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ANTIMICROBIAL ACTIVITY OF VINEGAR ON FOODBORNE PATHOGENS AND BACTERIAL SPECIES ISOLATED FROM RETAIL AND LOCAL CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

by

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A THESIS

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DEDICATION

This thesis is dedicated to my family. My parents, older brother and two younger sisters have always been there for me. I would not be where I am today if it wasn't for them. They supported, guided, motivated and believe that I can be very successful in life.

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ABSTRACT

In this study, the use of two vinegars was explored to reduce the growing number of illnesses caused by foodborne pathogens and spoilage bacteria. The antimicrobial activity of fish and chip vinegar and apple cider vinegar was studied on nine foodborne pathogens and various bacterial species isolated from domestic channel catfish fillets (*Ictalurus punctatus*). Ion chromatography was performed to confirm the presence of acetic acid in the vinegar samples using sodium acetate as the standard. The zone of inhibition and the minimum inhibitory concentration were used to measure how effective the vinegars are on the bacteria. Acetic acid was applied as the positive control to determine if the antimicrobial activity of the vinegars was due to acetic acid. Bacteria isolated from catfish fillets with the largest inhibition zone were identified through 16S rDNA sequencing to better understand the spoilage bacteria that could be inhibited by vinegar to increase the quality of fishery products. Microbial changes of catfish fillets were evaluated following treatments of vinegar marinades during storage at 4°C and sensory evaluation was performed with baked catfish fillets to evaluate consumer sensory

acceptability with respect to attributes including appearance (color), aroma, flavor and texture.

In this study, results indicate that the antimicrobial activity of the vinegar samples is due to acetic acid that inhibits foodborne pathogens and bacteria isolated from catfish fillets. Fish and chip vinegar is a stronger antimicrobial agent than apple cider vinegar on the foodborne pathogens and bacteria isolated from catfish fillets. Fish and chip vinegar exerted the highest antimicrobial activity on *E. coli* DH5α (9.82 mm) and *Pseudomonas fluorescens* (7.85 mm). This was the only study that evaluated the inhibitory effect of acetic acid from vinegar on bacteria isolated from catfish. Fish and chip vinegar was the most effective on *Shewanella putrefaciens* isolated from catfish fillets from the market and the pond with zone of inhibition measurement ranging from 10 to 15 mm in diameter. Microbial changes were evaluated and sensory evaluation was performed on different acetic acid dilutions of vinegar. Results showed that vinegar diluted to 0.5% acetic acid on catfish fillets would be suitable for prolonging shelf life and appealing to consumers. Vinegar as a natural antimicrobial product can improve the shelf life and safety of food products providing acceptable sensory quality at an affordable price and reducing economic lost due to spoiled seafood and other food products.

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GENERAL INTRODUCTION

There is an increasing interest in applying natural antimicrobial compounds in the food industry. Antimicrobial properties from vinegar have been reported previously in articles by scientists. Organic acids such as vinegar have been found as safe antibacterial agents (Smulders and Greer, 1998).Various researchers have proved the antibacterial effect of organic acids on different types of pathogenic bacteria. Chaff vinegar has been found to inhibit the growth of pathogenic bacteria such as *E. coli* (Makino et al., 2000). Vinegar assists in suppressing the anthracnose rot in tomatoes (Tzortzakis, 2010). It also assists in eliminating *Salmonella* Typhimurium in carrots (Sengun and Karapinar, 2004).

Fresh seafood has a short shelf-life. In most seafood products, microorganisms are the major cause of spoilage. High bacterial counts indicate storage abuse, thus increasing the presence of human pathogens. Specific spoilage organisms (SSOs) contribute to the unpleasant off-flavors associated with seafood spoilage (Gram and Dalgaard, 2002). Changes in sensory properties of a food product make the product unsuitable for human consumption.

Consumers are increasingly avoiding the consumption of foods treated with chemicals. Natural alternatives are needed to achieve a high level of safety with respect to foodborne pathogenic microorganisms (Rauha et al., 2000). The natural sanitizers, such as organic acids, have been investigated because of their bactericidal activity (Uyttendaele et al., 2004). Among the natural products, vinegar, also known as acetic acid, contains sanitizing properties.

Problem Statement

There is a concern about the growing number of illnesses caused by foodborne pathogens and spoilage bacteria in foods. "International agencies that monitor world food resources have acknowledged that one of the most feasible options for meeting future food needs is reduction of postharvest losses" (Kelman, 1984). The short life of fresh seafood causes problems for its distribution (Barakat, 2004). Freshness of fish deteriorates rapidly so maintaining the quality is of high importance. Food processors and consumers have expressed a desire to decrease synthetic chemicals used for food preservation. Applying natural antimicrobial agents, like the vinegar products used in this study, can improve food safety and increase shelf life of food products, thus having a positive influence on the economy.

In this study, two vinegar products, Crosse and Blackwell Old English Fish and Chip 100% Natural Malt vinegar and Bragg Raw-Unfiltered Organic Apple Cider vinegar were studied for their antimicrobial activities. The antimicrobial activity of the vinegars was determined on nine foodborne pathogens and bacteria isolated from catfish fillets. The catfish fillets used were from two sources: a local seafood market in Dover, Delaware and the Delaware State University Aquaculture Pond. Sensory evaluation was performed on catfish fillets with vinegar marinades to evaluate consumer acceptability.

OBJECTIVES

The primary objective of this research was to study the antimicrobial activity of vinegar on foodborne pathogens and various species derived from catfish fillets. The vinegar could be applied as an antimicrobial component in food in order to improve shelf

life and food safety. It could be used as a substitute ingredient in food that will decrease and inhibit bacterial growth.

Chapter 1: Identification of Acetic Acid in Vinegar and Antimicrobial Activity of Acetic Acid

The objectives were to confirm the presence and concentration of acetic acid in fish and chip vinegar and apple cider vinegar and to measure the antimicrobial activity of acetic acid.

$H_0$1: Acetic acid is not present in fish and chip vinegar and apple cider vinegar.

H_A1: Acetic acid is present in fish and chip and apple cider vinegar.

$H_0$2: Acetic acid does not exert antimicrobial activity on the bacteria.

H_A2: Acetic acid exerts antimicrobial activity on the bacteria.

Chapter 2: Antimicrobial Activity of Vinegar on Foodborne Pathogens

The objective was to measure the antimicrobial activity of different acetic acid dilutions of two vinegar samples on nine foodborne pathogens.

H_0: The two vinegar samples do not exert antimicrobial activity on the foodborne pathogens.

H_A: The two vinegar samples exert antimicrobial activity on the foodborne pathogens.

Chapter 3: Antimicrobial Activity of Vinegar on Bacterial Species Isolated from Retail and Local Channel Catfish (*Ictalurus Punctatus*)

The objectives of this study were: (1) To measure the antimicrobial activity of different acetic acid dilutions of two vinegar samples on bacteria isolated from catfish from a local seafood market and the Delaware State University Aquaculture Pond. (2) The bacteria derived from catfish fillets were identified by 16S rDNA to better understand which spoilage bacteria could be inhibited by vinegar to increase the quality of fishery products. (3) Microbial changes were evaluated following treatments of different acetic acid dilutions of vinegar marinades on domestic catfish fillets. (4) Sensory evaluation was performed with catfish fillets with different acetic acid dilutions of vinegar marinades to evaluate consumer sensory acceptability.

