5-1989

The Use of Modified Atmosphere Packaging and Phosphate-Citric Acid dip to Extend the Shelf life of Fresh Catfish, *Ictalurus punctatus*

Brian Preston Moore

*University of Tennessee, Knoxville*

---

**Recommended Citation**


https://trace.tennessee.edu/utk_gradthes/4420

---

This Thesis is brought to you for free and open access by the Graduate School at Trace: Tennessee Research and Creative Exchange. It has been accepted for inclusion in Masters Theses by an authorized administrator of Trace: Tennessee Research and Creative Exchange. For more information, please contact trace@utk.edu.
To the Graduate Council:

I am submitting herewith a thesis written by Brian Preston Moore entitled "The Use of Modified Atmosphere Packaging and Phosphate-Citric Acid dip to Extend the Shelf life of Fresh Catfish, *Ictalurus punctatus*." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

John R. Mount, Major Professor

We have read this thesis and recommend its acceptance:

P. Michael Davidson, Curtis C. Melton

Accepted for the Council:

Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
To the Graduate Council:

I am submitting herewith a thesis written by Brian Preston Moore entitled "The Use of Modified Atmosphere Packaging and Phosphate-Citric Acid dip to Extend the Shelf life of Fresh Catfish, Ictalurus punctatus." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

John R. Mount, Major Professor

We have read this thesis and recommend its acceptance:

Michael Davidson

Curtis E. Mulkey

Accepted for the Council:

Vice Provost
and Dean of The Graduate School
STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master's degree at The University of Tennessee, Knoxville, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of the source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his absence, by the Head of Interlibrary Services when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature __________________________

Date 28 FEB 1989
The Use of Modified Atmosphere Packaging and Phosphate-Citric Acid Dip to Extend the Shelflife of Fresh Catfish

(Ictalurus punctatus)

A Thesis
Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

Brian Preston Moore
May 1989
DEDICATION

To my parents, Donald P. and Lataine T. Moore, for without their love, support and inspiration this thesis would not have been possible.
ACKNOWLEDGEMENTS

There are many people who had an integral part in helping me complete my research and this thesis. I would like to express my appreciation to the entire Faculty and Staff of the The University of Tennessee, Food Technology and Science Department for their kindness and support.

A special thanks to Dr. Mount who served as my Major Professor and also for his assistance in preparing these data for statistical analysis. I would also like to thank my committee members Dr. P. Michael Davidson and Dr. Curtis C. Melton for their advise and assistance in preparing this thesis.

I owe thanks to Mr. Ed Williams of Harriman, TN who supplied the fish for my research and went to extra efforts to grade the fish to the proper size for my needs. Thanks to Dr. J.L. Wilson, Dr. T.K. Hill, and John Taylor of the Fisheries Department for their assistance, advice and the use of their equipment.

Thanks to Bobby Grindstaff and the Meats Lab Staff Todd Trivette, David Grindstaff and also Boyd Lee who helped in slaughtering the fish for my research.
Thanks is also due to Keith Moore of Cryovac Div., W.R. Grace Corp., Duncan, SC for the use of their facilities and his assistance in packaging the first trial of the experiment.

Thanks to Marvis Fryer of the Animal Science Department for the use of their facilities.

I would also like to thank J. Wm. Wempe and Kim Payne for their assistance in the microbiology lab and also Rachel Huff and Martha Davis for their help in preparing samples for chemical analysis. And thanks to my fellow graduate students for their friendship and encouragement.

Most of all thanks to my parents for their support and inspiration.
ABSTRACT

Ninety market size catfish (*Ictalurus punctatus*) 1 - 2 pounds live weight were slaughtered, dipped in one of three solutions (phosphate, phosphate + citric acid, no dip) then packaged in one of three atmospheres (100% CO₂, 70% CO₂ + 5% O₂ + 25% N, Air) and were stored at 1°C or 5°C. The catfish were evaluated for aerobic plate count, anaerobic plate count, lactic acid count, coliform count, pH, oxidation, drip loss, color and odor at 0, 4, 8, 12 and 16 days. The 100% CO₂ and 70% CO₂ atmospheres effectively reduced the rates of microbial growth compared to control samples stored in air. The microbial counts on fish stored at 1°C were 1 log lower than at 5°C. Color and odor scores deteriorated more rapidly for samples stored in air than in the CO₂ containing atmospheres. Phosphate or phosphate + citric acid dips were not effective in reducing drip loss or microbial growth. TBA values although quite low for all treatments tended to increase steadily with time of storage.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2. LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>Modified Atmosphere Packaging</td>
<td>3</td>
</tr>
<tr>
<td>Definition</td>
<td>3</td>
</tr>
<tr>
<td>History</td>
<td>4</td>
</tr>
<tr>
<td>Methods of Application</td>
<td>5</td>
</tr>
<tr>
<td>Gases Used--Physical and Chemical Effects</td>
<td>6</td>
</tr>
<tr>
<td>Important Properties of Catfish</td>
<td>8</td>
</tr>
<tr>
<td>Color</td>
<td>8</td>
</tr>
<tr>
<td>Odor</td>
<td>9</td>
</tr>
<tr>
<td>Microbiology</td>
<td>9</td>
</tr>
<tr>
<td>Lipid Oxidation-TBA</td>
<td>10</td>
</tr>
<tr>
<td>Processing Effects and Storage Characteristics</td>
<td>11</td>
</tr>
<tr>
<td>Temperature</td>
<td>11</td>
</tr>
<tr>
<td>Microbiology</td>
<td>11</td>
</tr>
<tr>
<td>Hazards</td>
<td>12</td>
</tr>
<tr>
<td>pH</td>
<td>14</td>
</tr>
<tr>
<td>Dip Solutions</td>
<td>14</td>
</tr>
<tr>
<td>Lipid Oxidation - TBA</td>
<td>14</td>
</tr>
<tr>
<td>Drip</td>
<td>15</td>
</tr>
<tr>
<td>Antioxidants, Acidulants and Sequesterants</td>
<td>16</td>
</tr>
<tr>
<td>Marketing and Economics</td>
<td>17</td>
</tr>
<tr>
<td>Potential</td>
<td>17</td>
</tr>
<tr>
<td>Cost Benefit Relationship</td>
<td>17</td>
</tr>
<tr>
<td>Requirements for Commercial Application</td>
<td>18</td>
</tr>
<tr>
<td>3. MATERIALS AND METHODS</td>
<td>19</td>
</tr>
<tr>
<td>Sample Preparation</td>
<td>19</td>
</tr>
<tr>
<td>Raw Materials and Preparation</td>
<td>19</td>
</tr>
<tr>
<td>Trials - Location</td>
<td>20</td>
</tr>
<tr>
<td>Dipping Procedure</td>
<td>20</td>
</tr>
<tr>
<td>Packaging Procedure</td>
<td>21</td>
</tr>
<tr>
<td>Storage</td>
<td>21</td>
</tr>
<tr>
<td>CHAPTER</td>
<td>PAGE</td>
</tr>
<tr>
<td>-----------------</td>
<td>------</td>
</tr>
<tr>
<td>Analysis of Samples</td>
<td>22</td>
</tr>
<tr>
<td>Microbiological Analysis</td>
<td>22</td>
</tr>
<tr>
<td>pH</td>
<td>23</td>
</tr>
<tr>
<td>Oxidation (TBA)</td>
<td>23</td>
</tr>
<tr>
<td>Drip Loss</td>
<td>25</td>
</tr>
<tr>
<td>Color and Odor</td>
<td>25</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>25</td>
</tr>
<tr>
<td>4. RESULTS AND DISCUSSION</td>
<td>27</td>
</tr>
<tr>
<td>Effects of Atmosphere, Dip and Storage Temperature on Aerobic Plate Count</td>
<td>27</td>
</tr>
<tr>
<td>Effects of Atmosphere, Dip and Storage Temperature on Anaerobic Plate Count</td>
<td>32</td>
</tr>
<tr>
<td>Effects of Atmosphere, Dip and Storage Temperature on Lactic Acid Plate Count</td>
<td>34</td>
</tr>
<tr>
<td>Effects of Atmosphere, Dip and Storage Temperature on Coliform Count</td>
<td>36</td>
</tr>
<tr>
<td>Effects of Atmosphere, Dip and Storage Temperature on pH</td>
<td>37</td>
</tr>
<tr>
<td>Effects of Atmosphere, Dip and Storage Temperature on TBA Values</td>
<td>41</td>
</tr>
<tr>
<td>Effects of Atmosphere, Dip and Storage Temperature on Drip Loss</td>
<td>43</td>
</tr>
<tr>
<td>Effects of Atmosphere, Dip and Storage Temperature on Color</td>
<td>44</td>
</tr>
<tr>
<td>Effects of Atmosphere, Dip and Storage Temperature on Odor</td>
<td>46</td>
</tr>
<tr>
<td>5. SUMMARY</td>
<td>48</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>50</td>
</tr>
<tr>
<td>VITA</td>
<td>57</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

