEC/g (; for *L. monocytogenes* and *Salmonella*, the level is the presence of the organism (zero tolerance). For all fish, the *Staphylococcus aureus* safety level is equal to or greater than 10^4 /g. In many cases, these levels represent the point at or above

which the agency will take legal action to remove products from the market (FDA, 2001, Fish and Fishery Products Hazards & Controls Guidance manual).

The traditional method of examining microbiological safety, storage, stability, and sanitary quality of food is to test a representative portion (or samples) of the final product for the presence of some pathogens (e.g., *Salmonella*) or the number or level of certain pathogens (e.g., *Staphylococcus aureus*), different microbial groups (e.g., aerobic plate counts and yeast and molds), and indicator bacteria (e.g., coliforms are used as an indicator of sanitation) per gram or milliliter of product.

Aerobic counts (log CFU/g) of fresh and smoked catfish samples plated on selective and nonselective media are shown in Figures 1, 2, 3, 4, and Table 1. Figure 1 represents the microbial counts of fresh catfish treated with antimicrobial agents and antioxidants before smoking and storage. Total plate count (TPC) of the fresh non-treated catfish was 4.6 log CFU/g (control), but after the samples were subjected to treatments with 25% NaCl and 1% ascorbic acid, and 5% sorbic acid, the TPC reduced to a ca. 2.6 log CFU/g for both samples. In addition the TPC of fresh samples after treated with 3% sodium lactate reduced to 2.4 log CFU/g. Coliform and *E. coli* counts of fresh non-treated sample (control) were 2.8 log CFU/g and 2.0 log CFU/g, respectively. Treatment of fresh catfish with salt reduced the coliform and *E. coli* counts to 2.4 log CFU/g and 1.3 log CFU/g. Sodium lactate reduced the coliform and *E. coli* counts to 2.0 and 1.7 log CFU/g, respectively. Coliform and *E. coli* counts of the samples treated with sorbic acid were 2.3 and 1.8 log CFU/g, correspondingly. *Staphylococcus* count was reduced after the fresh catfish steaks were subjected to treatments with salt (1.3 log CFU/g), sodium lactate (1.2 log CFU/g) and sorbic acid (1.5 log CFU/g), compared to that of the control (2.0 log

CFU/g). There was not a difference in *Salmonella* count (2 log CFU/g) between the fresh catfish control and samples treated with salt. However, there was a reduction in the samples treated with sodium lactate (1.2 log CFU/g) and sorbic acid (1.8 log CFU/g). The yeast and mold count of fresh catfish steaks ranged from 1.3 log CFU/g for the sample treated with sodium lactate and rosemary extract, to 2.3 log CFU/g for the control sample.

Smoking sharply reduced the total plate count (Figure3) in all samples, but the sample treated with 3% sodium lactate showed the greatest reduction and maintained a low level throughout 6 weeks of storage, especially on day 0 (1.2 log CFU/g). TPC of sodium lactate treated sample was 2.6 log CFU/g after 6-week storage. The TPC of the smoked catfish samples treated with 3% sodium lactate and 5% rosemary extract was similar to that of the samples treated with 5% sorbic acid after 2 weeks of storage. Both had 1.3 log CFU/g on day 0. At week 2, the TPC of the sample treated with 3% sodium lactate and 5% rosemary extract was 1.6 log CFU/g, and 1.7 log CFU/g for the sample treated with 5% sorbic acid. Significant increases in TPC occurred in all smoked catfish samples at the 6th week of storage, except for the control samples. The smoked control (untreated) samples were completely covered by mold after the 4th week of storage; therefore, no further microbial analysis was conducted. The above results were similar with those reported by Williams et al. (1995) where the APC for fresh catfish fillets treated with 2% and 1% sodium lactate were lower ($p < 0.05$) than controls after 8 days storage. Goktepe and Moody (1998) also reported that aerobic plate counts in raw catfish fillets were 4.03 log CFU/g prior to brining and 3.61 log CFU/g after brining. However, after hot smoking the APC was zero.

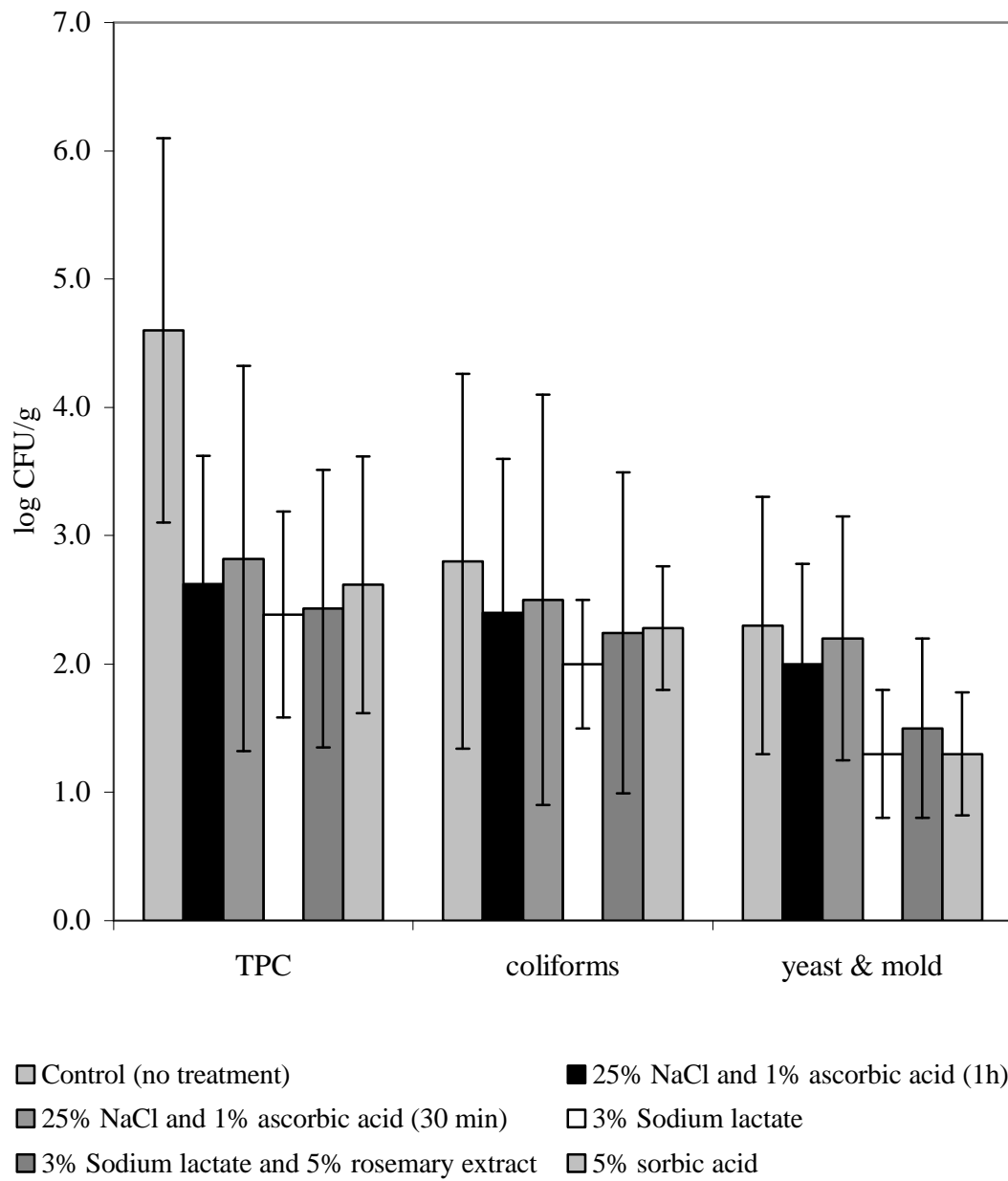


Figure 1: Total Plate counts (TPC), coliform and yeast and mold counts in fresh catfish steaks

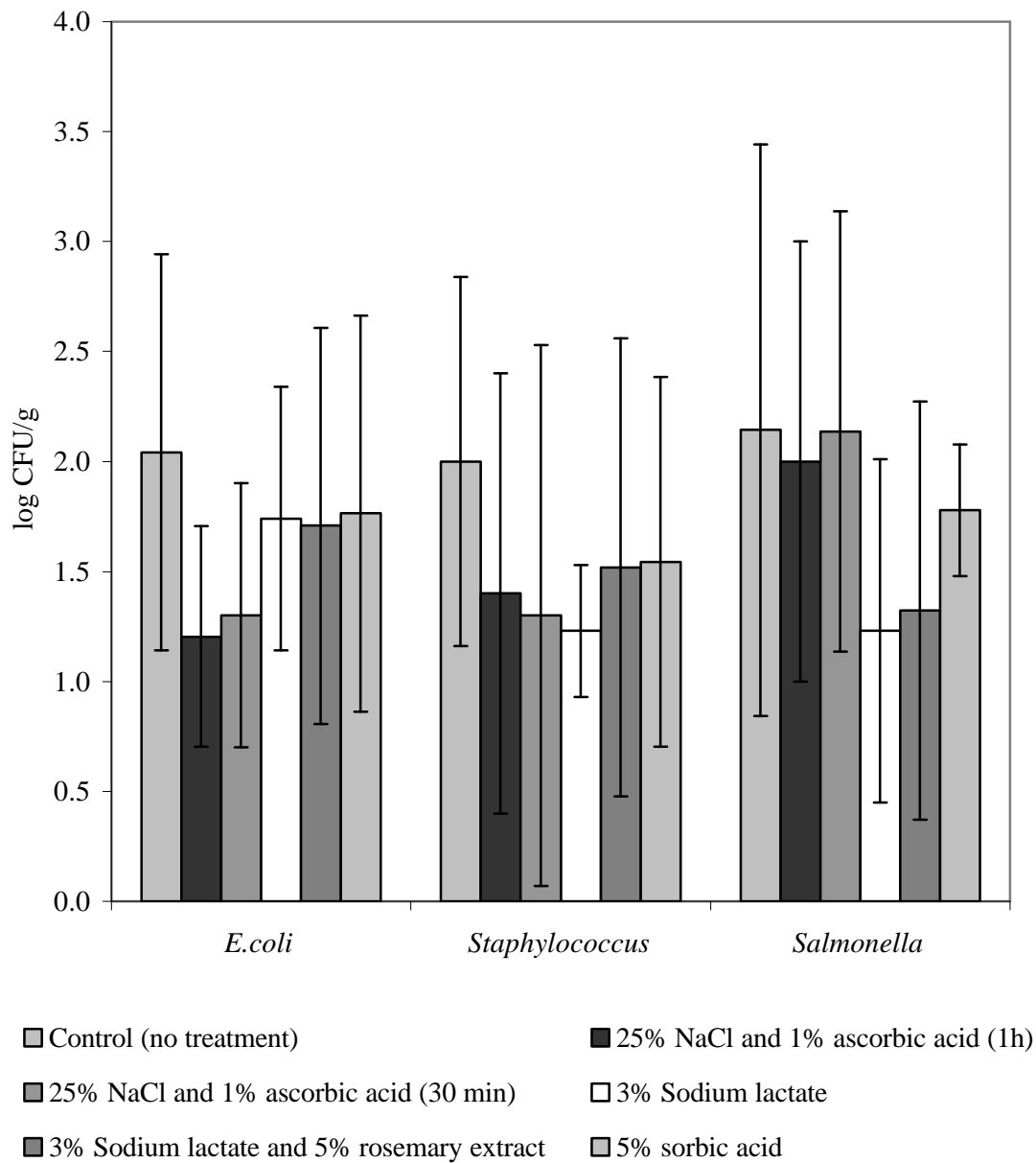


Figure 2: *E. coli*, *Staphylococcus*, and *Salmonella* counts in fresh catfish steaks

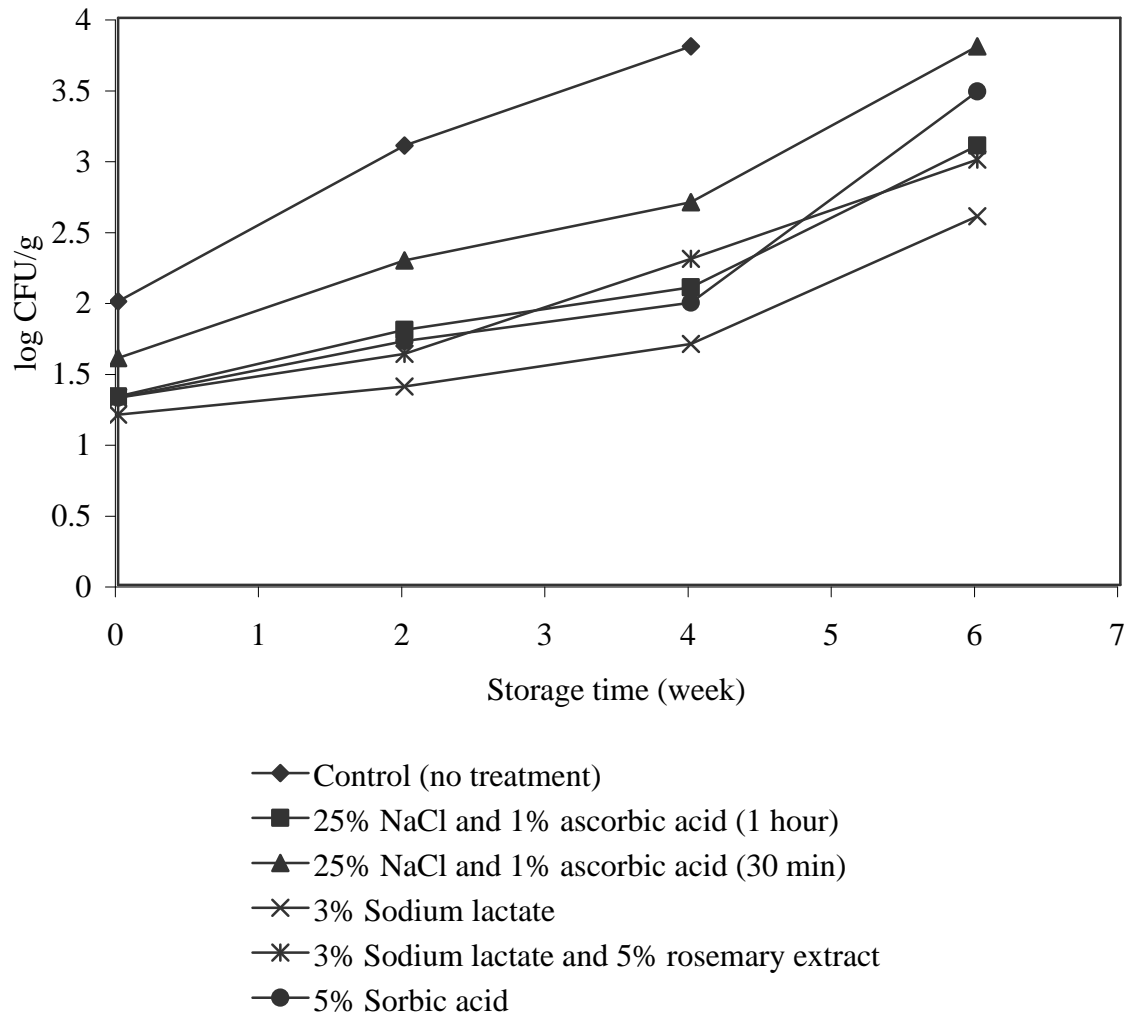


Figure 3: Total plate counts of smoked catfish steaks stored for 0, 2, 4, and 6 weeks at room temperature.

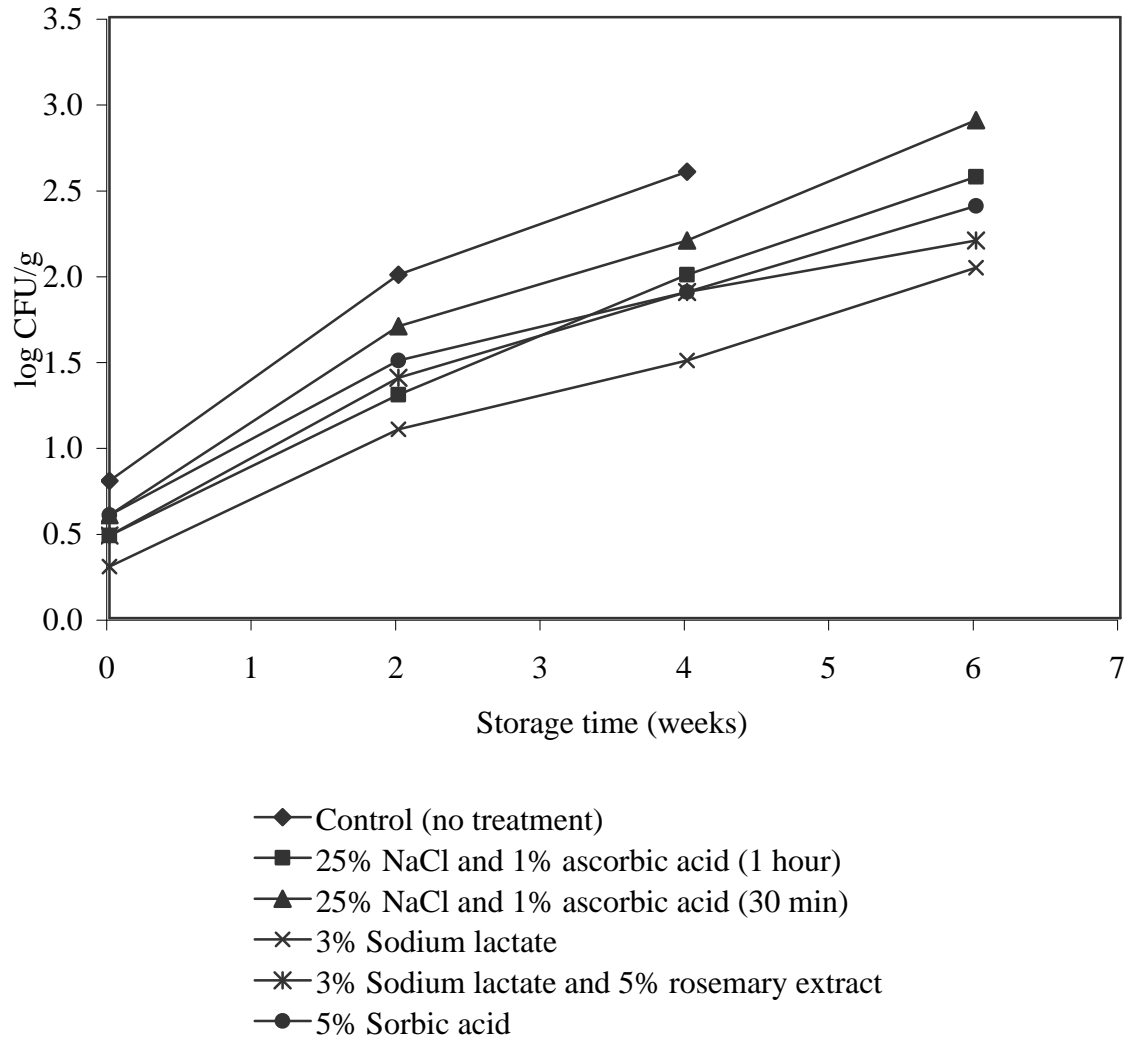


Figure 4: Coliform counts of smoked catfish steaks stored for 0, 2, 4, and 6 weeks at room temperature.

Table 1: *E. coli*, *Staphylococcus* and yeast and mold counts (Mean \pm standard deviation of log CFU/g) in smoked catfish stored for 0, 2, 4, and 6 weeks at room temperature.