$H_0$1: Two vinegar samples do not exert antimicrobial activity on bacteria isolated from a local seafood market and the Delaware State University Aquaculture Pond.

H_A1: Two vinegar samples exert antimicrobial activity on bacteria isolated from a local seafood market and the Delaware State University Aquaculture Pond.

$H_0$2: There is a not a difference in microbial change following treatments of different acetic acid dilutions of vinegar marinades on domestic catfish fillets.

H_A2: There is a difference in microbial change following treatments of

different acetic acid dilutions of vinegar marinades on domestic catfish fillets.

$H_0$3: Consumers will not accept the sensory attributes of the acetic acid dilutions of vinegar marinades on baked catfish fillets.

H_A3: Consumers will accept the sensory attributes of the acetic acid dilutions of vinegar marinades on baked catfish fillets.

Table 1: *List of foodborne pathogens used for antimicrobial tests.*

Foodborne pathogen	Designation
Escherichia coli DH5α	ATCC[1] 67876
Escherichia coli 0111	JB[2] 1-95
Pseudomonas aeruginosa	ATCC 10145
Pseudomonas fluorescens	ATCC 13525
Pseudomonas fluorescens	Carolina bio[3] 155255
Pseudomonas putida	Carolina bio 155265
Salmonella Typhimurium	SA[4] 35
Serratia marcescens	SM[5] 51
Staphylococcus aureus	ATCC 12598

*Foodborne pathogens were used for zone of inhibition and minimum inhibitory concentration.
[1]**ATCC**: American Type Culture Collection
[2]**JB**: Received from USDA in Wyndmoor, PA
[3]**Carolina bio**: Carolina Biological Supply Company
[4]**SA**: Received from USDA in Wyndmoor, PA
[5]**SM**: Received from USDA in Wyndmoor, PA

LITERATURE REVIEW

Foodborne pathogen and spoilage bacteria

Meat, eggs and poultry carcasses are the major sources of human foodborne infections associated with pathogenic bacteria (Skrivanova, 2011). Meat pathogens can cause diseases or fatal infections of the immunocompromised, the elderly, and children (Marshall and Bal'a, 2001). A wide range of microorganisms from different sources are transferred onto nutrient rich meat surfaces (Marshall and Bal'a, 2001). Animal hide, hair, and hooves are widespread sources of bacterial contamination (Mies, 2004). Foods can be contaminated with a wide range of microorganisms during harvesting, processing and handling. The number of microorganisms on surfaces of fresh meat changes during chilled storage following a normal microbial growth pattern. Bacterial counts in meat range from 10^2-10^5 CFU/cm^2 but only around 10% are able to initiate growth (Nychas et al., 1988). Microorganisms adapting to changes in conditions, such as temperature and surface desiccation, occurs in the lag phase. Logarithmic growth takes place after cells have adapted to their environment and optimize their metabolism. Bacterial numbers exceeding 10^7 cells per cm^2 elicits off odors, signs of spoilage (Raftari, 2009). Bacterial slime is noticeable around 10^8 cells per cm^2 (Gill, 1982).

Food spoilage can be considered as "any change which renders a product unacceptable for human consumption" (Hayes, 1985). It can be obvious; for example, physical damage, visible growth of microorganisms, slime formation or insect damage (Huis in't Veld, 1996). The exact figures of economic losses due to food spoilage are unknown. It is estimated that one-fourth of the world's food supply is lost through

microbial activity alone (Anonymous, 1985). Psychotrophic microorganisms, yeasts and molds are the main cause of food spoilage in developed countries and in less developed countries, rodents and other animals are of concern (Huis in't Veld, 1996). Thus, food spoilage is an economical problem despite the food technology and preservation techniques available.

Spoilage is mostly evident is proteinaceous foods such as meat, poultry, fish, shellfish and dairy products. They are highly nutritious, retain a neutral or slightly acidic pH and high moisture content, thus resulting in the growth of a wide range of microorganisms (Huis in't Veld, 1996). The pattern of microbial spoilage of proteinaceous foods is similar. SSO's are specific spoilage organisms that are usually present in low numbers and constitute a small fraction of the natural microflora on newly processed seafood (Gram, 2002). During storage, the SSOs grow faster than the microflora and are responsible for the production of off-odors, off-flavors and slime (Huis in't Veld, 1996). According to Huis in't Veld (1996), a change in extrinsic conditions such as refrigeration is the only way to delay the spoilage. The first signs of spoilage are caused by the formation of sweet-smelling esters followed by rancid sulfur compounds (Dainty et. al, 1983). Rancid odors are produced due to proteins and amino acids decomposing by anaerobic bacteria producing indole, methanethiol, dimethyl disuphide and ammonia volatiles (Dainty, 1996). Rancid odors are not associated with microbial growth; they are due to oxidation of unsaturated lipids. The second major cause of the reduced quality of fresh meat is oxidative rancidity. It occurs when unsaturated fats react with oxygen (Cheng et al., 2007; Kramlich et al., 1973). Heat, light and pro-oxidant compounds influence this reaction (Price and Schweigert, 1978). According to Springett

(1993), off-flavors in foods are divided into the three following categories: "(i) off-flavors preformed in the food; (ii) off-flavors formed as a result of cellular disruption; and (iii) off-flavors formed as a consequence of endogenous or microbial enzymes."

The microorganisms that colonize a particular food depend on product characteristics and how it is stored and processed. The parameters affecting the growth of microoorganisms in food 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 in food which include water activity, redox potential, available nutrients and natural antimicrobial agents. Extrinsic parameters include factors such as food storage, temperature and atmospheric composition. Modes of processing and preservation are the physical or chemical treatments that effect changes in a food product's characteristic including the microflora that is associated with the food product. Implicit parameters are synergistic or antagonistic influences that result from the other parameters; therefore, they result 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.

Escherichia coli are Gram-negative, rod shaped bacteria. They are facultative anaerobic and non-spore forming. Optimal growth temperature is at 37°C (Fotadar et al., 2005). Most *E. coli* strains are harmless but some serotypes cause food poisoning and are occasionally responsible for food recalls (CDC Division of Bacterial and Mycotic Diseases, 2011).

Staphylococcus aureus is a Gram-positive bacterium that is spherical, a facultative anaerobe and is non-motile (Raftari, 2009). Symptoms of *Staphylococcus aureus* food poisoning include nausea, vomiting and abdominal cramping (Raftari, 2009). It is one of the most frequent pathogens that contaminates meat (Raftari, 2009). It can be transferred to the carcass surface from skin of cattle, hide, equipment and infected personnel (Gill, 1988). *Staphylococcus aureus* food poisoning outbreaks have been associated with consuming meat, especially undercooked ground beef (Raftari, 2009).