Catfish production has increased steadily over the past two decades. Prior to the 1970's, commercial catfish production was virtually non-existent. In 1969 less than 1,600 tons were processed commercially, however, by 1984 the quantity of processed catfish had increased to an amount in excess of 77,000 tons annually (Ammerman, 1986). Catfish are now processed at an estimated 4,000 tons per week (Anon., 1988a).

Although catfish can be produced in most areas of the United States, Tucker (1985) reported that the major catfish producing areas have been limited to the southeastern U.S. This is due to requirements for an abundant groundwater supply and a favorable climate which allows for a longer growing season (generally in excess of 200 days) therefore making production more economically feasible. Commercial production areas are highly centralized and are usually located close to large processing plants. The largest growth of the industry has taken place in the areas of the northwest Mississippi "Delta", eastern Arkansas, west central Alabama, and parts of Louisiana where large amounts of relatively flat land with sufficient amounts of surface water exists, most of which is marginally
suited for other agricultural crops.

Tucker (1985) stated that commercially produced catfish are usually marketed through one of three distribution channels: retail grocery stores, food service distributors, and catfish specialty restaurants. Surveys also indicated that grocery outlets handled primarily fresh fish, while food service distributors and restaurants handled primarily frozen fish.

The use of carbon dioxide enriched atmospheres can extend the shelflife of various types of fish as much as 50 - 100% (Statham, 1984). Since the commercial production of catfish is so highly centralized, the ability to increase the shelflife of fresh catfish through modified atmosphere packaging, would bring about an exponential increase in the potential market distribution area as well as increase the time available for 'market exposure' in the current areas of distribution.

The purpose of this research was to determine the effects of different modified atmospheres and the effects of a phosphate or phosphate-citric acid dip on the shelf life of refrigerated fresh catfish.
The growing and processing of catfish in the U.S. has developed as a highly centralized industry. The continued growth of the catfish industry will depend more on market development and expansion rather than production improvements (Johnsen et al., 1987). The concept of modified atmosphere packaging would allow for a highly perishable product such as catfish to have a longer shelflife and the ability to spend a longer time in transit to more distant market areas.

I. Modified Atmosphere Packaging

Definition

Modified atmosphere packaging (MAP) is a packaging method by which a product is enclosed in a high barrier packaging material and is surrounded with a gaseous mixture that has a composition different from that of air, with the purpose of extending the shelflife of a product. Increased shelflife is accomplished by inhibiting bacterial growth, enzymatic processes, respiration and changes in color or moisture content.
Modified atmosphere packaging differs from a similar method of packaging, controlled atmosphere packaging (CAP), in that the atmosphere is continuously maintained throughout the storage life of the product in CAP, whereas, in MAP the desired atmosphere is introduced into the container at the time of packaging and may change over time (Wheaton and Lawson, 1985; Wolfe, 1980, Statham, 1984; Young et al., 1988). Although modified atmosphere packaging generally refers to a gas flushed package, vacuum packaging by definition is a type of modified atmosphere packaging. Since the product is surrounded by no atmosphere, it is an atmosphere that is different than air.

History

The benefits of using modified atmosphere packaging have been known for over 50 years as Coyne's (1932) early experiments indicated that carbon dioxide inhibited the growth of bacteria isolated from fish. One of the early applications of MAP involved transoceanic shipments of fresh meat from New Zealand to the United Kingdom in the 1930's (Lawrie, 1974). MAP technology has gained tremendous popularity in the U.S. the last few years and is now used in the fruit, vegetable and meat
industries. Only recently have the economics and technology come together to justify the application of this technique to fish and seafood.

Methods of Application

Young et al. (1988) reported three basic types of packaging systems that can be used to apply MAP technology to meat products. One process developed for sub-primal cuts of red meats, often referred to as 'pouch in a bag,' involves placing multiples of cuts in a high barrier pouch with bone protection material, flushing with an appropriate atmosphere, sealing the pouch then placing it in a corrugated box. Another method for pre-packaging red meats designed to increase distribution time, is to wrap the meat in a high oxygen permeable film, flushing the primary package with the desired atmosphere then sealing a number of the packages in a high barrier pouch master pack for distribution. The master pack is then opened by the retailer as needed and the meat is allowed to be in bloom for 2 - 3 days of retail display. A third method for packaging consumer cuts or portions of meat makes use of a rigid thermoformed tray with a flexible high-barrier lidding material. In this procedure the air around the product is replaced with the desired atmosphere as the lidding material is sealed to the tray. Statham (1984) noted that MAP could also be used for
bulk shipment of products by using refrigerated seavans, railcars or trailers where the container is loaded with the product to be shipped and the atmosphere is injected and sealed in.

Gases Used--Physical and Chemical Effects

The most commonly used gases for modified atmosphere packaging are carbon dioxide, oxygen, and nitrogen.

Carbon dioxide-- is used because of its ability to inhibit bacterial growth. Several theories have evolved as to why CO₂ has an inhibitory effect on bacterial growth. One idea is that the CO₂ is dissociated into carbonic acid resulting in a slight drop in pH. Weak acids in their undissociated form are known to have antimicrobial effects (Statham, 1984). CO₂ is probably most effective by penetrating the cell and altering the intracellular pH rather than by external acidification which would be more likely to be buffered by the organism (Wolfe, 1980). pH is not the lone factor for the inhibitory effect of CO₂, however, as reductions in enzymatic activity necessary for microbial growth may also occur. Another theory is that CO₂ acts on the cell membrane thus redistributing lipids at the interface and altering the contact between the cell and its aqueous environment.
Daniels et al. (1985) also noted that CO₂ may have negative effects on various enzymatic and biochemical pathways, causing a stress on the cell, resulting in a slowing of the growth rate. Gram negative bacteria are especially sensitive to carbon dioxide, while lactic acid bacteria appear to be less affected. Young et al. (1988) recommended that a level of at least 25% CO₂ be used to inhibit microbial growth and higher levels be used when practical. However, in red meats extremely high levels of CO₂ may cause some discoloration.

**Oxygen**-- is used on red meats to maintain the desirable "cherry red" bloomed color of the oxymyoglobin pigment. It may also be used in low levels so that a completely anaerobic state does not exist in order to prevent the growth of toxin producing bacteria such as Clostridium botulinum.

