THE EFFECTS OF NATURAL PRESERVATIVES ON THE SENSORY PROPERTIES AND SHELF LIFE OF CHANNEL CATFISH *Ictalurus punctatus*

by

Cassandra T. Eyong

A THESIS

Submitted in partial fulfillment of the requirements
for the degree of Masters of Science in the
Food Science Graduate Program of
Delaware State University

DOVER, DELAWARE
MAY 2018

This thesis is approved by the following members of the Final Oral Review Committee:

Dr. Jung-Lim Lee, Committee Chairperson, Department of Human Ecology, Delaware State University.

Dr. Bettina G. Taylor, Committee member, Department of Human Ecology, Delaware State University.

Dr. Bertrand Hankoua, Committee member, Department of Agriculture and Natural Resource, Delaware State University.

Dr. Gulnihal Ozbay, External Committee member, Department of Agriculture and Natural Resources, Delaware State University.

DEDICATION

This thesis is dedicated to my family. My father, whose undying love has kept me going through the most tough times in this journey, I love you Daddy. My two younger brothers and three younger sisters who have always been there for me. My cousin Dr. Julius Enoru who has always believed in me even at times when I did not belief in myself. My former boss Dr. Fonderson George who took it upon himself to make sure I never lacked anything and supported me throughout this journey. The Besongs who gave me all the love I could ever need away from home. I would not be where I am today if it wasn't for you all. You have supported, guided, motivated and believed that I can be very successful in life.

ACKNOWLEDGEMENT

I would like to give special thanks to my advisor, Dr. Jung-lim Lee and committee members: Dr. Bettina G. Taylor, Dr. Bertrand Hankoua, and Dr. Gulnihal Ozbay for providing their continuous support, assistance, and advice. I would like to thank Dr. Samuel Besong for giving me this opportunity and for guiding me through this journey. I would also like to thank my fellow graduate colleagues who have been there encouraging me in this journey. I would like to thank all faculty, staff, and students in Human Ecology and Agriculture and Natural Resources and the DSU Aquaculture Research and Demonstration Facility staff who helped me with my research, participated in my sensory evaluation sessions at very short notice and have given valuable advice.

THE EFFECTS OF NATURAL PRESERVATIVES ON THE SENSORY PROPERTIES AND SHELF LIFE OF CHANNEL CATFISH *Ictalurus punctatus*

Cassandra T. Eyong

Advisor: Dr. Jung- Lim Lee

ABSTRACT

This study explored the effect of three natural preservatives, lemon juice, vinegar and grapefruit seed extract (GFS), on reducing foodborne illnesses caused by food pathogens and spoilage bacteria on channel catfish (*Ictalurus punctatus)*. The minimum inhibitory concentrations (MIC) of these preservatives were determined using six specific spoilage bacteria species previously isolated from channel catfish. The zone of inhibition test confirmed the antimicrobial activity of these natural products. Microbial changes of catfish fillets were evaluated after marinating the catfish in the preservatives during storage at 4°C. Bacteria enumeration was done every other day for 27 days. Consumer groups performed sensory evaluation on baked marinated catfish evaluate acceptability with respect to color (appearance), aroma, flavor, and texture on days 1 and day 5 (after storage at 4°C and -20°C). The application of GFS, lemon juice, and vinegar extended shelf life by 7 days, 9 days, or 27 days, respectively compared to the control. The acceptable bacteria threshold value was defined as10^6 Log CFU/g. The sensory analysis of the catfish samples resulted in no significant differences (p>0.05) between the treatments and the control on day 1 and day 5 (4°C). Storage at -20°C resulted in a significant color difference (p=0.018) in catfish samples. Synergy was found between lemon and vinegar at 2%*2% using the checkerboard method of fractional inhibitory concentration (FIC). Protein analysis on SDS-PAGE gel showed protein denaturation. In conclusion, natural preservatives extended the shelf life of channel catfish with vinegar extending the most by 27days and retained the sensory properties.

Table of Contents

LISTS OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

GFS	Grapefruit sees extract
MIC	Minimum inhibitory concentration
FIC	Fractional inhibitory concentration.

CHAPTER I: INTRODUCTION

Channel catfish is one of the species of catfish including blue catfish and white catfish which are lean and highly nutritious. It is the most abundant and harvested species in North America. In the United States, Alabama, Arkansas, Louisiana and Mississippi account for over 95% of the nation's catfish production (Antonia da Silva et al.,2008). Due to its high nutrient content and the health benefits, it is in high demand all over the United States. This has resulted to the cultivation of channel catfish in aquacultures to meet the increasing demand. Associated with this increase in production is the significant economic loss due to the perishable nature of catfish caused by microbial spoilage.

Microbial activity is responsible for the spoilage of 25% of all post-harvest food with fish being most affected on a worldwide basis. (Hickey et al.,2013). Microbial spoilage limits the shelf life of fish and fish products beginning immediately after death due to the absence of normal body regulatory mechanisms. Bacteria enter postmortem fish through body cavities, intestines, and vascular tissue, resulting in tissue degradation and consequently changes in sensory properties of fish. In addition, microbial activity can harm consumer health and negatively impacts overall economic and commercial viability of fish and fish related products (Manju et al,2007). Typical spoilage organisms of fish include *Shewanella*, *Pseudomonas*, *Photobacterium*, *Aeromonas* and/or *Enterobacteriaceae* (Fuentes-Amaya et al.,2015) Of which Aeromonas spp. have been the predominant bacteria isolated from catfish fillets. In addition, some spoilage bacteria like *Pseudomonas* spp. strive at refrigeration temperature.

Many preservation techniques have been employed to suppress the growth of psychotropic bacteria able to survive in very cold environments and other spoilage bacteria. Chilling at refrigeration temperatures (4C°-6°C) and super chilling at temperatures below freezing have accomplished growth reduction in some bacteria but psychrotrophs are not affected and the sensory changes result from using these methods. The successful combination of salts such as phosphates and modified atmosphere have been used to increase shelf life and quality of fish (Masniyom et al.,2005) but, according to Sherman and Mehta (2009) phosphorus and potassium additives have implications on dialysis patients.

Fresh seafood has a short shelf life. In most seafood products, microorganisms are the major cause of spoilage. High bacteria count indicates storage abuse thus increasing the risk of human pathogens and consequent illness. Specific spoilage organisms contribute to the unpleasant flavors associated with seafood spoilage (Gram & Dalgaard,2002). Changes in the sensory properties of a food can result in rejection by consumers.

With consumers increasingly avoiding foods treated with preservatives of chemical origin, natural alternatives are needed to achieve high degree of safety with respect to foodborne pathogenic microorganisms (Sengun & Karapinar, 2004). Organic acids such as acetic acid, ascorbic acid, malic acid, propionic acid, succinic acid, tartaric acid, lactic acid, and citric acid, found naturally in fruits or fermented foods, have been extensively researched as antimicrobial agents.

Lemon, vinegar and grapefruit seed extract have shown success in inhibiting spoilage bacteria and foodborne pathogens in food in previous studies. These natural products inhibited the growth of food pathogens and spoilage bacteria thus extending the shelf life of these foods, while retaining their sensory properties.

Problem Statement

There is a great concern about the growing number of illnesses and death caused by foodborne pathogens and spoilage bacteria. These undesirable yet capable of control situations can be prevented. International bodies that monitor world food resources acknowledge that one of the most feasible options for meeting these needs is to eradicate post-harvest loss due in part to the short shelf life of fresh seafood. With the increase in sensitization of chemical preservatives being potential carcinogens and Geno toxins, consumer and food production units have shifted their preference towards natural antimicrobials. Applying natural antimicrobials like lemon, vinegar, grapefruit seed extract as used in this study will improve food safety, increase shelf life and improve food security.

In this study, fresh lemon juice with measured acidity, natural malt vinegar, and grapefruit seed extract were used to extend the shelf life by inhibiting growth of spoilage and pathogenic bacteria while retaining the sensory properties of Channel catfish (*Ictalurus punctatus)*. The antimicrobial activity of the natural products was studied on six bacteria including *Pseudomonas aeruginosa* which is also known for its antimicrobial resistance causing deathly infections

I.A. OBJECTIVES

The primary goal of this research was to study the effects of natural preservatives (lemon juice, vinegar, and grapefruit seed extract) on the shelf life and sensory properties of channel catfish.

SPECIFIC OBJECTIVES

1. Identification of antimicrobial activity of natural preservatives using minimum inhibitory concentration tests.

2. Extend the shelf life of channel catfish *Ictalurus punctatus* by inhibiting spoilage bacteria and food pathogens.
3. Evaluation of the sensory properties of channel catfish upon application of the natural preservatives.
4. Identification of synergy in the antimicrobial properties between natural preservatives; vinegar and lemon juice using the Fractional Inhibition Concentration analysis.
5. Determination of the protein stability upon application of the natural preservatives of channel catfish *Ictalurus punctatus*.

I.B. RESEARCH HYPOTHESES

H_o1: There will be no extension of the shelf life of Channel catfish *Ictalurus punctatus*

H_A1: There will be an extension of the shelf life of channel catfish *Ictalurus punctatus*

H_o2: The sensory properties of channel catfish *Ictalurus punctatus* will be altered by the preservatives.

H_A2: The sensory properties of channel catfish *Ictalurus punctatus* will not be altered by the preservative

CHAPTER II: LITERATURE REVIEW

Foods have been subject to microbial invasion for centuries. It is to be noted that different foods harbor different microbes depending on the nutrient availability. Just as human's microbes needs micronutrients for survival and proliferation, food high in protein content tend to attract most microbes because of the organic content. Meat, poultry and poultry products and most fresh foods tend to harbor human pathogenic and food spoilage microorganisms (Skrivanova et al., 2011). Meat contamination usually causes fatal diseases to the elderly, immunocompromised and children (Marshall & Bal'a, 2001). Contamination of foods usually results from pre- and postharvest handling (Vijayakumar & Wolf-Hall, 2002). Bacterial counts in meat usually range from 10^2 - 10^5CFU/g but only around 10% can initiate growth (Nychas et al., 1988). The growth of microorganisms follows a growth pattern of lag, log, stationary and death phases. The adaptation of microorganisms to changes in external conditions such as temperature and nutrient availability occurs in the lag. Logarithmic growth occurs when the cells have adapted to their environment and optimized their metabolism. The acceptable limit for bacterial numbers in food is 10^6CFU/g (Fernández et al., 2009). Above this number, there is existences of off odors and rancidity at 10^7 CFU/g (Raftari, 2009) which are signs of spoilage. Slime production is also noticeable when bacteria numbers reach 10^8CFU/g (Gill, 1982).

Food is considered spoiled when it is unaccepted by humans for consumption (Lingham et al., 2012). Food spoilage is slime, off odors, physical damage or insect damage (Huis in't Veld, 1996). Economic loss of foods is huge but cannot be predicted exactly because spoilage varies on the conditions under which food is subjected to. These conditions can be physical or chemical conditions. It is estimated that one-fourth of the world food supply is lost through microbial activity alone. Twenty-five percentage of all postharvest food is spoiled due to microbial activity with fish having the largest proportion of lost food in the world (Hickey et al., 2013). Psychotropic microorganisms, yeast, and molds are the main cause of food spoilage in developed countries and in less developed countries as well while rodents and other animals are also of concern (Huis in't Veld, 1996). These microorganisms do not only spoil foods but also cause deadly infections to humans upon consumption (Sengun at al., 2005). The first signs of spoilage include the formation of sweet-smelling esters followed by rancid sulfur compounds (Dainty et al., 1983). Rancid odors are produced by bacterial decomposition of proteins and amino acids, produce indoles, methanethiol, dimethyl disulfide and ammonia volatiles (Dainty, 1996). Rancidity is not caused by microbial breakdown of food but by oxidation of unsaturated lipids. Heat, light and pro-oxidant compounds influence this reaction (Price & Schweigert, 1978).

The microorganisms that colonize a food depend on the product characteristics; how it is stored and processed. The parameters affecting the growth of microbes in foods can be characterized into four groups: intrinsic parameters, extrinsic parameters, modes of processing, and preservation, and implicit parameters (Huis in't Veld, 1996). Intrinsic parameters are the physical, chemical and structural properties of food which include water activity, redox potential, nutrient availability and natural antimicrobial agents. Extrinsic parameters include factors such as food storage, temperature, and atmospheric composition. Modes of processing and preservation are the physical and chemical treatments that alter product characteristic and the microflora that is associated with the food product. Implicit parameters are synergistic or antagonistic influences of the parameters, resulting from the development of a synergistic or antagonistic effect from a microorganism on the microbial activity of other microorganisms that are present in the food product.

Aeromonas hydrophila is a facultatively anaerobic, gram-negative, rod-shaped bacterium currently classified in the family Vibrionaceae (Palumbo,1986). Early studies revealed the ability of this bacterium to grow at 1 to 5°C (Palumbo, 1986). This bacterium, found in fresh or brackish water, can grow rapidly on produce at refrigeration temperature (Beuchat, 1995). *A. hydrophila* is associated with diseases mainly found in freshwater fish and amphibians, because these live in aquatic environments. Fish develop ulcers, tail rot, fin rot, and hemorrhagic septicemia which causes lesions that lead to scale shedding, hemorrhages in the gills and anal area, ulcers, exophthalmia, and abdominal swelling.

Escherichia coli, gram-negative, rod shaped coliform bacteria, facultatively anaerobic and non-spore forming, commonly inhabit the lower intestines of warm-blooded organisms. They have an optimal growth temperature of 37°C. Most species of E. coli are harmless, but some serotypes produce toxins resulting in food poisoning and are occasionally responsible for food recalls. Food is mainly contaminated by feces through improper handling during pre-harvest and post-harvest.

II.A. Channel catfish

The channel catfish (*Ictalurus punctatus*), the official fish of Kansas, Missouri, Iowa, Nebraska, and Tennessee, is North America's most abundant catfish species. A healthy food option, it enjoys wide consumer acceptance (Lingham et al., 2012). This led to a rapid expansion of aquaculture in the United States for accessibility to consumers all-round the country. Consuming fish at least one to two times per week is important for human health

(Lingham et al., 2012), increasing the demand for high-quality fresh seafood. With the freshness of fish deteriorating rapidly, the shelf life of fresh fish ranges from seven to eleven days with traditional packaging. Modern technologies are needed to extend this shelf life (Fernandez et al., 2009). The aquaculture of freshwater fish depends on water sources. Lakes, rivers, streams, reservoirs, and surface run-off can contaminate ponds where fish are commercially raised for human consumption (Suhalim et al., 2007). One of the sources of contamination are pathogenic microbes whose survival is affected by temperature fluctuations and other environmental factors (Suhalim et al., 2007).

The quality deterioration of fish after harvest follows a four-phase pattern (Huss, 1995; Huss & Gram, 1996). The initial microbial degradation is caused by aerobes that cause the release of water and carbon dioxide from carbohydrates. As the surface becomes covered with microbes due to the desirable atmosphere created by the aerobes, slime build-up then creates favorable conditions for the growth of anaerobic microbes. Due to the increase of microbial habitation on fish tissue and subsequent oxygen depletion, trimethylamine oxide is broken down to trimethylamine, causing a fishy odor (Lingham et al., 2012) Off-flavors also arise from the breakdown of sulfur-containing amino acids.