Salmonella is an enteric Gram-negative bacterium that is a genus of the Enterobacteriaceae family. It's motile, facultative anaerobic and non-spore forming. It grows between 8 and 45°C (Guthrie, 1992). They are heat-sensitive and generally killed at 70°C. It is a foodborne pathogen that has been repeatedly occurring in fecally contaminated foods such as peanut butter, sprouts, almonds, eggs, meat and poultry products (Mani-Lopez, 2011). Symptoms of salmonellosis include gastroenteritis, bacteremia and typhoid fever, abdominal pain and diarrhea (Darwin and Miller, 1999). Salmonellosis has caused significant economic losses in many countries due to illness, deaths, medical costs, litigations and recalls (Buzby et al., 2001). Salmonellosis has remained unchanged during 2005-2009 and is consistently the most common bacterial pathogen in laboratory confirmed food-borne illness (Centers for Disease Control and Prevention [CDC], 2005-2009). *Salmonella* Typhimurium is the most common serotype associated with laboratory-confirmed illnesses.

Serratia marcesens is a Gram-negative rod-shaped bacterium in the Enterobacteriaceae family. It is a human pathogen that is involve in nosocomial

infections (Hejazi and Falkiner, 1997). According to Holmes and Gross (1990), Serratia is highly distributed in nature, including vegetables and products of animal origin.

Channel Catfish (*Ictalurus punctatus*)

The health benefits of seafood are well known. Channel catfish contains health benefits and a wide consumer acceptance. Consuming fish at least one to two times per week results is a positive effect on one's health (De Deckere et al., 1998; Marckmann and Gronbaek, 1999; Thorsdottir et al., 2004). Studies show that fish consumption increases antioxidation activity (Sveinsdottir et al., 2009). If not preserved in a certain way, significant economic losses could result from the highly perishable nature of catfish (Wood, 1981). The culture system of many freshwater fish depends on water sources such as lakes and reservoirs and flooding could reach into ponds where fish are commercially raised for human consumption (Suhalim, 2007). Thus, outdoor fish ponds are continually exposed to contamination by pathogens. Temperature fluctuations and other environmental factors may also influence the survival of pathogens (Suhalim, 2007).

Huss (1995) and Gram and Huss (1996) described a four-phase pattern for the quality of fish after harvest. Aerobes cause the initial growth, which acts on carbohydrates to give up water and carbon dioxide. As the surface becomes covered with microbes, slime builds up, resulting in favorable conditions for anaerobic growth. Trimethylamine oxide is catalyzed to trimethylamine-N-oxide reductase. The breakdown of sulfur-containing amino acids is associated with the off-flavors in fish products. Volatile products include hydrogen sulfide, methyl marcaptan and dimethyl sulfide.

Spoilage of fish is a major problem. Fish have low carbohydrate content and high levels of amino acids (Barmstedt and Auerbach, 1961; Jacquot, 1961). The carbohydrates and amino acids are used to produce spoilage products which contain ammonia, biogenic amines, organic acids and sulfur compounds (Jorgensen et al., 2000; Rodrigues et al., 2003). Trimethylamine (TMA) is responsible for the "fishy" odor (Dainty, 1996). When oxygen levels are depleted, trimethylamine oxide (TMAO) is reduced to TMA by serving as a terminal electron receptor for anaerobic respiration (Easter et al., 1983).

Pathogenic microorganisms may also contaminate fish during processing. According to Kim and Marshall (2002), skin and gut of catfish are critical sources of microbial contamination. The bacteria can spread from the skin and gut to contaminate the entire catfish processing environment (Bal'a et al., 1999). Fish secrete mucus on their surfaces as a natural defense mechanism. The mucus contains immunoglobins and lysozyme, which can inhibit bacteria on the skin of fish (Speare and Mirasalimi, 1992). Although live catfish have defense mechanisms, it is unknown if this function remains after harvesting of the catfish (Suhalim, 2007). The mucus may lose its antibacterial activity after slaughtering. During processing, fish could be contaminated with blood such that inhibitors in the blood serum reduce the antibacterial activity (Hjelmeland et al., 1983; Smith & Ramos, 1976). Robinette et al. (1998) states proteases in the mucus of fish could also inactivate the antibacterial compounds. Temperature abuse, high bacterial count and health condition could also affect this fish mucus function (Suhalim, 2007).

Bacteria isolated from channel catfish (*Ictalurus punctatus*)

Pseudomonas is a Gram-negative rod shaped bacterium. *Pseudomonas* species are the most common spoilage organisms especially in aerobically stored foods with high water content and natural 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, 1996), poultry (Lahellec and Colin, 1979) and dairy products (Walker and Stringer, 1990; Craven and Macauly, 1992a; Craven and Macauly, 1992b; Crave and Macauly, 1992c). They comprise 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 are capable of using a wide range of materials as substrates for growth. In foods of animal origin, non-protein nitrogen factor (NPN) is metabolized and lipases and proteases are produced to release fatty acids and amino acids. The metabolism results in off-odors, off-flavors and rancidity. At later stages, extracellular slime is produced and pigmented growth becomes visible (Dainty and Mackey, 1992; Dainty, 1996).

Aeromonas and *Shewanella* are Gram-negative rod shaped bacteria. They grow rapidly at chilled temperatures and on spoiled foods. They contribute to spoilage of chilled red meat, cured meat, poultry, fish, shellfish and dairy products (Huis in't Veld, 1996). Their spoilage is caused by NPN metabolism. Enzymes from *Aeromonas*, *Shewanella* and other bacteria cause spoilage resulting in off-odors, off-flavors, and slime (Walker and Stringer, 1990).

Techniques that prevent or inhibit bacteria growth

It is very important to reduce the microbial population on food products because of the health and economic problems that are caused by these bacteria. Various strategies have been developed such as chilling, freezing, smoking, sterilization and the addition of food grade acids.

Chilling fresh fish is the most important approach to delay bacteria growth and improve the shelf life further (Randell et al., 1999). CO_2 also has a positive effect on shelf life. CO_2 and MAP (modified atmosphere packaging) reduces the growth of *Shewanella* and *Pseudomonas* (Farber 1991; Hansen et al., 2007; Mendez and Goncalves, 2008; Sivertsvik et al., 2002). Using CO_2 alone would cause it to dissolve in meat and fish resulting in package shrinking and deformation (Gill, 1988; Zhao et al., 1995). A disadvantage of MAP is that the increased package volume would increase transportation costs (Farber, 1991). With vacuum packaging, there is less volume but it is less effective than MAP (Hansen et al., 2007).

Freezing influences fish quality causing changes in shelf life and sensory quality as well as physicochemical changes in lipids and proteins (Kolakowska, 1976). Freezing of fish muscle protein changes the solubility of protein fractions (Connell, 1962), water-holding capacity (Paredi, 1996), activity of proteolytic enzymes (Dixon and Webb, 1971) and texture and sensory quality (Simeonidou, 1997). During freezing, pro-oxidative (Apgar and Hultin, 1982) and high polyunsaturated fatty acid concentrations (Kolakowska, 1981) cause hydrolytic and oxidative changes of fish lipids. During freezing, the tissue is partly damaged by ice crystals facilitating protein diffusion (Szymczak, 2010).