Brody (1988) stated that at least 3% O₂ is necessary to prevent the growth of Clostridium botulinum.

**Nitrogen**-- is an inert gas and is used primarily as a filler and is said to have no effect on the color of food products or microbial growth (Young et al. 1988).

Other gases such as carbon monoxide, ozone, ethylene oxide, nitrous oxide, and others that have a bacteriostatic or bactericidal properties have
been evaluated in various experiments but these gases have not been considered practical for modified atmosphere packaging of fish due to either their regulatory status or toxic effects that they may exhibit (Wilhelm, 1982).

II. Important Properties of Catfish

There are a number of factors to be considered in applying MAP to prevent the spoilage and deterioration of fresh catfish.

Color

The color of fresh catfish should not be a limiting factor in applying modified atmosphere packaging techniques. Catfish have a very light colored flesh, apparently low in myoglobin concentration, which should be affected very little by high concentrations of carbon dioxide. Young et al. (1988) and others have reported that off colors can develop, especially with red meats which are high in myoglobin, when using elevated levels of CO₂ and low levels of O₂. This is due to the fact that meat color is largely determined by the oxidation state of the myoglobin pigment of the muscle.
**Odor**

Fresh chilled catfish has a very clean, barely detectable odor. The development of "fishy" or undesirable odors is caused primarily by an increase in numbers of bacterial spoilage organisms. Wheaton and Lawson (1985) reported that in fish and seafood products, *Pseudomonas* produces putrefactive and ammoniacal odors, *Achromabacter* results in a fruity odor that over time will turn ammoniacal, and lactic acid bacteria will produce stale and sour odors. Consumers generally use odor as an indicator for spoilage and usually do not consume products with off odors.

**Microbiology**

The flesh of fish or any muscle flesh of a live animal is essentially sterile and therefore most of the microbial contamination occurs post slaughter (Price and Schweigart, 1987; Jay, 1986). Tucker (1985) reported that the bacterial load on catfish is a function of initial contamination and multiplication. The predominant surface microflora of fresh catfish as reported by Byrd (1973) were found to be *Flavobacterium*, *Enterobacter*, *Escherichia*, coagulase negative *Staphylococcus*, and *Aeromonas*. When spoilage occurred the predominant bacteria were *Pseudomonas* species. No *Salmonella* were detected. Byrd (1973) also reported that the surface
analysis of dressed catfish surveyed at a processing plant had a mean level of bacteria of 3,190 per cm² total count, a coliform level of 9 per cm², and a fecal coliform level of 1 per 3 cm². An FDA bacteriological survey of fresh catfish at the retail level as reported by Andrews et al. (1977) resulted in a wide range of median values. Aerobic plate count (APC) values were $6.9 \times 10^3$ to $1.9 \times 10^8$ per g, total coliform most probable number (MPN) values were $<3$ to $2.4 \times 10^6$ per g and fecal coliform MPN values were $<3$ to $2.4 \times 10^3$ per g. *Salmonella* was isolated from 15 of 335 units collected from 41 processors. Andrews et al. (1977) reported that fish rarely, and catfish never, have been definitely incriminated in human outbreaks of salmonellosis, although a possibility for such an occurrence may exist.

**Lipid Oxidation-TBA**

According to Tucker (1985) the shelf-life of catfish is primarily dependant on lipid stability, however, fish may be stored frozen for up to one year and still be acceptable. Thiobarbituric acid (TBA) values reported ranged from 0.435 to 1.41 over 12 months storage at -18°C or below. Not much is known about oxidation rates in fresh refrigerated catfish, since microbial spoilage usually occurs before oxidation can
become a problem. Mustafa and Medeiros (1985) have shown that catfish are high in mono- and some poly- unsaturated fatty acids, which, according to Melton (1983), are susceptible to oxidation.

III. Processing Effects and Storage Characteristics

Temperature

Maintaining temperature and sanitation are the two most important factors for success with modified atmosphere packaging (Brody, 1988). Although maintaining a low temperature during storage may be mandatory to prevent the growth of dangerous toxin producing bacteria there are several other advantages. Refrigeration temperatures help to reduce most types of microbial growth, slow the rate of fat oxidation, and retard enzymatic activity. Low temperatures also increase the solubility of CO$_2$ in fluids (Wolfe, 1980; Statham 1984).

Microbiology

Christopher et al. (1980) reported that there were decreases in the percentages of Pseudomonas spp. and increases in the amount of lactobacilli in pork loins stored in CO$_2$ + N$_2$ or in vacuum packages compared to pork loins stored in air. They also reported that after 28 days
lactobacilli became the predominant microflora of the loins. Thomas et al. (1984) reported similar results with CO₂ and vacuum packaged chickens. Oberlender et al. (1983) showed a similar growth trend in swordfish but the change in microflora occurred sooner as *Lactobacillus* became a major part of the microflora by the 14th day of storage. Contrary to Christopher's report, Spahl et al. (1981) found that lactobacilli remained at low levels and that *Pseudomonas* growth was more significant, resulting in 25 - 60% of the total psychrotrophic flora.

**Hazards**

The reduction or elimination of oxygen from the package atmosphere may disturb the equilibrium of the microflora. Aerobic bacteria that normally flourish and cause spoilage may be inhibited allowing anaerobic bacteria which are normally suppressed to be at an atmospheric advantage and may produce dangerous toxins while not causing any noticeable organoleptic degradation.

Silliker and Wolfe (1980) stated that the use of elevated CO₂ atmospheres did not seem to increase the hazard of *C. Botulinum* Types A and B in fresh meat and poultry, however, in the case of fish, extra precautions should be made due to the possibility of *C. Botulinum* Type E.
Eyles (1986) noted that fresh fish have historically been low risk foods with regards to botulism primarily because of the activity by spoilage microflora. Eyles (1986) also indicated that some types of *C. botulinum* may produce toxin at refrigerated temperatures, however growth of these types at below 10°C is so slow that spoilage will occur before a detectable amount of toxin is produced. Additionally, normal cooking of raw fish will inactivate botulinum toxins. Since treatments such as modified atmosphere alter the spoilage microflora, the integrity of the cold chain must be maintained during storage as there is no hazard with respect to botulism if temperature is maintained at or below 3°C.

Inspection of fish and seafood products is not required by law. The FDA does not prohibit modified atmosphere packaging of fish, however, the National Marine Fisheries Service (NMFS) has raised concern over *Clostridium Botulinum*, Type E which can grow at temperatures exceeding 3°C. The NMFS has placed a moratorium on inspecting MAP fish which can only be lifted if the packer provides a detailed report of sanitation requirements, product holding temperatures, atmospheres, film permeability and shelf life studies. NMFS inspections are optional and are used on less than 15% of the seafood consumed in the U.S. (Anon., 1988b).
pH

According to Wang and Brown (1983) the pH of cooked crawfish tissue stored in modified atmospheres decreased over time of storage and was significantly lower than samples stored in air. Oberlender et al. (1983) reported that pH of swordfish stored in controlled atmospheres of CO₂ + O₂ remained about the same during a 22 day storage period, while pH of samples stored in air steadily increased with time.

Dip Solutions

Cormier and Léger (1987) indicated that the use of phosphate solutions on frozen Cod fillets resulted in significantly less thaw drip and a higher expressible moisture index. All the fillets that were dipped in the phosphate solutions picked up 2-3% of their total weight. Fillets that were dipped for 60s picked up significantly more weight than those dipped for 30s while those that were dipped for 120s picked up only slightly more than those dipped for 60s but not a beneficial amount.