Pathogenic microorganisms can contaminate fish during processing. Improper handling of fish during pre-harvest and post-harvest activities also results in microbial contamination. (Vijayakumar and Wolf-Hall, 2002). The skin and gut of catfish are common sources of microbial contamination (Kim and Marshall, 2002) as bacteria spread from the skin and gut to contaminate the entire processing environment (Bal'a et al., 1999). Fish secrete mucus containing lysozymes on their body surfaces as a natural defense mechanism when alive (Hickey et al., 2013). This mucus inhibits bacterial thriving on the fish skin (Spear and

Mirasalimi, 1992). External conditions such as temperature abuse, high bacterial count and other health condition of the fish can affect the antibacterial properties of the fish mucus (Suhalim et al., 2007). After death, these regulatory mechanisms are lost (Hickey et al., 2013)

II.B. Bacteria Isolated from Channel catfish (*Ictalurus punctatus*)

Pseudomonas is a gram-negative rod-shaped bacterium that is the most common spoilage organisms, especially in aerobically stored foods with high water activity and neutral pH, such as red meat (Dainty and Mackey, 1992; Dainty,1996; Borch et al., 1996; Gallo et al., 1988; Regez et al., 1988), fish (Gram and Huss, 1990), poultry (Lahellec and Colin, 1979) and dairy products (Walker and Stringer, 1990; Craven and Macauly, 1992a; Craven and Macauly, 1992b; Craven and Macauly, 1992c). A small fraction of the initial microflora of foods (Huis in't Veld, 1996) they are widely distributed in the environment, contaminate food from many sources, and can use a wide range of substrate for growth. In foods of animal origin, non-protein nitrogen (NPN) is metabolized and lipases and proteases release fatty acids and amino acids producing off flavors, off-odors and rancidity. Extracellular slime and pigmented growth become visible in the last stages of deterioration (Dainty & Mackey, 1992; Dainty, 1996).

Aeromonas and *Shewanella* are gram-negative rod-shaped bacteria that grow rapidly at chilled temperatures, causing spoilage of chilled red meat, cured meat, poultry, fish, shellfish, and dairy products through the NPN metabolism (Huis in't Veld, 1996). Enzymes from *Aeromonas,*

Shewanella, and *Serratia* cause spoilage resulting in off-odors, off-flavors and slime (Walker & Stringer, 1990).

II.C. Popular Techniques to Inhibit Bacterial growth

The reduction of microbial populations on food products has health and economic implications. Various strategies have been employed such as super chilling, freezing, smoking, heating, sterilization and the use of natural preservatives.

Super chilling is one of the techniques that helps inhibit most autolytic and microbial reactions and thereby extend the shelf life and quality of fish (Fernandez et al., 2009). It consists of several types of cooling systems such as the use of slurry ice or ice flakes (-4°C to 0°C) to subzero temperatures during storage (-2°C) (Fernandez et al., 2009). It is usually coupled with modified atmosphere packaging (MAP) which is the replacement of packaged air with different, fixed gas mixture with carbon dioxide being the most important gas used because of its bacteriostatic and fungistatic properties (Feranandez et al., 2009). Carbon dioxide only specifically targets *Shewanella* and *Pseudomonas* species (Fernandez et al., 2009; Hansen et al., 2007; Farber ,1991; Mendez and Goncalves, 2008; Sivertsvik et al., 2002) causing carbon dioxide tolerant microbes to dominate the microflora. Carbon dioxide dissolves in meat and fish changes the pH

(Fernandez et al.,2009) in addition to package shrinking and deformation (Gill,1988; Zhao et al., 1995). A disadvantage of MAP is that it increases the package volume thus increasing transportation cost (Farber, 1991). Vacuum packaging uses less volume but is less effective than MAP (Hansen et al., 2007). Another disadvantage is the loss in the water holding capacity of fish but this fall back can be counteracted by adding phosphate compounds to fishery products to improve the functionality by reducing drip loss (Masniyom et al., 2005).

Freezing influences fish quality causing changes in shelf life and sensory quality as well as physicochemical changes in lipids and proteins (Kolakowska, 1976). Quality deterioration is seen during freezing and frozen storage due to the osmotic removal of water, denaturation of protein and mechanical damage. Freezing of fish muscle protein changes the solubility of protein fractions (Connell, 1962), water holding capacity (Paredi, 1996), the activity of proteolytic enzymes (Dixon & Webb, 1971) and the texture and sensory quality (Simeonidou, 1997). The severity of quality change of frozen food greatly depends on how the food was handled before and during frozen storage.

Smoking has been used as one of the techniques to improve the shelf life of fish by improving the microbial safety (Antonia da Silva et al., 2008). This method is widely used in developing countries to preserve up to 70% of total caught fish by a process where volatiles from the thermal combustion of wood penetrate fish flesh (Antonia da Silva et al., 2008). In industrialized countries, smoking is primarily a tool to enhance flavor and texture of fish. Efiuvwevwere and Ajiboye (1996) stated "Smoking usually extends the shelf life of fish due to the reduced moisture content and the effects of an imparted phenolic compound." High heat results

in microbial destruction.

Hurdle technology is the deliberate combination of existing or novel techniques to establish a series of preservative factors to improve the microbial stability and the sensory quality of food as well as their nutritional and economic properties (Mani -Lopez et al., 2011). Sous vide packaging is a method that was developed involves the hurdle effect (Jang et al. 2006). It involves vacuum packaging raw meat or partially cooked food, pasteurizing in hot water, cooling followed by chilled storage (Creed & Reeve, 1998). According to Leistner and

Gould (2002), this hurdle effect approves the storage stability of food products. This method has a better sensory quality to foods compared to the labor-intensive conventional method (Creed, 1998; Werlein, 1998). In a study by Manju et al. (2007), vacuum packaging with sodium acetate was found to delay the spoilage of pearl spot at refrigerated temperatures, therefore, extending the shelf life.

In microbiology, sterilization refers to the complete destruction or elimination of all viable organisms in or on a substance being sterilized (Todar, 2009). The procedures involve heat, radiation, chemicals or removal of cells physically by filtration. Heat is the most important and widely used. Examples of heat sterilization include incineration, boiling and autoclaving. Ethylene oxide is commonly used for chemical sterilization. It prevents microbial reproduction by reacting with amino acids, proteins, and DNA. Gamma-rays interact with electrons of atomic constituents; therefore, breaking chemical bonds and electron beam radiation are like gamma- rays in that alters chemical and molecular bonds. Irradiation destroys microorganisms using ultraviolet, X-rays and gamma irradiation. Pasteurization involves mild heat to reduce microorganisms in a food product.

II.D. Antimicrobial Agents

Antimicrobials are substances that exert bacteriostatic and/or bactericidal effects on microorganisms. They could be chemical/ synthetic in nature or from natural origin. Bacteriostatic refers to inhibiting bacterial cell growth while bactericidal refers to killing bacteria. Curing with chemical preservatives or salting with sodium chloride have been strategies used to promote shelf life of fish (Antonia da Silva et al. ,2008). Sodium benzoate (Efiuvwevwere and Ajiboye, 1996), lactic acid (Fernandez et al. 1998), sorbic acid (Sofos, 2000), sodium lactate (William et al., 1995) are common food preservatives used as antibacterial and antifungal agents on catfish.

Synthetic antimicrobials have successfully used their chemical properties such as their ionic strength, pH, and alcohol content to inhibit and kill microorganisms thus, extending the shelf life of food. However, theses chemical/synthetic preservatives have negative effects on the consumer. Sulfites, nitrates (converted to nitrous acid), benzoates, and sorbates have been suspected to cause headaches, palpitations, allergies, urticaria and even cancers (Sharmar, 2015).

Nuclear radiation is another effective means of preservation as it doesn't make food radioactive but cause changes in the texture and color of food (Sharma, 2015).

Organic acids were approved by the Food Safety and Inspection Service of the United States Department of Agriculture (Skrivanova, 2011). Theron and Lues (2007) stated "the use of organic acids is a promising method of meat decontamination because processing, sanitizing practices and Hazard Analysis and Critical Control Points (HACCP) programs in the meat industry are often insufficient for preventing the presence or inhibiting the growth of pathogens." HACCP is a management system that identifies and controls hazards of food products from production to consumption of the finished products. Antioxidants such as ascorbic acid and rosemary slow down lipid oxidation (Antonia da Silva et al., 2008).

The pH of a substance is it hydrogen ion concentration or a measure of the acidity or alkalinity of the substance. It is the most important chemical factor that influences bacteria growth. Bacteria growth thrives at neutral pH (Jay et al., 2005) thus reducing or increasing the pH limits growth. Malicki et al. (2004) explained that the bactericidal action of organic acids decreases the pH of bacteria cells. Organic acids such as tartaric, citric, lactic, malic, propionic, and acetic acids have been used for years for decontamination of bacteria on beef, pork, and poultry (Mani-Lopez et al., 2011). Organic acids that are used to inhibit foodborne pathogens and spoilage bacteria in meat are applied by spraying and dipping (Dincer & Baysal, 2004). In a study conducted by Bradley et al. (2011), the addition of citric acid and acetic acid each reduced the growth of Enterobacteriaceae.

II.E. Vinegar

Vinegar is an acidic liquid that is made from the fermentation of alcoholic beverages. The principal component of vinegar is acetic acid meanwhile other components include organic acids, volatile compounds and other fermentation products also play a role in the organoleptic and microbiological properties of vinegar (Ozturk et al. 2015). Acetic acid is a monocarboxylic weak acid, which is the major organic acid found in vinegar. It is generally regarded as safe with a pungent odor (Lingham et al., 2012) for general purpose and miscellaneous usage. According to Malicki (2004), organic acids are considered weak acids meaning the antimicrobial effect of organic acids is mainly caused by its undissociated forms. They passively diffuse through the bacteria cell wall and internalizing into neutral pH dissociating into anions and protons. Release of the protons causes the internal pH to decrease which exert inhibitory effects on the bacteria (Ricke, 2003). Substrate transport mechanisms are inhibited due to the release of proton ions causing the internal pH to decrease to disrupt the proton motive force (Russel, 1991; Cherrington et al., 1990).

Previous studies have shown that vinegar, as well as other organic acids exhibit antimicrobial properties. The total acidity of vinegar is expressed as acetic acid which is considerably responsible for antimicrobial activity against bacteria and yeast (Ozturk et al., 2015). Vinegar has been used to suppress anthracnose rot in tomatoes (Tzortzakis, 2010) and eliminating *Salmonella typhimurium* in carrots (Sengun & Karapinar, 2004). It is used in salad dressings to increase flavor alone or as a mixture with other natural flavorings and provide a harsh environment for foodborne pathogens such as *Salmonella spp.* and *E. coli* (Lingham et al., 2012). In the United States, salad dressings maintain a good safety record and are widely used (Smittle, 2000). Acetic acid was also found to be effective against *Botrytis* and *Penicillium* molds in post-harvest decay of apples by fumigation (Vijakakumar &Wolf-Hall, 2002). The antimicrobial activity of the different organic materials depends on the presence of the fermentation products in addition to the number of hydrogen ions present. Vijayakumar and Wolf-Hall (2002) showed that apple cider vinegar with pH of 5.1 produced a stronger bactericidal effect than white vinegar with pH of 4.1. However, excessive consumption of apple cider vinegar has been said to cause mineral loss such as potassium, increase bone softening in patients with osteoporosis and gastroparesis in diabetics (www.healthydietbase.com).

II.F. Grapefruit Seed Extract

Grapefruit seed extract (GFS), also known as citrus seed extract, is a liquid extract derived from the seeds, pulp, and white membrane of the grapefruit. Laboratory preparation of GFS is obtained by grinding the seeds and juiceless pulp prior to mixing it with glycerin. It is rich in nutrients and phytochemicals, and is a major source of plant antioxidants, one of which is hesperidin, a natural immune system stimulator. GFS contains vitamin sterols, tocopherols, citric acid, limonoids and trace minerals.

In agriculture, GFS has been used as an antimicrobial agent to kill bacteria and fungus, fight mold growth, kill parasites in animal feed, preserve food, and disinfect water. According to Corbo et al. (2008), GFS was the most successful active natural preservative in slowing the growth of specific fish spoilage bacteria. *Coliforms* were reduced on alfalfa by 1-2 Log CFU/g while

Salmonella and *Vibrio* were reduced by up to 1.5 Log CFU/g using 100ppm citricidal as treatment (Juneja et al., 2005). Although GFS has this strong antimicrobial activity, some concerns exist because of the drug-nutrient interaction between numerous pharmaceuticals with grapefruit. GFS interrupts the body's normal regulation of drugs absorption, thereby making increasing the effect or side effects of drugs. This is due to the phytochemicals in GFS (Cristóbal-Luna et al., 2018). The furanocoumarins that block cytochrome P450 3A4 (CYP3A4) enzymes, enzymes that break down many drugs for excretion in the urine. According to the University of Illinois Hospital & Health Science System, "The most commonly identified mechanism is irreversible inhibition of intestinal cytochrome (CYP) P450 3A4 enzymes by furanocoumarins, a group of constituents contained in grapefruit. The CYP3A4 enzymes metabolize the furanocoumarins to reactive intermediates which irreversibly inactivate the enzymes. This increases the bioavailability of medications metabolized by CYP3A4 and the likelihood of increased drug exposure and adverse effects" (https://pharmacy.uic.edu/sites/default/files/Jan_Feb2013_0.pdf). Therefore, patients need to be made aware when GFS is used as an antimicrobial.

II.G. Lemon

Lemon is an important medicinal plant from the family Rutaceae, cultivated mainly for its alkaloids, which have anticancer and antibacterial activities. These chemical compositions of lemons in the crude form have shown significant difference clinically against bacterial strains (AlRousan et al., 2018). Citrus flavonoids have a wide biological activity against bacteria, cancers, fungi, diabetes and viruses (Burt, 2004; Sohn et al., 2004).

Flavonoids from plant antimicrobials can function as antioxidants or free radical scavengers and have the capacity to modulate enzymatic activities thus inhibiting cell proliferation (Duthie & Crozier, 2000). Flavonoids are generally present in glycosylated forms with the glucose moiety playing a key role in the bioavailability of the compounds. Extracts from the peel, leaves, flowers and the bitter oranges have been used to minimize central nervous system disorders (Pultrini et al., 2006). The peel of citrus fruits is a rich source of flavonoids glycosides, coumarins, β and γ-sitosterol, glycosides and volatile oils (Shahnah et al., 2007). The fibers of citrus fruits contain bioactive compounds such as polyphenols which have a strong antimicrobial effect. The antimicrobial properties of these peels and juices are directly related to its composition. The bactericidal effects of lemon are expressed as the percent concentration of citric acid present. Sengun and Karapinar (2005) showed that 4.5%,4.2% and 3.3% citric acid v/v were very effective against *Yersinia enterocolitica* in carrots. There was a 2 LogCFU/g reduction at 0 minute. In 2004, they had shown a similar effectiveness of lemon on *Salmonella typhimurium* at 0 minute.

These information on the effect of natural products on spoilage and pathogenic bacteria helped in carrying out our objective in extending the shelf life and organoleptic properties of catfish.

CHAPTER III: MATERIALS AND METHODS

III. A. Materials

Natural malt vinegar: Natural malt vinegar was used in this study. It was purchased from a retail store (Food Lion, Dover, DE). It contains 5% acetic acid by volume. This vinegar product was chosen because of its natural origin from malt.