Smoking is another method that improves the microbial safety of fish. In this process, thermal combustion volatiles of wood penetrate fish flesh (Ward, 1995). In industrialized countries, smoking is primarily done to enhance flavor and texture of fish (Dillion et al, 1994). According to Efiuvwevwere and Ajiboye (1996), it prolongs the shelf-life of fish by reducing moisture content. Microbial destruction results from direct heat.

Hurdle technology "advocates the deliberate combination of existing and/or novel preservation techniques to establish a series of preservatives factors to improve the microbial stability and the sensory quality of food as well as their nutritional and economic properties" (Mani-Lopez, 2011). *Sous vide* packaging is a method that was developed by the authors of a study that involves the hurdle effect (Jang, et al., 2006). It involves vacuum-packing raw meat or partially cooked food, pasteurizing in hot water, cooling followed by chilled storage (Creed and 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 Pearlspot 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 (ɣ)-rays interact with electrons of atomic constituents; therefore breaking chemical bonds and electron beam radiation are similar to ɣ-rays in that it alters chemical and molecular bonds. Irradiation destroys microorganisms using ultraviolet (UV), X-ray and ɣ-irradiation. Pasteurization involves mild heat to reduce microorganisms in a food product.

Antimicrobial agents

Antimicrobial agents are chemicals that inhibit or kill microorganism growth. They include chemical preservatives and antiseptics and may be of synthetic or natural origin. They may have a static or cidal effect on microorganisms. Bacteriostatic refers to inhibiting bacterial cell growth. Bactericidal refers to killing bacteria.

Organic acids were approved by the Food Safety and Inspection Service of the United States Department of Agriculture (Skrivanova, 2011). According to Theron and Lues (2007), "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 product.

The pH of a substance is its hydrogen ion concentration or a measure of the acidity or alkalinity of a solution. It is an important factor in influencing the growth of bacteria. According to Jay et al. (2005), microorganisms grow best at a pH around 7, thus

reducing pH can limit bacterial growth. It has been shown that bactericidal action of organic acids decreases the pH in bacterial cells (Malicki, 2004). 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, 2011). Organic acids that are used to inhibit foodborne pathogens and spoilage bacteria in meat are applied by spraying and dipping (Dincer and Baysal, 2004). In a study conducted by Bradley (2011), the addition of citric acid and acetic acid each reduced the growth of Enterobacteriaceae.

Vinegar

Vinegar is an acidic liquid that is made from the fermentation of an alcoholic beverage mainly wine (Nascimento et al., 2003). The principal component of vinegar is acetic acid. The total acidity of vinegar is expressed as acetic acid which is the major organic acid in vinegar. Acetic acid is a monocarboxylic acid. It has a pungent odor and flavor. It is generally regarded as safe (GRAS) 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).

Vinegar may be used as a mixture or alone as a natural flavoring in some salads (Sengun and Karapinar, 2005). These salad dressings provide a harsh environment for

foodborne pathogens such as *Salmonella* and *E. coli* to survive because of the acetic or citric acids (Beuchat et al., 2006). In a study by Frederick et al. (1994), results indicated that 2% acetic acid reduced the incidence of *Salmonella* on pork. In the United States, salad dressings maintain a good safety record and are widely used (Smittle, 2000).

CHAPTER 1

Identification of Acetic Acid in Vinegar and Antimicrobial Activity of Acetic Acid

INTRODUCTION

Ion chromatography has been used to determine the concentration and types of organic acids in vinegar. It's useful for separating organic acids by using a strong ion-exchange resin as the stationary phase (Morales et al., 1998). The stationary phase is an ion-exchange resin. It enables the non-ionic species to penetrate the resin. It's a valuable technique for separating ionic compounds of neutral and weakly acidic or basic substances and is based on ion-exclusion rather than ion-exchange. Weak acids with a low pH acting as nonelectrolytes and are retained strongly by the resin and the stronger acids are eluted first, therefore the pH is an important factor in the separation. In a study by Ashoor and Welty (1984), in order to perform a rapid monitoring of acetic acid in vinegar, a diluted sulphuric aqueous solution was used as the eluent followed by UV detection at 210 nm. Bertolini et al. (1994) used a sulphuric acid solution as the mobile phase at 210 nm to separate the organic acids in vinegar. Acetic acid is the main component in vinegar contributing to its antimicrobial activity.

OBJECTIVES

The objectives were to confirm the presence and concentration of acetic acid in fish and chip vinegar and apple cider vinegar and to measure the antimicrobial activity of acetic acid.

$H_0$1: Acetic acid is not present in fish and chip vinegar and apple cider vinegar.

H_A1: Acetic acid is present in fish and chip and apple cider vinegar.

$H_0$2: Acetic acid does not exert antimicrobial activity on the bacteria.

H_A2: Acetic acid exerts antimicrobial activity on the bacteria.

MATERIALS AND METHODS

Fish and Chip (FC) Vinegar

Crosse and Blackwell Old English Fish and Chip Vinegar 100% Natural Malt (FC) Vinegar was used in this study. It was purchased from a retail store. It contains 5% acetic acid by volume. This vinegar product was chosen because it contains 100% natural malt vinegar.

Apple Cider (AC) Vinegar

Bragg- Unfiltered Apple Cider (AC) Vinegar was used in this study. This product was diluted to 5% acetic acid. It was purchased from a retail store. This product was chosen because of its natural ingredients which include certified organic, raw apple cider vinegar and purified water.

Foodborne pathogens

Nine foodborne pathogens were used in this study. [Refer to General Introduction, Table 1, Page 6].

Ion Chromatography

Components of the vinegar products were separated to identify acetic acid in the samples. The vinegar samples were filtered with 0.22 μm filters (Millex GS Filter Unit, Corrigwohill Co., Cork, Ireland). The pH of FC vinegar was 2.95 and AC vinegar was 3.25 using a pH meter (pH/mV Meter, UltraBASIC, Denver Instrument). The vinegars were neutralized with NaOH using a pH meter. FC vinegar was neutralized to pH 7.35. AC vinegar was neutralized to pH 7.10. The vinegar samples were diluted to 1:10, 1:100 and 1:500 in H_2O. The standard solution used was sodium acetate. Sodium acetate samples were diluted to 10 mM, 1 mM, 0.5 mM, 0.1 mM and 0.02 mM. The instrument used was a Shimadzu LC-20AB Ion Chromatograph (Kyoto, Japan) with a CDD-10 conductivity detector. The filtered vinegar samples were diluted 5000 times and 20 μl were injected into an anion column (Shodex 1C SI-50 4E [Showa Denko, Munich, Germany, Europe]) operating at a temperature of 40°C. The mobile phase was 3.5 mM Na_2CO_3+1.0 mM $NaHCO_3$ and the mobile flow rate was 1 ml/min.