Lipid Oxidation - TBA

The 2-thiobarbituric acid test has been widely used for measuring oxidative rancidity in fat containing foods, especially fish and meat.
products, with the distillation method being the most popular. Rhee (1978) recommended that for the distillation TBA test to add propyl gallate (PG) and ethylenediaminetetraacetic acid (EDTA), especially to fish, to minimize further lipid oxidation during the test.

Wang and Brown (1983) found that the TBA value of cooked crawfish stored in CO₂ atmospheres increased over time, but that the absorbance values remained quite low. They also demonstrated that a CO₂ atmosphere had little effect on lipid oxidation. Fey and Regenstein (1982) reported that red hake stored in modified atmospheres did not show measurable increases in TBA numbers. Brown et al. (1980) found that, for rockfish and salmon stored in different modified atmospheres, there was a general trend of increasing TBA values with no particular effect of atmospheres. They also noted that all TBA values were quite low and that lipid oxidation was not a major problem in these fish.

Drip

According to Statham (1984) elevated CO₂ levels resulted in the development of excessive amounts of drip loss which may be caused by a reduction of pH. Statham and Bremner (1985) found that drip loss of
trevella stored in CO$_2$ correlated with pH and concluded that there was a
decrease in water binding capacity of fish muscle as pH approaches the
isoelectric point. However, Parkin et al. (1981) observed no difference in
weight loss between rockfish fillets stored in air or 80% CO$_2$. The
problem of excess drip may be reduced by using an atmosphere of no
higher than 40% CO$_2$, the use of high concentrations of polyphosphates or
by placing absorbent pads in the packages (Statham, 1984).

**Antioxidants, Acidulants and Sequesterants**

Certain acid materials such as ascorbic acid and citric acid are used to
enhance the stability of vegetable oils. These compounds are ineffective
when used alone, but have a synergistic effect when used with fats
containing a phenolic antioxidant, and can chelate metals such as copper
and iron that catalyze the oxidation of fats (Price and Schweigart, 1987).
Wheaton and Lawson (1985) list several acidulants that may be used on fish
malic, fumaric, adipic, succinic, citric, propionic, sorbic, acetic and lactic
acids. These acids can be used to lower pH or act as antimicrobial agents
and thus reduce bacterial growth. Several may also function as
sequesterants or chelating agents.
IV. Marketing and Economics

Potential

Lioutas (1988) reported that higher prices have forced many food companies, growers, retailers, and distributors to look for more efficient methods to reduce the tremendous amount of fresh food spoilage and quality loss that occurs in the current food distribution chain. In the long run, MAP should allow food companies to be more efficient, and deliver higher quality products to the customer under stricter specifications and therefore be more profitable.

Cost Benefit Relationship

Several advantages of MAP are extended transit time, higher quality maintenance, inhibition of microbial growth, less waste and reduced economic loss. Some of the disadvantages are added cost to packaging, variable product requirements (if more than one type of product is being packaged), extra capital requirements for new equipment and employee training and the need for temperature monitoring throughout the storage period. Wolfe (1980) estimated the additional cost of the portion bag system to be approximately 2 - 5 cents per pound. MAP could be justified for more valuable products such as meat and fish, as this would be only a
small percentage of total price and could be easily absorbed as part of total marketing cost. Lower valued products such as produce and seasonal products present a less clear cut economic justification.

Requirements for Commercial Application

MAP cannot improve product quality, only maintain it, therefore starting with high quality raw products will enhance the marketability of a finished product. Lioutas (1988) noted that the quality of the raw ingredients and proper temperature control are the two most significant factors in attaining maximum shelflife using MAP. A high microbial load, poor hygiene or excessive temperature during processing may cut shelf life by as much as 50 - 70 %. Other factors important in producing a commercially successful MAP product include proper employee training and knowledge among distributors, retailers, and consumers of proper handling requirements for the product.
CHAPTER 3

MATERIALS AND METHODS

I. Sample Preparation

Raw Materials and Preparation

Live catfish, approximately 1 to 2 pounds live weight were purchased from a local catfish farmer and transported to the University of Tennessee Meats Lab, Knoxville, TN. The fish were held off feed in aerated holding tanks for approximately three days before slaughter.

Slaughtering was done in the University of Tennessee Meats Lab. Initially, the fish were electrically shocked to render them unconscious for humane slaughter and ease of handling. The fish were then skinned by hand, deheaded with a bandsaw, eviscerated, washed and chilled on ice. The spinous dorsal, pelvic, pectoral and adipose fins were removed, however, the caudal and anal fins were left intact. The fish were chilled in ice water for approximately one hour, then packed on ice and held overnight (approximately 15 hours) before being packaged according to the respective treatments.
Trials - Location

Two trials of the experiment were performed at two locations. For
the first trial the fish were transported on ice to, and packaged at, the
facilities of Cryovac Division, W.R. Grace Corp. Duncan, SC. Upon
completion of packaging the fish were immediately transported back to
The University of Tennessee Food Technology & Science Department for
storage. In the second trial the fish were packaged at the University of
Tennessee Food Technology Department using the same type of equipment
as for the first trial.

Dipping Procedure

The chilled fish were randomly separated into three groups of 30
fish. The first group served as the control (no dip) while the two
remaining groups were dipped into the following solutions for 60 seconds:

1. 10% Sodium Tripolyphosphate (STPP), (FMC, Philadelphia, PA)
2. 10% STPP + 0.25% Citric Acid (CA)
Packaging Procedure

Each group of 30 fish was divided into three subgroups of 10 fish, with each subgroup packaged in one of the following atmospheres:

1. Air (Control)
2. 100% CO₂
3. 70% CO₂, 5% O₂, 25% N (mixture)

Each fish was placed on a white styrofoam tray with an absorbent meat pad, and placed in a high barrier bag (Cryovac Division, W. R. Grace Corp., Duncan, SC). A Multivac packer was used to pull a vacuum on the bag (ca. 40 cm Hg) and then backflush the package with the appropriate atmosphere to a gas:fish ratio of approximately 2:1 (v:v). The bag was then heat sealed.

Storage

One half of each subgroup (5 fish) were stored at 1 ± 2 °C and the other half at 5 ± 2 °C under incandescent lighting for 0, 4, 8, 10, 12, and 16 days.
II. Analysis of Samples

Microbiological Analysis

A 2.5 cm x 4 cm foil template and a sterile dacron swab was used to swab a 10 cm$^2$ area from each fish selected for the specified sampling period. The tip of the dacron swab was broken off into a 10 ml aliquot of 0.1% peptone water (Difco Laboratories, Detroit, MI).

From the 10 ml aliquot, a Spiral Systems™ Plater, model C (Spiral Systems Instruments, Bethesda, MD) was used to perform aerobic plate counts (APC), anaerobic plate counts (AnPC), and lactic acid bacteria counts. Coliform counts were performed by pour plate method.

The following agar and incubation conditions were used to determine the CFU/cm$^2$ from each fish:

<table>
<thead>
<tr>
<th>Test</th>
<th>Agar</th>
<th>Method</th>
<th>Incubation Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>SMA</td>
<td>Spiral Plater</td>
<td>48 hrs @ 32°C, aerobic</td>
</tr>
<tr>
<td>AnPC</td>
<td>SMA</td>
<td>Spiral Plater</td>
<td>48 hrs @ 32°C, anaerobic</td>
</tr>
<tr>
<td>Lactic Acid</td>
<td>MRS</td>
<td>Spiral Plater</td>
<td>48 hrs @ 32°C</td>
</tr>
<tr>
<td>Coliform</td>
<td>VRB</td>
<td>Pour Plate</td>
<td>24 hrs @ 32°C</td>
</tr>
</tbody>
</table>

SMA - Standard Methods Agar
MRS - Specific for lactic acid bacteria
VRB - Violet Red Bile Agar

Anaerobic conditions for incubation were obtained by using an anaerobe jar under vacuum.
Surface pH was determined by using a Fisher Accumet® model 600 pH meter equipped with an Orion Combination gel-filled flat surface electrode model 91-35. The pH meter was calibrated to a final pH of 7.0. An average of four measurements was recorded for each fish.