Microbes	Storage time (week)	Treatments ^a					
		1	2	3	4	5	6
<i>E. coli</i>	0	0.6 \pm 0.3	0 ^b	0.5 \pm 0.3	0	0	0
	2	0.7 \pm 0.3	0	0	0	0	0
	4	1.6 \pm 0.5	0	0	0	0	0
	6	*	0	0	0	0	0
<i>Staphylococcus</i>	0	1.3 \pm 0.5	0.3 \pm 0.3	0.3 \pm 0.3	0.5 \pm 0.3	0.9 \pm 0.5	0.9 \pm 0.3
	2	1.8 \pm 0.3	0	0	0	0	0
	4	2.2 \pm 0.3	0	0	0	0	0
	6	*	0	0	0	0	0
Yeast and mold	0	0.6 \pm 0.3	0	0	0	0	0
	2	0.8 \pm 0.3	0	0	0	0	0
	4	1.0 \pm 0.6	0	0	0	0	0
	6	*	0.4 \pm 0.3	0.4 \pm 0.3	0	1.2 \pm 0.5	0

^a1=Control (no treatment); 2=25% NaCl and 1% ascorbic acid (1 hour); 3=25% NaCl and 1% ascorbic acid (30 min); 4=3% Sodium lactate; 5=3% Sodium lactate and 5% rosemary extract; and 6= 5% Sorbic acid.

^b0 = no detected.

*Control sample was completely covered with mold on the 6th week of storage.

Catfish steaks were exposed to a temperature of 312⁰F to reach an internal temperature of 232⁰F for 5 hours. This temperature was high enough to destroy all the viable microorganisms in the final product. However, after smoking, the microbial count was not higher than 2.0 log CFU/g (control) on day 0. The presence of microbes in the smoked catfish steaks on day zero might be due to post processing contamination during packaging and storage, or recovery of injured cells.

Similar to TPC, coliform population (Figure 4) of the smoked samples treated with 3% sodium lactate was sharply reduced and showed 0.3 log CFU/g on day 0. Coliform population of the samples treated with 25% NaCl and 1% ascorbic acid for 30 min increased (1.7 log CFU/g) in the second week of storage and stayed high until the end of the storage period (2.9 log CFU/g after 6 weeks). Significant increases in coliform population of all samples occurred after 4 weeks of storage. Coliform count of all treated samples was less than 3.0 log CFU/g throughout the 6-week storage. However, the control sample showed 2.6 log CFU/g on the 4th week and the sample was completely covered by mold on the 6th week of storage. Williams et al. (1995) also reported that the fecal coliforms of fresh catfish fillets were similarly low ($p < 0.05$) for all treatments (1 and 2% sodium lactate), and remained at < 2 log counts throughout 8 days storage. In addition, smoking also reduced *E. coli*, *Staphylococci*, and yeast and mold (Table 1) counts. The smoked control and the sample treated with 25% NaCl and 1% ascorbic acid showed 0.6 and 0.5 log CFU/g of *E. coli* (Table 1), respectively at day 0 and the counts reduced to 0 on week 2 and remained 0 until the end of 6-week storage for the sample treated with salt. *E. coli* bacteria count for the control slightly increased to 0.7 log CFU/g in the second week and reached 1.6 log CFU/g in the 4th week of storage. The sample

treated with 25% NaCl and 1% ascorbic acid for 30 min, 3% sodium lactate, and/or 3% sodium lactate and 5% rosemary extracts showed similar results of 0 CFU/g after smoking and throughout 6-week storage time. The isolation of *Staphylococcus* in smoked samples on day 0 can be attributed to postprocessing contamination. However, *Staphylococcus* was killed by the treatments (salt, sodium lactate, and sorbic acid) and the count dropped to 0 on the second week. The treated samples were maintained *Staphylococcus* free during storage due to low a_w (less than 0.85). *Salmonella* population was reduced to 0 log CFU/g after smoking and stayed stable until the end of storage time. All the smoked samples tested negative for *Listeria monocytogenes*. The fresh control sample contained *L. monocytogenes* and *L. innocua*. The fresh samples treated with 25% NaCl and 1% ascorbic acid for 1 h contained *L. innocua*. The samples treated with 5% sorbic acid contained *L. welshimeri* and *L. innocua*. The fresh samples treated with 25% NaCl and 1% ascorbic acid for 30 min., sample treated with 3% sodium lactate, and sample treated with 3% sodium lactate and 5% rosemary were negative for *Listeria*. Goktepe and Moody (1998) reported that *Listeria spp.* counts of raw catfish fillets were 4.37 log CFU/g; after brining the count decreased slightly to 3.24 log CFU/g. After hot smoking no *Listeria spp.* were detected in samples.

The population of the yeast and mold (Table 1) increased at the end of the 6-week storage time; however, the sample treated with sorbate showed 0 count throughout 6-weeks storage. Yeast and mold count for the sample treated with 3% sodium lactate was also 0. The control sample was completely covered by mold at the end of the 6-week storage. The sample treated with 3% sodium lactate and 5% rosemary extract showed 1.2 log CFU/g, which was greater than all the other treatments, except for the control sample.

The results obtained were similar to those reported by Efiuvwevwere and Ajiboye (1996), where the samples treated with 0.4% potassium sorbate showed the minimum fungal load throughout storage. Efiuvwevwere and Ajiboye (1996) attributed the initial marked decrease in microbial population of the smoked catfish to the destruction or inhibition of the Gram-negative flora but the subsequent dramatic increase may be attributed to recovery of sublethally injured cells and limited competition among the surviving more heat-resistant microorganisms such as *Bacillus spp.* and staphylococcus.

Microbial counts of the sample treated with 3% sodium lactate and 5% rosemary were higher than that treated with 3% sodium lactate alone. This difference can be attributed to the fact that rosemary extract was oily and greasy, which can prevent the sample from drying. The sample treated with 3% sodium lactate had lower moisture content and lower water activity, consequently it had lower microbial load. In addition all treated samples had a_w lower than 0.85, however some microbial growth was observed. This increase in microbial count can be due to the increase in surface water activity of the smoked catfish steaks during storage. The bacterial contamination of hot smoked fish just out of the smokehouse, is usually below 10^3 per gram. In hot smoked eel, just after smoking, the TPC is usually less than 20 per gram (Doe, 1998).

The APCs of the samples were all below 5×10^5 CFU/g (Figure 1), which is below m in a three-class attribute plan and signifies good quality. Low levels of coliform bacteria were detected and *E. coli* counts were below 11 CFU/g. The pathogens *Staphylococcus aureus* counts were below 10^3 , and *Salmonella* were not isolated from any of the smoked samples. The range of tests and specified microbiological limits recommended by the ICMSF (1986) for smoked fish reflected the potential hazard of the

product. The microbial results were, however, within the ICMSF limits, so the samples were of acceptable microbial quality.

Physico-Chemical Analysis

pH

Water activity and pH are among the most critical factors affecting microbial growth and spoilage of foods. The pH of living fish, although generally between 6.7 and 7.0, is subjected to variation with time of year, feeding, and degree of activity of the fish (Woyewoda, 1986). After death, glycogen stored in the muscle is broken down by glycolysis to produce lactic acid and in turn pH is lowered. This reduction accompanies the onset of rigor mortis where the fish muscle stiffens and muscle filaments shorten temporarily. Once rigor has passed, bacterial activity produces a gradual increase of pH through the production of ammonia and other bases. Therefore, pH first decreases with the onset of rigor but then progressively increases from microbial activity. For some fish, a pH value greater than 7.0 indicates spoilage (Woyewoda, 1986). The pH in fish tissues drops due to smoking, generally to 6.5 or less (Doe, 1998).

In this study the pH value of fresh catfish samples ranged from 6.33 for 5% sorbic acid treated fish to 6.92 for sodium lactate treated fish (Figure 5). The pH value of the control was 6.8 and the pH value of samples treated with 3% sodium lactate and rosemary was 6.83. The pH value of the two samples treated with NaCl and ascorbic acid were 6.6. Salt treatment affected ($p < 0.05$) the pH value of fresh and smoked catfish steaks (Figures 5 and 6). Moreover, salt concentration affects water activity and pH (Hernandez-Herrero et al. 1999). After smoking, the pH of the catfish samples dropped ($p < 0.05$), except for the control, whose pH remained the same at day 0. The pH of the

sample treated with sorbic acid increased 0.2 units (Figure 6). Figure 6 represents the pH changes of smoked catfish stored for 6 weeks. The pH value was not significantly affected by storage time ($p \geq 0.05$). The pH value for the smoked control sample at week 6 was not measured as it was completely covered by mold. The addition of sodium lactate also had no significant effect on pH ($p \geq 0.05$). Williams (1995) found no effects of sodium lactate on the pH of fresh catfish fillets. No significant differences were revealed between surface pH and corresponding total pH for control fillets and those treated with 1 and 2% sodium lactate throughout 8 days storage (Williams, 1995).

Water Activity

Dried products are usually considered shelf stable and are, therefore, often stored and distributed unrefrigerated. The characteristic of dried foods that makes them shelf stable is their low a_w . Water activity (a_w) is a measure of the amount of water in a food that is available for growth of microorganisms including pathogens. A a_w of 0.85 or below will prevent the growth and toxin production of all pathogens, including *Staphylococcus aureus* and *Clostridium botulinum*, and is necessary for a shelf-stable dried product. It is recommended by the FDA to scientifically establish a drying process that reduces a_w to 0.85 or below, if the product will be stored and distributed unrefrigerated (Hazards and Controls Guidance, FDA, 2001). In the present study, a_w mean values of smoked catfish varied from 0.48 to 0.94 (Figure 7). The minimum a_w (0.48) occurred in smoked samples treated with 25% NaCl and 1% ascorbic acid, soaked for 1 hour, and the maximum value (0.94) occurred in the control sample. The a_w of the control was very high until week 2 then gradually dropped and reached 0.75 on week 4. Water activity values decreased sharply in samples treated with salt or sodium lactate

compared to the control sample. Salt was very effective in reducing a_w of smoked catfish. However, the sample treated with salt and soaked for 1 hour had undesirable hardness texture. It is widely known that reducing a_w will result in a reduction of microbial activity; at low a_w , however, other effects such as rancidity become important (Olley et al., 1988). Both storage time and treatments affected significantly ($p < 0.05$) the a_w value of smoked catfish. Although, the a_w of the samples treated with salt and sodium lactate were lower than 0.85, increases were observed during the later part of storage in the sample treated with salt. This may have been attributed to the absorption of ambient moisture since the samples were stored at room temperature. There were no significant ($p \geq 0.05$) changes in a_w of samples treated with sodium lactate, and sorbic acid throughout 6 weeks of storage.

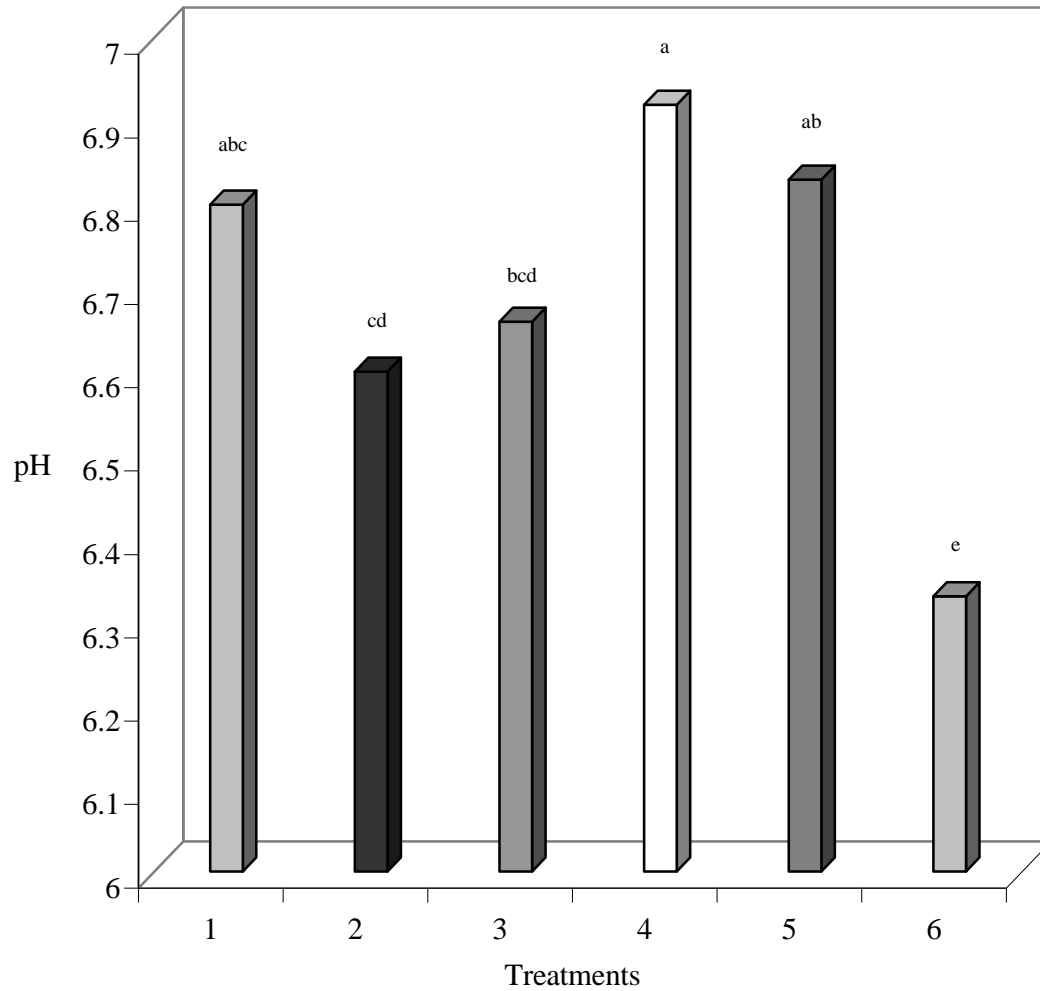
Color

The color characteristics of smoked fish depend not only on pigmentation of the skin but also on quality and composition of the smoke deposit and their interaction with the tissue components (Doe et al. 1998). Data for external and internal color of smoked catfish steaks are presented in the Tables 2 and 3. L^* indicates color lightness (0 = black and 100 = white); a positive a^* value represents redness and negative a^* represents greenness; a positive b^* represents yellow and a negative b^* represents blue. The hue angle represents an actual color, chroma evaluates the purity of the color and ΔE represents total color difference.

In the present study, the internal L^* values of smoked catfish steaks was lower than those of raw samples, and varied from 21.09 to 30.75 (Table 2), indicating the darkening of the steaks due to cooking and smoking. The external L^* value was lower

than the internal L^* and ranged from 12.68 to 19.61, which meant that the catfish steak was externally darker (Table 3). High variation in internal color characteristics of smoked catfish was observed. The actual internal color of smoked catfish varied from dark to dull brown by appearance. The treatments significantly ($p < 0.05$) affected the internal lightness of the smoked catfish. The samples treated with salt and ascorbic acid (1 hour soaking) were internally lighter than other treatments at week 0. Storage time had no effect on internal chroma and hue angle of samples treated with salt and ascorbic acid. However, the ΔE value decreased due to a significant ($p < 0.05$) decrease of L^* value (darkening) in the 4th week of storage. The internal ΔE and chroma values of the samples treated with sodium lactate (treatment 4) increased significantly in the 6th week of storage due to increased lightness.

The actual external color of smoked catfish varied from dark to very dark-grayish brown. Storage time did not significantly ($p > 0.05$) affect the external color of the control (non treated) sample. External chroma and ΔE value of the sample treated with salt and ascorbic acid (treatment 2) significantly ($p < 0.05$) decreased in the 4th week. After 4 weeks of storage, the external chroma value of the sample treated with sodium lactate decreased significantly ($p < 0.05$). The ΔE of the sample treated with sodium lactate and rosemary decreased in the 6th week. The external ΔE of the sample treated with sorbic acid decreased significantly after 4 weeks of storage. However, there were no changes in the lightness of the sample. There were generally no differences in the external color of smoked catfish among the treatments for week 0. In the 2nd week, sample treated with salt appeared to be darker than all others treatments.



- 1- Control (no treatment)
- 2- 25% NaCl and 1% ascorbic acid (1 hour)
- 3- 25% NaCl and 1% ascorbic acid (30 min)
- 4- 3% Sodium lactate
- 5- 3% Sodium lactate and 5% rosemary extract
- 6- 5% Sorbic acid

Figure 5: pH of fresh catfish steaks. Bars with different letters are significantly different ($p \leq 0.05$).