Zone of Inhibition

The zone of inhibition test measured the antimicrobial activity of acetic acid (Acetic acid, Glacial Certified A.C.S. PLUS, Fisher Scientific, Fair Lawn, NJ) on the foodborne pathogens. The pH of 5% acetic acid was 2.38. Each bacterium was inoculated in Tryptic Soy Broth (TSB) for 14 to 16 hours prior to the test to ensure that fresh bacteria were to be tested. The optical density of the bacteria was obtained on a Genesys 105 UV-VIS spectrophotometer (Thermo Scientific, Madison, WI). Saline solution of 0.85% was used to fix the optical density to 0.3 at 600 nm. One hundred μl of each

bacterium was spread onto nutrient agar (NA) plates. Two layers of 6 mm discs were placed onto the NA plates. Discs consisted of distilled H_2O as a negative control and treatments with the acetic acid dilutions of 0, 0.5%, 1%, 2.5% and 5%. Twenty µl of acetic acid was placed onto the discs with a micropipette. Each plate was incubated at the appropriate temperature overnight (*Pseudomonas fluorescens, Pseudomonas putida,* and *Serratia marcescens* at 28°C; *E. coli* DH5 α, *E. coli* 0111, *Pseudomonas aeruginosa, Salmonella* Typhimurium, and *Staphylococcus aureus* at 37°C). The zones of inhibition were measured in diameter with an Ultra-Tech Carbon Fiber Composite Digital caliper (Penn Tool Co., Maple, NJ). The acetic acid dilutions were the same acetic acid dilutions of vinegar used in the next chapter to determine if the antimicrobial activity of the vinegars is due to acetic acid.

RESULTS

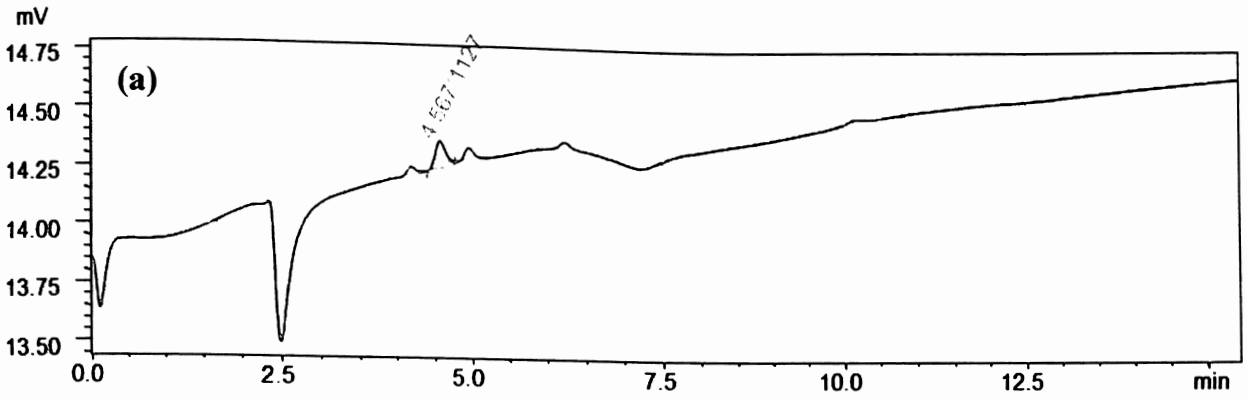
Ion Chromatography

The vinegar products were separated to identify acetic acid by ion chromatography. The standard sodium acetate at different dilutions came in at a peak ranging from 4.57 to 4.61 min. (Figure 1-1). The vinegar samples came in at a peak at 4.58 min. (Figure 1-2). The acetate concentration is calculated based on the peak area. Table 1-1 shows the different concentrations of the standard of acetate ions (0.020, 0.050, 0.100, 0.500, and 1.00 mM) and peak areas. Table 1-2 shows the concentrations of the vinegar samples and peak areas after being diluted 5000 times. FC vinegar had a concentration of 0.175 mM and AC vinegar had a concentration of 0.200 mM acetate ion.

The concentration of FC vinegar was 8.75×10^2 mM (52.5 g/L) acetic acid (Table 1-3). The concentration of AC vinegar was 1.00×10^3 mM (60 g/L) acetic acid.

Zone of Inhibition

The antimicrobial activity of acetic acid was quantified on foodborne pathogens by using the zone of inhibition test. Acetic acid dilutions tested included 0, 0.5, 1, 2.5 and 5%. Negative control consisted of distilled water. The order of the pathogens sensitivity to acetic acid was ranked by 5% in Table 1-4. Acetic acid dilution of 0.5% was not effective against *E. coli* DH5α, *Pseudomonas fluorescens*, *Serratia marcescens*, and *Salmonella* Typhimurium at 0.5% acetic acid and *Staphylococcus aureus* was not effective at all acetic acid dilutions used. Although *E. coli* DH5α was one of the bacteria not affective in all acetic acid dilutions used, it was the bacteria with the largest clear zone with the 5% acetic acid treatment (Figure 1-3). The most sensitive strains tested were *E. coli* DH5α, *Pseudomonas fluorescens* and *Serratia marcescens* with inhibition zones ranging from 5.45 to 7.09 mm in diameter (Figure 1-3). The zone of inhibition of *Pseudomonas* strains ranged from 4.81 to 6.07 mm in diameter. The smallest inhibition zone was produced by *Salmonella* Typhimurium with 5% acetic acid inhibition zone measuring at 1.09 mm in diameter (Table 1-4). *Staphylococcus aureus* showed no clear zones. *Staphylococcus aureus* was the only Gram positive bacterium tested in this study and the only bacterium that is resistant to the antimicrobial activity of acetic acid. The acetic acid dilutions tested in this study did not seem to inhibit the growth of *Staphylococcus aureus.*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14.25
14.00
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(a)
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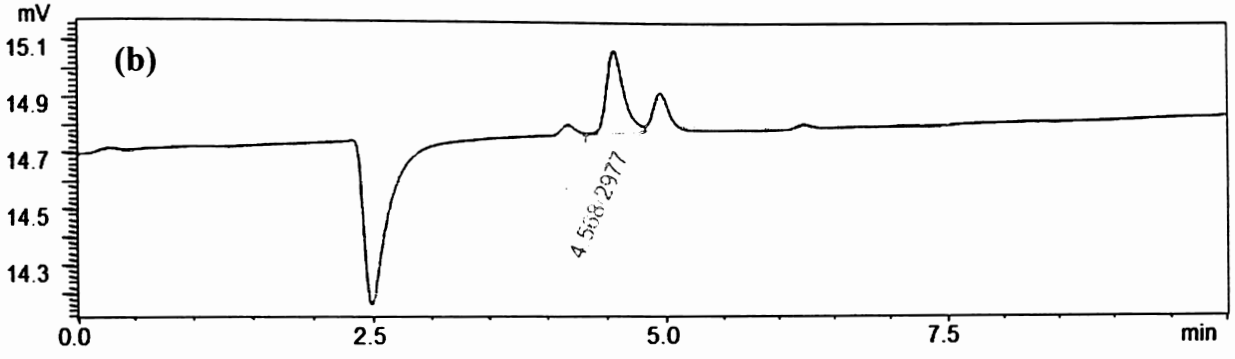
10.0
12.5
min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14.5
14.3
(b)
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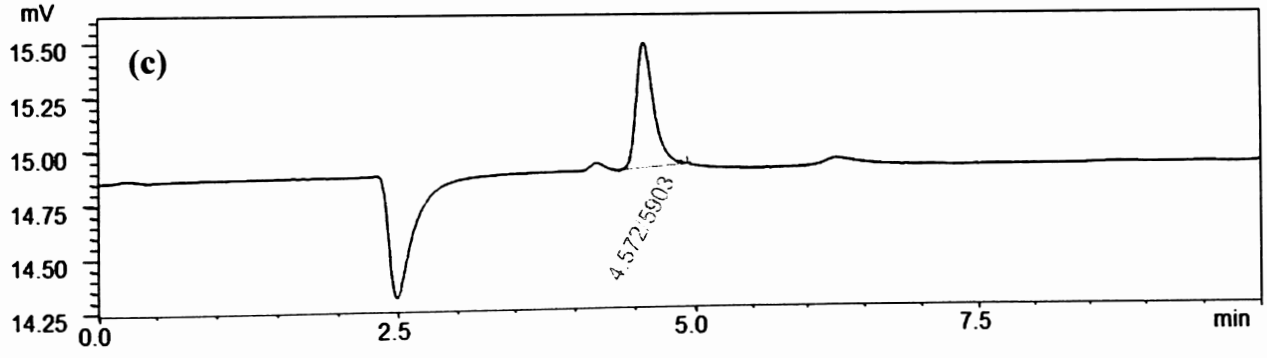
2.5
5.0
7.5
min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(c)
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7.5
min