Oxidation (TBA)

The modified steam distillate method of Rhee (1978) was used to determine malonaldehyde concentration which is directly related to oxidation in meat products.

Approximately 30 g of flesh was removed from each fish at the designated sampling period then frozen in liquid nitrogen and powdered in a Waring blender. The powdered sample was placed in a Whirl-Pak™ bag and stored at -28°C until analyzed. All of the samples from each sampling period were placed in a master pack and flushed with nitrogen before being placed in frozen storage.

For analysis, 10 g of the powdered sample was blended with 5 ml of 0.5% propyl gallate (PG), 5 ml of 0.5% sodium ethylenediaminetetraacetic acid (Na₂EDTA) and 40 ml of deionized H₂O for 2 min. Following
blending, 2.5 ml of 4N HCl was added. The homogenate was transferred to an 800ml Kjeldahl flask and the homogenizing jar rinsed with 47.5 ml of deionized H₂O which was added to the flask. Five drops of Antifoam B® antifoaming agent "Baker" grade (J.T. Baker Chemical Co., Phillipsburg, NJ) was also added to the flask. The samples were distilled until 50 ml of distillate was collected, within 5-15 minutes after the onset of boiling. To analyze for malonaldehyde concentration (MA), 5 ml of distillate and 5 ml of 2-thiobarbituric acid (TBA) were placed in a test tube and heated in a boiling water bath for 30 - 35 minutes. The test tube was allowed to cool for 10 minutes and the absorbance was measured at 532 nm using a Hitachi model 100-60 double beam spectrophotometer. MA concentration was determined using a standard curve and was expressed as mg MA/ kg fish.

A standard curve was developed by placing 5 ml of 10⁻⁴ M tetraethoxypropane (TEP), 2.5 ml of 4N HCl and 92.5 ml of deionized water in a kjeldahl flask and distilling to yield 10⁻⁵ M MA. Then 1, 2, 3, 4 and 5 ml of the10⁻⁵ M TEP distillate was pipetted into 5 test tubes. Deionized water was added to the tubes to bring the total volume of each tube to 5ml resulting producing concentrations of 1x10⁻⁵, 2x10⁻⁵, 3x10⁻⁵, 4x10⁻⁵ and 5x10⁻⁵ moles MA respectively. Five ml of 0.02M TBA was added to each tube; the tubes were heated in a boiling water bath for 30-35
minutes then allowed to cool for approximately 10 minutes. Absorbance of each solution was measured at 532 nm. Linear regression was used to derive a standard curve equation for absorbance versus MA concentration.

**Drip Loss**

Percent drip loss or percent shrinkage was determined by weighing each fish just prior to packaging and at the designated sampling period. Drip loss or shrinkage was calculated according to the following equation:

\[
\text{percent drip} = \left(\frac{\text{initial weight} - \text{final weight}}{\text{initial weight}}\right) \times 100.
\]

**Color and Odor**

Color and odor were subjectively evaluated by an untrained panelist at each sampling period. A separate score of 1 - 10 was given for each, where 1 = very poor and 10 = extremely good.

**III. Statistical Analysis**

The Statistical Analysis System, Cary, NC was used to calculate analysis of variance (ANOVA) with the following design. A randomized complete block of 2 trials (rep) x 5 storage periods (day) x 3 atmospheres (atm) x 3 dip solutions (dip) x 2 storage temperatures (temp), with all
possible combinations of two way interactions, was utilized to analyze log aerobic plate counts, log anaerobic plate counts, log lactic acid counts, coliform counts initial and final pH, TBA, drip, color and odor. The General Linear Models procedure was performed for all data, and the Student-Newman-Keuls test was used to separate the range of the means when the treatment was significant at $p \leq 0.05$. For significant interactions ($p \leq 0.05$) involving days of storage, atmosphere, dip, or temperature ANOVA was determined for individual treatments.
CHAPTER 4

RESULTS AND DISCUSSION

I. Effects of Atmosphere, Dip and Storage Temperature on Aerobic Plate Count

The summary of the analysis of variance (ANOVA) for aerobic plate count is presented in Table 1. Aerobic plate count (APC) was used as an indication of total microbial population and overall microbial quality. Days of storage, atmosphere, and storage temperature all had significant (p<0.05) effects on aerobic plate counts of catfish, however, dip had no effect. The interaction between day and atmosphere also was significant. Significant effects of trial (rep) as shown by ANOVA are not discussed because the fish were handled differently the first day resulting in differences in initial counts. Trial was included in the ANOVA to remove that variation from the error term.

The mean log count for the air atmosphere of 7.43 CFU/cm² was higher than the counts for the CO₂ atmosphere (100% CO₂) and the mixed atmosphere (70% CO₂, 5% O₂, 25% N) at 5.41 CFU/cm² and 5.30
Table 1. Analysis of variance for the effect of treatment on microbial growth.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>D.F.</th>
<th>LAPC</th>
<th>LANPC</th>
<th>LMRS</th>
<th>VRB</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAY</td>
<td>4</td>
<td>222.29**</td>
<td>281.04**</td>
<td>259.75**</td>
<td>639.22 N.S.</td>
</tr>
<tr>
<td>ATM</td>
<td>2</td>
<td>85.80**</td>
<td>102.72**</td>
<td>9.54**</td>
<td>288.76 N.S.</td>
</tr>
<tr>
<td>DIP</td>
<td>2</td>
<td>0.09 N.S.</td>
<td>1.01 N.S.</td>
<td>1.15 N.S.</td>
<td>76.47 N.S.</td>
</tr>
<tr>
<td>TEMP</td>
<td>1</td>
<td>18.24**</td>
<td>32.68**</td>
<td>11.69**</td>
<td>564.06**</td>
</tr>
<tr>
<td>DAY-ATM</td>
<td>8</td>
<td>28.18**</td>
<td>27.94**</td>
<td>9.09**</td>
<td>1089.75 N.S.</td>
</tr>
<tr>
<td>DAY-DIP</td>
<td>8</td>
<td>3.04 N.S.</td>
<td>5.27 N.S.</td>
<td>2.53 N.S.</td>
<td>251.38 N.S.</td>
</tr>
<tr>
<td>DAY-TEMP</td>
<td>3</td>
<td>1.86 N.S.</td>
<td>6.47*</td>
<td>4.15*</td>
<td>724.85*</td>
</tr>
<tr>
<td>ATM-DIP</td>
<td>4</td>
<td>1.47 N.S.</td>
<td>0.72 N.S.</td>
<td>0.55 N.S.</td>
<td>324.49 N.S.</td>
</tr>
<tr>
<td>ATM-TEMP</td>
<td>2</td>
<td>1.21 N.S.</td>
<td>0.75 N.S.</td>
<td>1.26 N.S.</td>
<td>317.54 N.S.</td>
</tr>
<tr>
<td>DIP-TEMP</td>
<td>2</td>
<td>0.92 N.S.</td>
<td>0.85 N.S.</td>
<td>0.23 N.S.</td>
<td>184.62 N.S.</td>
</tr>
<tr>
<td>REP</td>
<td>1</td>
<td>5.49**</td>
<td>0.61 N.S.</td>
<td>4.94**</td>
<td>65.34 N.S.</td>
</tr>
<tr>
<td>REP-DAY</td>
<td>3</td>
<td>27.97**</td>
<td>9.53**</td>
<td>7.61**</td>
<td>454.68 N.S.</td>
</tr>
<tr>
<td>REP-ATM</td>
<td>2</td>
<td>4.77*</td>
<td>7.56**</td>
<td>0.53 N.S.</td>
<td>181.43 N.S.</td>
</tr>
<tr>
<td>REP-DIP</td>
<td>2</td>
<td>0.17 N.S.</td>
<td>0.66 N.S.</td>
<td>0.80 N.S.</td>
<td>25.09 N.S.</td>
</tr>
<tr>
<td>REP-TEMP</td>
<td>1</td>
<td>7.05**</td>
<td>1.54 N.S.</td>
<td>6.73**</td>
<td>60.06 N.S.</td>
</tr>
<tr>
<td>ERROR</td>
<td></td>
<td>68.20 (107)</td>
<td>72.07 (106)</td>
<td>36.07 (102)</td>
<td>7523.82 (107)</td>
</tr>
</tbody>
</table>