Lemon juice: Organic lemon fruits were purchased from a local grocery store. The organic and non-genetically modified were chosen because of natural nature of the produce lemon.

Grapefruit seed extract: The unfiltered formula of grapefruit seed extract was purchased from a retail store (Vitamin Shoppe in Dover, DE). This product contains 100% grapefruit seed extract. **Food spoilage microorganisms:** The food spoilage microorganisms in Table 1 were selected because they had been identified on catfish fillets.

Table 1. Test bacteria used for sampling.

Organism	Type	Origin	Characteristics
Aeromonas hydrophila	Gram negative	ATCC	Food spoilage
Aeromonas jandaei	Gram negative	Wild	Food pathogen
Pseudomonas aeruginosa	Gram negative	ATCC	Opportunistic pathogen
Pseudomonas lurida	Gram negative	Wild	Food spoilage
Serratia grimesii	Gram negative	Wild	Food spoilage
Shewanella baltica	Gram negative	Wild	Food spoilage

* ATCC = American Type Culture Collection

Fish Sample: Channel catfish (*Ictalurus punctatus*) was collected from the Delaware State University Aquaculture Research and Demonstration Facility. The fish were filleted by the pond manager under aseptic conditions and the fillets were transported to the laboratory on ice slurry for microbial analyses.

III.B. Method

III.B.1. Zone of inhibition.

I measured the clear zones stipulating antimicrobial activity of the preservatives on the bacteria as listed on Table 1. Each bacterium was inoculated in Tryptic Soy Broth (TSB) for 16-18 hours prior to test to ensure fresh bacteria were tested. The optical density of the bacteria was obtained by measuring on a Genesys 10S UV-VIS Spectrophotometer (Thermo Scientific, Madison, WI). TSB was used as blank to fix the optical density to 0.5 at 600nm. Each bacterium was spread on Tryptic

Soy Agar plates. A 100% of each preservative was used for this test and TSB as a negative control. The 20 µl of the different treatments were added to the wells. Each plate was incubated at 28°C for 24 hours. The zone of inhibition was measured by measuring the diameter of the clear regions around the wells using a ruler.

III.B.2. Minimum Inhibitory Concentration (MIC)

This procedure was adopted from Lingham et al. (2012). The MIC test was done to determine the lowest inhibitory concentration of each of the preservatives. These MIC's was then applied to the fish fillet to determine the shelf life against the control. The preservatives were diluted in Tryptic Soy Broth (TSB) (Carolina Biological Supply, Burlington, NC, USA) v/v by a range of 0.5% to

10%. Bacteria, as shown in Table 1, were inoculated in TSB and incubated for 18 hours. The optical densities were fixed to 0.05. The inoculum was the added to the different dilutions of preservative (v/v) and incubated for 24-48 hours. The MIC was measured by checking for turbidity.

III.B.3 Fractional Inhibitory Concentration (FIC)

The procedure was used to determine the synergy between lemon and vinegar. The checkerboard method to evaluate synergy was used and dilutions were made for both lemon and vinegar in the ranges 0,2,4,6,8,10 and 12%. Vinegar was placed on the x-axis while lemon on the y-axis. The bacteria in the table where used in this analysis. Different volumes of lemon and vinegar were added to the wells according to their percent concentrations and a standard number of bacteria cells $5*10^5$ CFU/g were added into the wells. The plates were then incubated at 27°C for 24 hours to check for bacteria growth or turbidity.

III.B.4. Bacterial Enumeration.

The procedure for determining the shelf life of catfish (*Ictalurus punctatus*) was adopted and modified from Lingham et al. (2012). Catfish fillets were used for isolation every other day for 30 days. Catfish tissue diced in approximately 2 cm cubes for the first day and 20 g from the second to the last day were added into stomacher bags as follows: control, vinegar, grapefruit seed extract and lemon The MIC of the preservatives were used to determine the shelf life of catfish fillet (*Ictalurus punctatus*). Each preservative was added into appropriate stomacher bags. The stomacher bags including the control were stored at 4°C for 30 minutes to marinate the fillets in the preservatives. The excess preservative was drained out, the bags were kept in the refrigerator at 4°C until sampling days. The 80-ml saline water at room temperature was poured into each stomacher bag on each sampling day, the sample were homogenized at 2 speeds for 40seconds (Stomacher 3500; Seward Inc., Bohemia, NY, USA). The homogenate was collected into two falcon tubes and was centrifuged in a swing bucket. Preventing to collect any precipitate, 200µl of the supernatant was collected for spread plating. Ten-fold serial dilutions were performed using TSB, spread plated on TSA plates and incubated for 48 hours at 27°C. Total bacteria were enumerated and recorded. This procedure was done for each preservative and control every other day for 27 days. A bacterial growth curve was drawn to show when the samples reach the spoilage point and stationary phase of bacterial growth.

III.B.5. Sensory Evaluation.

Fresh catfish fillets were purchased from a retail local store in Dover (Redner's, Dover, DE). Sampling was done twice; at zero-day and after five days. The sampling on the fifth day was divided into two groups; fillets stored at 4°C and -20°C. Fillets were placed in zip lock bags in the following order: control, lemon, vinegar, and grapefruit seed extract (Lingham et al., 2012). This order was followed for all the sampling groups. The control had no treatments added to it. Into each zip lock bag, treatment was added. The bags were kept in the refrigerator at 4°C for 30 minutes to marinate in the treatments. Excess treatments were drained and were backed at 350°F for 15 minutes. Ten panelists consisting of students and staffs from the Delaware State University participated in the sensory evaluation. Each panelist received a sensory evaluation sheet including a brief survey, the five samples and distilled water to cleanse their pallet after each sample evaluation. Affective testing of appearance (color), aroma, flavor, and texture were the attributes evaluated using the Hedonic seven-point scale; 1=Totally unacceptable and 7=perfectly acceptable.

III.C. Statistical Analysis

All sensory evaluation data were analyzed using the statistical model SPSS 25.0 (IBM SPSS, Armonk, NY, USA). A rating was the independent variable. The analysis was based on the Hedonic seven-point scale of attributes including appearance, aroma, flavor and texture per sample (control, lemon, vinegar, and grapefruit seed extract) according to (Lingham et al.,2012).

A confident interval of 95 % and an alpha level of 0.05 were used for the statistical analysis. Values of $p < 0.05$ were significant.

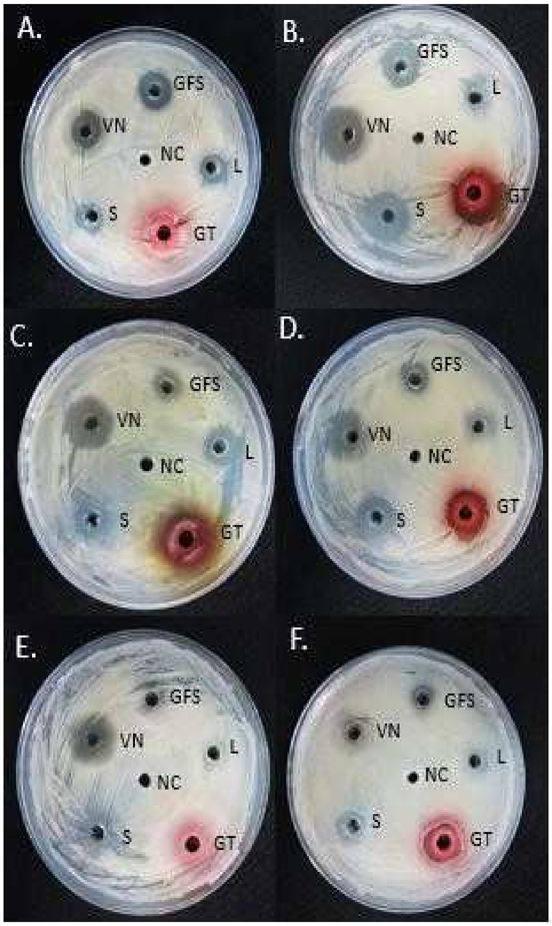
III.D. Protein Evaluation

Fish protein analysis was done using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Catfish fillets were purchased from a retail store (Redner's, Dover, DE). Sampling was done twice at Day 1 and Day 5. Three fish samples and one control were analyzed. The fish pieces were kept in the refrigerator to marinate for all the treatments along with the control. The treated fish samples were placed in micro centrifuge tubes. The incubated samples in Laemmli sample buffer were boiled on a heat block and were centrifuged to pellet debris. Supernatant for each sample was collected and used for SDS-PAGE.

CHAPTER IV: RESULTS.

IV.A. *Zone of Inhibition* and *Minimum Inhibitory Concentration*

The antimicrobial activity of the treated catfish was evaluated on the foodborne microbes as shown in Figure 1. Almost all the microbes were susceptible and showed clear zones ranging from 0.8 to 1.86 cm. *Serratia grimesii* showed very little clear zones for lemon and none for GFS but was susceptible to vinegar with a clear zone of 1.60 cm. Overall, vinegar showed the highest antimicrobial activity with a mean value of 1.48 (lemon-1.12 and GFS-0.97) compared to the other treatments with 1.86 cm inhibition zone followed by GFS; 1.53cm. (Figure 1 and Table 3). A statistical analysis of 95% confidence interval for paired sample T-test showed no significant difference between GFS-Lemon (p=0.452), GFS-Vinegar(p=0.104) but there was a significant difference between vinegar-lemon(p=0.016) as can be seen in table 3. The lowest effective working concentration of the treatments was determined by using a range from 0.5 to 10% v/v for each natural preservative. Vinegar had a minimum inhibitory concentration of 4%, lemon 5% and grapefruit seed extract (GFS) 7%, respectively. These MIC's were effective for *Aeromonas hydrophila* (ATCC), *Aeromonas jandaei*, *Pseudomonas aeruginosa* (ATCC), *Pseudomonas lurida*, *Shewanella baltica*. It is important to note that *Serratia grimesii* showed a little more turbidity than the other bacteria with the MIC's above except for vinegar.



A . *Aeromonas* hydrophila ATCC 7966
B . *Aeromonas* jandaei P60
C . *Pseudomonas* aeruginosa ATCC 10145
D . *Pseudomonas* lurida D19
E. *Serratia* grimesii D28
F. *Shewanella* baltica D10
*NC negative control, GFS grapefruit seed (33%), L lemon (6.75% acidity), GT green tea (50 mg), S salt (50%), V vinegar (5% acidity).

Fig 1: Zone of Inhibition of 100% natural preservatives on six bacteria species.

Table 2: Zone of inhibition of natural preservatives on test strains

Test strains	Inhibition in cm		
	GFS	V	L
Aeromonas hydrophila ATCC 7966	1.26±0.12	1.43±0.12	1.13±0.26
Aeromonas jandaei P60	1.53±0.29	1.86±0.23	1.33±0.29
Pseudomonas aeruginosa ATCC 10145	0.9±0.39	1.76±0.28	1.35±0.07
Pseudomonas lurida D19	1.06±0.26	1.30±0.17	1.20±0.34
Serratia grimesii D28	0	1.60±0.14	0.87±0.29
Shewanella baltica D10	1.1±0.34	0.95±0.35	0.85±0.35

GFS- grapefruit seed extract
V- vinegar
L- lemon

Mean value was obtained from two independent trials. ± STDV.

IV.B. ***Bacterial Enumeration and Shelf Life Determination***.

Domestic catfish fillets without and with treatments of 5% lemon, 4% vinegar, or 7% GFS were stored at 4ºC for 27 days. Figure 2 illustrates changes in total bacteria enumerated every two days. The number of bacteria decreased slightly less than the control for lemon, vinegar, and GFS for day 1 after marinating for 30 minutes by about 0.5log CFU/g. As the days progressed, the bacteria growth pattern for the control gradually increased and crossed the threshold count of 6 LogCFU/g on day 5. There was a lag phase for lemon, vinegar and grapefruit seed extract after which there was exponential growth for GFS and lemon (Figure 2). The growth curve of GFS crossed the threshold value on day 12 while lemon crossed on day 14. Vinegar maintained a fairly plateau curve from day 9 to day 27 below the threshold count. Bacterial counts in the control, lemon and GFS treated samples gradually increased to an endpoint of approximately 9 LogCFU/g at day 27.

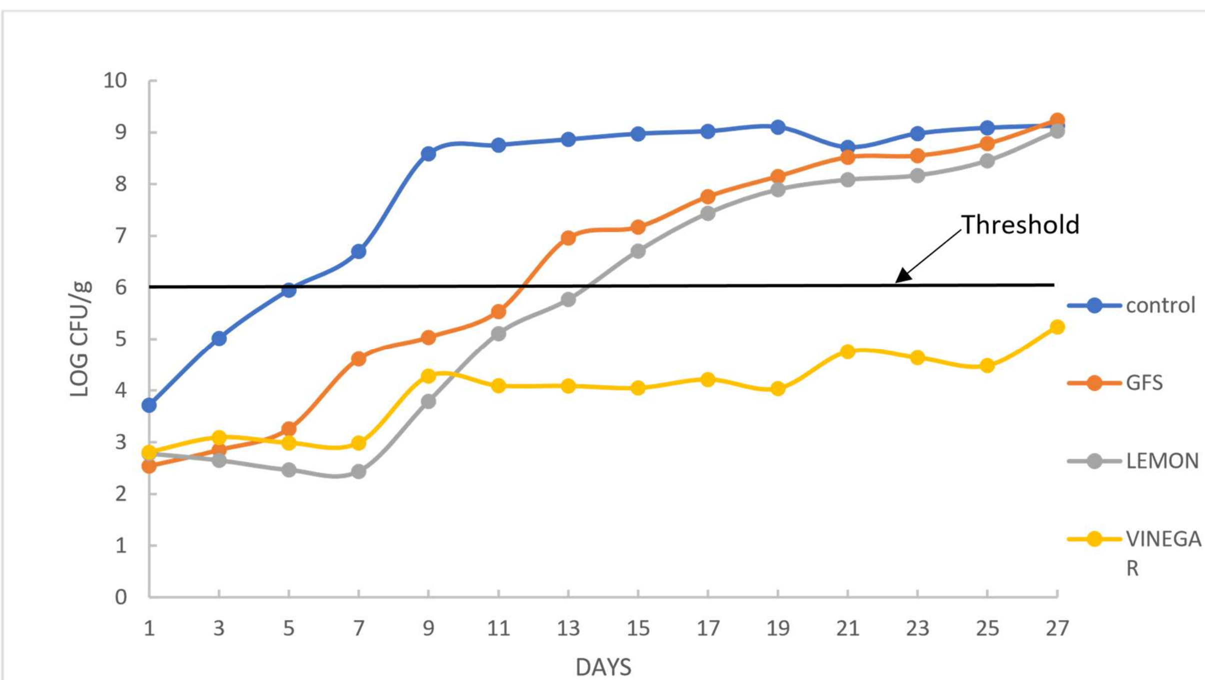


Fig 2: Bacteria Enumeration curve

IV.C. *Sensory Evaluation*

Ten panelists evaluated four sensory attributes in four samples using the seven-point Hedonic scale. The attribute rating frequencies for each sample were identified (Fig 15-18) and the rating average calculated (Fig 3-14). Among the samples analyzed were control, lemon 5%, vinegar 4% and grapefruit seed extract 7%. Evaluation of catfish fillet was done twice at three different sampling conditions; before storage (day 1), after five days storage at 4 °C, and after five days storage at -20 degrees. The overall least liked attributes for all treatments on day 1 and day 5 (4°C) were aroma and flavor. However, we notice that at day 5 after storage at -20°C, the least liked for all treatments were flavor and texture implying the freezing temperature had an effect on these attributes.