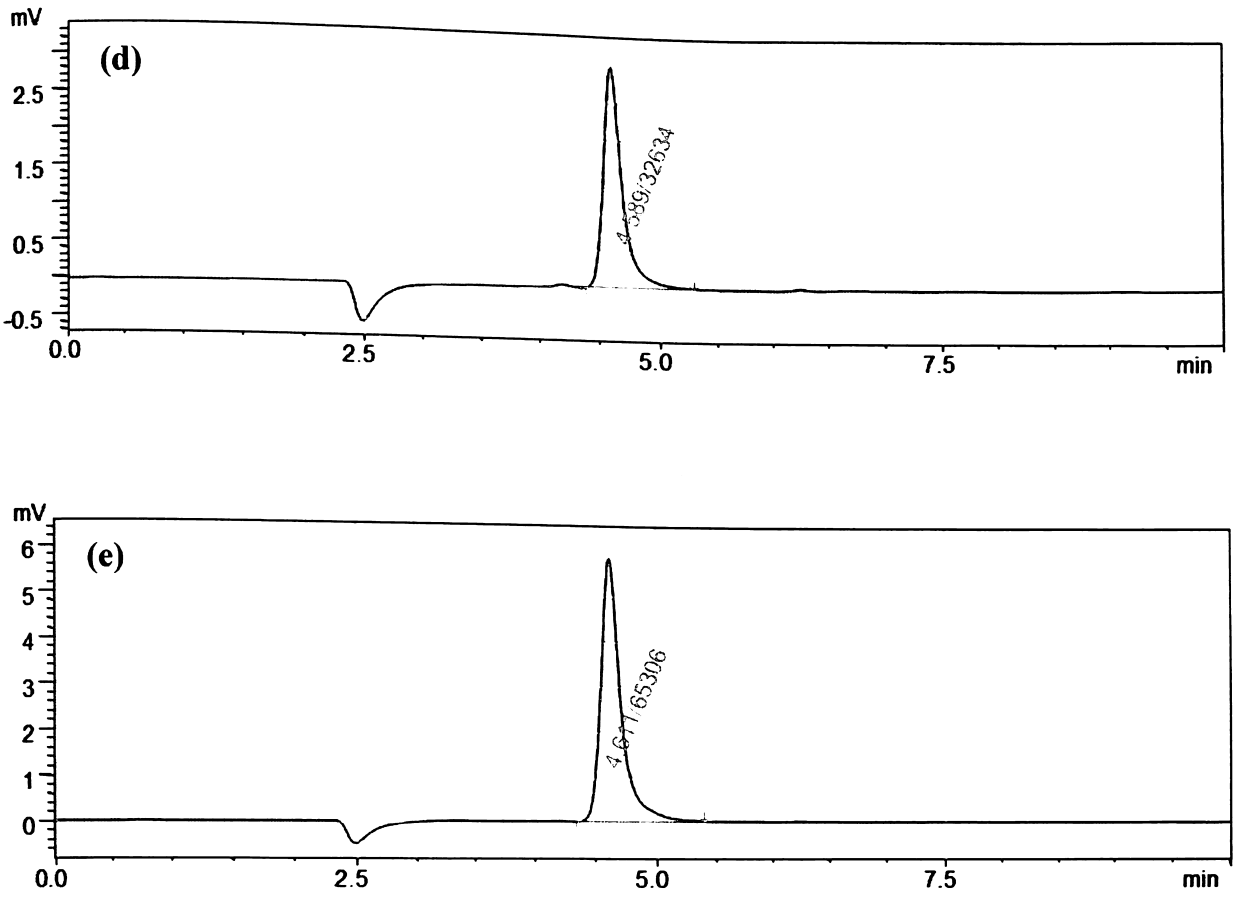


Figure 1-1: *Chromatograph of the standard with different dilutions.* (a) 0.020 mM acetate with a peak at 4.57 min with 1127 mM concentration. (b) 0.050 mM acetate with a peak at 4.57 min with 2977 concentration. (c) 0.10 mM acetate with a peak at 4.57 min with 5903 mM concentration. (d) 0.500 mM acetate with a peak at 4.59 min with 32634 concentration. (e) 1.00 mM acetate with a peak at 4.61 min with 65306 concentration.