** P< .01
*P< .05
N.S. No Significant Difference
( ) Indicates Degrees of Freedom

LAPC - log aerobic plate count
LANPC - log anaerobic plate count
LMRS - log lactic acid count
VRB - coliform count
CFU/cm² respectively. As shown in Figure 1, the counts on fish stored in air increased more rapidly than on fish stored in either of the other two atmospheres. The APC for air atmosphere fish peaked above log 7 and had reached spoilage by day 8. Spoilage occurs approximately at log 7 CFU/cm² and is indicated in Figure 1 by the dotted line. Microbial growth did not increase significantly after day 8. The APC’s on fish in CO₂ and mixed atmospheres were, on the average, 2 log counts lower than in the air atmosphere up to day 16. This indicated that the CO₂ was effective in reducing the rate of microbial growth. This agrees with Banks et al. (1980), who found that rockfish in CO₂ remained 2 logs lower than in air.

Comparing the time required to reach log 7 CFU/cm², catfish stored in air reached this point by day 8 whereas the fish stored in the CO₂ containing atmospheres required at least 16 days. Gray et al. (1983) reported similar results comparing ice-packed and MAP perch.

APC on fish stored at 1ºC remained one log lower than on fish stored at 5ºC (Fig. 1) until spoilage occurred. The fact that a difference was detected with a spread of only 4ºC agrees with Brody (1988) on the importance of maintaining low temperatures. In order for catfish to be put on retail display in a modified atmosphere package and attain maximum
Figure 1. Effects of atmosphere and temperature on aerobic plate count
shelf life there must be tight control of refrigeration temperatures. For instance where 32 - 40°F may be considered an adequate refrigeration temperature range for many products, a narrower range of approximately 32 - 35°F may be required for MAP fish.

While there was little difference between the growth curves of the mixed atmosphere and the CO₂ atmosphere at 5°C, at 1°C the growth curve of the mixed atmosphere was about one half log lower than the CO₂ atmosphere. The mixture of CO₂ and O₂ gases may be more effective in controlling growth when CO₂ is more soluble which occurs at the lower temperature. The significant interactions between days and atmosphere may be explained by rapid acceleration of growth and then leveling off of the growth curve for the fish in the air atmosphere, while the fish stored in the elevated CO₂ atmospheres exhibited more linear growth curves.

The dipping of the fish into a phosphate or phosphate-citric acid solution did not significantly effect the mean log counts. The fish that were not dipped had a mean log count of 6.09 CFU/cm². The phosphate and phosphate + citric acid dipped fish had mean APC of 6.04 CFU/cm² and 6.02 CFU/cm², respectively.
II. Effects of Atmosphere, Dip, and Storage Temperatures on Anaerobic Plate Count

The analysis of variance for anaerobic plate counts (AnPC) is presented in Table 1. Similar to the aerobic plate counts, days of storage, atmosphere, and storage temperature all had significant (p<0.05) effects on anaerobic plate count while dip had no effect. Significant interactions occurred between day and atmosphere, and between day and temperature.

The mean log count of 7.38 CFU/cm$^2$ for fish stored in air atmosphere was higher than the mean log count of 5.18 CFU/cm$^2$ for the CO$_2$ atmosphere and 5.19 CFU/cm$^2$ for the mixture. Figure 2 shows growth curves of the anaerobic plate counts. In comparison, the aerobic and anaerobic growth curves showed nearly identical mean log counts for each treatment over days of storage. This indicated that a large portion of the microflora were probably facultative anaerobes. It was also noted that colonies on SMA plates of the same sample had similar morphology whether they were incubated in aerobic or anaerobic conditions.

One difference between the aerobic count and anaerobic counts was a decrease in the AnPC from 0 to 4 days at 10°C in both CO$_2$ atmospheres. No lag phase was observed on the aerobic plate count.
Figure 2. Effects of atmosphere and temperature on anaerobic plate count
III. Effects of Atmosphere, Dip, and Storage Temperature on Lactic Acid Plate Count

The analysis of variance of lactic acid plate count on MRS agar is presented in Table 1. Days of storage, atmosphere, and storage temperature had significant (p<0.05) effects on lactic acid count, dip had no effect. There were significant interactions between day and atmosphere, and between day and temperature.

The growth curves for the lactic acid count are shown in Figure 3. Lactic acid counts increased steadily over days of storage and peaked above log 6 CFU/cm² at day 16. Catfish stored in the air atmosphere were at least one log higher than those stored in CO₂ or the gas mixture through day 8. By day 12, similar counts were observed for all atmospheres. In comparison to the APC, lactic acid bacteria comprised a larger portion of the total microflora of catfish stored in CO₂ and the gas mixture than those stored in air, especially at 1°C. It appears that lactic acid bacteria became the predominant species as early as day 8. Huffman (1974) reported that lactic acid counts on pork chops stored in CO₂ increased more rapidly than on chops stored in air. Mokhele et al. (1983) also found that lactic acid bacteria were predominant on rockfish fillets stored under CO₂. A number
Figure 3. Effects of atmosphere and temperature on lactic acid count
of other researchers have reported similar occurrences. Mokhele et al. (1983) also reports that this phenomenon may be beneficial since lactic acid bacteria have been shown to have antagonistic effects on some types of harmful bacteria.

For catfish stored in air, lactic acid counts at 1°C remained at least one log lower than at 5°C until day 12. Catfish stored in CO2 and the gas mixture had similar counts at 1°C and 5°C through day 4. From day 4 through day 16, the fish at 1°C had lactic acid counts one half to one log lower.

IV. Effects of Atmosphere, Dip, and Storage Temperature on Coliform Count

The analysis of variance for coliform count from VRB agar is presented in Table 1. There was no significant difference (p>0.05) in coliform count among atmosphere, dip, or temperature; only days of storage had a significant effect.

Coliform counts were generally low at less than 15 CFU/cm² for all treatments and from day 12 had steadily declined throughout the storage period. Coliform counts are usually performed as an indicator of sanitary
quality, and these counts indicated the fish were processed under sanitary conditions.

V. Effects of Atmosphere, Dip, and Storage Temperature on pH

The analysis of variance for pH is presented in Table 2. Days of storage, and dip solution had significant (p<0.05) effects on final pH of catfish, while atmosphere and storage temperature appeared to have no effect.