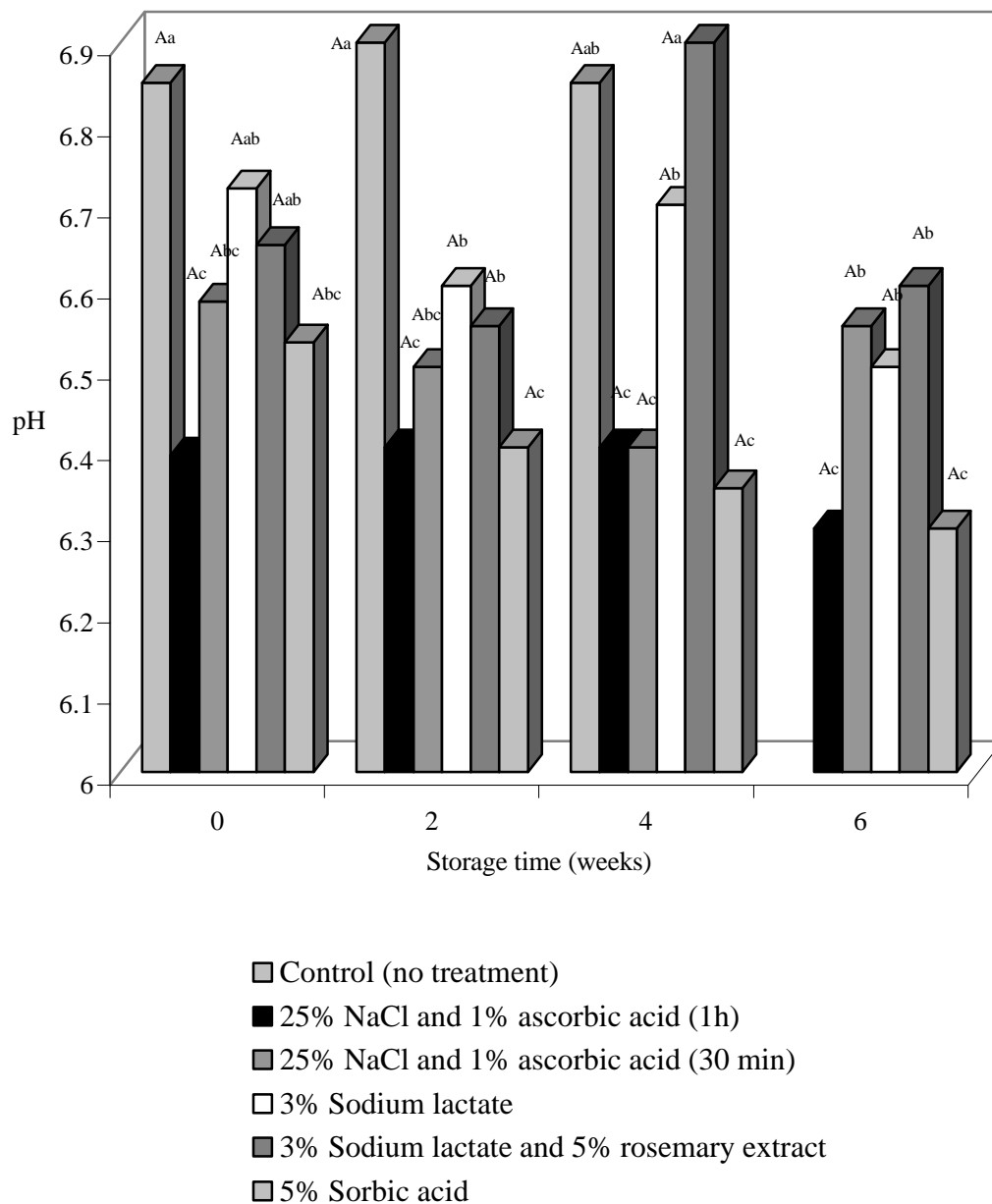
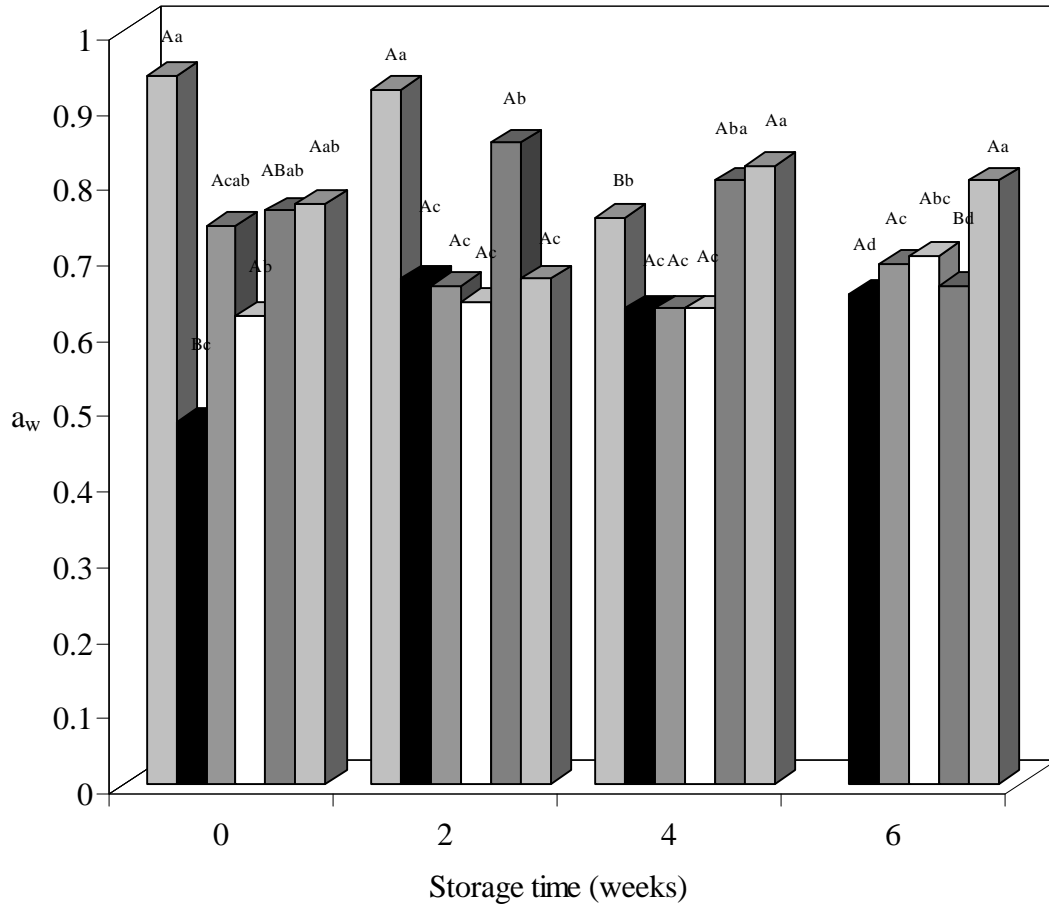


Figure 6: Changes in pH of smoked catfish stored for 0, 2, 4, and 6 weeks at room temperature. Bars with different letters are significantly different ($p < 0.05$). Capital letters represent the storage time effects and lower case letters represent treatments effects.



- Control (no treatment)
- 25% NaCl and 1% ascorbic acid (1 hour)
- ▒ 25% NaCl and 1% ascorbic acid (30 min)
- 3% Sodium lactate
- ▓ 3% Sodium lactate and 5% rosemary extract
- ▒ 5% Sorbic acid

Figure 7: Water activity of smoked catfish stored for 0, 2, 4, and 6 weeks at room temperature. Bars with different letters are significantly different ($p < 0.05$). Capital letters represent the storage time effects and lower case letters represent treatment effects.

Table 2: Internal color changes in smoked catfish steaks during 6-week storage.

Week	Treatment	L*	a*	b*	Chroma	Hue angle	ΔE
0	1	22.3 ± 2.4 ^{Bb}	4.44±2.38 ^{Ba}	3.94±6.00 ^{Ab}	9.81±2.52 ^{Abc}	294±31.3 ^{Aa}	0 ^{Cd}
	2	30.7 ± 7.4 ^{Aa}	5.80±2.82 ^{Aa}	17.22±2.37 ^{Aa}	15.92±6.26 ^{Aa}	159±124 ^{Ab}	16.86±5.10 ^{Aa}
	3	24.0 ± 7.0 ^{ABb}	4.51±1.10 ^{Ba}	13.95±5.79 ^{Aa}	14.73±5.70 ^{Aab}	70.7±6.7 ^{Ab}	10.28±7.77 ^{Ab}
	4	23.2 ± 3.1 ^{Bb}	3.84±2.62 ^{Bca}	6.76±8.49 ^{Bb}	11.23±2.92 ^{Bbc}	161.8±121 ^{Ab}	3.78±2.07 ^{Ccd}
	5	24.86±6.9 ^{Aab}	3.24±2.46 ^{Aba}	6.18±6.67 ^{ABb}	8.99±4.02 ^{Bc}	168.1±132 ^{Bb}	8.81±5.99 ^{Abc}
	6	21.09±4.03 ^{ABb}	4.26±2.32 ^{Aa}	3.92±5.71 ^{Bb}	8.08 ± 2.11 ^{Bc}	158 ± 130 ^{Ab}	4.46±3.90 ^{Ac}
2	1	31.79±2.60 ^{Aa}	9.07±1.16 ^{Aa}	9.09±2.40 ^{Aa}	12.88±2.41 ^{Aa}	44.52±5.00 ^{Bb}	12.31±3.54 ^{Aa}
	2	30.93± 4.09 ^{Aa}	8.75±2.37 ^{Aa}	9.06±3.66 ^{Ca}	12.91±3.04 ^{Aa}	44.82±14.86 ^{Ab}	12.01±3.66 ^{ABa}
	3	28.86±2.29 ^{Aab}	6.42±0.79 ^{Abc}	4.93±1.99 ^{Cab}	8.24±1.28 ^{Ab}	36.31±12.23 ^{Bbc}	9.49 ± 1.82 ^{Aa}
	4	25.59±1.24 ^{Bbc}	7.51±0.48 ^{Aab}	3.23±1.43 ^{Bb}	8.25±0.82 ^{BCb}	22.78±8.48 ^{Ac}	8.48 ± 0.78 ^{Ba}
	5	22.37±0.90 ^{Ac}	4.57±0.37 ^{ABc}	-1.12±0.65 ^{Bc}	4.73±0.4 ^{BCc}	346.7± 6.84 ^{Aa}	10.30±0.59 ^{Aa}
	6	26.07±2.1 ^{ABbc}	6.17±0.68 ^{Abc}	3.12±1.74 ^{Bb}	7.05±0.99 ^{Bb}	25.79±12.4 ^{Abc}	8.41 ± 0.20 ^{Aa}
4	1	26.04±1.59 ^{Ba}	3.99±0.99 ^{Bcd}	8.64±0.35 ^{Aab}	8.95±1.11 ^{Bbc}	61.84±12.93 ^{Ba}	5.13±0.89 ^{Bab}
	2	20.63±2.39 ^{Bbc}	7.92±0.68 ^{Aa}	10.08±2.35 ^{BCa}	12.86±2.19 ^{Aa}	51.27±4.57 ^{Aab}	5.43±1.02 ^{Bab}
	3	18.44±2.28 ^{Bbc}	6.15±0.95 ^{Ab}	5.81±2.34 ^{BCb}	8.50±2.33 ^{Ac}	42.13±6.19 ^{Bb}	6.22±0.66 ^{Aab}
	4	22.45±1.93 ^{Bab}	2.93± 0.23 ^{Cd}	6.40±1.58 ^{Bb}	7.06±1.40 ^{Cc}	64.43±6.70 ^{Aa}	3.74±1.16 ^{Cb}
	5	16.98± 0.14 ^{Ac}	2.68± 0.17 ^{Bd}	1.31±0.53 ^{Bc}	3.02±0.25 ^{Cd}	25.59±9.67 ^{Bc}	8.83±0.52 ^{Aa}
	6	26.78± 5.21 ^{Aa}	5.38±1.37 ^{Abc}	11.25±2.22 ^{Aa}	12.51±2.61 ^{Aab}	64.77±2.18 ^{Aa}	6.67±4.19 ^{Aab}
6	1	**	**	**	**	**	**
	2	25.15±2.19 ^{ABb}	7.87±1.73 ^{Aa}	13.9±2.78 ^{ABab}	14.83±3.60 ^{Aab}	60.56±1.77 ^{Ab}	7.79±3.82 ^{Bab}
	3	24.27±3.17 ^{ABb}	4.91±0.49 ^{ABb}	12.19±2.80 ^{ABb}	12.60±2.15 ^{Abc}	66.70± 2.64 ^{Aab}	5.65±3.28 ^{Ab}
	4	31.48±4.04 ^{Aa}	6.51±1.36 ^{ABab}	17.65±2.77 ^{Aa}	18.85±2.78 ^{Aa}	69.61±3.85 ^{Aa}	13.90±4.44 ^{Aa}
	5	24.49±3.03 ^{Ab}	5.8 ±0.37 ^{Aab}	14.13±3.21 ^{Aab}	15.29±3.23 ^{Aab}	67.24±2.44 ^{Bab}	6.79±3.96 ^{Ab}
	6	20.16±2.45 ^{Bb}	5.68± 0.82 ^{Ab}	6.42±1.90 ^{ABb}	8.61±1.89 ^{Bc}	47.58± 5.92 ^{Ac}	4.52±1.81 ^{Ab}

Mean \pm standard deviation of triplicate experiments and 5 replicates of each sample (15 readings of each sample). Mean values with different superscripts are significantly different ($p < 0.05$). Capital letters represent the storage time effects and lower case letters represent treatment effects.
1= control (no treatment); 2= 25% NaCl and 1% ascorbic acid (1 hour); 3= 25% NaCl and 1% ascorbic acid (30 min); 4= 3% sodium lactate; 5= 3% sodium lactate and 5% rosemary extract; and 6= 5% sorbic acid. **Moldy

Table 3: External color changes in smoked catfish steaks during 6-week storage.

Week	Treatment	L*	a*	b*	Chroma	Hue angle	ΔE
0	1	17.50±3.31 ^{Aa}	2.70±2.81 ^{ABa}	-3.19±3.7 ^{Aa}	4.55±4.40 ^{ABa}	283.6±39.97 ^{Aa}	0 ^{Cb}
	2	19.61±4.94 ^{Aa}	2.46±2.27 ^{ABa}	-1.96±3.7 ^{Aa}	4.52± 2.92 ^{Ba}	146.5±121.1 ^{Bb}	4.83±1.65 ^{Ba}
	3	12.68±1.06 ^{Cb}	0.08± 0.85 ^{Ca}	-0.28±0.5 ^{Aa}	0.94±0.31 ^{Da}	226±90.97 ^{Aab}	3.79± 2 ^{Aa}
	4	17.08±4.49 ^{Aab}	2.04±2.90 ^{ABa}	-1.7±4.1 ^{ABa}	4.08±3.92 ^{ABa}	193.9±106.3 ^{Aab}	4.29±3.08 ^{Ba}
	5	19.59± 3.2 ^{Aa}	2.35±2.91 ^{ABa}	-2.52±4.25 ^{Aa}	3.91± 4.79 ^{Aa}	143.5±120.6 ^{Bb}	3.48± 3.37 ^{ABa}
	6	19.49±6.31 ^{Aa}	2.34±2.72 ^{ABa}	-1.46±3.30 ^{Aa}	3.76±3.42 ^{ABa}	213.4 ± 115 ^{Aab}	6.33±3.79 ^{ABa}
2	1	18.96±0.48 ^{Aa}	5.85±0.18 ^{Aa}	-6.47±0.44 ^{Ab}	8.73± 0.33 ^{Aa}	312.1 ± 2.26 ^{Ab}	8.56±0.56 ^{Aa}
	2	19.18±1.70 ^{Aa}	4.97±0.36 ^{Ab}	-6.48±0.67 ^{Bb}	8.10±0.69 ^{Aab}	307.6 ± 1.33 ^{Ac}	8.48±0.69 ^{Aa}
	3	20.75±0.86 ^{Aa}	5.11±0.25 ^{Ab}	-5.91±0.30 ^{Cb}	7.81±0.37 ^{Aabc}	310.9 ± 0.70 ^{Abc}	5.14±1.05 ^{Ab}
	4	20.65±1.09 ^{Aa}	5.13±0.20 ^{Ab}	-6.0±0.68 ^{Bb}	7.90±0.60 ^{Aab}	310.7±2.82 ^{ABbc}	8.55±0.76 ^{Aa}
	5	20.81±1.27 ^{Aa}	4.91±0.34 ^{Ab}	-4.56±0.7 ^{Aa}	6.71±0.67 ^{Ac}	317.3 ± 3.24 ^{Aa}	7.51±1.31 ^{Aa}
	6	21.07±1.79 ^{Aa}	4.93±0.32 ^{Ab}	-5.6±0.67 ^{Bab}	7.48±0.72 ^{Abc}	311.3±1.62 ^{Abc}	8.29±1.12 ^{Aa}
4	1	18.02±2.72 ^{Aa}	2.20±0.21 ^{Ba}	-2.51±0.29 ^{Ab}	3.23 ± 0.30 ^{Ba}	314.6±13.0 ^{Aab}	3.51± 0.52 ^{Ba}
	2	15.65±0.83 ^{Aab}	1.84±0.35 ^{Bab}	-2.14±0.33 ^{Ab}	2.83 ± 0.41 ^{Ba}	310.5 ± 5.07 ^{Ab}	3.41 ± 0.65 ^{Ba}
	3	15.39±1.7 ^{BCab}	1.89±0.10 ^{Bab}	-2.53±0.47 ^{Bb}	3.16 ± 0.40 ^{Ba}	307.1 ± 4.62 ^{Ab}	4.47 ± 1.14 ^{Aa}
	4	15.97±1.06 ^{Aab}	1.71±0.17 ^{Bb}	-2.1±0.57 ^{ABb}	2.77±0.38 ^{Ba}	309.7±9.86 ^{ABb}	3.56 ± 0.94 ^{Ba}
	5	14.75±1.43 ^{Bb}	1.63±0.09 ^{ABb}	-2.08±0.61 ^{Ab}	2.66±0.53 ^{Aa}	309.3± 6.84 ^{Ab}	3.87±1.78 ^{ABa}
	6	14.35±0.62 ^{Ab}	1.59±0.24 ^{Bb}	-0.95±0.36 ^{Aa}	1.89±0.11 ^{Bb}	329.1±12.61 ^{Aa}	3.10±1.54 ^{Ba}
6	1	**	**	**	**	**	**
	2	18.33±2.02 ^{Aa}	1.7±0.48 ^{Bb}	-0.6±0.38 ^{Aab}	1.84±0.41 ^{Bb}	339.3±14.5 ^{Aa}	3.23±0.91 ^{Ba}
	3	16.15±2.84 ^{Bab}	1.53±0.25 ^{Bb}	-1.82±0.39 ^{Bc}	2.38±0.43 ^{Cab}	310.3±3.93 ^{Ab}	2.72±0.70 ^{Aa}
	4	16.07±1.50 ^{Aab}	2.38±0.16 ^{ABa}	-0.3±0.31 ^{Aa}	2.42±0.13 ^{Bab}	350.7±6.70 ^{Aa}	2.28±0.45 ^{Ba}
	5	15.50±1.18 ^{Bab}	1.42±0.25 ^{Ba}	-1.82±0.25 ^{Ac}	2.31±0.34 ^{Ab}	307.9±3.17 ^{Ab}	3.21±1.37 ^{Ba}
	6	14.43±0.74 ^{Ab}	2.64±0.18 ^{ABb}	-1.2±0.63 ^{Abc}	2.95±0.20 ^{Ba}	335.8±11.8 ^{Aa}	3.57 ± 1.33 ^{Ba}

Mean \pm standard deviation of triplicate experiments and 5 replicates of each sample (15 readings of each sample). Mean values with different superscripts are significantly different ($p < 0.05$). Capital letters represent the storage time effects and lower case letters represent treatment effects.
1= control (no treatment); 2= 25% NaCl and 1% ascorbic acid (1 hour); 3= 25% NaCl and 1% ascorbic acid (30 min); 4= 3% sodium lactate; 5= 3% sodium lactate and 5% rosemary extract; and 6= 5% sorbic acid. **Moldy

Thiobarbituric Acid Reactive Substances (TBARS)

The Thiobarbituric acid (TBA) test measures a secondary product of lipid oxidation, malonaldehyde. It involves the reaction of malonaldehyde (or malonaldehyde type products) with TBA to yield a colored compound that can be measured spectrophotometrically. Because the reaction is not specific to malonaldehyde, results sometimes are reported as TBA reactive substances (TBARS). The food sample may react directly with TBA, but is often distilled to eliminate interfering substances, and then the distillate is reacted with TBA (Pike, 1998). Absorbance of the solution (distilled and TBA reagent) was determined at 535 nm, and then the absorbance readings were converted to milligrams of malonaldehyde (or TBARS) per kilogram of sample by using a standard curve (Figure 8).

The TBA values of raw catfish steaks subjected to different treatments (Table 4) were 0.009 to 0.042 mg TBARS/ kg. Samples treated with 3% sodium lactate showed the lowest TBA value (0.009 mg TBARS/kg) followed by the samples treated 5% sorbic acid (0.010mg TBARS/kg). The mean TBA value of the samples treated with 3% sodium lactate and 5% rosemary extract was 0.011 mg TBARS/kg, and the two samples treated with 25% NaCl and 1% ascorbic acid and soaked for 1hour or for 30 min showed 0.026 and 0.020 mg TBARS/kg, respectively. Control samples showed the highest TBA value of 0.042 mg TBARS/kg. Williams and others (1995) reported that sodium lactate retarded rancidity by maintaining lower TBA values for fresh catfish fillets treated with 2% sodium lactate throughout 8 days storage at 1.11°C. Similarly, Calhoun (1999) reported that sodium lactate appears effective in controlling lipid oxidation in ground pork patties stored at 4°C.