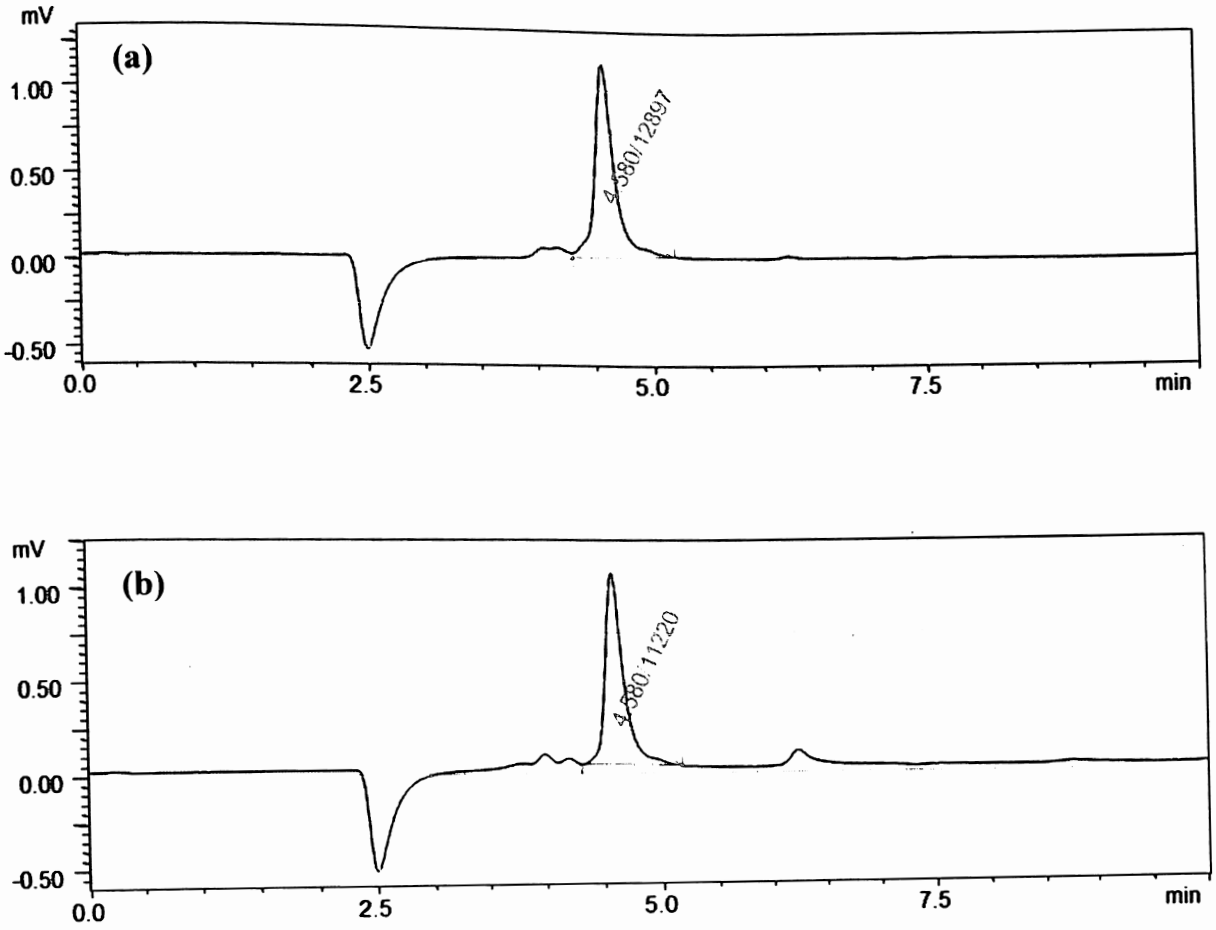


Figure 1-2: *Chromatograph of fish and chip and apple cider vinegar.* (a) Chromatograph of fish and chip vinegar diluted 5000 times with a peak at 4.58 min with 12897 concentration. (b) Chromatograph of the apple cider vinegar diluted 5000 times with a peak at 4.58 min with 11220 mM concentration.

Table 1-1: *Standards of acetate ion concentrations and peak areas.*

Concentration (mM)*	Peak area
1. 0.020	1127
2. 0.050	2977
3. 0.100	5903
4. 0.500	32634
5. 1.00	65306

*Calibration curve: (mM)=0.0040+0.0000152*Area, r=0.9999

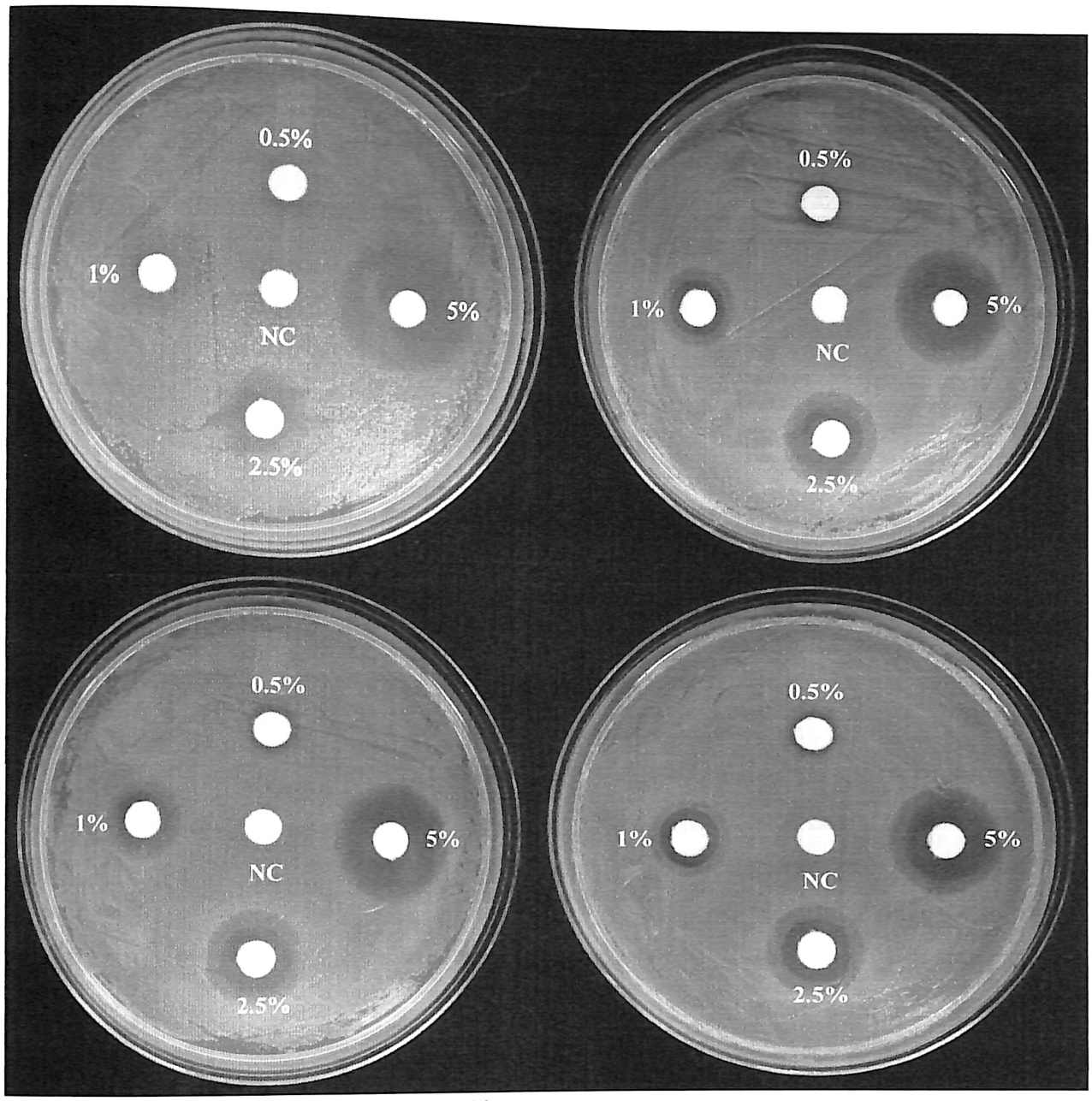
Table 1-2: *Vinegar samples diluted 5000 times and peak areas.*

Sample	Concentration (mM)	Peak area
1. Fish and Chip Vinegar	0.175*	11220
2. Apple Cider Vinegar	0.200*	12897

*Vinegar samples were diluted 5000 times.

Table 1-3: *Vinegar samples acetic acid concentrations.*

Sample	Acetic acid concentration (mM)	Acetic acid concentration (g/L)
1. Fish and Chip Vinegar	8.75×10^2	52.5
2. Apple Cider Vinegar	1.00×10^3	60.0



*Diameter of the zones of inhibition measured in mm.
*Ranked from largest to smallest zone of inhibition by 5% acetic acid.