Initial pH for all samples was approximately 6.7 prior to treatment. The effects of the dip solutions on the pH of catfish flesh is shown in Figure 4. Samples that were not dipped (ND) as a control remained at the initial pH of 6.7 ± 0.1 for the entire storage period. Catfish that were dipped in the phosphate or phosphate + citric acid solution showed a slight reduction of pH to approximately 6.5 on day 4. By day 8 the pH of these samples had increased to just below the initial pH and remained just slightly below the ND samples for the duration of the storage period. pH for all treatments ranged from 6.5 to 6.8. There are conflicting reports by other researchers on the effect of dip solutions on the pH of fish flesh. In this study there was little difference in pH observed throughout the storage
Table 2. Analysis of variance for the effect of treatment on chemical and organoleptic scores.

<table>
<thead>
<tr>
<th>D.F.</th>
<th>pH</th>
<th>TBA</th>
<th>DRIP</th>
<th>COLOR</th>
<th>ODOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAY</td>
<td>6</td>
<td>0.66 *</td>
<td>37.38 **</td>
<td>92.85 **</td>
<td>172.85 **</td>
</tr>
<tr>
<td>ATM</td>
<td>2</td>
<td>0.08 N.S.</td>
<td>12.32 **</td>
<td>32.81 **</td>
<td>96.44 **</td>
</tr>
<tr>
<td>DIP</td>
<td>2</td>
<td>0.35 *</td>
<td>4.42 **</td>
<td>28.38 **</td>
<td>33.49 **</td>
</tr>
<tr>
<td>TEMP</td>
<td>1</td>
<td>0.05 *</td>
<td>0.33 N.S.</td>
<td>0.04 N.S.</td>
<td>9.50 N.S.</td>
</tr>
<tr>
<td>DAY-ATM</td>
<td>8</td>
<td>0.30 N.S.</td>
<td>6.79 *</td>
<td>14.33 N.S.</td>
<td>132.65 **</td>
</tr>
<tr>
<td>DAY-DIP</td>
<td>8</td>
<td>0.14 N.S.</td>
<td>1.97 N.S.</td>
<td>32.24 N.S.</td>
<td>41.95 *</td>
</tr>
<tr>
<td>DAY-TEMP3</td>
<td>4</td>
<td>0.46 *</td>
<td>1.27 N.S.</td>
<td>18.30 *</td>
<td>11.52 N.S.</td>
</tr>
<tr>
<td>ATM-DIP</td>
<td>4</td>
<td>0.16 N.S.</td>
<td>3.21 N.S.</td>
<td>17.31 N.S.</td>
<td>2.61 N.S.</td>
</tr>
<tr>
<td>ATM-TEMP2</td>
<td>4</td>
<td>0.02 N.S.</td>
<td>0.06 N.S.</td>
<td>3.16 N.S.</td>
<td>0.18 N.S.</td>
</tr>
<tr>
<td>DIP-TEMP2</td>
<td>4</td>
<td>0.07 N.S.</td>
<td>2.79 *</td>
<td>1.57 N.S.</td>
<td>7.09 N.S.</td>
</tr>
<tr>
<td>REP</td>
<td>1</td>
<td>2.15 **</td>
<td>0.09 N.S.</td>
<td>339.44 **</td>
<td>0.01 N.S.</td>
</tr>
<tr>
<td>REP-DAY</td>
<td>4</td>
<td>0.49 *</td>
<td>0.62 N.S.</td>
<td>6.79 N.S.</td>
<td>6.79 N.S.</td>
</tr>
<tr>
<td>REP-ATM</td>
<td>2</td>
<td>0.13 N.S.</td>
<td>2.85 *</td>
<td>78.09 **</td>
<td>10.26 N.S.</td>
</tr>
<tr>
<td>REP-DIP</td>
<td>2</td>
<td>0.15 N.S.</td>
<td>0.23 N.S.</td>
<td>14.71 N.S.</td>
<td>6.68 N.S.</td>
</tr>
<tr>
<td>REP-TEMP1</td>
<td>2</td>
<td>0.00 N.S.</td>
<td>0.30 N.S.</td>
<td>8.46 N.S.</td>
<td>14.06 *</td>
</tr>
</tbody>
</table>

| ERROR | 5.26 (122) | 41.13 (115) | 241.71 (107) | 263.29 (107) | 266.86 (107) |

** P<.01
*P<.05
N.S. No Significant Difference
( ) Indicates degrees of freedom
Figure 4. Effect of dip solution on pH
period. Cormier and Léger (1987) reported that cod fillets dipped in polyphosphate solution were 0.4 pH units higher than the non-dipped control samples. In contrast, Statham et al. (1985) found that dipping morwong fillets in polyphosphate lowered the pH by 0.4 units.

There was no significant (p>0.05) effect of storage atmosphere on the pH of catfish observed in this study. Scott et al. (1984) reported similar results with similar pH values for snapper fillets stored in air and CO2 atmospheres. Some controversy exist over the ability of CO2 to reduce pH and subsequently reduce microbial growth. Parkin et al. (1981) reported that the pH of rockfish fillets stored in CO2 remained low while samples stored in air consistently increased in pH until spoilage occurred, and Oberlender et al. (1983) reported a similar trend for pH of swordfish steaks. In both cases it was presumed that the drop in pH resulted from the dissolution of CO2 as carbonic acid. Conversely, Huffman (1975) found that beef samples stored in CO2 and a gas mixture of 25% CO2 had identical pH's at 23 and 27 days of storage, yet had significant differences in microbial plate counts and thus concluded that depression of microbial growth in CO2 stored samples is not the result of lower pH. In this study it
was found that CO₂ was effective in reducing microbial growth, however, little difference in pH was observed among treatments. In all noted cases CO₂ has proven effective in reducing microbial growth yet there is inconclusive evidence to indicate this is caused by lowering pH.

VI. Effects of Atmosphere, Dip, and Storage Temperature on TBA Values

The analysis of variance for TBA (thiobarbituric acid) values is presented in Table 2. Days of storage, atmosphere, and dip solution had significant (p<0.05) effects on TBA values of fresh catfish, while storage temperature had no effect. Significant interactions were observed between day and atmosphere, and between dip and temperature.

The mean TBA values or TBA number, expressed as mg malonaldehyde (MA)/kg sample, was 0.42 at day 0 and had a general trend of steadily increasing with days of storage for all treatments with a mean TBA number of 2.00 at day 16. Catfish stored in the air atmosphere exhibited the lowest mean TBA value of 1.08, while catfish in the CO₂ and gas mixture had significantly higher mean TBA numbers of 1.66 and 1.82 respectively.
Comparing the effectiveness of dip solution, surprisingly, the phosphate dip, with a mean TBA value of 1.74 was significantly higher than either the no dip (control) at 1.35 or the phosphate + citric acid dip at 1.48. While the phosphate dip alone was not effective in reducing the rate of oxidation, when used in combination with citric acid significantly lower TBA values were achieved. This was possibly due to the ability of citric acid to chelate heavy metals which are prooxidants. The most successful combination of atmosphere and dip was the air atmosphere with no dip which had a mean TBA value of 0.96 while the gas mixture with the phosphate dip produced the least desirable results with a mean TBA value of 2.29.