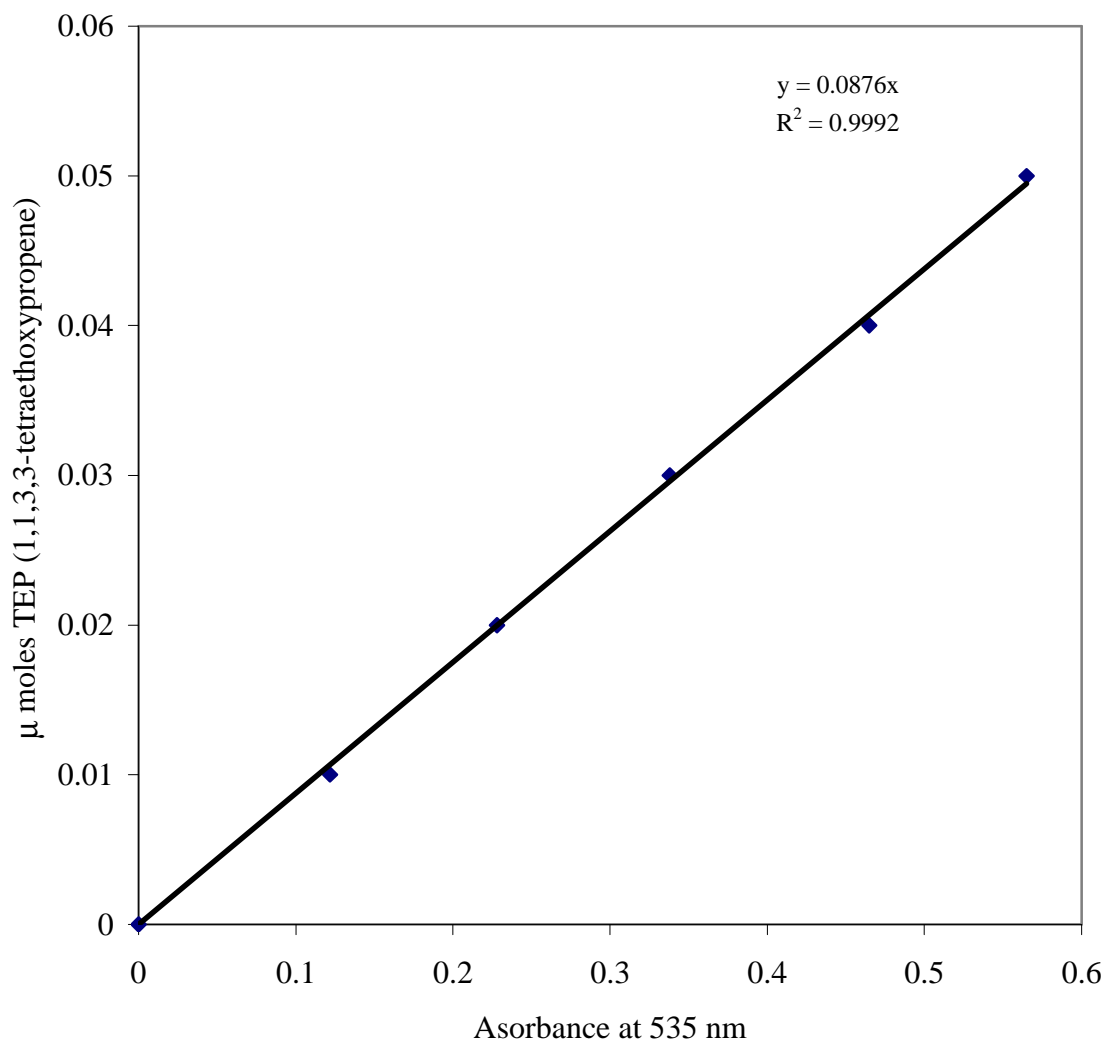


Figure 8: The TBARS Standard curve.

The TBA values of catfish samples increased after the smoking process at day 0 (Figure 9). The TBA value of the control increased from 0.042 to 0.102 mg TBARS/kg. The TBA value of the samples treated with 25% NaCl and 1% ascorbic acid at both soaking times increased to 0.04 mg TBARS/kg. The samples treated with 5% sorbic acid increased to 0.041 mg TBARS/kg and the samples treated with 3% sodium lactate and 5% rosemary extract increased to 0.025. However, the samples treated with 3% sodium lactate showed the lowest TBA value among the treatments, which increased to 0.018 mg TBARS/kg after the smoking process. The TBARS (0.009 to 0.042 mg TBARS/kg) did not exceed 0.58mg (Doe et al., 1998), which was well within acceptable limits. The sensory threshold level for detecting rancidity in fresh meat was reported to be between 1 and 2 TBARS (Calhoun and others, 1999).

Guntensperger and others (1998) reported that addition of rosemary extract in heat sterilized pork meat could stabilize the product over extended shelf-life periods. Therefore, the addition of the antioxidant after precooking was more effective than addition before cooking, probably due to losses of part of the rosemary extract with animal fat, which deposited on cooking equipment during precooking. Similarly, Langourieux and Escher (1998) found that the addition of rosemary extract led to effective stabilization of a heat-sterilized model system consisting of pork meat, fat and water.

The increased TBA values in the smoked catfish probably originated from the breakdown of oxidation products, mainly malonaldehyde, during smoking due to the high temperature (Goktepe and Moody, 1998). Beltran and Moral (1991) reported that high TBA values are correlated with the degree of oxidation of fats in hot smoked sardines.

The TBA values of all the sample treatments (including control) gradually declined during storage (Figure 9). TBARS decreased at week 2 and somewhat increased at week 4 then decreased again at week 6. This characteristic time course of TBARS can be due to storage conditions because the samples were air packed and stored at room temperature, which indicated high susceptibility to oxygen. TBA value attained a maximum value and declined on further storage, and this value depends on species, accessibility to oxygen (packaging), and storage temperature (Woyewoda, 1986).

Salt and soaking time had no significant effect on oxidation of smoked catfish. The two samples treated with 25% NaCl and 1% ascorbic acid solutions, soaked for 1 hour and 30 min, respectively, were not significantly different ($p>0.05$), and both of them were not significantly different from the control after 4 weeks of storage. Vara-Ubol and Bowers (2001) reported that salt (NaCl) had no significant effect on lipid oxidation of stored, cooked, ground turkey and ground pork.

Peroxide Value (PV)

Peroxides are the primary products of oxidation. However, since they are relatively short lived, their usefulness as oxidation indicators is limited to an early stage of rancidity development. As oxidation proceeds, peroxides break down to aldehydes or combine with proteins (Woyewoda et al., 1986).

The peroxide values (PV) results are similar in pattern to TBARS. The PV of raw catfish steaks subjected to different treatments (Table 5) ranged from 1.03 to 9.8 milliequivalent (mEq) peroxide/kg fat. Samples treated with 3% sodium lactate showed the lowest PV value (1.03 mEq/kg fat) and the control samples showed the highest PV value (9.8 mEq/kg fat) (Table 5). The PV of the sample treated with 3% sodium lactate

and 5% rosemary extract was 1.73 mEq/kg, and the PV of the two samples treated with 25% NaCl and 1% ascorbic acid were 1.9 and 3.5 mEq/kg fat. The sample treated with 5% sorbic acid had a PV of 4.83 mEq/kg.

After smoking the peroxide value decreased for all treatments at day 0 of storage at room temperature. In week 0, the treatments were not significantly different ($p \geq 0.05$) (Figure 10). Along the six weeks of storage the PV of smoked catfish steaks treated with 3% sodium lactate increased ($p < 0.05$) significantly from 2.03 (week 0) to a maximum of 28.00 mEq/kg. The increase in the PV can be attributed to low (0.62) water activity of the sample. Oxidation is maximized at very low a_w probably because of the concentration of metal catalysts (Burt et al., 1988). Pacheco-Aguilar et al. (2000) found an increase in PV during storage, for which initial values ranged from 15.0 to 26.1 mEq/kg lipid. The maximum values from 31.8 to 33.9 mEq/kg occurred at day 15 (Pacheco-Aguilar et al., 2000). Awad and others (1969) correlated sensory evaluation with oxidation in muscle of white-fleshed fish (*Caulolatilus princeps*) and reported values of 19.6 and 26.2 mEq/kg lipid for initial and moderate oxidation, respectively. The observed PV tendency in this study suggested that smoked catfish were not oxidized even at week 6, except the samples treated with 3% sodium lactate that started to be oxidized before week 6 of storage.

The addition of rosemary extract to catfish before smoking kept the PV lower in the smoked product throughout 6 weeks of storage. There were no significant ($p \geq 0.05$) changes in PV of samples treated with 3% sodium lactate and 5% rosemary extract up to 4 weeks of storage. However, the PV of samples treated with sodium lactate and rosemary dropped from 3.5 mEq/kg at week 2 to a minimum of 0.05 mEq/kg at week 6.

Similar to TBARS, the PV of all samples fluctuated during a 6-week storage. Hernandez-Herrero et al. (1999) reported that this characteristic time course of PV was suggested to derive from the preferential oxidation of phospholipids during the early stages of autoxidation. Break and Fenema (1999) also reported an increase in TBA and PV with increasing storage time at -5, -15, and -20°C in frozen minced mackerel. Goktepe and Moody (1996) also found that the smoked catfish fillets stored in the air at 2 and 8°C had a significant increase in TBA values (1.188 and 1.489 mg TBRS/kg, respectively) over time.

Table 4: Mean and standard deviation of TBA values of raw catfish steaks.

Treatments	mg TBARS/kg
Control (no treatments)	0.042 ± 0.040 ^a
25% NaCl and 1% ascorbic acid (1hour)	0.026 ± 0.018 ^{ab}
25% NaCl and 1% ascorbic acid (30 min)	0.020 ± 0.001 ^{ab}
3% Sodium lactate	0.009 ± 0.006 ^b
3% Sodium lactate and 5% rosemary extract	0.011 ± 0.009 ^b
5% Sorbic acid	0.010 ± 0.006 ^b

Mean values with different letters are significantly different (p< 0.05).

Table 5: Mean and standard deviation of peroxide values of raw catfish steaks

Treatments	mEq peroxide / kg
Control (no treatment)	9.80 ± 8.44 ^a
25% NaCl and 1% ascorbic acid (1hour)	1.90 ± 0.91 ^b
25% NaCl and 1% ascorbic acid (30 min)	3.50 ± 2.89 ^b
3% Sodium lactate	1.03 ± 0.08 ^b
3% Sodium lactate and 5% rosemary extract	1.73 ± 0.99 ^b
5% Sorbic acid	4.83 ± 2.98 ^{ab}

Mean values with different letters are significantly different (p< 0.05).

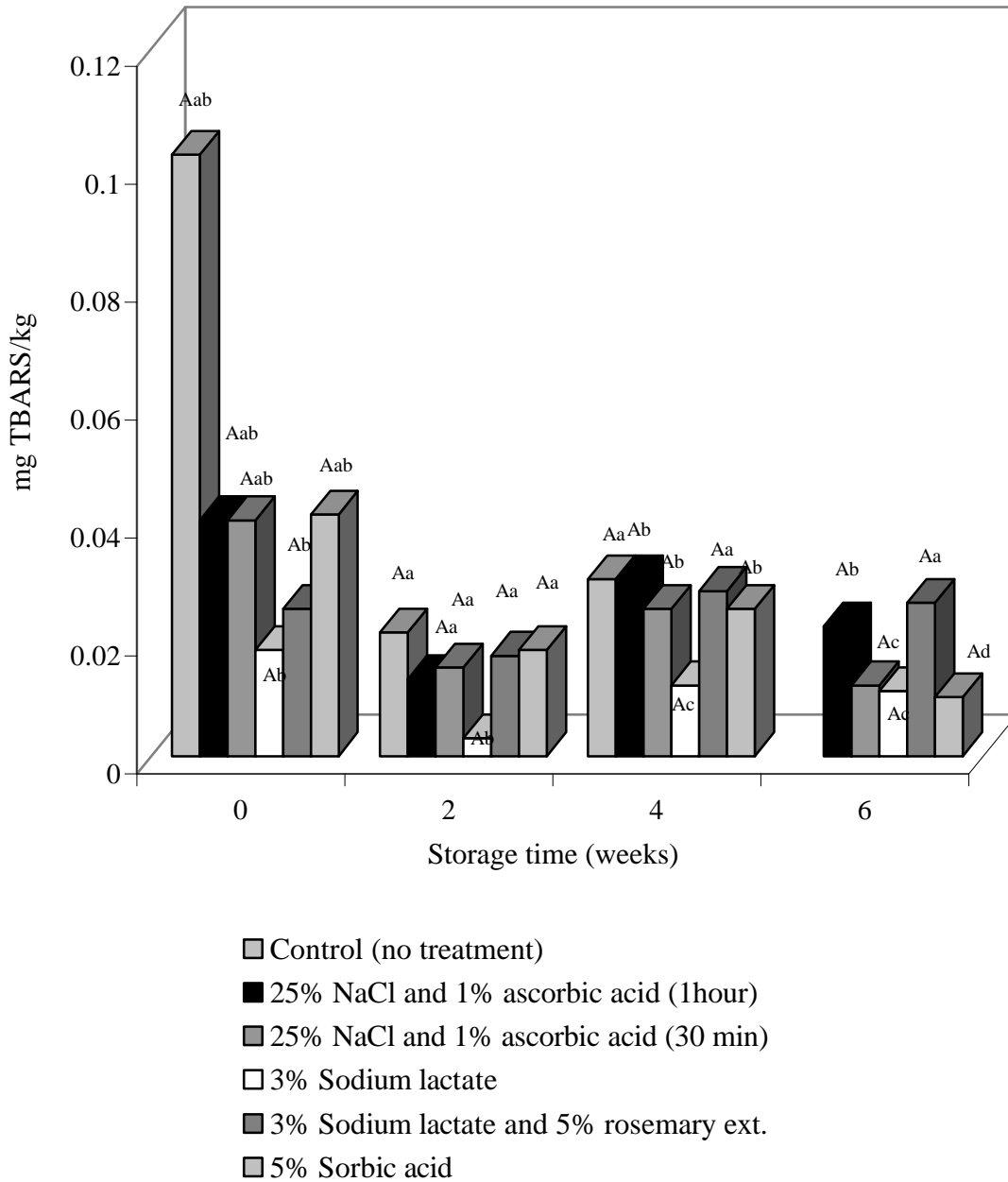


Figure 9: Changes in TBARS of smoked catfish during 6 weeks of storage. Bars with different superscripts are significantly different ($p < 0.05$). Capital letters represent the storage time effects and lower case represent effects.

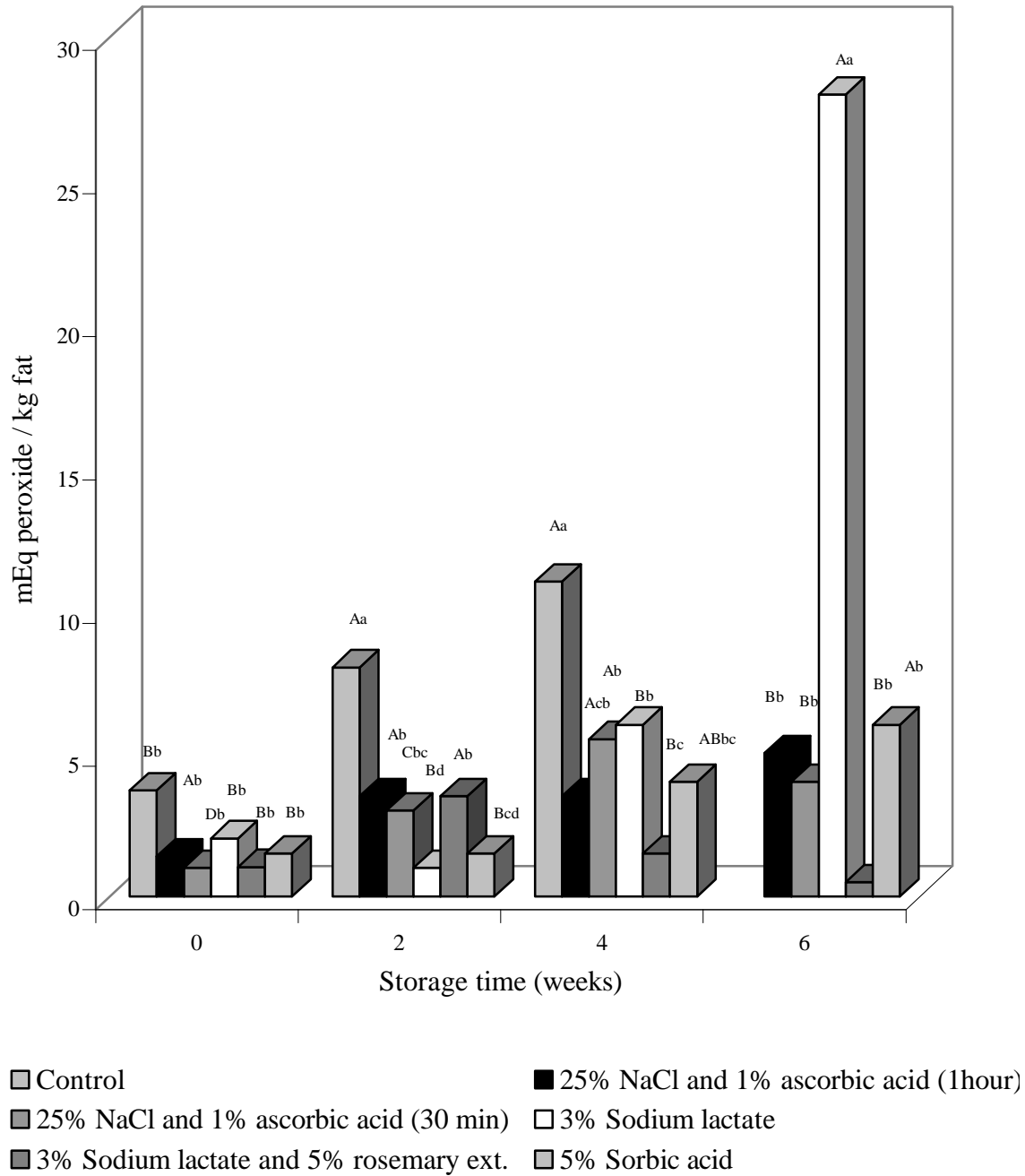


Figure 10: Changes in PV values of smoked catfish during 6 weeks of storage. Bars with different letters are significantly different ($p < 0.05$). Capital letters represent the storage time effects and lower case letters represent treatment effects.

Proximate Analysis

The proximate analysis of raw catfish steaks is presented in Figure 10. There were no significant ($p \leq 0.05$) differences in protein (16.4-18%), fat (1.7-4.3%), ash (0.9-1.9) and moisture contents (76.9-79.6%) of fresh catfish steaks subjected to different treatments.

Fat Content

The fat content of raw catfish ranged from 1.7 to 4.3%. After the smoking process, the percent of fat increased significantly due to loss of moisture and an increase in the dry matter content per unit of weight following sample dehydration. The fat content of smoked catfish varied from 13.11 to 22.02% (Figure 12). Sample treated with 3% sodium lactate showed low fat content 2.54 and 6.89% on the 2nd and 4th week of storage. The reduction in the fat content can be due to a sampling problem, since on the 6th week of storage this sample (3% sodium lactate treated sample) showed a result similar to that of the day 0. Storage time had no significant effect on the fat content of smoked catfish, except for the control, which decreased in the 4th week. The sample treated with sorbic acid showed a sharp decrease from 22.02% to 9.95% on the second week (Figure 12). The fat content of this sample increased again to 21.32% in the 4th week, which can be due to sampling variation.

Protein Content

The protein levels, averaging 15 to 20% in lean fish, varied among and within species (Woyewoda, 1986). Protein in farm-raised catfish usually ranges from 15% to 18% (Forrester, 1999). In the present study, the protein content of raw catfish ranged from 16.4% to 18% (Figure 13). There was no significant ($p > 0.05$) difference in the

protein content of raw catfish among the treatments. After smoking, the protein content increased to 56.19% and 67.53%. There was an inverse relationship between the moisture and protein content in the smoked catfish steaks. Protein content in smoked catfish increased (Figure 13) due to an increase in the dry matter content per unit of weight following sample dehydration during smoking (Goktepe, 1996). Storage time did not affect ($p>0.05$) the protein content of smoked catfish. In addition, treatments did not affect the protein content of the smoked catfish steaks (Figure 13).