For sampling condition at day 1, the panelists preferred the aroma of vinegar (4.7), and the texture of lemon (5.4). For sampling condition at day 5 after storage at 4°C, the panelists preferred the color of grapefruit seed extract (4.8), the aroma of control (4.7), and the flavor (4.7) and texture (5.4) of lemon, respectively. For sampling condition at day 5, after storage at -20°C, the panelists had high preference for vinegar over the other samples for all the attributes color (4.8), aroma (4.7), flavor (4.7) and texture (5.1), respectively.

The statistical analysis using one-way ANOVA, showed no significant difference ($p > 0.05$) in the attributes for all samples at day 1 (before refrigeration) and day 5 at 4°C storage conditions. However, there was a significant difference ($p=0.018$) in the color after storage at -20°C on day 5 for all samples. Multiple Comparison Post Hoc test for color showed a significant difference between control and lemon ($p=0.031$), grapefruit seed extract and lemon ($p=0.004$), lemon and vinegar ($p=0.002$).

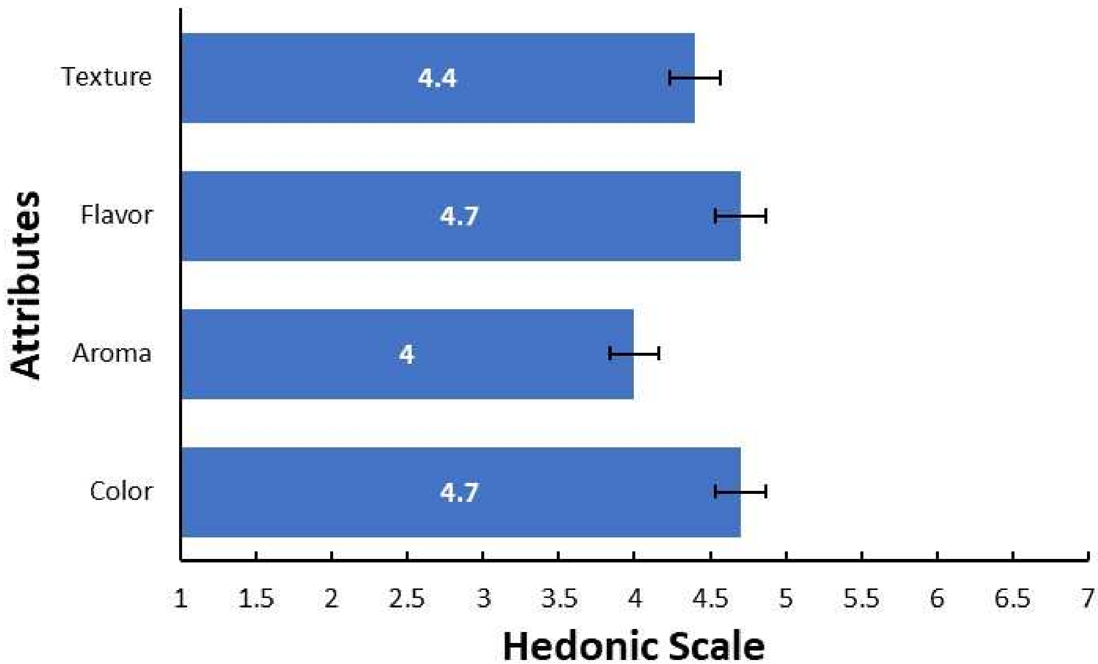


Fig 3: Sensory attributes rating average for catfish control on day 1.

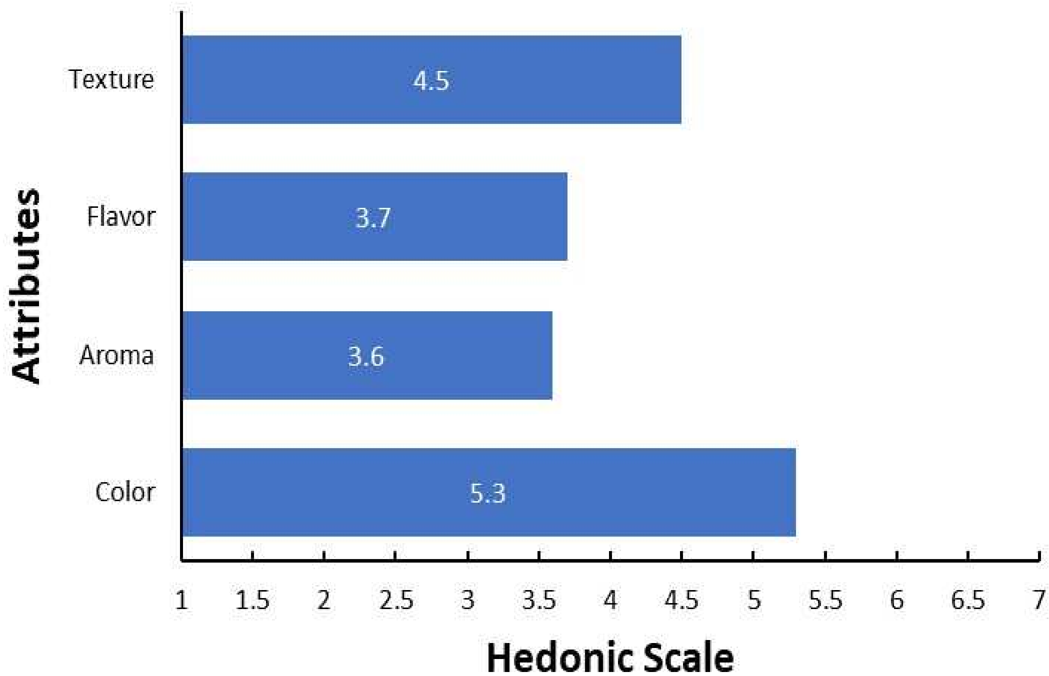


Fig 4: Sensory attributes rating average for catfish treated with GFS on day 1.

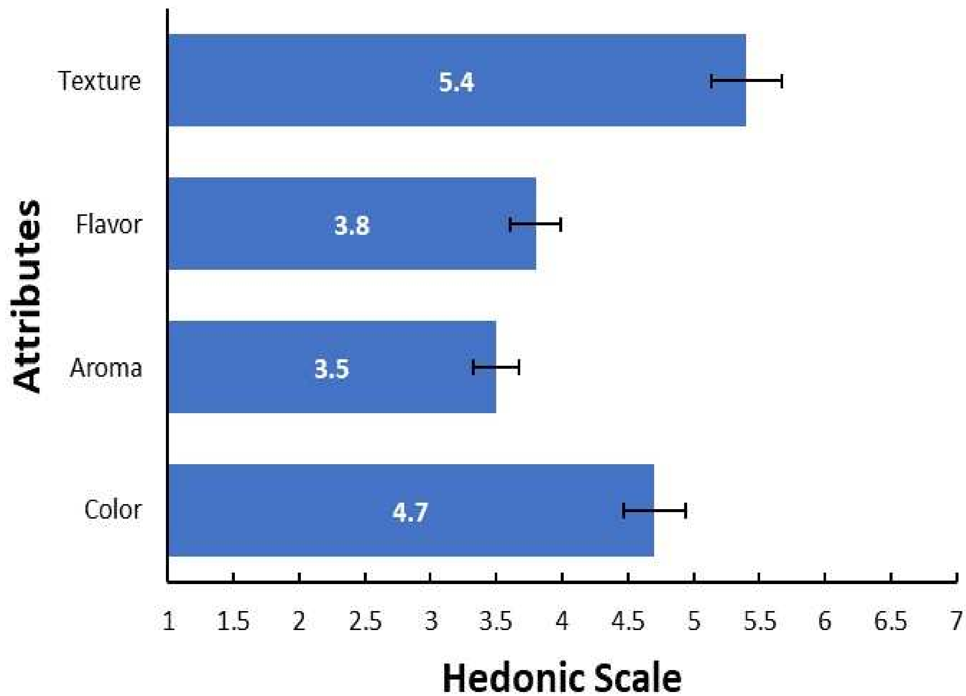


Fig 5: Sensory attributes rating average for catfish treated with lemon on day 1.

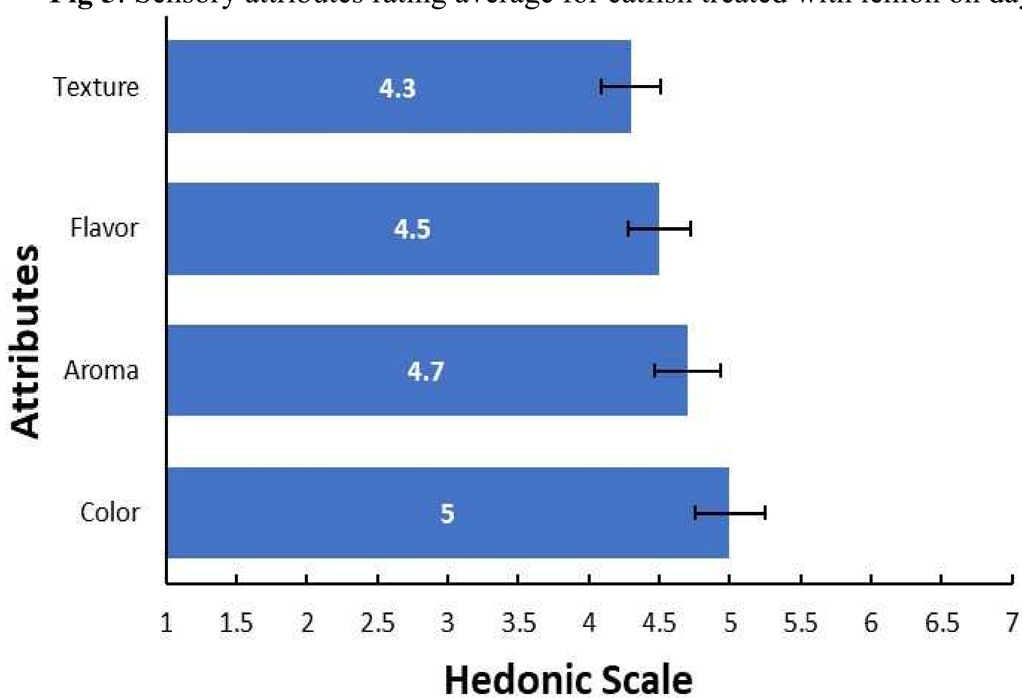


Fig 6: Sensory attributes rating average for catfish treated with vinegar on day 1.

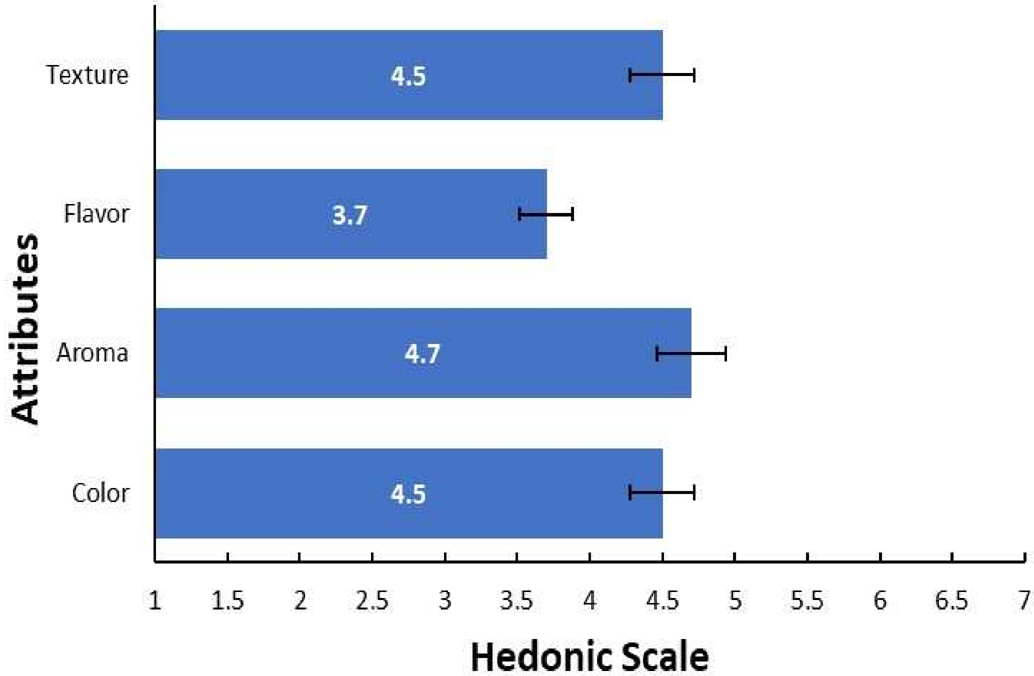


Fig 7: Sensory attributes rating average for catfish control after five days of storage at 4°C.

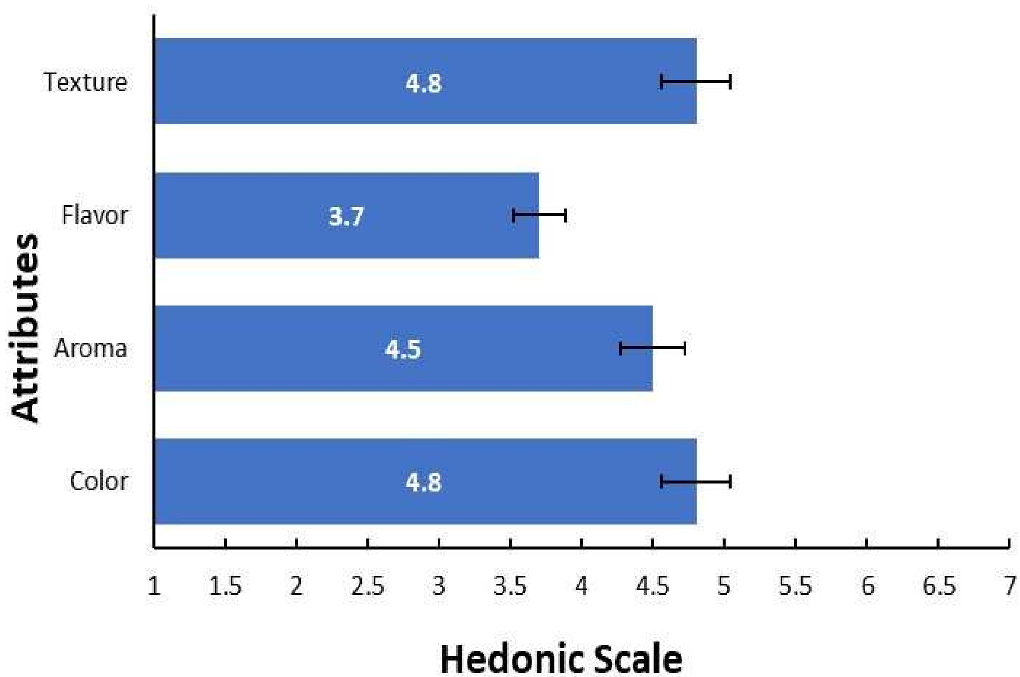


Fig 8: Sensory attributes rating average for catfish treated with GFS after five days of storage at 4°C.