Yu and Sinnhuber (1958) reported that canned and frozen fish of good quality had TBA numbers (mg MA/kg sample) of less than 3; while poorer quality products had TBA numbers ranging from 4 - 27. Although ANOVA for this study showed significant differences among treatments, the mean TBA numbers were quite low, almost all below 3, as microbial spoilage occurred before oxidative rancidity could become a problem. These results agree with Brown et al. (1980) who found low TBA values for MAP rockfish with no particular effect due to atmospheres. Fey and Regenstein (1982) found that, although a potassium sorbate as an
VII. Effect of Atmosphere, Dip and Storage Temperature on Drip Loss

The analysis of variance for drip loss is presented in Table 2. Days of storage, atmosphere, and dip solution had significant (p<0.05) effects on drip loss or shrinkage from catfish, while storage temperature had no effect. A significant interaction was observed between days of storage and storage temperature.

The largest amount of drip loss occurred in the very early stages of the storage period. At day 4 the mean drip loss was 5.29% and increased at a slow steady rate for the remainder of the storage period reaching a mean of 7.04% at day 16. Catfish stored in the gas mixture atmosphere had the lowest mean drip loss at 5.80%. The fish stored in the air atmosphere was only slightly higher at 6.09%, while the fish stored in 100% CO₂ had significantly greater drip loss at 6.86%. Excessive drip loss has been reported previously for fish packaged in atmospheres containing high
levels of CO₂ (Statham et al., 1985). The use of polyphosphate dip solutions to reduce drip loss did not achieve the desired results. The non dipped (control) fish had a mean drip loss of 5.59% while the fish dipped in the phosphate and phosphate + citric acid solutions had significantly higher mean drip loss at 6.62% and 6.54% respectively. This was not the expected result because phosphates have been reported to increase the water holding capacity in meats. At 1°C, drip loss changed very little from day 4 (5.79%) to day 16 (6.67%). Meanwhile, at 5°C drip loss increased more rapidly over time from 4.78% at day 4 to 7.41% at day 16. This is possibly caused by water purging more readily at the higher temperature.

**VIII. Effects of Atmosphere, Dip, and Storage Temperature on Color**

Subjective evaluation of color / appearance was performed at each sampling period and each fish received a score of 1 - 10. Scores between 5 and 6 were considered borderline acceptable, while scores below 5 were unacceptable. The analysis of variance for the color / appearance of catfish is presented in Table 2. Days of storage, atmosphere, and dip had significant (p<0.05) effects on color, while temperature had no effect.
Significant interactions were observed between days of storage and atmosphere, between days of storage and dip.

The mean color score was 8.7 on day 0 and steadily decreased over time for all treatments with a mean score of 5.7 on day 16. Catfish stored in the air atmosphere had the lowest mean color score at 5.7 while the catfish stored in the CO\textsubscript{2} and gas mixtures received significantly higher color scores of 7.9 and 8.0 respectively. The samples stored in the air atmosphere received lower color scores earlier in the storage period most likely because of the rapid acceleration of microbial growth in the air atmosphere. As samples became spoiled, slime was present on the surface of many samples.

Catfish that were not dipped (control) received significantly higher mean color scores (7.8) than those that were dipped in phosphate or phosphate + citric acid (6.8 and 6.5 respectively). The dip solutions appeared to have a slight bleaching effect on some of the catfish samples. This was most noticeable in areas along and around the belly flaps which is very undesirable since this would be unappealing to the consumer when displayed on the retail shelf.
IX. Effects of Atmosphere, Dip, and Storage Temperature on Odor

Subjective evaluation of odor was performed at each sampling period, and each fish received a score of 1 - 10. Scores below 5 were considered unacceptable. The analysis of variance for the odor of catfish is presented in Table 2. Days of storage, atmosphere, and storage temperature had significant (p<0.05) effects on odor, while dip solution had no effect. Significant interactions were observed between atmosphere and days of storage.

The mean odor score was 8.8 at day 0, and similar to the color score, decreased over time for all treatments 4.8 at day 16. Catfish stored in the air atmosphere had the lowest odor scores, with a mean value of 4.6, while the fish stored in CO₂ and the gas mixture received higher mean odor scores of 8.2 and 7.9, respectively. These samples stored in CO₂ and the gas mixture received higher scores most probably due to inhibition or delay of growth of spoilage type organisms.

At 1°C catfish had a mean odor score of 7.5 while at 5°C the odor score was significantly lower at 6.2. The effect of temperature on odor
scores, like atmosphere, was probably related to the growth of spoilage microorganisms.
CHAPTER 5

SUMMARY

The object of this study was to determine the effects of modified atmospheres containing high levels of CO$_2$ and the effects of phosphate + citric acid dip on the shelf life of fresh catfish. Market size catfish ranging from 1 - 2 pounds live weight were used for this experiment. The fish were slaughtered, dipped in one of three treatments (phosphate, phosphate + citric acid, no dip), packaged in one of three atmospheres (70% CO$_2$ + 5% O$_2$ + 25% N, 100% CO$_2$, Air) and were stored at 1°C or 5°C. After packaging the catfish were evaluated for aerobic plate count, anaerobic plate count, lactic acid count, coliform count, pH, oxidation, drip loss, color, and odor at 0, 4, 8, 12 and 16 days.

These data indicated that catfish stored in CO$_2$ or the gas mixture had significantly (p<0.05) lower aerobic, anaerobic, and lactic acid plate counts than those stored in air. These plate counts were also significantly lower for catfish stored at 1°C than at 5°C. The lowest counts were observed for fish stored in the gas mixture at 1°C. TBA values, although quite low,
tended to increase steadily with storage time. However, microbial spoilage occurred before oxidation could become a problem. The catfish stored in the gas mixture with no dip had the lowest amount of drip loss. At 1°C drip loss occurred less rapidly than at 5°C. Color and odor scores for catfish stored in air deteriorated more rapidly than in CO₂ or the gas mixture. Color and odor scores were also lower for catfish stored at 5°C.

The best results were obtained using the gas mixture with no dip at 1°C. This treatment had the lowest microbial counts. This atmosphere-temperature combination (containing 5% O₂) should remove any risks of *Clostridium botulinum* Type E growth. This treatment also resulted in high color and odor scores which is important for consumer acceptance through extended storage. It was concluded that the shelf life of fresh catfish could be extended from one week to two weeks by using modified atmospheres with elevated levels of CO₂ if low temperatures (< 3°C) were maintained throughout the storage period.

Implementing this technology would increase costs due to equipment requirements and the education of retailers and consumers concerning the packaging handling requirements. However, the benefits obtained, such as longer shelf life, increased market exposure and the ability to transport the product into new market areas, should be cost effective.
REFERENCES
REFERENCES


53


Yu, T.C. and Sinnhuber, R.O. 1957. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. Food Technol. 11(2):104.
VITA

Brian Preston Moore was born in Opelika, Alabama, on May 23, 1963 to Donald P. and Lataine T. Moore. He attended Huguley Elementary School in Lanett, Alabama and attended high school at Valley High School in Fairfax, Alabama and graduated on May 26, 1981. The following September he entered Southern Union State Junior College located in Wadley, Alabama. In September 1983 he transferred to Auburn University located in Auburn, Alabama. On August 29, 1984 he received a Associate in Science degree from Southern Union State Junior College, and on June 7, 1985 he received a Bachelor of Science degree in Agricultural Business and Economics from Auburn University.

After graduation from Auburn University he completed a year of post graduate study at Auburn University and concurrently enrolled in the pilot training program in the Auburn University Aviation Department. He received an FAA Private Pilot Certificate on January 6, 1987.

In January 1987 he entered The University of Tennessee Graduate School. From January 1987 to February 1989 he has been working to complete the requirements for the Master of Science degree in Food Technology and Science.
The author is a member of Alpha Gamma Rho Fraternity, Alpha Zeta honor society, and the Institute of Food Technologists.