In the present study, the fish was subjected to a temperature higher than 115°C, which can cause protein degradation. At higher temperatures protein suffers thermal degradation. Thus at 115°C losses of cysteine occur both at low (14%) and high (70-80%) moisture content. Heating of proteins at temperatures above 115°C causes extensive destruction of several amino acids, which can cause a severe depression of protein utilization (Burt et al., 1988).

Moisture Content

The moisture content of fresh catfish was 76.9-79.6% (Figure 11). In contrary to protein, fat, and ash, the moisture content of catfish decreased sharply after the smoking process (Figure 14). This decrease was due to loss of water during smoking (Asiedu et al., 1991). Moisture content of smoked catfish ranged from 10.55-21.5%. The moisture content of samples treated with sodium lactate was 10.55%, which was lowest value of all treatments. The maximum value was observed with the control (21.5%). The moisture content of all treatments remained similar throughout 6 weeks of storage.

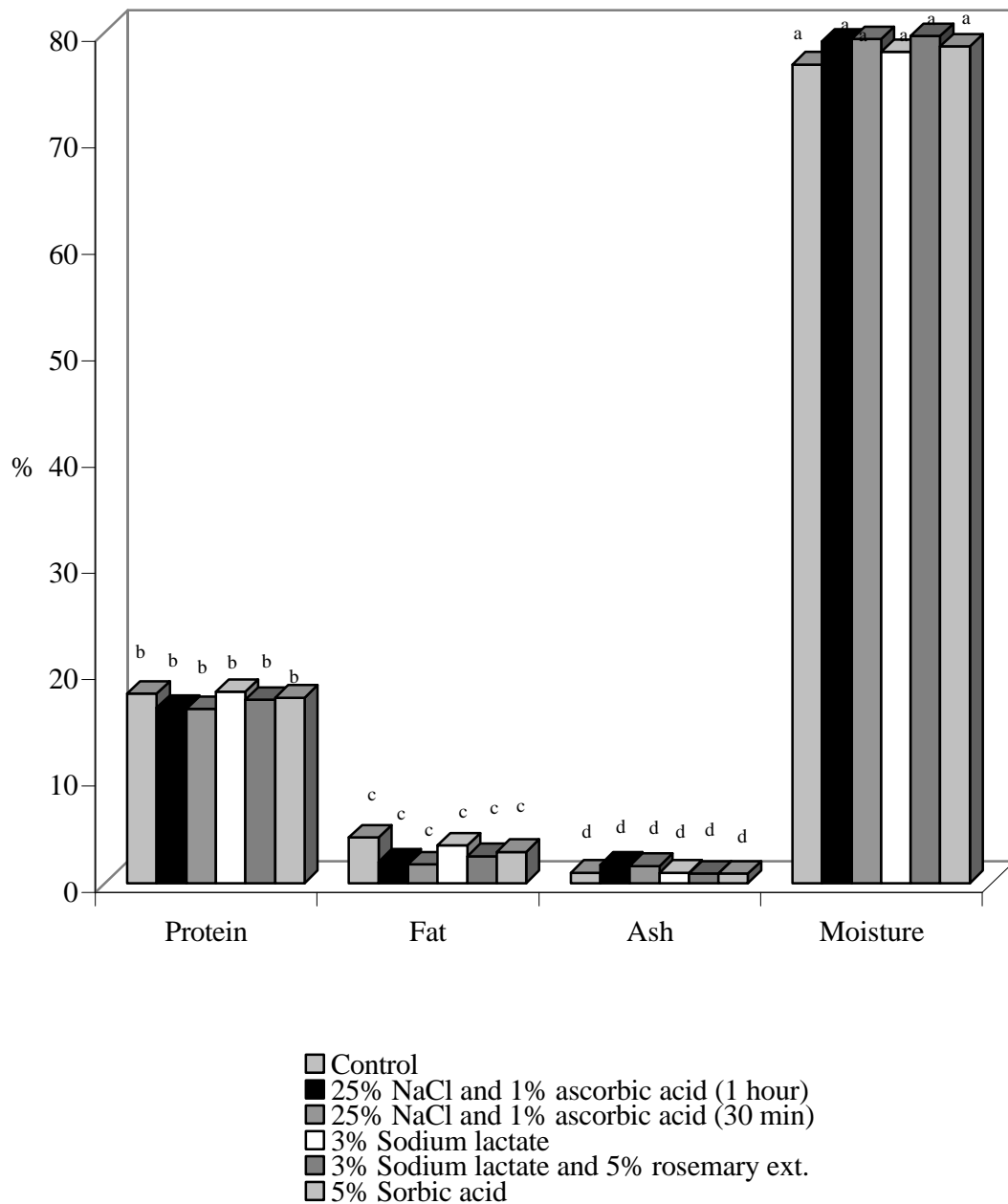


Figure 11: Proximate composition of fresh catfish steaks. Bars with different letters are significantly different ($p < 0.05$).

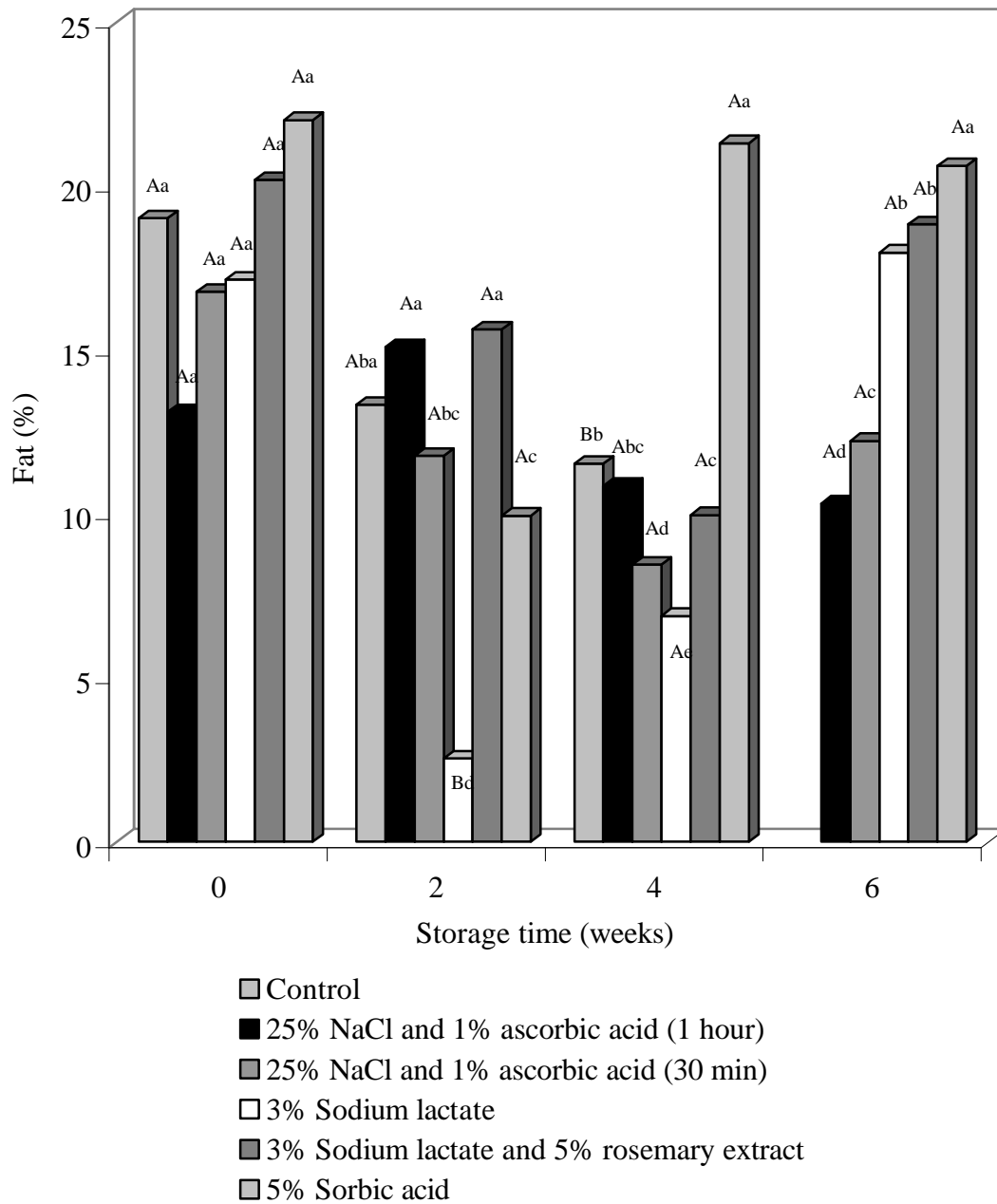


Figure 12: Changes in the fat content of smoked catfish steaks during 6 weeks of storage at room temperature. Bars with different letters are significantly different ($p < 0.05$). Capital letters represent the storage time effects and lower case letters represent treatment effects.

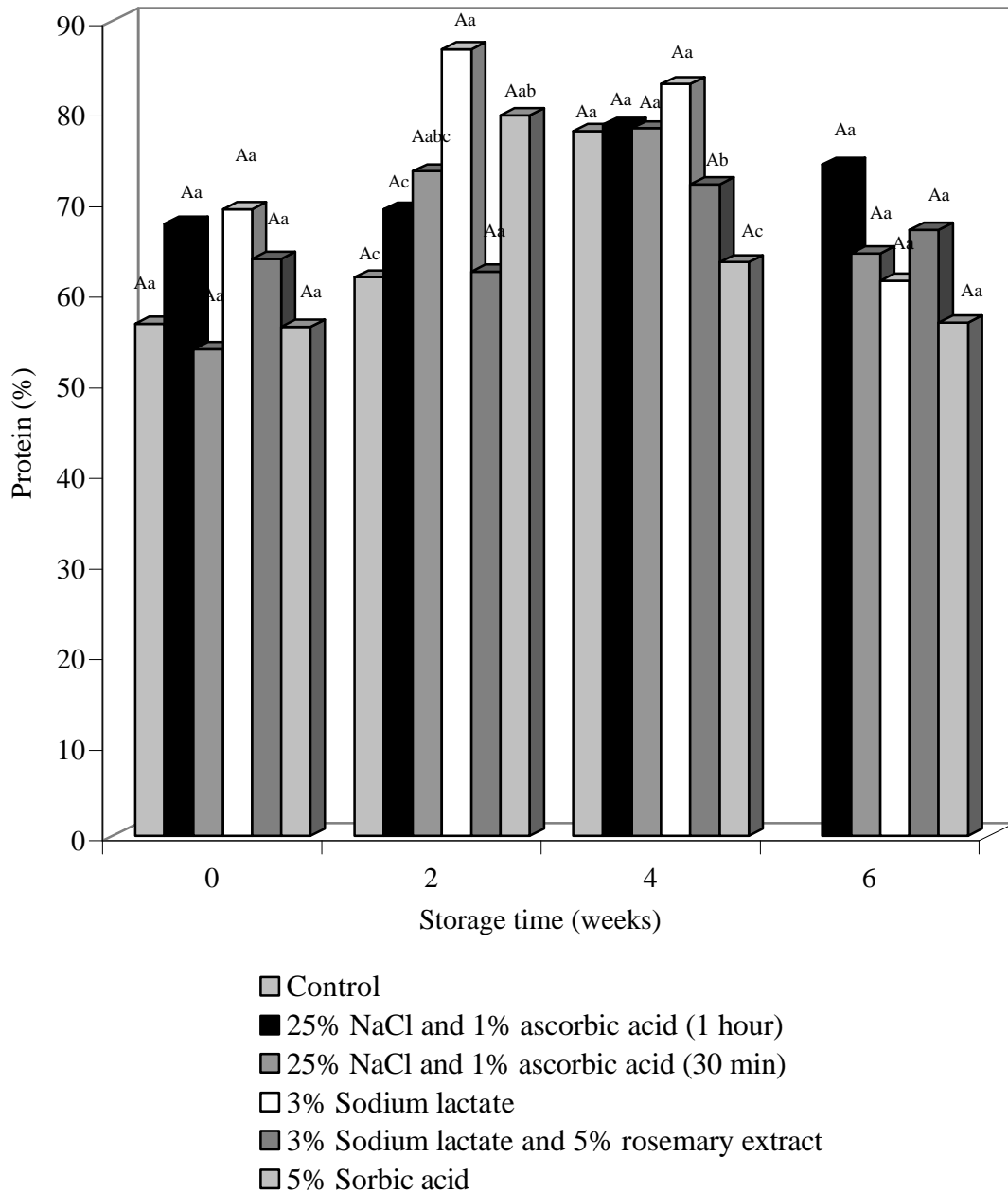


Figure 13: Changes in protein content of smoked catfish during 6 weeks of storage at room temperature. Bars with different letters are significantly different ($p < 0.05$). Capital letters represent the storage time effects and lower case letters represent treatment effects.

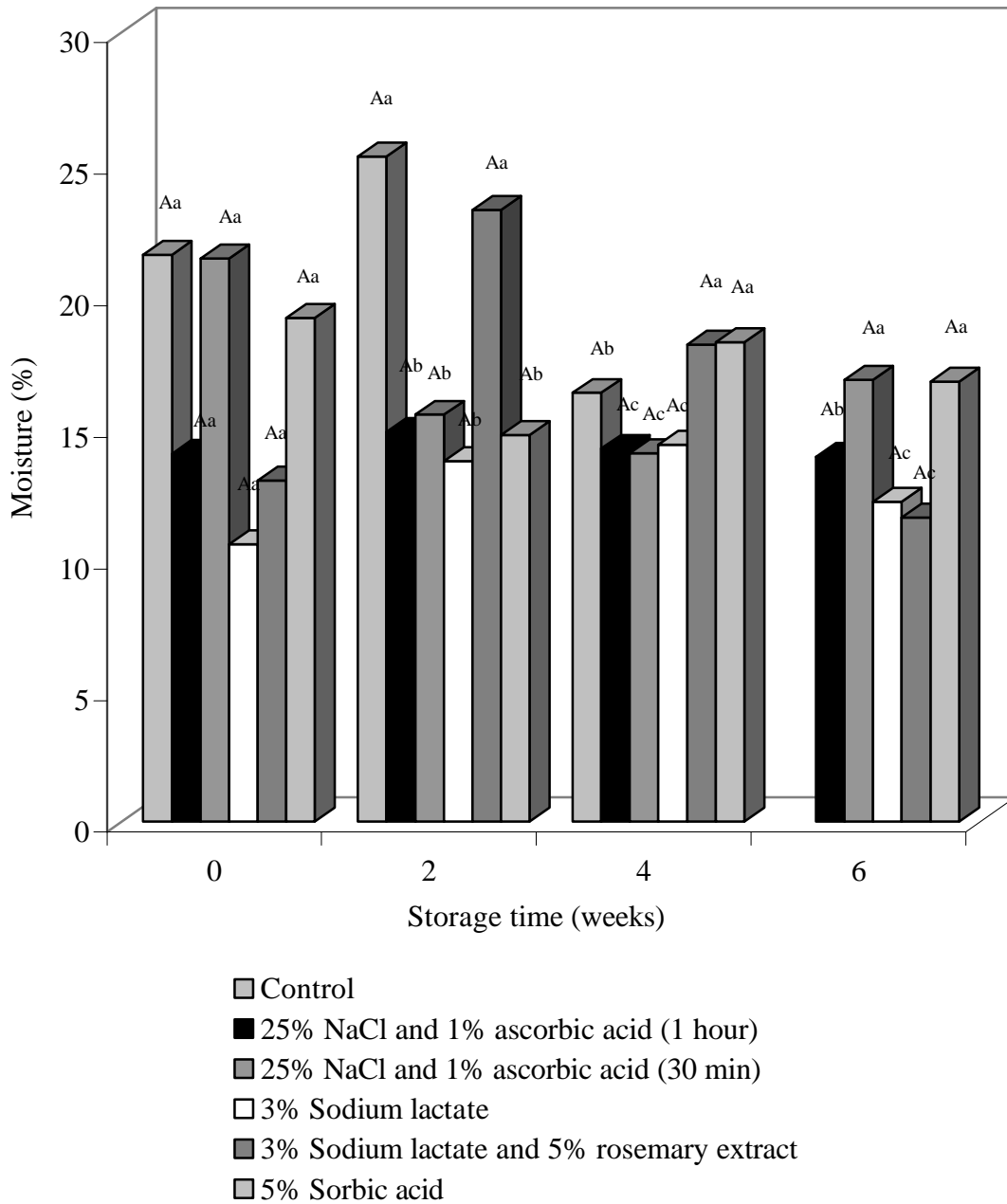


Figure 14: Changes in moisture content of smoked catfish during 6 weeks of storage at room temperature. Bars with different letters are significantly different ($p \leq 0.05$). Capital letters represent the storage time effects and lower case letters represent treatment effects.

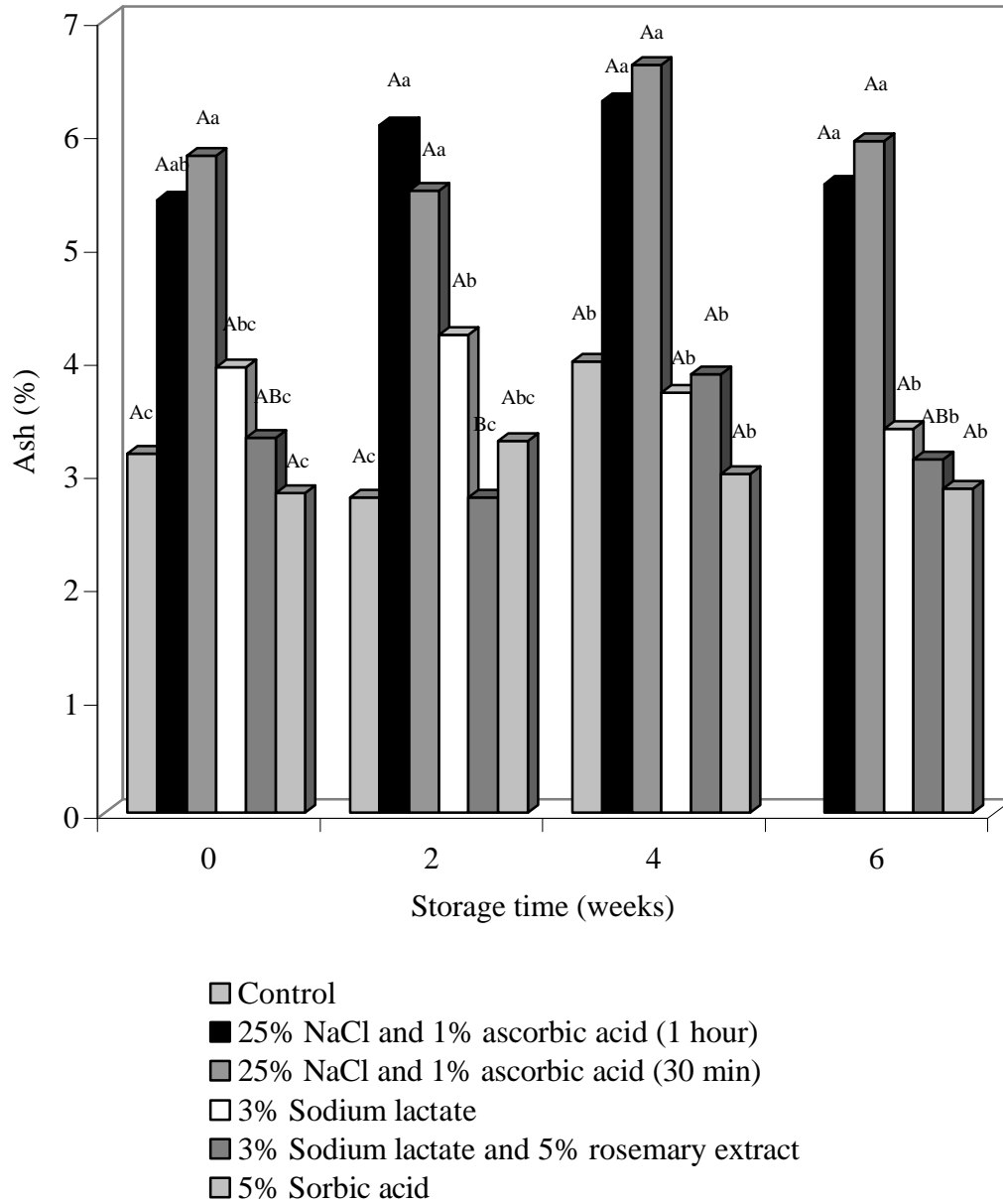


Figure 15: Changes in ash content of smoked catfish during 6 weeks of storage at room temperature. Bars with different letters are significantly different ($p < 0.05$). Capital letters represent the storage time effects and lower case letters represent treatment effects.