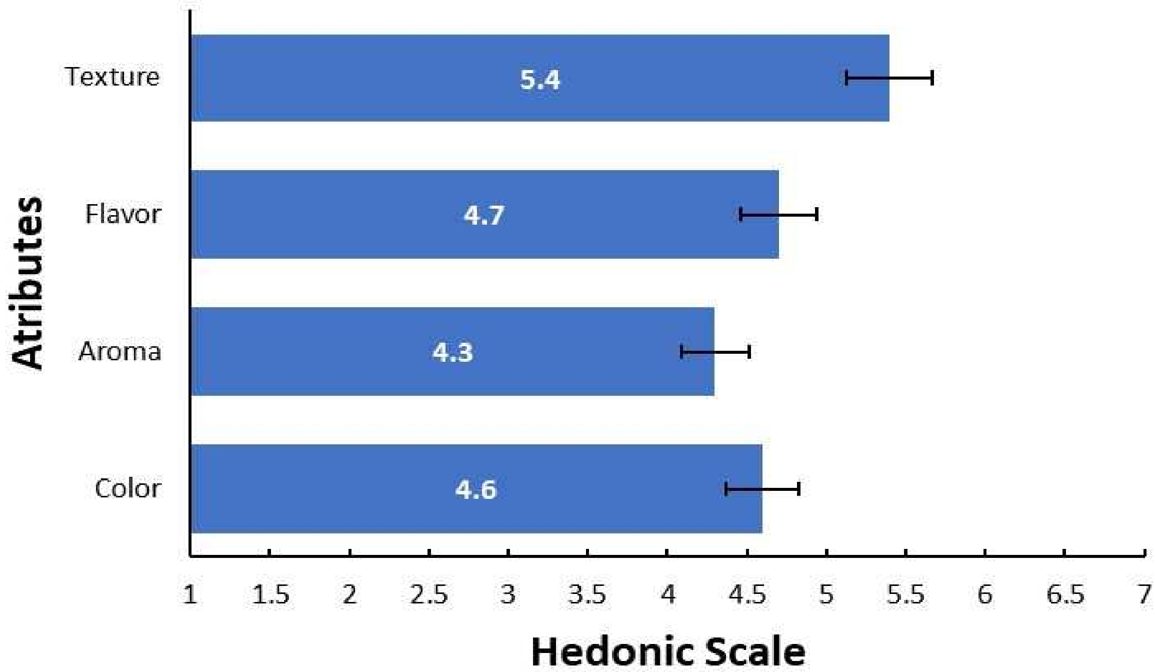


Fig 9: Sensory attributes rating average for catfish treated with lemon after five days of storage at 4°C.

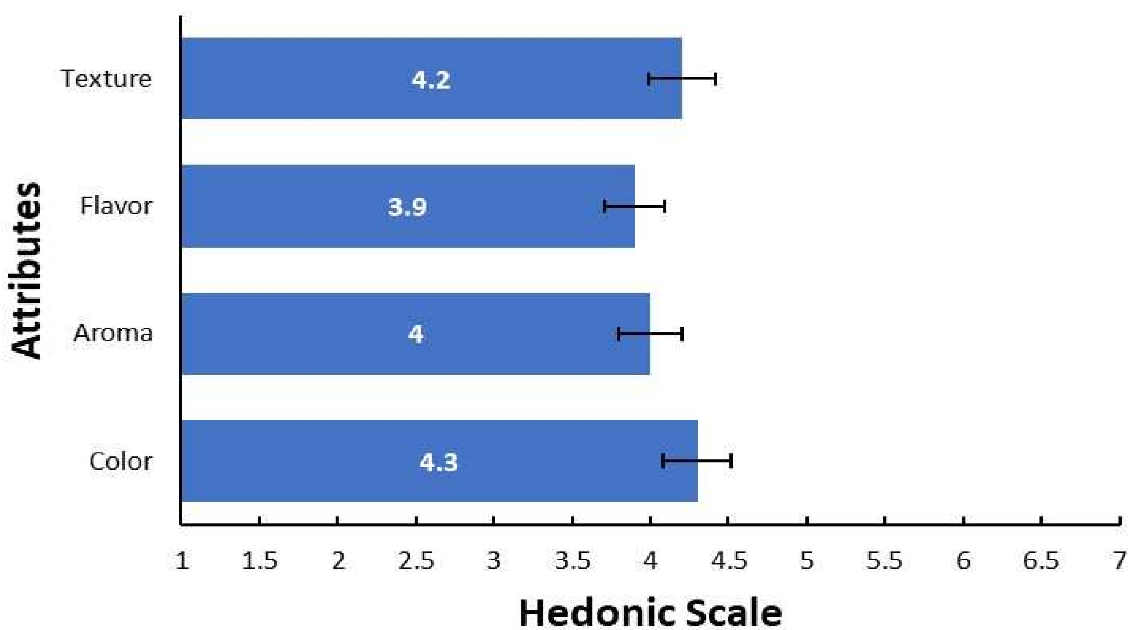


Fig 10: Sensory attributes rating average for catfish treated with vinegar after five days of storage at 4°C.

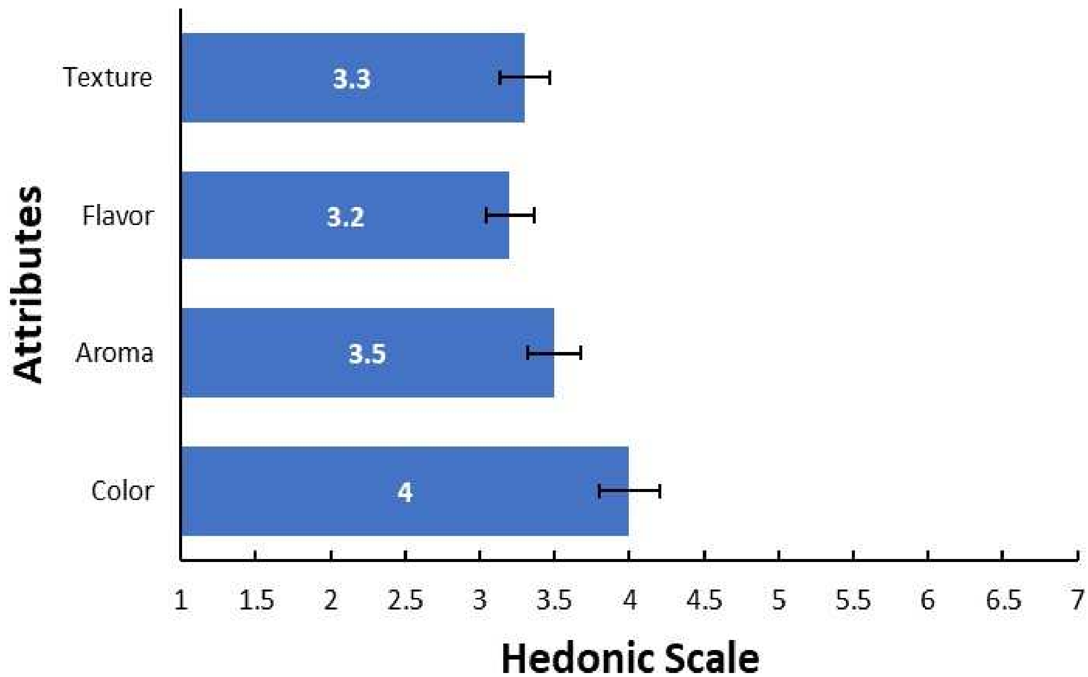


Fig 11: Sensory attributes rating average for catfish control after five days of storage at -20°C.

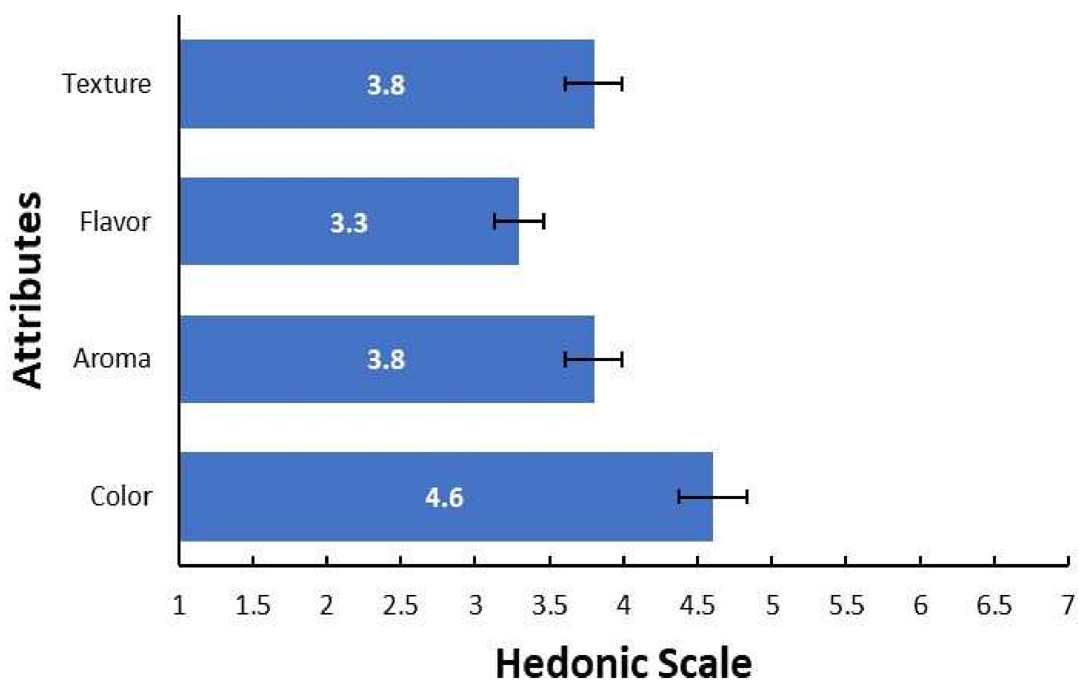


Fig 12: Sensory attributes rating average for catfish treated with GFS after five days of storage at -20°C

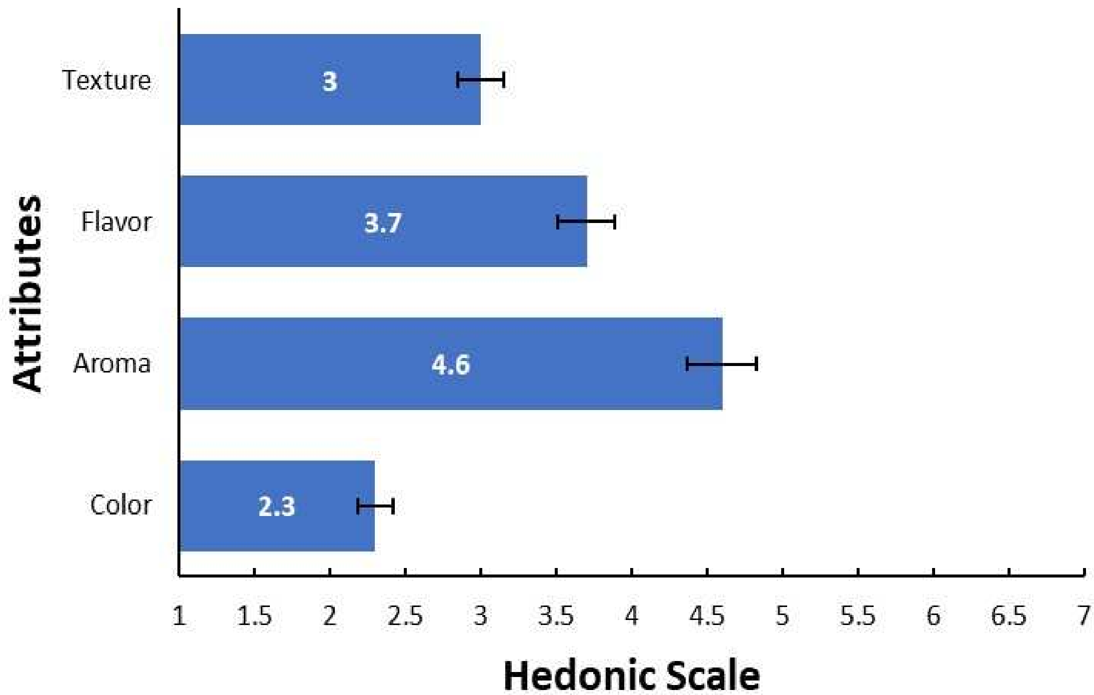


Fig 13: Sensory attributes rating average for catfish treated with lemon after five days of storage at -20°C.

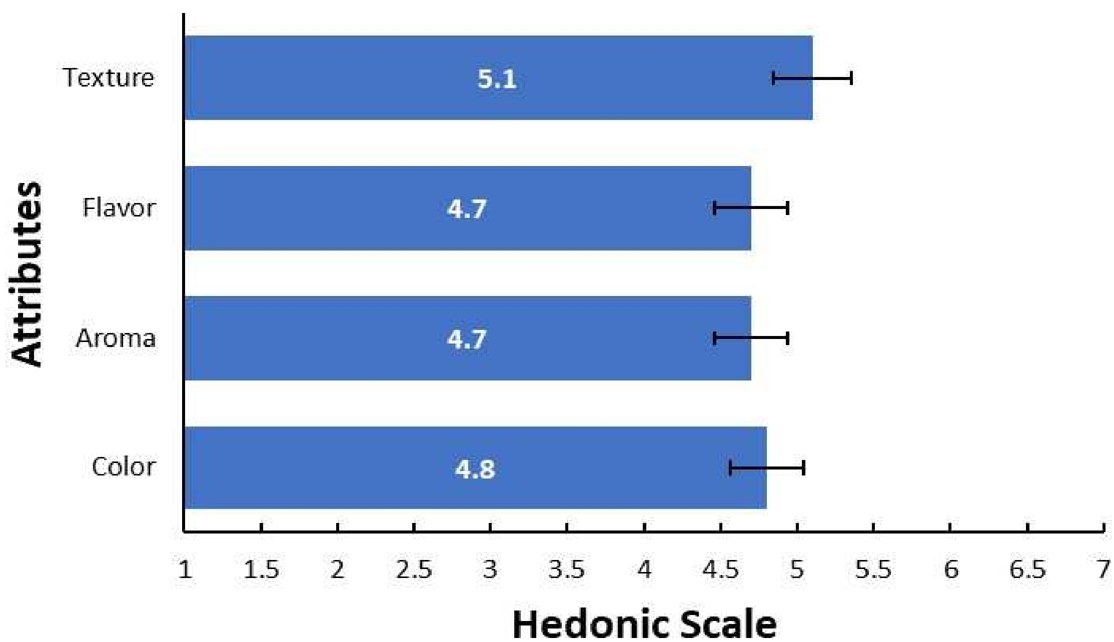


Fig 14: Sensory attributes rating average for catfish treated with vinegar after five days of storage -20°C.

IV: D. *Fractional Inhibitory Concentration (FIC)*

The FIC pf preservatives on the foodborne microbes is shown in Table 4. Lemon and vinegar were tested for synergy or antagonism because they had a greater antimicrobial effect on the catfish fillet, extending shelf life more than the control. Synergy was observed at the addition of 2% of lemon and vinegar. The MIC of lemon and vinegar for all the test bacteria were measured in percent v/v. Vinegar had MIC at 4% while lemon had MIC at 6% for all test bacteria except for *Serratia grimesii* where the MIC of lemon fell at 10%.