Ash Content

The ash content of fresh catfish varied from 0.9 to 1.7% (Figure 11). Suvanich et al. (1998) reported that ash content of fresh catfish was $0.9 \pm 0.1\%$. After smoking the ash content of smoked catfish increased and it ranged from 2.82 to 5.80% (Figure 15). The increase in mineral content can be attributed to an increase in the dry matter content per unit of weight following sample dehydration, the addition of NaCl, sodium lactate, sorbic acid and smoke during the smoking process. In addition, the ash content of samples treated with NaCl was higher than that of all other treatments. Storage time had no significant effect on ash content of smoked catfish.

Temperature Profile

The temperature profile of the smoking process is illustrated in Figures 16 and 17. Fish samples were smoked in two stages and in different ovens. The first oven was electric and temperature was controlled, while the second oven was a firewood smoker. Chamber temperatures in both ovens were recorded with a datalogger and the data were downloaded to a portable computer. The temperature was recorded every 5 minutes.

The cooking was done in the first oven for 5 hours and the temperature was set to 325°F. The chamber temperature reached 324°F after 30 minutes. After 1 hour of processing the chamber temperature dropped to 270.3°F and reached the minimum of 68.8°F after 2 hours, then slightly increased to 74.1°F and was stable until the end of the cooking cycle. After 1 hour, the internal temperature (heat penetration) of the fish was 199.6°F.

After cooling the cooked fish were transferred to the second oven. The initial temperature in the chamber and internal temperature in the fish were 75°F. The chamber

temperature increased and reached 311.6°F after 2 hours, and internal fish temperature increased to 231.82°F after 18 hours. After 15 hours of processing, the temperature started to decline and reached 72.4°F. The temperature increased again after the smoker had been lighted. After the fire extinguish during the night, the smoker had to be lighted and the wood had to be added in the following morning. The Chamber temperature reached 367.6°F and internal fish temperature was 239.9°F, which were the maximum temperatures for all smoking processes. The minimum internal fish temperature was 168.6°F. The temperature and time were adequate to kill all the possible pathogens during the smoke-drying process.

Processing Flow Chart and the HACCP Plan

The primary tool used by regulatory agencies to ensure food safety by commercial food processors, especially muscle food processors, is Hazard Analysis and Critical Control Point (HACCP) principles. Some food processors especially those who use heat treatment to destroy pathogens (cooked ready-to-eat products), are especially impacted by this regulation. This particular operation is especially complex because heat treatment and water activity are used to control pathogens and toxin production. The purpose of this study was to evaluate those parameters using HACCP principles to ensure the safety of those products.

As with any food product, a HACCP program is recommended to ensure that the product is safe for consumption (Corlett, 1998). The results from this study provided important information needed for the development and implementation of HACCP. HACCP is used as a management tool to protect a food product against microbial, chemical, and physical safety hazards (Lyon and Milliet, 2000). The data from this study

were used as a basis for identifying the critical control points needed in the HACCP plan, and provided microbial data needed for identifying microbial hazards associated with smoked catfish. Before the HACCP plan can be developed, the smoking-process flow chart for catfish steaks needs to be established.

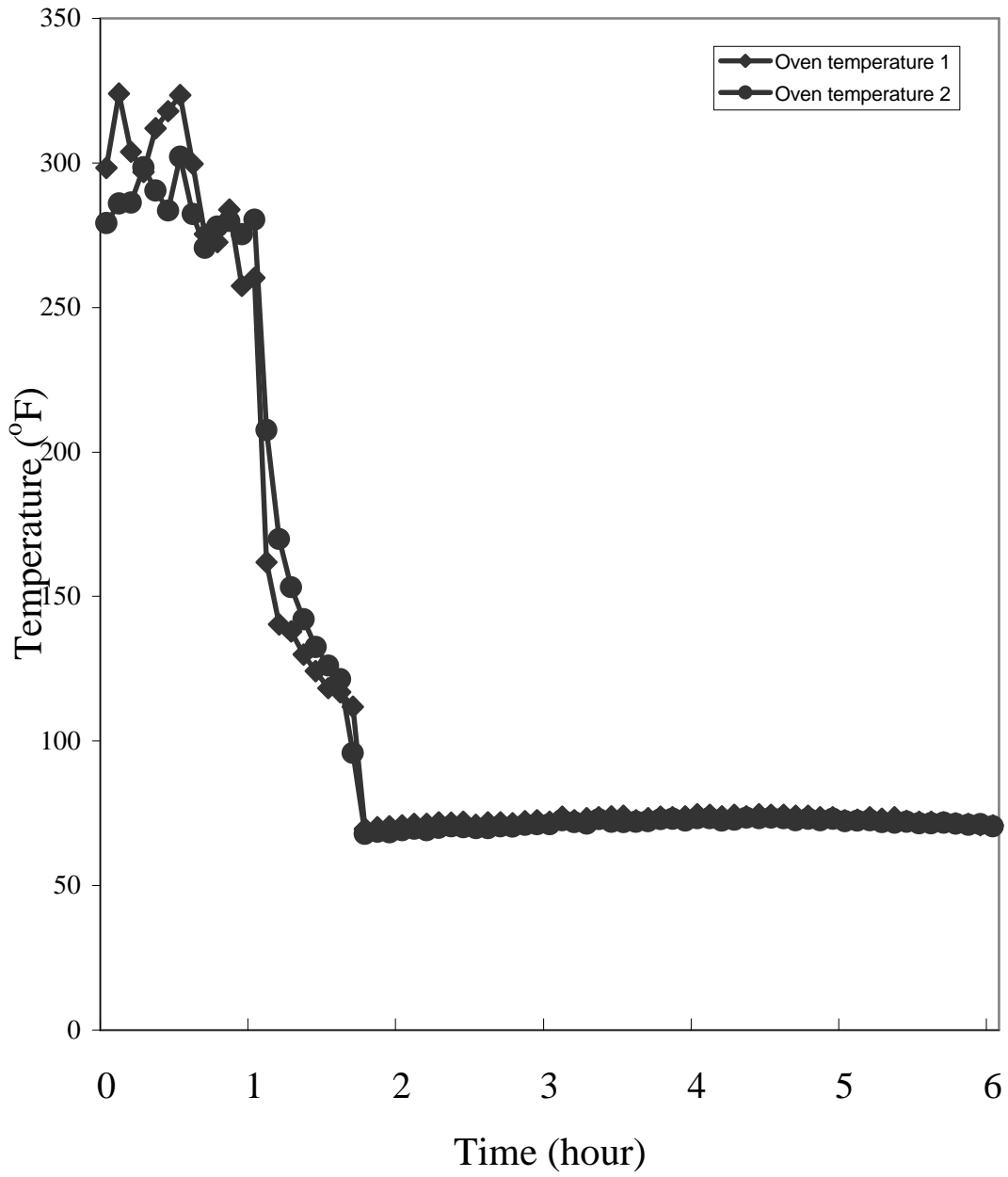


Figure 16: The temperature profile in oven #1 used for cooking.

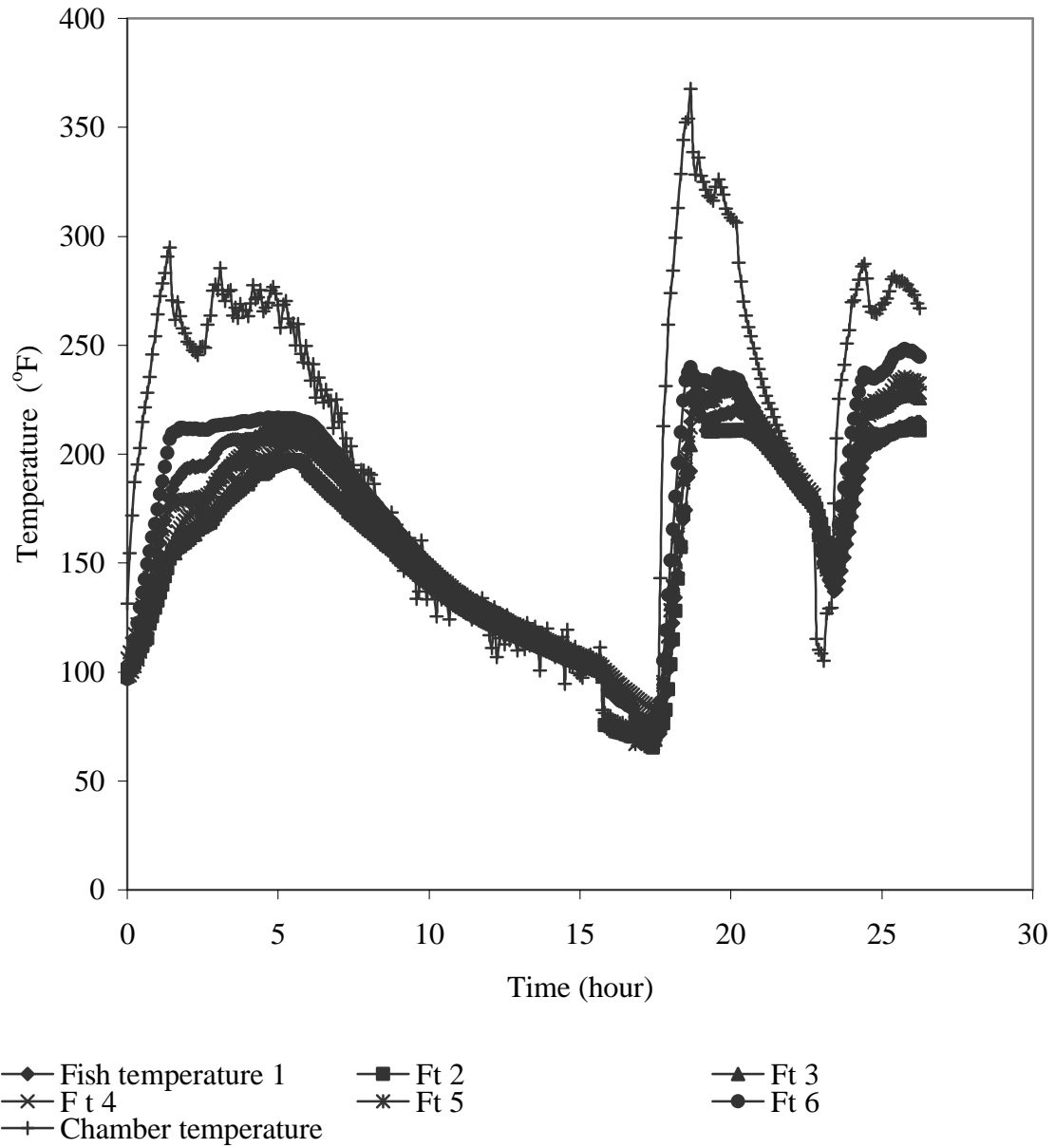


Figure 17 A: The temperature profile in oven #2 used for smoking and the internal fish temperature (Ft) during smoking (Batch 1).

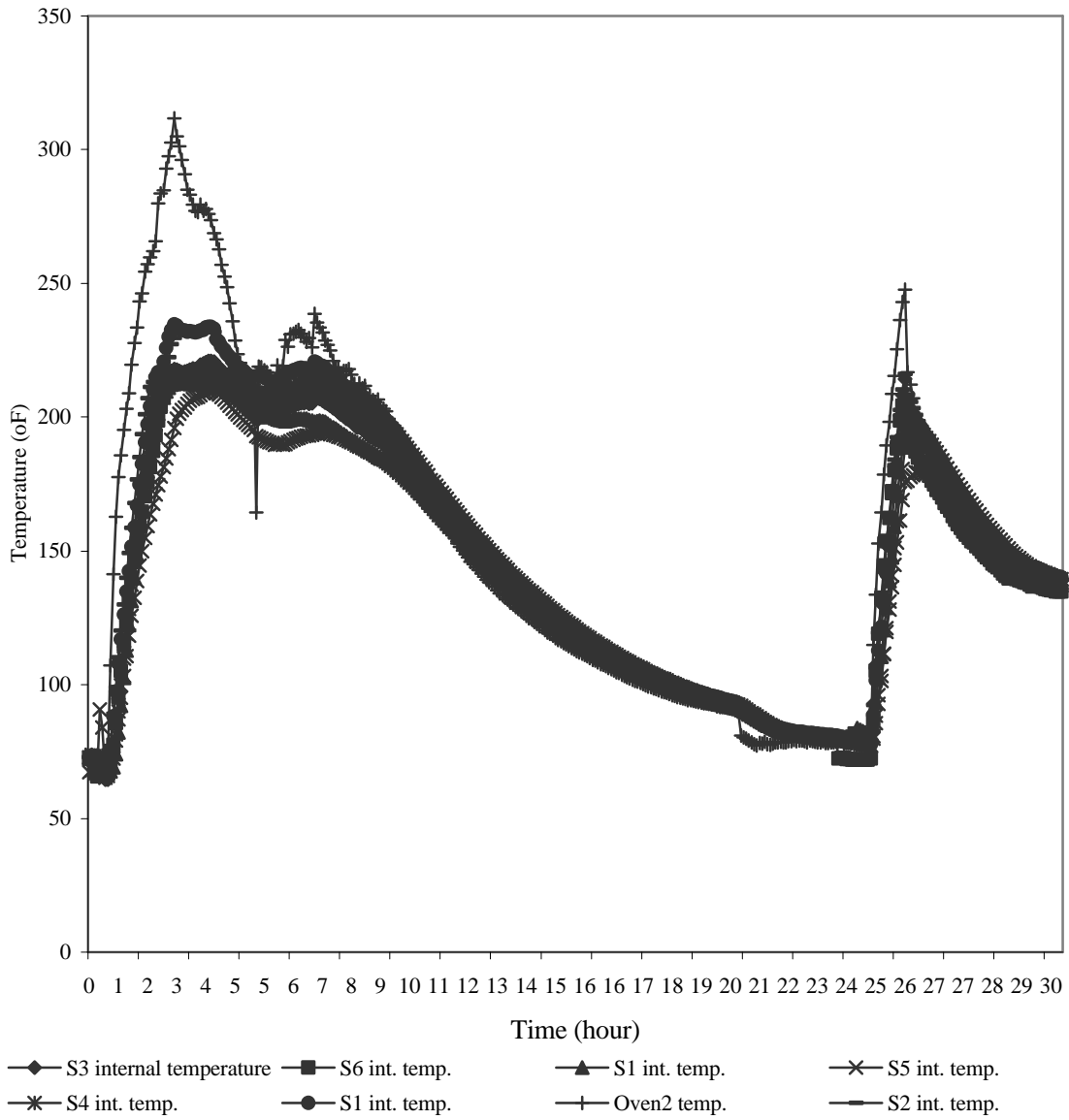


Figure 17 B: The temperature profile in oven #2 used for smoking and the internal fish temperature during smoking (Batch 2)

Smoking Process

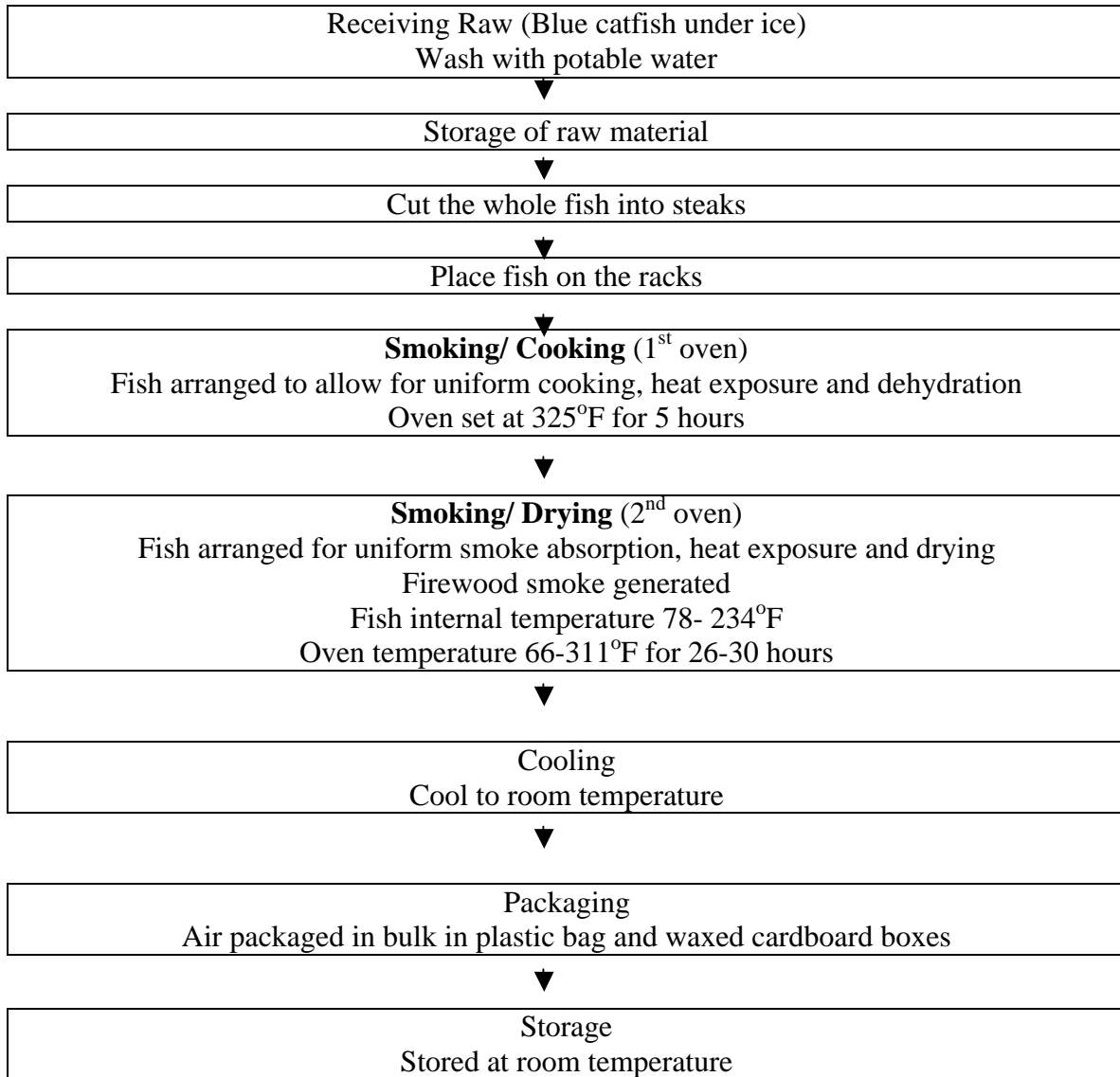


Figure 18: Smoking processing flow chart

Table 6: HACCP plan

Product Description: Smoked air packed smoked catfish

Method of Distribution and Storage: Stored and transported at ambient temperature or under refrigeration

Intended Use and Consumer: Further cooking and eating by general public

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Critical Control Point (CCP)	Significant Hazard(s)	Critical Limits for each Preventive Measure	Monitoring				Corrective Action(s)	Records	Verification
			What	How	Frequency	Who			
Cooking	Pathogen growth in final product (target, <i>C. botulinum</i> type E)	Achieve an internal temperature of 200°F and hold for 5 min.	Time/temperature (cook temperature and center temperature of the fish)	Clock and data logger	Every batch	Smoke house operator	*Recook *Destruct product *Achieve equivalent cook time during smoking and drying steps	Cooking record	*Calibrate equipments *Review records
Smoking and drying	Surface pathogen (<i>S. aureus</i>)	Achieve oven temperature 300 °F each day	Temperature and in/out process times	Data logger	Daily for each batch	Smoke-house operator	*Raise oven temperature *Hold and evaluate product	Smoking and drying records	*Calibrate equipment *review records

(Table 6 continued)

Table 6: HACCP plan (cont.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Critical Control Point (CCP)	Significant Hazard(s)	Critical Limits for each Preventive Measure	Monitoring				Corrective Action(s)	Records	Verification
			What	How	Frequency	Who			
Storage	Pathogen growth (surface phenomena)	0.85 a _w or less	a _w measurement	a _w meter	Largest fish portion per batch	Operator	*Refrigerated at 38 °F or less *Redry product	a _w log	*Calibrate equipment *Review records
OR: Storage	Pathogen growth (surface phenomena)	0.85 a _w or more, refrigerated at 38°F	Temperature (cold storage)	Thermometer	Read thermometer at least twice a day	Operator	Hold and evaluate /adjust cold room temperature	Refrigerator storage record *Review records	Calibrate thermometer *Review

Signature of Company Official: _____ Date: _____

SUMMARY AND CONCLUSIONS

The popularity of smoked fish is bound to increase with growing appeal for “old fashioned food” as processing is being done without chemicals or any additives. Smoking of fish is a traditional practice of fish preservation in many countries, mainly in Africa. On the other hand, smoking as practiced in industrialized countries is not a form of preservation since potential invasion by pathogenic microorganisms is high at various processing stages. Thus, smoked fish should be handled with caution. Preservation of smoked fish can be achieved by using chemical preservatives and antioxidants, which include sodium lactate, sorbic acid, salt, rosemary extract, and ascorbic acid.