Table 3: Fractional Inhibitory Concentration (FIC) of lemon and vinegar on six bacteria species.

Test Bacteria	Minimum Inhibitory Concentration (MIC) %v/v		Fractional Inhibitory Concentration (FIC) %v/v
	Vinegar	Lemon	
Aeromonas hydrophila ATCC	4	6	2
Aeromonas jandaei	4	6	2
Pseudomonas aeruginosa ATCC	4	6	2
Pseudomonas lurida	4	6	2
Serratia grimesii	4	10	2
Shewanella baltica	4	6	2

IV: E. *Protein Separation on SDS-PAGE with Coomassie Blue Stain.*

The Coomassie blue stains on gel revealed the presence of proteins in all catfish samples. Lanes of proteins of different molecular weights had varying band brightness due to the different preservative treatments of catfish samples. The headings A and B, C and D, E and F, and G and H from fig 19 represent control catfish samples (A and B), and catfish samples treated with vinegar (C and D), or lemon (E and F), or GFS (G and H) respectively. Although both A and B showed bright bands A, the day 1 control sample was brighter than B, the day 5 control sample. The day 1 vinegar treated sample (C) was brighter than the day 5 vinegar treated sample (D) while the day 1 lemon treated sample (E) was less bright than the day 5 lemon treated sample (F).G, the day 1 treated GFS treated sample was also less bright than the day 5 treated GFS treated sample H. The control samples contained the largest amount of protein independent of day followed by vinegar treated samples. The lemon treated sample stored for 5 days contained more protein than any of the remaining samples.

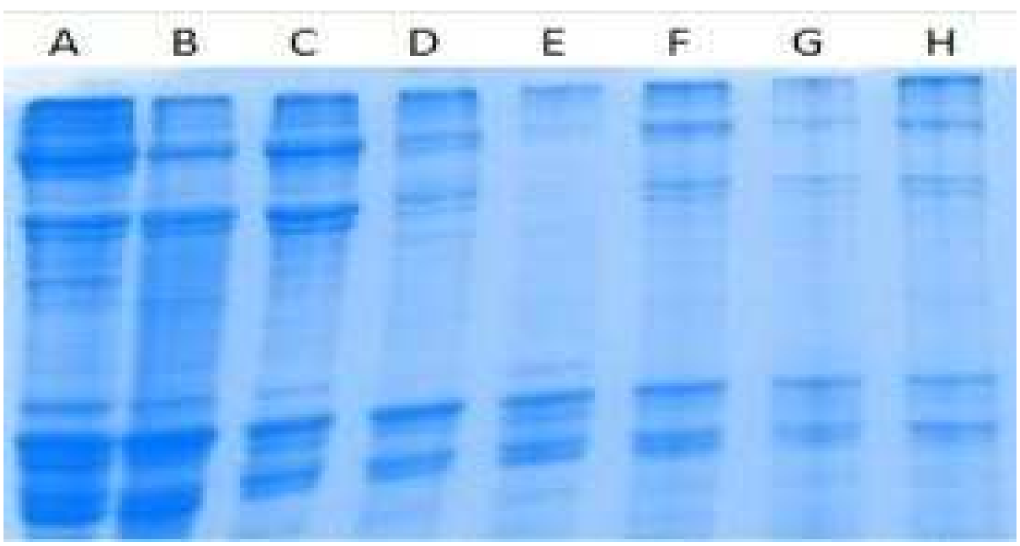


Fig 15. Protein separation on SDS-PAGE of five catfish samples. **A:** control day 1, **B:** control day 5, **C:** vinegar day 1, **D:** vinegar day 5, **E:** lemon day 1, **F:** lemon day 5, **G:** GFS day 1, **H:** GFS day 5.

CHAPTER V: DISCUSSION AND CONCLUSION

V.A. DISCUSSION

The present study assessed the effects of natural antimicrobials such as lemon, vinegar and grapefruit seed extract on the shelf life and sensory properties of channel catfish *Ictalurus punctatus*. The minimum inhibitory concentrations were for lemon 5%, vinegar 4%, and GFS 7%. The zone of inhibition tests showed the antimicrobial activity of the natural preservatives with vinegar having the largest clear zones. *Serratia grimesii* belonging to the family Enterobacteriaceae showed the largest clear zone of 1.60 cm with vinegar. This result of vinegar was like the study by Sengun and Karapinar (2005) in the inhibition of *Salmonella* Typhimurium on rocket and spring onion.

Domestic catfish fillets with no treatment and treatments of vinegar, lemon and grapefruit seed extract were stored at 4°C for 27days. Changes in total bacteria were enumerated every two days. The bacteria populations for the treated samples reduced by 0.5 log CFU/g as compared to the control at day 1. Grapefruit seed extract and lemon maintained the bacteria growth below the threshold value of 10^6CFU/g until the 12th and 14th days of the sampling period. Extending the shelf life of catfish by 7 and 9 days, respectively from the control whose growth exceeded the threshold value on day 5 of the sampling period. The initial bacteria population for vinegar gradually increased to approximately 5.2 LogCFU/g for the total sampling period and the bacteria growth curve was below the threshold value for the total sampling period of 27 days. A variety of factors such as temperature, substrate, organism type and concentration of organic acids cumulatively affect the inhibitory activity of antimicrobials (Al-Rousan et al., 2018).

The fractional inhibition concentration analysis was done using the checkerboard method to check for synergy between lemon and vinegar. This analysis was done on six bacteria species.

Synergy was found at 2%*2% v/v for lemon and vinegar respectively for all the bacteria species. The percentages, which are lower than the MIC of the single preservatives showed synergy between the citric acid activity in lemon and the acetic acid activity of vinegar. This result was similar to the study by Sengun and Karapinar (2005) on elimination of *Yersinia enterolitica* on carrots using household sanitizers.

Sensory evaluation was performed on baked catfish fillet marinated with treatments vinegar, lemon, grapefruit seed extract for 30 minutes at 4°C. The control was catfish fillets without treatments. Sampling was done on day 1 and day 5 at storage 4°C and -20°C. Ten students at Delaware State University evaluated the fish samples after storage at the different temperatures. Evaluation of the sensory attributes color, aroma, flavor and texture was done using the sevenpoint Hedonic scale.

Natural antimicrobials have been known to add the sensory properties of food, the reason why lemon and vinegar are commonly added to food. The least preferred was the GFS treatment with a score of 4.2 which can be approximated to be “neutral”. The attribute with the highest score was the color with a 5.06 “slightly acceptable” followed by the texture with a score of 4.74. These results showed that the fish was still fresh and there was no evidence of spoilage as the color and texture were appreciated by the panel. Statistically, there was no significant difference ($p>0.05$). between the treatments and the control.

At storage 4°C after 5 days, the most preferred sample was lemon with a score of 4.75 “slightly acceptable”. Most spoilage bacteria are psychotropic and cause spoilage at refrigerated temperatures. The attribute with the highest score was the texture with a score of 4.56 “slightly acceptable” followed by the color with a score of 4.44 “neutral”. Statistically, there was no significant difference ($p>0.05$) between the treatments.

At storage -20°C on day 5, the most preferred sample was vinegar with a score of 4.82 “slightly acceptable” while the least preferred was lemon 3.4 “slightly unacceptable”. This low score of lemon can be supported with the claim quality deterioration is seen during freezing and frozen storage due to the osmotic removal of water, denaturation of proteins and mechanical damage (Baygar et al., 2013). It is important to note that thawing in the refrigerator helps retain the sensory properties but these changes after multiple freezing and thawing. The attribute with the highest score was the aroma 4.04 “neutral”. This result can be supported with the claim that the main purpose of freezing seafood is to protect the initial sensory and chemical properties of the fresh products (Baygar et al., 2013).

Overall, the panel preferred the catfish samples of day 1 with a score of 4.51 “slightly acceptable” followed by storage 4°C after 5 days with a score of 4.3 “neutral” and lastly storage at -20°C after 5 days with a score of 3.7 “slightly acceptable”. Statistically, there was no significant difference between the sensory properties of the samples except for storage at -20°C, which had a significant difference in color between the samples. There was a significant difference between control/lemon with ($p=0.031$), GFS/lemon with ($p=0.004$) and lemon/vinegar with ($p=0.002$).

A protein analysis was done using SDS-PAGE gels with Coomassie Blue Stain to evaluate the effect of the natural preservatives on protein of channel catfish with the control. The results showed a difference in band brightness between the treatments and the control. Two sampling conditions were analyzed; samples with treatment on day 1 and samples with treatment after 5 days storage at 4°C. The effect of lemon was stronger on day 1 than on day 5 especially from the beginning to the center of the lane E, but the effect was reduced on day 5 as the bands was brighter on lane F from the beginning to the end. GFS (Lanes G and H) affected the proteins but the difference between day 1 and day 5 was minimal. The effect of vinegar (lane C and D) were strongest at day 5, showing faint bands. These differences could be because of the chemical properties of the treatments on the proteins taking into consideration the pH of the gel and treatments. In addition, the ionic strength of the treatments could alter the solubility of the proteins causing the faintness of the treatment bands.

Limitations in this study include limited panels for sensory evaluation, more samples used for the sensory evaluation. Additional comparisons using citric and acetic acids will warrant responses on the lemon and vinegar treatments and will provide some comparisons between the treatments and chemicals themselves.

V.B. CONCLUSION

The purpose of this study was to evaluate the antimicrobial activity of vinegar, lemon and grapefruit seed extract to retain the sensory properties and extend the shelf life of channel catfish *Ictalurus punctatus*. These natural antimicrobials have shown bactericidal and bacteriostatic effects on many food spoilage bacteria and food pathogens. The antimicrobial capacity of these natural products is different due to their different chemical composition. The activity of lemon and vinegar is based on its citric acid and acetic acid composition, respectively while that of grapefruit seed extract is mainly due to its antioxidant capability and the presence of polyphenols. These natural preservatives can be used separately or combined in extending the shelf life of fish and food in general.

REFERENCES

Al-Rousan, W.M., Olaimat, A.N., Osaili, T.M., Al-Nabulsi, A.A., Ajo, R.Y. & Holley, R.R. (2018). Use of acetic and citric acid to inhibit Escherichia coli O157:H7, Salmonella Typhimurium and Staphylococcus aureus in tabbouleh salad. *Food Microbiology* 73,61-66

Bal'a, M.F.A, Podolak, A.R., & Marshall, D.L. (1999). Steam treatment reduces the skin microflora population on de-headed and eviscerated whole catfish. *Food Microbiology,* 16, 495-501.

Bal'a, M.F.A. and Marshall, D.L. (1998). Organic Acid Dipping of Catfish Fillets: Effect on
Color, Microbial Load, and *Listeria monocytogenes*. *Journal of Food Protection*, 61(11), 1470-1474

Baygar, T., Alparslan, Y. & Cakli, S. (2013). Effects of multiple freezing and refrigerator thawing cycles on the quality changes of sea bass (*Dicentrarchus labrax*). *Iranian Journal of Fisheries Sciences*.12(2)289-300.

Beuchat, L. R. (1995). Pathogenic microorganisms associated with fresh produce. Journal of food protection, vol 59 No 2,204-216.,

Borch. E., Kant-Muermans, M.L., & Blixt, Y. (1996) Biochemical spoilage of meat and cured meat products. *International Journal of Microbiology*.

Bradley, E.M., Williams, J.B., Schilling, M.W., Coggins, P.C., Crist, C., Yoder, S., & Campano, S.G. (2011). Effects of sodium lactate and acetic acid derivatives on the quality and sensory characteristics of hot-boned pork sausage patties. *Meat Science* 88, 145-150.

Burt, S.A., 2004. Essential oils: Their antibacterial properties and potential applications in foods: A review. *International Journal of Food Microbiology*, 94: 223-253.

CDC (2005). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 sites, United States, 2004. *Morbidity and Mortality Weekly Report,* 54, 352–356.

CDC (2006). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, United States, 2005. *Morbidity and Mortality Weekly Report,* 55, 392–395.

CDC (2007). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2006. *Morbidity and Mortality Weekly Report,* 56, 336–339.

CDC (2008). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2007. *Morbidity and Mortality Weekly Report,* 57, 366-370.

CDC (2009). Preliminary FoodNet Data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2008. *Morbidity and Mortality Weekly Report,* 58, 333-337.

Cherrington C.A., Hinton M., & Chopra I. (1990). Effect of short-chain organic acids on macromolecular synthesis in *Escherichia coli*. *Journal of Applied Bacteriology*, 68, 69–74.

Connell, J.J. (1962). Changes in amount of myosin extractable from cod flesh during storage at -14 degrees C. *Journal of Science and Food Agriculture,* 13, 607-617.

Corbo, M.R., Speranza, B., Filippone, A., Granatiero, S., Conte, A., Sinigaglia, M. & Del Nobile, M.A. (2008). Study on the synergic effect of natural compounds on the microbial quality decay of packed fish hamburger. *International Journal of Food Microbiology*, 127,261267.

Craven, H.M. & Macauly, B.J. (1992) Microorganisms in pasteurized milk after refrigerated storage I. Identification of types. *Australia Journal of Dairy Technology*, 47, 38-45.

Craven, H.M. & Macauly, B.J. (1992b) Microorganisms in pasteurized milk after refrigerated storage 2. Seasonal variation. *Australia Journal of Dairy Technology,* 47, 46-49.

Craven, H.M. & Macauly, B.J. (1992c) Microorganisms in pasteurized milk after refrigerated storage 3. Effect of milk processor. *Australia Journal of Dairy Technology*, 47, 50-25

Creed, P. G., & Reeve, W. (1998). Principles and application of sous vide processed foods. In: S. Ghazala (Ed.), Sous vide and cook-chill processing for the food industry (pp. 25–56). Gaithersburg, MD: Aspen Publishers.

Creed, P.G. (1998). Sensory and nutritional aspects of *sous vide* processed foods. In*:* S. Ghazala (Ed.), Sous vide and cook-chill processing for the food industry (57-88*).* Gaithersburg, MD: Aspen Publishers.

Cristóbal-Luna, J.M., Ặlvarez-Gonzalez, I., Madrigal-Bujaidar, E. & Chamorro-Cevallos, G. (2018). Grapefruit and its biomedical, antigenotoxic and chemo preventive properties. Review. *Food and Chemical Toxicology*, 112,224-234.

Dainty, R.H. & Mackey B.M. (1992). The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. *Journal of Applied Bacteriology,* 73, 103S-114S.

Dainty, R.H. (1996). Chemical/biochemical detection of spoilage. *International Journal of Food Microbiology.* 33, 19-33.

Dainty, R.H., Shaw, B.G., & Roberts, T.A. (1983). Microbial and chemical changes in chillstored red meats. In: Roberts, T.A. and F.A. Skinner (editors). *Food Microbiology: Advances and Prospects. Academic Press, London,* 151-178.

Darwin, K.H., and Miller, V.L. (1999). Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. *Clinical Microbiology Reviews,* 12, 405-428.

Dinçer, A.H. & Baysal, T. 2004. Decontamination techniques of pathogen bacteria in meat and poultry. *Critical Reviews in Microbiology* 30, 197–204.Y Dixon, M. & Webb, E.C. (1971). Enzymes. Longman, London.

Duthie, G. & Crozier, A., 2000. Plant-derived phenolic antioxidants. A Review. *Current Opinion in Lipidology*., 11: 43-47.