From this study, smoking significantly reduced the total plate count in all samples of catfish steaks; however, the samples treated with 3% sodium lactate showed the lowest count throughout 6 weeks of storage. Sodium lactate can be used as a preservative in smoked catfish without adversely affecting quality in terms of lipid oxidation, microbial quality, color, and nutritional quality. Treating catfish prior to smoking with sodium lactate extended the shelf life of smoked catfish up to 6 weeks when stored at room temperature. Sodium lactate was effective not only in inhibiting microbial growth, but controlling the lipid oxidation of smoked catfish stored at room temperature for 6 weeks. The TPC of the samples treated with sodium lactate was 1.2 log CFU/g at week 0.

The TPC of the fresh control was 4.6 log CFU/g. After treatment with sodium lactate the TPC was reduced to 2.4 log CFU/g and after smoking the count was significantly reduced to the minimum of 1.2 log CFU/g and the low microbe count was maintained until the end of storage time (6 weeks) with 2.6 log CFU/g. Sodium lactate also reduced the coliform count of fresh catfish steaks to 2.0 log CFU/g and after

smoking the coliform count was 0.3 log CFU/g on day 0. At end of storage time, the coliform count remained low with 2.0 log CFU/g. In addition, sodium lactate reduced the *E. coli*, *Staphylococcus*, *Salmonella*, *Listeria* and yeast and mold counts to 0 on smoked catfish samples throughout 6 weeks of storage. Sodium lactate not only reduced the microbial load in smoked catfish but also kept the TBARS and PV stable throughout 6-week storage.

Water activity of samples treated with sodium lactate was 0.62 on day 0 and stayed low throughout 6 weeks of storage, and consequently the microbial load in these samples was low. Sodium lactate did not significantly affect pH and composition of smoked catfish steaks. It, however, significantly reduced the moisture content of smoked catfish to 10.55%, compared to 21.5% of the control sample on day zero. The external chroma value of the samples treated with sodium lactate decreased significantly at the end of the storage time. Samples treated with 3% sodium lactate had lowest microbial loads and were shelf-stable up to 6 weeks without refrigeration. Sodium lactate treatment was most effective in controlling microbial quality and extending the shelf-life of smoked catfish. The experimental protocol used in this study can serve as a model for other smoked fish and the data from microbial analysis will be used for development of generic HACCP plans for smoked catfish processing.

The primary tool used by regulatory agencies to ensure food safety by commercial food processors, especially muscle food processors, is Hazard Analysis and Critical Control Point (HACCP) principles. Some food processors, especially those who use heat treatment to destroy pathogens (cooked ready-to-eat products), are especially impacted by this regulation. This particular operation is especially complex because heat treatment

and water activity are used to control pathogens and toxin production. This study evaluated those parameters using HACCP principles to ensure the safety of smoked catfish products.

REFERENCES

- AOAC International. 1995. *Official Methods of Analysis*, 16th ed. AOAC International, Gaithersburg, MD.
- AOCS, 1996. *Official Methods and Recommended Practices, Physical and Chemical Characteristics of Oils, Fat and Waxes, Section I*, American Oil Chemists' Society, Champaign, IL.
- Asiedu, M.S., Julshamn, K., and Lie, O. 1991. Effect of local processing methods (cooking, frying, and smoking) on three fish species from Ghana: Part I, Proximate composition, fatty acids, minerals, trace elements, and vitamins. *Food Chem.* 40:309-321.
- Austin, B. and Austin, D.A. 1989. General introduction. *In Methods for the Microbiological Examination of fish and Shellfish*, B. Austin and D.A. Austin (Ed.), Ellis Horwood Limited, England, p19-24.
- Awad, A., Powriw, W.D., Fennema, O. 1969. Deterioration of fresh-water whitefish muscle during frozen storage at -10°C . *J. Food Sci.* 34:1-9.
- Banwart, G. 1989. *Basic Food Microbiology*, 2nd ed. Van Nostrand Reinhold, New York, NY.
- Beltran, A. and Moral, A. 1991. Changes in fatty acid composition of fresh and frozen sardine (*Sardinella pilchardus*) during smoking. *Food Chem.* 42:99-109.
- Ben Embarek, P.K. 1994. Presence, detection and Growth of *Listeria monocytogenes* in seafoods: a review. *Int. J. Food Microbiol.* 23:17-34.
- Bonnell, A.D. 1994. *Quality Assurance in Seafood Processing: A practical Guide*, Chapman & Hall, New York.
- Brake, N.C. and Fennema, O.R. Lipolysis and lipid oxidation in frozen minced mackerel as related to Tg' molecular diffusion, and presence of gelatin. *J. Food Sci.* 64:25-32.
- Bryan, F.L. 1988. Risk associated with vehicles of foodborne pathogens and toxins. *J. Food Prot.* 51:498-508.
- Burt J.R. 1988. *Fish Smoking and Drying. The Effect of Smoking and Drying on the Nutritional Properties of Fish.* Elsevier Applied Science. London and New York.
- Calhoun, C.M., Gaebler, D.M., and Mandingo, R.W. 1999. Storage stability of ground pork containing meat from an advanced meat recovery system. *J. Food Sci.*, 64: 69-75.

- Davies, A.R. 1997. Modified-atmosphere packaging of fish and fish products. In *Fish Processing Technology*, 2nd ed., G.M Hall (Ed.), Chapman & Hall, New York, NY, p. 200-223.
- Dillon, R. Patel, T.R., and Martin, A.M. 1994. Microbiological control of fish smoking operations. In *Fisheries Processing*. Martin, A.M. (Ed.) p 51-81. Chapman and Hall, London, U.K.
- Doe P.E., 1998. *Fish Drying and Smoking Production and Quality*. Technomic Publishing Co., Inc. Lancaster, Pennsylvania.
- Efiuvwevwere, B. J. O. and Ajiboy, M. O. 1996. Control of microbiological Quality and Shelf-life of Catfish (*Clarias gariepinus*) by Chemical preservatives and smoking. *Journal of Applied Bacteriology* vol. 80 p.465-470.
- El-Shenawy M.A. and Marth E.H. Inhibition and inactivation of *Listeria monocytogenes* by sorbic acid. *J. Food Prot.* 51:842-847.
- Faber, J.M. 1991. Microbiological aspects of modified-atmosphere packaging technology: a review. *J. Food Prot.* 54: 58-70.
- FDA, Department of Health and Human Services, 2001. Pathogen Growth & Toxin Formation as a Result of inadequate Drying. In *Fish & Fisheries Products Hazards & Controls Guidance: Third Ed.* Chapter 14, p. 191.
- FDA, Department of Health and Human Services, 2001. FDA & EPA Safety Levels in Regulations and Guidance. In *Fish & Fisheries Products Hazards & Controls Guidance: Third Ed.* Appendix 5, p. 285.
- Fernandez, C.F., Flick, G.J., Cohen, J., and Thomas, T.B. 1998. Role of organic acids during processing to improve the quality of channel catfish fillets. *J. Food Prot.* 61(4): 495-498.
- Fernandez, C.F., Flick, J.G., Silva, J.L., and McCaskey, T.A. 1997a. Comparison of quality in aquacultured fresh catfish fillets II. Pathogens *E. coli* O157:H, *Campylobacter*, *Vibrio*, *Plesiomonas*, and *Klebsiella*. *J. Food Prot.* 60(10): 1182-1188.
- Fernandez, C.F., Flick, J.G., Silva, J.L., and McCaskey, T.A. 1997b. Influence of processing schemes on indicative bacteria and quality of fresh aquacultured catfish fillets. *J. Food Prot.* 60(1): 54-58
- Fernandez, P.S., George, S.M., Sills, C.C., and Peck M.W. 1997. Predictive model of the effect of CO₂, pH, temperature and NaCl on growth of *Listeria monocytogenes*. *Int. J. Food Microbiol.* 37:37-45.

- Fleming, D.W., S.L. Cochi, K.L. MacDonald, J. Brondum, P.S. Hayes, B.D. Plikaytis, M.B. Holmes, A. Audurier, C.V. Broome, and A.L. Reingold. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *N. Engl. J. Med.* 312:404-407.
- Forester. P.G.N. 1999. Reduction of off-flavor compounds in catfish fillets by acid treatment. Department of Food Science, Louisiana State University.
- Garthwaite, G.A. 1997. Chilling and freezing of fish. In *Fish Processing Technology*. G.M. Hall (Ed.). VCH Publisher. New York, p93-119.
- Goktepe, I. and Moody, M.W.1998. Effect of modified atmosphere package on the quality of smoked catfish. *Journal of Muscle Foods*, 9:375-389.
- Greenberg, R.A., Silliker, J.H., and Fata, L. D. 1959. The influence of sodium chloride on toxin production and organoleptic breakdown in perishable cured meat inoculated with *Clostridium botulinum*. *Food Technol.* 13:509.
- Guer, S., and Jemmi. 1991. Behavior of *Listeria monocytogenes* during fabrication and storage of experimentally contaminated smoked salmon. *Appl. Environ. Microbiol.* 57:1523-1527.
- Guntensperger, B., Hammerli-Meier, D.E., and Escher, F.E. 1998. Rosemary extract and precooking effects on lipid oxidation in heat-sterilized meat. *J. Food Sci.*, 63: 955-957.
- Guyer, S. and Jemmi, T. 1991. Behavior of *Listeria monocytogenes* during fabrication and storage of experimentally contaminated smoked salmon. *Appl. Environ. Microbiol.* 57:1523-1527.
- Heintz, M.L., and Johnson, J.M. 1998. The incidence of *Listeria* spp., *Salmonella* spp., and *Clostridium botulinum* in smoked fish and shellfish. *Journal of Food Protection*, 61(3): 318-323.
- Hernandez-Herrero, M.M., Roig-Sagues, A.X., Lopez-Sabater, E.I., Rodriguez-Jerez, J.J., and Mora-Ventura M.T. 1999. Total volatile basic nitrogen and other physico-chemical and microbiological characteristics as related to ripening of salted anchovies. *J. Food Sci.* 64:344-347.
- Hintlian, C.B. and Hothckiss, J.H. 1986. The safety of modified atmosphere packaging. *Food Technol.* 40 (12), 70-76.
- Hu A.C. and Shelef L.A.1996. Influence of fat content and preservatives on the behavior of *Listeria monocytogenes* in beaker sausage. *J. Food Saf.* 16:175-181.

- ICMSF (International Commission on Microbiological Specification for Foods) (1986) Microorganisms in Foods 2, Sampling for Microbiological Analysis. Principles and Specifications, 2nd edn. Oxford: Blackwell Science.
- English, V., Richard, R.H., and Wodward, K.N. 1993. Public health aspect of bacterial infections of fish. In *Bacterial Diseases of Fish*, Valery English, Ronald Roberts, and Niall Bromage (Ed.), Halsted Press, New York, p.284-303.
- Jay, J.M. 1992. Modern Food Microbiology. 4th Ed. P76. Avi Pub., N.Y.
- Kim, J. and Foegeding, P.M. 1993. Principles of control. In: Hauschild A.H.W., Dodds K.L., editors. *Clostridium botulinum*: Ecology and control in foods. New York: M. Dekker. P 121-176.
- Kuhn, H., B. Gericke, M. Klepp, G. Fellmann, and W. Rabsch. 1994. Outbreak of *Salmonella paratyphi B* infections in connection with consumption of smoked fish. *Gesundheitswesen* 56:211-214.
- Langourieux, S. and Escher, F.E. 1998. Sulfurous off-flavor formation and lipid oxidation in heat-sterilized meat in trays. *J. Food Sci.* 63:716-720
- Lee, J.S. 1991. Commercial Catfish Farming (3rd Ed.). Interstate Pub. Inc., p. 3-15, 293-297.
- Li, Y., Slavik, M. F., Walker, J.T., and Xiong, H. 1997. Pre-chill spray of chicken carcasses to reduce *Salmonella typhimurium*. *J. Food Sci.* 62:605-607.
- Lone Gram, 2001. Potential hazard in cold-smoked fish: *Clostridium botulinum* type E. In Processing Parameters Needed to Control Pathogens in Cold-smoked Fish. Especial Supplement to the Journal of Food Science 66(7) S-1082-1087.
- Lone Gram, 2001. Potential hazard in cold-smoked fish: *Listeria monocytogenes*. In Processing Parameters Needed to Control Pathogens in Cold-smoked Fish. Especial Supplement to the Journal of Food Science 66(7) S-1072-1081.
- Meng, J. and Genineorgis C.A. 1993. Modeling lag phase of nonproteolytic *Clostridium botulinum* toxigenesis in cooked turkey and chicken breast as affected by temperature, sodium lactate, and sodium chloride and spore inoculum. *Int. J. Food Microbiol* 19:109-122.
- Mitsuda, H. Nakajima, K., Mizuno, H., and Kawai, F. 1980. Use of sodium chloride solution and carbon dioxide for extending shelf life of fish fillets. *J. Food Sci.* 45:661-666.
- Nickelson, R. and Finne, G. 1992. Fish, crustaceans, and precooked seafoods. In Compendium for the Microbiological Examination of Foods, American Public

- Health Association, 3rd ed., Ch. 47, Carl Vanderzant and Don Splittstoesser (Ed.), Washington, DC.
- Olley, J., Doe, P.E., and Heruwati, E.S. 1988. The influence of drying and smoking on the Nutritional properties of fish: an introductory overview. *In Fish Smoking and Drying*. p1-21. Elsevier Science, New York.
- Pacheco-Aguilar, R., Lugo-Sanchez, M.E., and Robles-Burgueno M.R. 2000. Postmortem biochemical and functional characteristic of Monterey sardine muscle storage at 0°C. *J. Food Sci.* 65:40-47.
- Pastoriza, L., Sampedro, G., Herrera, J.J., and Cabo, M.L. 1998. Influence of sodium chloride and modified atmosphere packaging on microbiological, chemical, and sensorial properties in ice storage of slices of hake (*Merluccius merluccius*). *Food Chem.* 61: 23-28.
- Pelroy G.A., Peterson M.E., Holland P.J., and Eklund, M.W. 1994. Inhibition of *Listeria monocytogenes* in cold-process (smoke) salmon by sodium lactate. *J. Food Prot* 57(2): 108-113.
- Peterkin, P.I., Parrington, L.J., and Sharpe, A.N., 1998. Determination of aerobic colony counts in foods by Hydrophobic Grid-Membrane Filtration (HGMF) method. Health Protection Branch, Ottawa, Canada. Government of Canada.
- Pigott G.M. and Tuckker. B.W. 1990. Seafood Effects of Technology on Nutrition, Marcel Dekker Inc. N.Y. p 155-170.
- Pike, O. A. 1998. Fat characterization. *In Food Analysis* Nielsen S.S. (2nd ed) p. 229. Aspen Pub. Inc. Gaithersburg, Maryland.
- Purac America Inc.1997. Salt of the earth. <http://www.purac.com>
- Purac America Inc.1999. Lactate controls Listeria. <http://www.purac.com>
- Purac America Inc.1999. Shelf-life extenders. <http://www.purac.com>
- Ramos, M.R. 1999 Reduction of Endogenous Bacteria Associated with Catfish (*Ictalurus Punctatus*) Fillets Using The Grovac Process. A Dissertation Louisiana State University, Baton Rouge.
- Ravishankar, S. Juneja V.K. 2000. Sodium chloride. In Natural Food Antimicrobial Systems. Edited by A.S. Naidu. CRC Press LLC. N.W. Corporate Blvd., Boca Raton, Florida.
- Ray, B. 1996. Fundamental food Microbiology. CRC Press LLC, Boca Raton, FL.

- Rorvik, L.M., Skjerve, E. and Yndestad, M. 1991. Growth of *Listeria monocytogenes* in vacuum-packed, smoked salmon, during storage at 4°C. *Int. J. Food Microbiol.* 14: 111-118.
- Ryser, E.T. and Marth, E.H. 1988. Survival of *Listeria monocytogenes* in cold-pack cheese food during refrigerated storage. *J. Food Prot.* 51:615-621.
- Segner, W.P., Schmidt, C.F., and Boltz, J.K. 1966. Effect of sodium chloride and pH on outgrowth of spores of type E *Clostridium botulinum* at optimal and suboptimal temperature. *Appl. Microbiol.* 14:49-54.
- Simko, P. 1991. Changes of Benzo (a) Pyrene content in smoked fish during storage. *Food Chem.* 40:293-300.
- Sodium lactate has a deflationary effect on water activity (a_w) higher than other organic acids or sodium chloride at equimolar concentrations. (Bogaert and Naidu, 2000).
- Sofos, J.N. 1989. *Sorbate Food Preservatives*. Boca Raton, FL: CRC Press.
- Sofos, J.N. 2000. Sorbic acid. In *Natural Food Antimicrobial Systems*, ed. A.S. Naidu, pp.637-659. Boca Raton, FL: CRC Press.
- Sofos, J.N. and Busta, F.F. 1993. Sorbic acid and sorbates. In *Antimicrobial in Food*, ed. P.M. Davidson and A.L., pp. 49-94. New York: Marcel Dekker, Inc.
- Suvanich, V., Ghaedian, R. Chanamai, R. Decker E.A., and McClements D.J. 1998. Prediction of proximate fish composition from ultrasonic properties: Catfish, Cod, Flounder, Mackerel and Salmon. *J. Food Sci.* 63:966-968.
- Suvanich, V., Marshall, D.L., and Jahncke M.L. 2000. Microbiological and color quality changes of Channel catfish frame mince during chilled and frozen storage. *J. Food Sci.* 65: 151-154.
- Tarladgis, B.G., Watts, B.M., Younathan, M.T., and Dugan, L.R. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists' Society* 37 (1): 44-48.
- United States Department of Agriculture, National Agricultural Statistics Service 1997. *Catfish Production December 1997*. Washington D.C.
- Vara-Ubol, S. and Bowers, J.A. 2001. Effect of α -tocopherol, β -carotene, and sodium tripolyphosphate on lipid oxidation of refrigerated cooked ground turkey and ground pork. *J. Food Sci.* 66:662-667.
- Varnam, A.H. 1991. *Foodborne pathogens: an illustrated text*. Mosby-Year Book, Inc., London.

- Wang, D., Tang, J., Correia, L.R. and Gill, T.A. 1998. Postmortem changes of cultivated Atlantic salmon and their effects on salt uptake. *J. Food Sci.* 63:634-637.
- Warburton, D. 2001. Enumeration of total aerobic bacteria in food product and food ingredients using 3M™ Petrifilm™ aerobic counts plates. Health Products and Food Branch. Government of Canada. <http://www.hc-sc.gc.ca/food-aliment>.
- Ward, A. R. 1995. Fish smoking in the tropics. A review. *Trop. Sci.* 35, 103-112.
- Ward, Donn R. 2001. Description of the situation. In *Processing Parameters Needed to Control Pathogens in Cold-smoked Fish*. Especial Supplement to the *Journal of Food Science* 66(7) S-1067-1071.
- Williams, S.K., Rodrick, G.E., and West, R.L. 1995. Sodium lactate affects shelf life and consumer acceptance of fresh catfish (*Ictalurus nebulosus*, *marmoratus*) fillets under simulated retail conditions. *J. Food Sci.* 60: 636-639.
- Woyewoda, A.D., Shaw, S.J., Ke, P.J., and Burns, B.G. 1986. Quality indices-lipid related. In *Recommended Laboratory Methods for Assessment of Fish Quality*. Canadian technical report of fisheries and aquatic science, Canada.

APPENDIX 1. MICROBIAL COUNT IN FRESH CATFISH STEAKS

Table 7: Total plate count, coliform, and yeast & mold counts in fresh catfish steaks

Treatments	Total plate count	coliforms	yeast and mold
Control (no treatment)	4.6 ± 1.5	2.8 ± 1.5	2.3 ± 1.0
25% NaCl and 1% ascorbic acid (1h)	2.6 ± 1.0	2.4 ± 1.2	2.0 ± 0.8
25% NaCl and 1% ascorbic acid (30 min)	2.8 ± 1.5	2.5 ± 1.6	2.2 ± 1.0
3% Sodium lactate	2.4 ± 0.8	2.0 ± 0.5	1.3 ± 0.5
3% Sodium lactate and 5% rosemary extract	2.4 ± 1.1	2.2 ± 1.3	1.5 ± 0.7
5% Sorbic acid	2.6 ± 1.0	2.3 ± 0.5	1.3 ± 0.5

Mean ± standard deviation of log CFU/g

Table 8: *E. coli*, *Staphylococcus*, and *Salmonella* counts in fresh catfish steaks

Treatments	<i>E. coli</i>	<i>Staphylococcus</i>	<i>Salmonella</i>
Control (no treatment)	2.0 ± 0.9	2.0 ± 0.8	2.1 ± 1.3
25% NaCl and 1% ascorbic acid (1h)	1.2 ± 0.5	1.4 ± 1.0	2.0 ± 1.0
25% NaCl and 1% ascorbic acid (30 min)	1.3 ± 0.6	1.3 ± 1.2	2.1 ± 1.0
3% Sodium lactate	1.7 ± 0.6	1.2 ± 0.3	1.2 ± 0.8
3% Sodium lactate and 5% rosemary extract	1.7 ± 0.9	1.5 ± 1.0	1.3 ± 1.0
5% Sorbic acid	1.8 ± 0.9	1.5 ± 0.8	1.8 ± 0.3

Mean ± standard deviation of log CFU/g

APPENDIX 2. pH OF FRESH AND SMOKED CATFISH STEAKS

Table 9: pH of fresh catfish steaks

Treatments	pH
Control (no treatment)	6.80 ± 0.11 ^{abc}
25% NaCl and 1% ascorbic acid (1 hour)	6.60 ± 0.01 ^{cd}
25% NaCl and 1% ascorbic acid (30 min)	6.66 ± 0.05 ^{bcd}
3% Sodium lactate	6.92 ± 0.41 ^a
3% Sodium lactate and 5% rosemary extract	6.83 ± 0.52 ^{ab}
5% Sorbic acid	6.33 ± 0.52 ^e

Mean ± standard deviation

Table 10: pH of smoked catfish steaks during 6-week storage at room temperature

Treatments	Storage time (weeks)			
	0	2	4	6
Control (no treatment)	6.85 ± 0.13 ^{Aa}	6.90 ± 0.00 ^{Aa}	6.85 ± 0.07 ^{Aab}	*
25% NaCl and 1% ascorbic acid (1 hour)	6.39 ± 0.05 ^{Ac}	6.40 ± 0.00 ^{Ac}	6.40 ± 0.00 ^{Ac}	6.30 ± 0.00 ^{Ac}
25% NaCl and 1% ascorbic acid (30 min)	6.58 ± 0.10 ^{Abc}	6.50 ± 0.00 ^{Abc}	6.40 ± 0.00 ^{Ac}	6.55 ± 0.07 ^{Ab}
3% Sodium lactate	6.72 ± 0.14 ^{Aab}	6.60 ± 0.00 ^{Ab}	6.70 ± 0.00 ^{Ab}	6.50 ± 0.00 ^{Ab}
3% Sodium lactate and 5% rosemary extract	6.65 ± 0.06 ^{Aab}	6.55 ± 0.07 ^{Ab}	6.90 ± 0.00 ^{Aa}	6.60 ± 0.00 ^{Ab}
5% Sorbic acid	6.53 ± 0.21 ^{Abc}	6.40 ± 0.00 ^{Ac}	6.35 ± 0.07 ^{Ac}	6.30 ± 0.00 ^{Ac}

Mean ± standard deviation. *Moldy

APPENDIX 3. WATER ACTIVITY OF SMOKED CATFISH

Table 11: Water activity of smoked catfish steaks during 6-week storage at room temperature

Treatments	Storage time (weeks)			
	0	2	4	6
Control (no treatment)	0.94 ± 0.04 ^{Aa}	0.92 ± 0.02 ^{Aa}	0.75 ± 0.01 ^{Bb}	*
25% NaCl and 1% ascorbic acid (1 hour)	0.48 ± 0.08 ^{Bc}	0.67 ± 0.00 ^{Ac}	0.63 ± 0.01 ^{Ac}	0.65 ± 0.01 ^{Ad}
25% NaCl and 1% ascorbic acid (30min)	0.74 ± 0.20 ^{Aabc}	0.66 ± 0.01 ^{Ac}	0.63 ± 0.01 ^{Ac}	0.69 ± 0.01 ^{Ac}
3% Sodium lactate	0.62 ± 0.20 ^{Ab}	0.64 ± 0.01 ^{Ac}	0.63 ± 0.05 ^{Ac}	0.70 ± 0.01 ^{Abc}
3% Sodium lactate and 5% rosemary extract	0.76 ± 0.12 ^{Abab}	0.85 ± 0.01 ^{Ab}	0.80 ± 0.01 ^{Ba}	0.66 ± 0.01 ^{Bd}
5% Sorbic acid	0.77 ± 0.24 ^{Aab}	0.67 ± 0.02 ^{Ac}	0.82 ± 0.01 ^{Aa}	0.80 ± 0.01 ^{Aa}

Mean ± standard deviation. *Moldy

APPENDIX 4: PROXIMATE ANALYSIS OF FRESH AND SMOKED CATFIS STEAKS

Table 12: Proximate analysis of fresh catfish steaks

Treatments	Protein	Fat	Ash	Moisture
Control (no treatment)	17.8 ± 0.8 ^b	4.3 ± 0.9 ^c	0.99 ± 0.1 ^d	76.9 ± 1.1 ^a
25% NaCl and 1% ascorbic acid (1 hour)	16.5 ± 0.8 ^b	2.02 ± 1 ^c	1.7 ± 0.3 ^d	79.1 ± 1.3 ^a
25% NaCl and 1% ascorbic acid (30 min)	16.4 ± 0.6 ^b	1.7 ± 1.5 ^c	1.6 ± 0.1 ^d	79.3 ± 1.7 ^a
3% Sodium lactate	18.0 ± 0.4 ^b	3.5 ± 1.3 ^c	0.99 ± 0.1 ^d	78.1 ± 1.4 ^a
3% Sodium lactate and 5% rosemary extract	17.2 ± 0.5 ^b	2.5 ± 1.6 ^c	0.9 ± 0.13 ^d	79.6 ± 0.9 ^a
5% Sorbic acid	17.4 ± 0.6 ^b	2.9 ± 2.3 ^c	0.9 ± 0.2 ^d	78.6 ± 1.9 ^a

Mean ± standard deviation

Table 13: Protein content of smoked catfish steaks during 6-week storage at room temperature

Treatments	Storage time (weeks)			
	0	2	4	6
Control (no treatment)	56.48 ± 11.0 ^{Aa}	61.63 ± 5.88 ^{Ac}	77.75 ± 2.03 ^{Ab}	*
25% NaCl and 1% ascorbic acid (1 hour)	67.53 ± 11.9 ^{Aa}	69.10 ± 5.93 ^{Abc}	78.47 ± 1.15 ^{Aa}	74.06 ± 0.5 ^{Aa}
25% NaCl and 1% ascorbic acid (30 min)	53.71 ± 10.5 ^{Aa}	73.31 ± 2.86 ^{Aabc}	78.07 ± 1.76 ^{Aa}	64.28 ± 1.9 ^{Aa}
3% Sodium lactate	69.14 ± 13.5 ^{Aa}	86.79 ± 2.90 ^{Aa}	82.95 ± 1.02 ^{Aa}	61.26 ± 10.2 ^{Aa}
3% Sodium lactate and 5% rosemary extract	63.66 ± 2.14 ^{Ba}	62.24 ± 0.77 ^{Bc}	71.87 ± 2.10 ^{Ab}	66.87 ± 4.43 ^{Aa}
5% Sorbic acid	56.19 ± 8.24 ^{Ba}	79.50 ± 3.30 ^{Aab}	63.30 ± 0.34 ^{ABc}	56.62 ± 3.95 ^{Ba}

Mean ± standard deviation. *Moldy

Table 14: Fat content of smoked catfish steaks during 6-week storage at room temperature

Treatments	Storage time (weeks)			
	0	2	4	6
Control (no treatment)	19.03 ± 2.60 ^{Aa}	13.33 ± 0.26 ^{Aba}	11.54 ± 0.27 ^{Bb}	*
25% NaCl and 1% ascorbic acid (1 hour)	13.11 ± 4.75 ^{Aa}	15.10 ± 1.55 ^{Aa}	10.86 ± 0.21 ^{Abc}	10.32 ± 0.01 ^{Ad}
25% NaCl and 1% ascorbic acid (30 min)	16.78 ± 8.38 ^{Aa}	11.78 ± 0.29 ^{Abc}	8.46 ± 0.11 ^{Ad}	12.23 ± 0.11 ^{Ac}
3% Sodium lactate	17.16 ± 9.65 ^{Aa}	2.54 ± 0.08 ^{Ad}	6.89 ± 0.23 ^{Ae}	17.98 ± 0.38 ^{Ab}
3% Sodium lactate and 5% rosemary extract	20.20 ± 9.01 ^{Aa}	15.64 ± 0.47 ^{Aa}	9.97 ± 0.06 ^{Ac}	18.84 ± 0.18 ^{Ab}
5% Sorbic acid	22.02 ± 6.78 ^{Aa}	9.95 ± 0.37 ^{Ac}	21.32 ± 0.45 ^{Aa}	20.62 ± 0.81 ^{Aa}

Mean ± standard deviation. *Moldy

Table 15: Ash content of smoked catfish steaks during 6-week storage at room temperature

Treatments	Storage time (weeks)			
	0	2	4	6
Control (no treatment)	3.17 ± 0.56 ^{Ac}	2.78 ± 0.62 ^{Ac}	3.98 ± 0.24 ^{Ab}	*
25% NaCl and 1% ascorbic acid (1 hour)	5.41 ± 1.36 ^{Aab}	6.07 ± 0.30 ^{Aa}	6.28 ± 0.07 ^{Aa}	5.55 ± 0.01 ^{Aa}
25% NaCl and 1% ascorbic acid (30 min)	5.80 ± 2.12 ^{Aa}	5.49 ± 0.22 ^{Aa}	6.60 ± 0.05 ^{Aa}	5.93 ± 0.22 ^{Aa}
3% Sodium lactate	3.93 ± 0.40 ^{Abc}	4.22 ± 0.08 ^{Ab}	3.71 ± 0.19 ^{Ab}	3.39 ± 0.28 ^{Ab}
3% Sodium lactate and 5% rosemary extract	3.31 ± 0.21 ^{Abc}	2.78 ± 0.05 ^{Bc}	3.87 ± 0.76 ^{Ab}	3.12 ± 0.18 ^{ABb}
5% Sorbic acid	2.82 ± 0.37 ^{Ac}	3.28 ± 0.16 ^{Abc}	2.99 ± 0.23 ^{Ab}	2.86 ± 0.33 ^{Ab}

Mean ± standard deviation. *Moldy

Table 16: Moisture content of smoked catfish steaks during 6-week storage at room temperature

Treatments	Storage time (weeks)			
	0	2	4	6
Control (no treatment)	21.5 ± 11.60 ^{Aa}	25.26 ± 2.30 ^{Aa}	16.29 ± 0.18 ^{Ab}	-
25% NaCl and 1% ascorbic acid (1 hour)	13.97 ± 10.40 ^{Aa}	14.81 ± 0.30 ^{Ab}	14.17 ± 0.19 ^{Ac}	13.85 ± 0.27 ^{Ab}
25% NaCl and 1% ascorbic acid (30 min)	21.4 ± 8.20 ^{Aa}	15.46 ± 0.23 ^{Ab}	13.99 ± 0.00 ^{Ac}	16.78 ± 0.18 ^{Aa}
3% Sodium lactate	10.55 ± 6.99 ^{Aa}	13.69 ± 0.15 ^{Ab}	14.32 ± 0.32 ^{Ac}	12.16 ± 0.37 ^{Ac}
3% Sodium lactate and 5% rosemary extract	12.96 ± 8.34 ^{Aa}	23.23 ± 0.10 ^{Aa}	18.13 ± 0.78 ^{Aa}	11.55 ± 0.74 ^{Ac}
5% Sorbic acid	19.14 ± 11.8 ^{Aa}	14.69 ± 0.33 ^{Ab}	18.22 ± 0.36 ^{Aa}	16.72 ± 0.11 ^{Aa}

Mean ± standard deviation

**APPENDIX 5. THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS)
AND PEROXIDE VALUE (PV) OF SMOKED CATFISH STEAKS**

Table17: TBARS in smoked catfish steaks during 6-week storage at room temperature

Treatments	Storage time (weeks)			
	0	2	4	6
Control (no treatment)	0.102 ± 0.135 ^{Aab}	0.021 ± 0.002 ^{Aa}	0.03 ± 0.0014 ^{Aa}	*
25% NaCl and 1% ascorbic acid (1 hour)	0.04 ± 0.041 ^{Aab}	0.013 ± 0.002 ^{Aa}	0.03 ± 0.001 ^{Ab}	0.022 ± 0.001 ^{Ab}
25% NaCl and 1% ascorbic acid (30 min)	0.04 ± 0.027 ^{Aab}	0.015 ± 0.003 ^{Aa}	0.025 ± 0.001 ^{Ab}	0.012 ± 0.001 ^{Ac}
3% Sodium lactate	0.018 ± 0.011 ^{Ab}	0.003 ± 0.001 ^{Ab}	0.012 ± 0.001 ^{Ac}	0.011 ± 0.001 ^{Ac}
3% Sodium lactate and 5% rosemary extract	0.025 ± 0.013 ^{Ab}	0.017 ± 0.003 ^{Aa}	0.028 ± 0.001 ^{Aa}	0.026 ± 0.001 ^{Aa}
5% Sorbic acid	0.041 ± 0.039 ^{Aab}	0.018 ± 0.002 ^{Aa}	0.025 ± 0.001 ^{Ab}	0.01 ± 0.001 ^{Ad}

Mean ± standard deviation. * Moldy

Table 18: PV in smoked catfish steaks during 6-weeks storage at room temperature

Treatments	Storage time (weeks)			
	0	2	4	6
Control (no treatment)	3.70 ± 1.46 ^{Bb}	8.00 ± 0.00 ^{Aa}	11.00 ± 1.41 ^{Aa}	*
25% NaCl and 1% ascorbic acid (1 hour)	1.40 ± 0.47 ^{Ab}	3.50 ± 0.71 ^{Ab}	3.50 ± 0.71 ^{Abc}	5.00 ± 1.41 ^{Bb}
25% NaCl and 1% ascorbic acid (30 min)	1.00 ± 0.00 ^{Dd}	3.00 ± 0.00 ^{Cbc}	5.50 ± 0.71 ^{Ab}	4.00 ± 0.00 ^{Bb}
3% Sodium lactate	2.03 ± 1.53 ^{Bb}	1.00 ± 0.00 ^{Bd}	6.00 ± 0.00 ^{Bb}	28.0 ± 5.66 ^{Aa}
3% Sodium lactate and 5% rosemary extract	1.03 ± 0.08 ^{Bb}	3.50 ± 0.71 ^{Ab}	1.50 ± 0.71 ^{Bc}	0.05 ± 0.71 ^{Bb}
5% Sorbic acid	1.5 ± 0.55 ^{Bb}	1.50 ± 0.71 ^{Bcd}	4.00 ± 1.41 ^{ABbc}	6.00 ± 2.80 ^{Ab}

Mean ± standard deviation. *Mold

VITA

The author was born and grew up in Bissau, Republic of Guinea-Bissau. In 1987, she was awarded a scholarship by the Guinea-Bissau Government to study for a bachelor of science degree in biology. In 1992, she graduated from Tashkent State University, former Soviet Union, with a master of science degree in biochemistry. She was employed as a researcher in the Ministry of Public Health in the Center of Tropical Medicine of Bissau. In 1995, she was awarded a scholarship by the Guinea-Bissau Government to study a post-graduation course in Public Health at the Institute of Hygiene and Tropical Medicine of Universidade Nova de Lisboa, Portugal. In 1997, she was awarded a scholarship by the Guinea-Bissau Government to pursue a Master of Science degree in Food Science in the United States of America. In spring 1999, she was accepted to the master's program in the Department of Food Science at Louisiana State University. Her future plan includes completion of doctoral work in the same department.