Efiuvwevwere, B.J. & Ajiboye, M.O. (1996). Control of microbiological quality and shelf-life of catfish (*Clarias gariepinus)* by chemical preservatives and smoking. *Journal of Applied Bacteriology,* 80, 465-470

Farber, J.M. (1991). Microbiological aspects of modified-atmosphere packaging technology- a review. *Journal of Food Protection,* 54, 58-70.

Fernandez, K., Aspe, E., & Roeckel, M. (2009). Shelf life extension on fillets of Atlantic salmon (*Salmo salar*) using natural additives, super chilling and modified atmosphere packaging*. Journal of Food Control* 20,1036-1042.

Fuentes-Amaya, L.F., Munyard, S., Fernandez-Piquer, J. & Howieson, J. (2016). Sensory, Microbiological and Chemical Changes in Vacuum-Packages Blue Spotted Emperor (*Lethrnus* sp), Saddletail Snapper (*Lutjanus malabaricus*), Crimson Snapper (*Lutjanus erythropterus*), Barramundi (*Lates calcarifer*) and Atlantic Salmon (*Salmo salar*) Fillets Stored at 4°C.*Food Science & Nutrition* 4(3): 479-489.doi:10.1002/fsn3.309.

Gallo, L., Schmitt, R.E., & Schmidt-Lorenz. W. (1988). Microbial spoilage of refrigerated fresh broilers. I. Bacterial flora and growth during storage. *Lehensm. Wiss. u.-Technol*. 21, 216-223

Gill, C. O. (1982). Microbial Interaction with Meats. *In*: brown M.H, editor. *Meat Microbiology,* London: Applied Science, 225-264.

Gill, C.O. (1988). The solubility of carbon-dioxide in meat. *Meat Science*, 22, 65-71

Gram, L. & Huss, H.H. (1996). Microbial spoilage of fish and fish products. *International Journal of Food Microbial*, 33, 121-137.

Guthrie, R.K. (1992). *Salmonella.* Boca Raton, FL; CRC Press.

Gyawali, R. & Ibrahim, S.A. (2014). Natural products as antimicrobial agents. Review. *Food Control,* 46,412-429.

Hansen, A.A., Morkore, T., Rudi, K., Olsen, E., & Eie, T. (2007). Quality changes during refrigerated storage of MA-packaged pre-rigor fillets of farmed Atlantic cod (*Gadus* *morhua* L.) using traditional MAP, CO_2 emitter, and vacuum. *Journal of Food Science 72,* M423-M430

Hickey, M.E., Besong, S.A., Kalavacharla, V. & Lee J-L. (2013). Identification of extracellular DNase-producing bacterial populations on catfish fillets during refrigerated storage. *Journal of Science and Biotechnology* 22(1):87-92. Doi 10.1007/s10068-013-0012-1.

https://pharmacy.uic.edu/sites/default/files/Jan_Feb2013_0.pdf

Huis in't Veld, J.H.J. (1996). Microbial and biochemical spoilage of foods: an overview. *International Journal of Food Microbiology*, 33, 1-18.

Jang, J.D., Seo, G.H., Lyu, E.S., Yam, K. L., & Lee, D.S. (2006). Hurdle effect of vinegar and sake on Korean seasoned beef preserved by *sous vide* packaging. *Food Control* 17, 171-175.
Jay, J.M., Loessner, M.J., & Golden, D.A. (2005). *Modern Food Microbiology.* 7th. New York:
Springer Science and Business Media.

Juneja, V.K., Fan, X., Peῆa-Ramos, A.,Diaz-Cinco, M. & Pacheco-Aguilar, R.(2005).The effect of grapefruit extract and temperature abuse on growth of Clostridium perfringens from spore inocula in marinated, sous-vide chicken products. *Innovative Food Science & Emerging Technologies*, 7,100-106.

Kim, J. & Marshall D. L. (2002). Influence of catfish skin mucus on trisodium phosphate inactivation of attached *Salmonella Typhymurium, Edwardsiella tarda* and *Listeria monocytogenes. Journal of Food Protection,* 65, 1146-1151.

Kolakowska, A. (1976). Comparison of oxidative and hydrolytic changes of lipids in frozen minces, whole fish and fillets from Baltic herring hold and 253 degrees K [in Polish]. *Biul Cent Lab Chlod,* 1, 51.

Lahellec, C. & Colin, P. (1979) Bacterial flora of poultry. Changes due to variations in ecological conditions during processing and storage. Arch. Lebensm. *Hygiene*, 30, 95-98

Lingham, T., Besong, S., Ozbay, G. & Lee, J-L. (2012). Antimicrobial activity of vinegar on bacterial species isolated from retail and local channel catfish (*Ictalurus punctatus*). *Journal of Food Processing and Technology* S11-001.Doi 10.4172/2157-7110.S11-001.

Malicki, A., Zawadzki, W., Bruzewicz, S., Graczyk, S., & Czerski A. (2004). Effect of formic and propionic acid mixture on *Escherichia coli* in fish meal stored at 12ºC. *Pakistan Journal of Nutrition* 3, 353–356.

Mani-Lopez, E., Garcia, H.S., & Lopez-Malo, A. (2011). Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. *Food Research International.* doi: 10.1016/j.foodres.2011.04.043

Manju, S., Jose, L., Srinivasagopal, T.K., Ravishankar C.N., & Lalitha K.V. (2007). Effects of sodium acetate dip treatment and vacuum -packaging on chemical, microbiological, textural and sensory changes of pearlspot (*Etroplus suratensis*) during chill storage. *Food Chemistry*,102: 27-35.

Marshall, D. L. & Bal'a, M. F. A. (2001). Microbiology of Meat. In. Hui YH, editor. Meat Science and Applications. New York: Marcel Dekker Inc. 160-181

Masniyom, P., Benjakul, S. & Visessanguan, W., (2005). Combination effect of phosphate and modified atmosphere on quality and shelf life of refrigerated seabass slices. Swiss society of *Food Science and Technology* LWT 38,745-756.

Mendes, R. & Goncalves, A. (2008). Effect of soluble CO_2 stabilization and vacuum packaging in the shelf life of farmed sea bream and sea bass fillets. *International Journal of Food Science and Technology* 43, 1678-1687.

Nychas, G. J., Dillion, V. M., & Board, R.G. (1988). Glucose, the key substrate in the microbiological changes occurring in meat and certain meat products. *Biotechnology Applied Biochemistry,* 10, 203-231.

Ozturk, I., Caliskan, O., Tornuk, F., Ozcan, N., Yalcin, H., Baslar, M., & Sagdic, O. (2015). Antioxidant, antimicrobial, mineral, volatile, physicochemical and microbiological characteristics of traditional home-made Turkish vinegars. *LWT- Food Science and Technology* 63,144-151.

Palumbo, S.A. (1986). Is refrigeration enough to restrain foodborne pathogens? *Journal of Food Protection* vol 49 No. 12,1003-1009.

Paredi, M.E., De Vido de Mattio, N., & Crupkin, M. (1996). Biochemical properties of actomyosin and expressible moisture of frozen stores striated adductor muscle of *Aulacomya ate rater* (Molina): effects of polyphosphates. *Journal of Agriculture of Food Chemistry,* 10, 3108-3112.

Price, J.F. & Schweigert, B.S. (1978). The science of meat and meat products. Connecticut: Food and Nutrition Press.

Pultrini, A.M., Galindo, L.A., & Costa, M., 2006. Effects of the essential oil from Citrus aurantium L. in experimental anxiety models in mice. *Life Science*, 78(15): 1720-1725

Raftari, M., Jalilian, A., Abdulamir, A.S., Son, R., Sekawi, Z., & Fatimah, A.B. (2009). Effect of organic acids on *Escherichia coli* O157:H7 and *Staphylococcus aureus* contaminated meat. *The Open Microbiology Journal,* 3, 121-127.

Regez, P., L. Gallo, R. E. Schmitt, & W. Schmitt-Lorenz. (1988). Microbial spoilage of refrigerated fresh broilers. III. Effect of storage temperature on the microbial association of poultry carcasses. *Lebensmittel Wissenschaft Technologie*, 21, 229–233.

Ricke, S.C. (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science,* 82, 632–39.

Russel, J.B. (1991). Resistance of *Streptococcus bovis* to acetic acid at low pH: relationship between intracellular pH and anion accumulation. *Applied Environmental Microbiology,* 57, 255–259.

Sengun, I.Y., & Karapinar, M. (2004). Effectiveness of lemon juice, vinegar and their mixture in the elimination of *Salmonella typhimurium* on carrots (*Daucus carota L*). *International Journal of Food Microbiology*, 96,301-305.

Sengun, I.Y., & Karapinar, M. (2005). Elimination of *Yersinia enterocolitica* on carrots (Daucus carota L.) by using household sanitizers. *Food Control* 16,845-850.

Shahnah, S.M., Ali, S., Ansari, H., & Bagri, P., 2007. New sesquiterpene derivative from fruit peel of *Citrus limon* (Linn). *Food Science Pharmacology*, 75: 165-170.

Sharma, S. (2015). Food preservatives and their harmful effects. *International Journal of Scientific and Research Publications*. ISSN 2250-3153.

Sherman, R. A, & Mehta, O. (2009). Phosphorus and Potassium Content of Enhanced Meat and Poultry Products: Implications for Patients Who Receive Dialysis. Clinical Journal of American Society of Nephrology 4: 1370-1373.doi:10.2215/CJN.02830409.

Simeonidou, S., Govaris, A., & Vareltzis, K. (1997). Effect of frozen storage on the quality of whole fish and fillets of horse mackerel (*Trachurus trachrus)* and Mediterranean hake (*Merluccis Mediterranean). Lebensm Unters Forsch,* 204, 405-410.

Sivertsvik, M., Jeksrud, W.K., & Rosnes, J.T. (2002). A review of modified atmosphere packaging of fish and fishery products-significance of microbial growth, activities and safety. *International Journal of Food Science and Technology,* 37, 107-127.

Skrivanova, E., Molatova, Z., Matenova, M., Houf, K., & Marounek, M. (2011). Inhibitory effect of organic acids on acrobacters in culture and their use for control of *Acrobacter butzleri* on chicken skin. *International Journal of Food Microbiology,* 144, 367-371.

Skrivanova, E., Molatova, Z., Matenova, M., Houf, K., & Marounek, M. (2011). Inhibitory effect of organic acids on acrobacters in culture and their use for control of *Acrobacter butzleri* on chicken skin. *International Journal of Food Microbiology,* 144, 367-371

Smittle, R.B. (2000). Microbiological safety of mayonnaise, salad dressings, and sauces produced in the United States: a review. *Journal of Food Protection*, 63, 1144–1153.

Sofos, J.N. (2000). Sorbic acid. In: Naidu, A.S.(Ed.). Natural Food Antimicrobial Systems. CRC Press, Boca Raton, Fl.637-659.

Sohn, H.Y., Son, K. H., Know, C.S. & Kang, S.S., 2004. Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: *Morus alba L., Morus mongolica Schneider, Broussnetia papyrifera (L.) Vent, Sophora flavescens Ait* and *Echinosophora koreensis Nakai*. *Phytomedicine*, 11: 666-672.

Speare, D.J. & Mirasalimi, S.M. (1992). Pathology of the mucous coat of trout skin during an erosive bacterial dermatitis. *Journal of Comparative Pathology, 106,* 210-211.

Suhalim, R.R., Huang, Y., & Chen, J. (2007). Interaction of *Escherichia coli* O157:H7 cells with the mucus of harvested channel catfish (*Ictalurus punctatus*). *LWT,* 40, 1266-1270.

Theron, M.M. & Lues, J.F.R., (2007). Organic acids and meat preservation: a review. *Food Reviews International* 23, 141–158.

Tzortzakis, N., (2010). Ethanol, vinegar and origanum vulgare oil vapor suppress the development of anthracnose rot in tomato fruit. *International Journal of Food Microbiology* 96: 301-305.

Vijayakumar, C. and Wolf- Hall, C.E. (2002). Minimum bacteriostatic and bactericidal concentrations of household sanitizers for Escherichia coli strains in tryptic soy broth. *Journal of Food Microbiology*, 19,383-388.

Walker, S.J. & Stringer. M.F. (1990) Microbiology of chilled foods. In: T.R. Comely (editor). Chilled Foods. The State of the Art. Elsevier. Barking, 269-304.

Werlein, H.D. (1998). Comparison of the quality of *sous-vide* and conventionally processed carrots. *Zeitschrift fur Lebensmittel Untersuchung and Forschung A,* 207, 311-315.

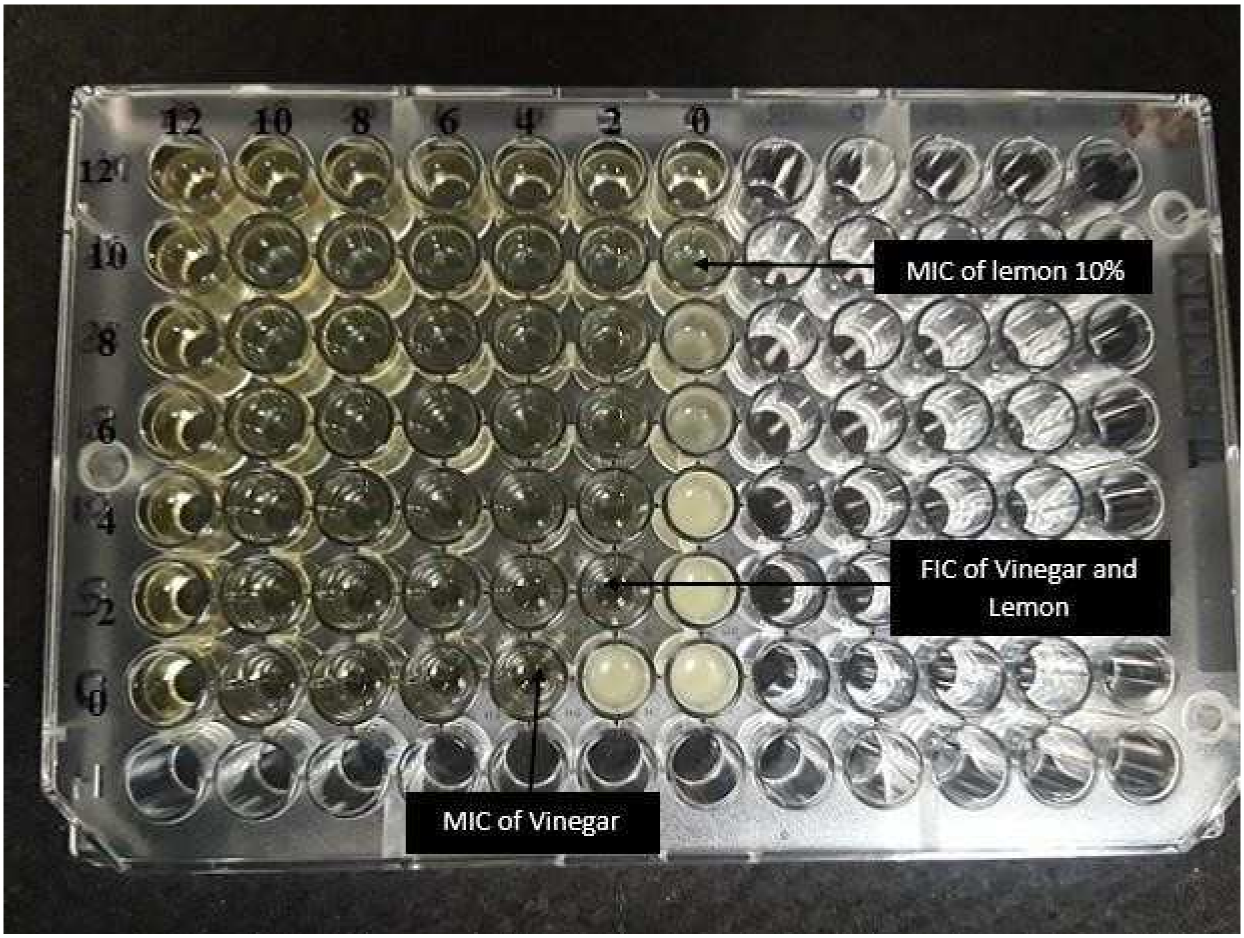
Williams, S.K., Rodrick, G.E., & West, R.L. (1995). Sodium lactate affects shelf life and consumer acceptance of fresh catfish (*Ictalurus nebulosus,marmoratus*).

www.healthydietbase.com/the-advantages-and-disadvantages-of-apple-cider-vinegar. Retrieved on March 09, 2018.

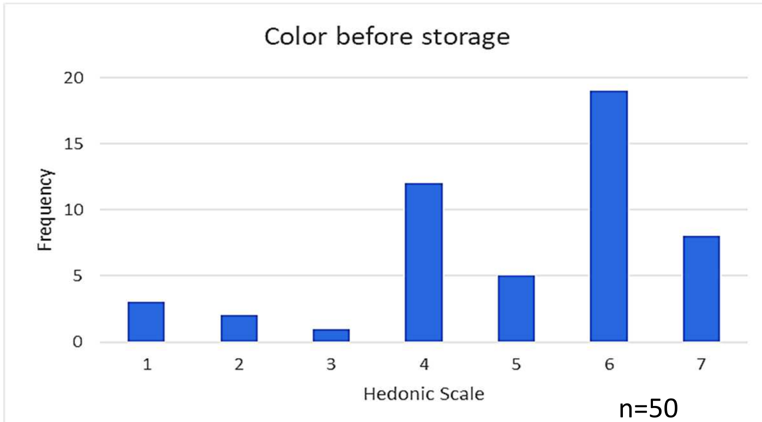
Zhao, Y.Y., Wells, J.H., & Mcmillin, K.W. (1995). Dynamic changes of headspace gases in CO_2 and N_2 packaged fresh beef. *Journal of Food Science,* 60, 571-575.

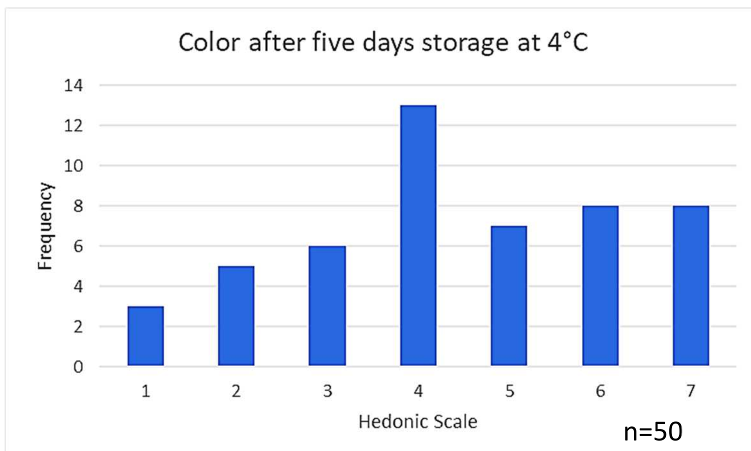
APPENDICES

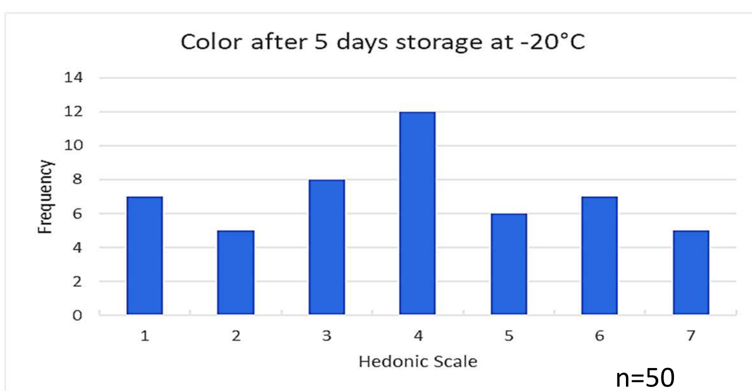
Appendix A. Fractional Inhibitory Concentration of Lemon and vinegar using the checkerboard method.



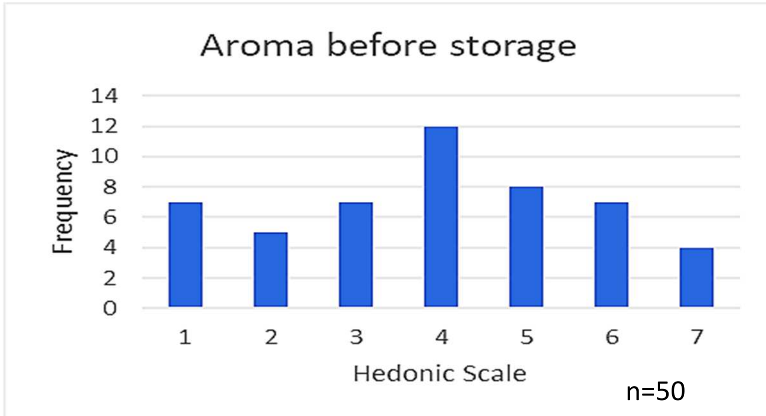
Appendix B: Bar charts of sensory evaluation attribute rating frequencies at different storage conditions

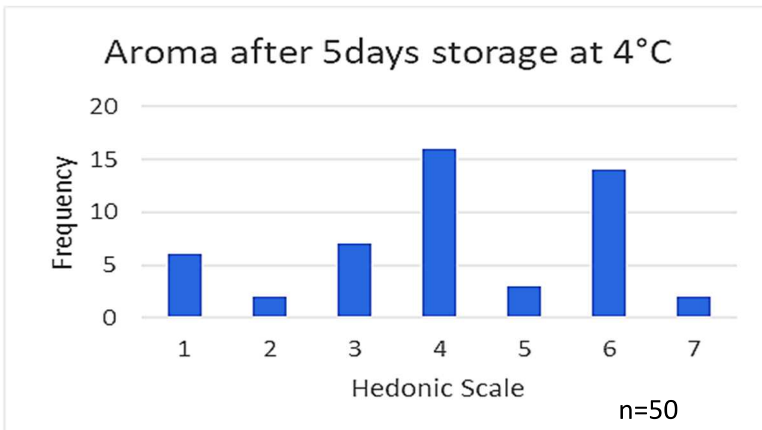


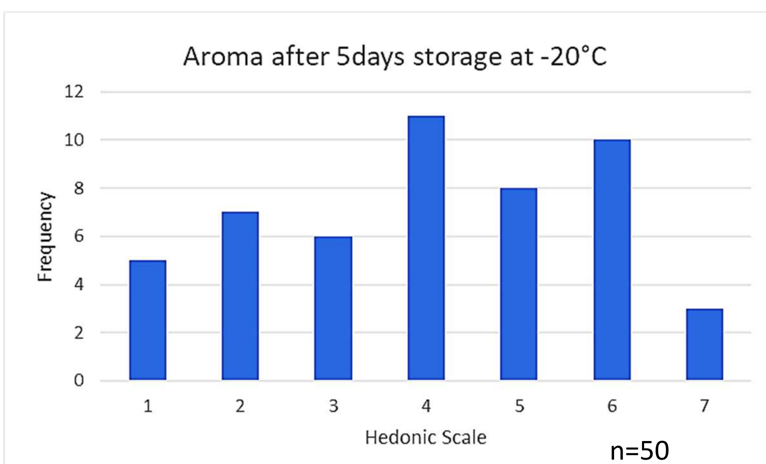




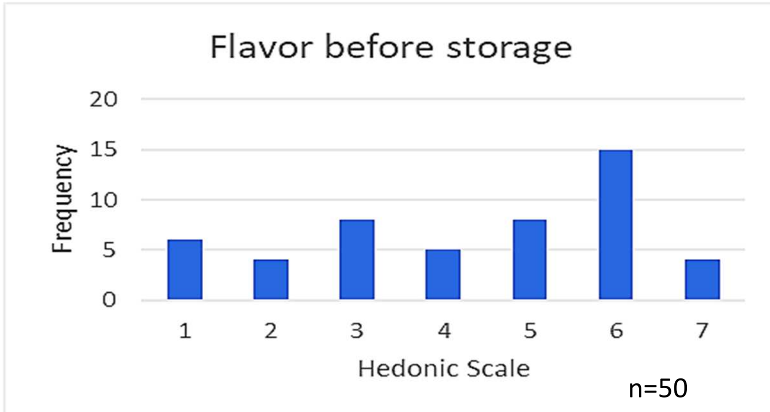
Color rating frequencies at before storage, 4 and -20°C.

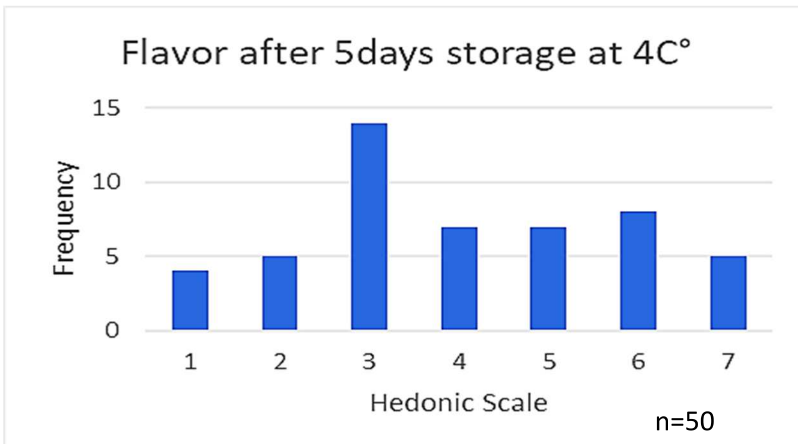


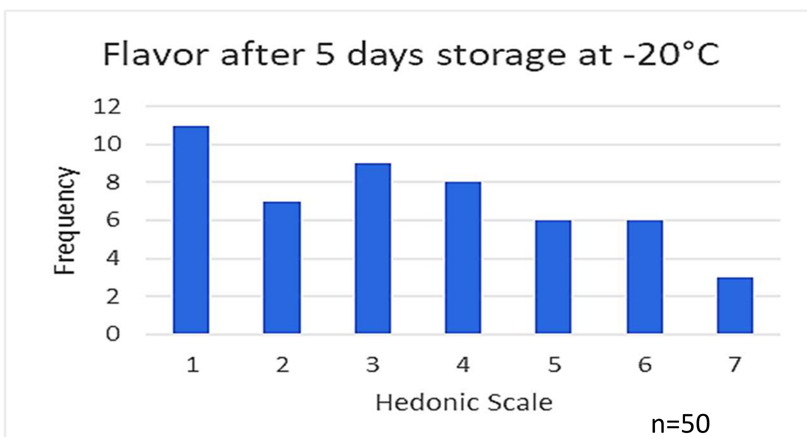




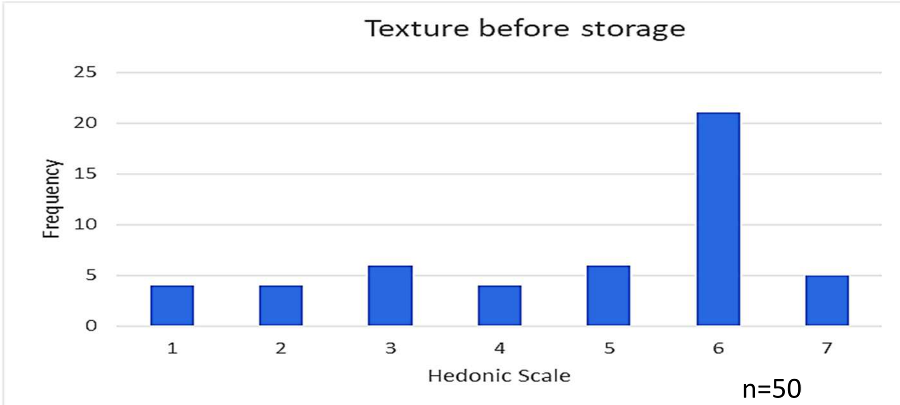
Aroma rating frequencies at before storage, 4 and -20°C

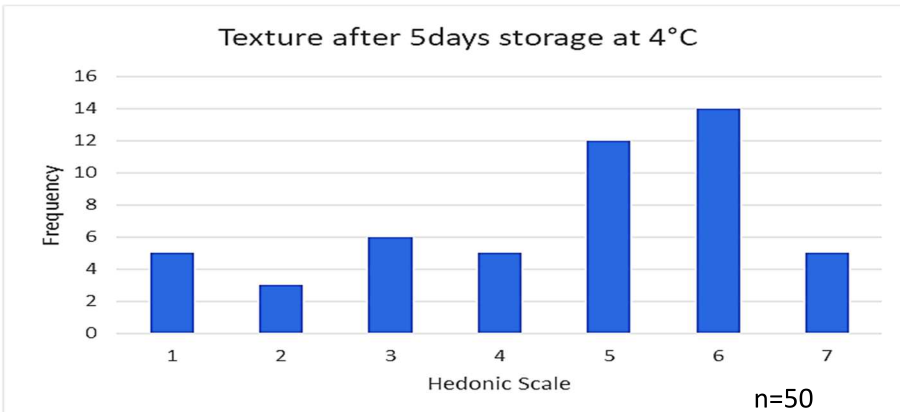


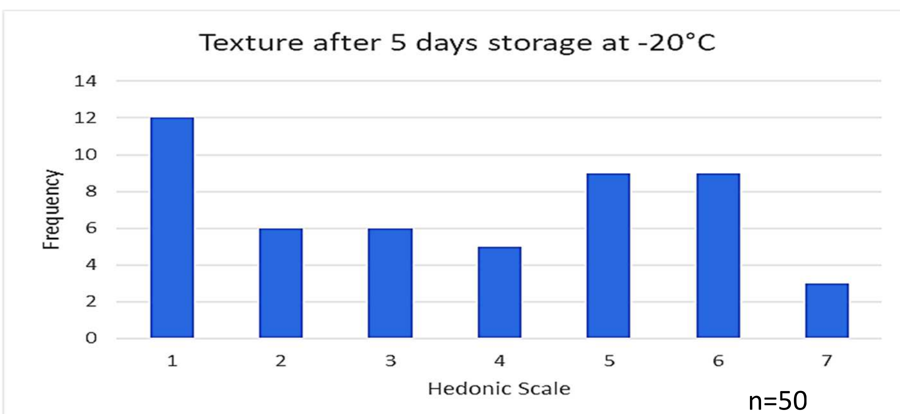




Flavor rating frequencies at before storage, 4 and -20°C







Texture rating frequencies at before storage, 4 and -20°C