Figure 1-3: *Foodborne pathogens with largest inhibition zone from acetic acid.* Top left: *Escherichia coli* DH5α - 7.09 mm. Top right: *Pseudomonas fluorescens* (Carolina bio) - 6.07 mm. Bottom left: *Serratia marcescens*- 5.82. Bottom right: *Pseudomonas fluorescens* (ATCC)- 5.45 mm. NC=Negative Control.

Table 1-4: *Zone of inhibition of foodborne pathogens from various dilutions of acetic acid.*

Foodborne pathogen	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%[1]
Escherichia coli DH5α	0	1	4.13	7.09
Pseudomonas fluorescens (Carolina Bio)	0	2.34	4.19	6.07
Serratia marcescens	0	2.37	3.75	5.82
Pseudomonas fluorescens (ATCC)	0	1.98	3.66	5.45
Pseudomonas putida	1.04	2.37	3.22	4.83
Pseudomonas aeruginosa	0.82	2.53	3.71	4.81
Escherichia coli 0111	0.89	2.25	2.24	4.33
Salmonella Typhimurium	0	0.40	0.87	1.09
Staphylococcus aureus	0	0	0	0

*Measurements were an average of two runs.
*Diameter of the zones of inhibition measured in mm.
[1]Ranked from largest to smallest zone of inhibition by 5% acetic acid.

DISCUSSION

A mobile phase consisting of sodium acetate at a flow rate of 1 ml/min and an anion column temperature of 40°C were the selected chromatographic conditions to develop the ion chromatography method. Various concentrations of the standard, sodium acetate (0.020, 0.050, 0.100, 0.500, and 1.00 mM) were used. Acetic acid in the vinegars was identified by peaks at 4.58 min. matching the spectrum of the standard, sodium acetate indicating the presence of acetic acid in the two vinegar samples. FC vinegar's acetic acid concentration was 0.175 mM (52.5 g/L). The acetic acid concentration of AC vinegar was 0.200 mM (60 g/L).

Various dilutions of acetic acid were tested on nine foodborne pathogens. Acetic acid served as an antimicrobial agent in all dilutions tested except *E. coli* DH5α, *Pseudomonas fluorescens*, *Serratia marcescens*, and *Salmonella* Typhimurium for 0.5%, and *Staphylococcus aureus* in all acetic acid dilutions. In a study by Frederick et al. (1994), the spraying of 2% acetic acid reduced the incidence of *Salmonella* by 67% on pork cheek meat and reduced aerobic plate and coliform counts. Anderson and Marshall (1989) demonstrated that the application of 3% acetic acid was effective in sanitizing beef muscle inoculated with *E. coli* and *Salmonella* Typhimurium. The bacteria with the largest inhibition zone included *E. coli* DH5α, *Pseudomonas fluorescens* and *Serratia marcescens*. Following were *Pseudomonas* strains ranging from a zone of inhibition of 4.81 to 6.07 mm and *E.coli* 0111 with a diameter measure of 1.09 mm. These strains included *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *Pseudomonas fluorescens*.

Staphylococcus aureus was the only bacteria that didn't result in any clear zones and was also the only Gram positive bacterium tested in this study. Gram negative

bacteria have an outer membrane and Gram positive bacteria lack an outer membrane. According to Todar (2009), bacterial structure surface of Gram negative bacteria may act as permeability barriers that allow selective passage of nutrients and exclusion of harmful substances such as antimicrobial agents. These results were different from other studies. In a study by Raftari et al. (2009), the spraying of acetic acid dilutions of 1, 1.5 and 2% were effective at reducing *Staphylococcus aureus* on meat tissues. According to Beuchat et al (2006), vinegar based salad dressings provide a harsh environment for *Staphylococcus* to survive due to the presence of acetic acid. Although this bacterial strain was not inhibited by acetic acid in this study, other techniques were performed in these studies resulting in acetic acid exerting antimicrobial activity and inhibiting the growth of *Staphylococcus aureus*.

CHAPTER 2

Antimicrobial Activity of Vinegar on Foodborne Pathogens

INTRODUCTION

"Zone of inhibition" is a commonly used method for evaluating antimicrobial agents (Zaika, 1987). The antimicrobial liquid is deposited onto a small filter paper disk which is then placed onto an agar plate inoculated with the test bacteria. The plates are incubated for a suitable period of time at the appropriate temperature and observed for microbial growth. If the antimicrobial agent exerts antimicrobial activity, the bacteria will not grow in the area surrounding the filter paper disk. The diameter of the clear area or "zone of inhibition" is measured in mm. The determination of the "zone of inhibition" by filter paper disk diffusion is a simple, convenient and widely used method (Zaika, 1987). In this study, the antimicrobial activity of fish and chip and apple cider vinegar was measured on nine foodborne pathogens.

Minimum Inhibitory Concentration (MIC) is the lowest concentration of the compound capable of inhibiting the growth of an organism as stated by Mann and Markham (1998). This measures the effectiveness of a compound. Broth and agar dilution methods are two methods commonly used to determine the MIC. In this study, the agar dilution method was used to determine the lowest concentration that exerted antimicrobial activity on nine foodborne pathogens.

OBJECTIVE

The objective was to measure the antimicrobial activity of different acetic acid dilutions of two vinegar samples on nine foodborne pathogens.

- **H_0:** The two vinegar samples do not exert antimicrobial activity on the foodborne pathogens.

H_A: The two vinegar samples exert antimicrobial activity on the foodborne pathogens.

MATERIALS AND METHODS

Fish and Chip (FC) Vinegar

Fish and chip vinegar was used in this study [Refer to Chapter 1, Materials and Methods, Fish and Chip Vinegar, Page 20]. Dilutions of fish and chip vinegar to 0.5, 1, 2.5, and 5% acetic acid were used.

- FC 5%: Fish and chip vinegar contains 5% acetic acid.
- FC 2.5%: Fish and chip vinegar diluted to 2.5% acetic acid.
- FC 1%: Fish and chip vinegar diluted to 1% acetic acid.
- FC 0.5%: Fish and chip vinegar diluted to 0.5% acetic acid.

Apple Cider (AC) Vinegar

Apple cider vinegar was used in this study [Refer to Chapter 1, Materials and Methods, Apple Cider Vinegar, Page 20]. Dilutions of apple cider vinegar to 0.5, 1, 2.5, and 5% acetic acid were used.

- AC 5%: Apple cider vinegar contains 5% acetic acid.
- AC 2.5%: Apple cider vinegar diluted to 2.5% acetic acid.
- AC 1%: Apple cider vinegar diluted to 1% acetic acid.
- AC 0.5%: Apple cider vinegar diluted to 0.5% acetic acid.

Foodborne pathogens

Nine foodborne pathogens were used in this study. [Refer to General Introduction, Table 1, Page 6].

Zone of Inhibition

The modified zone of inhibition method was used [Refer to Chapter 1, Materials and Methods, Zone of Inhibition, Page 21]. The zone of inhibition test measured the antimicrobial activity of the FC and AC vinegar on the foodborne pathogens. The vinegar samples were filtered prior to testing with 0.22 µm filters (Millex GS Filter Unit, Corrigwohill Co., Cork, Ireland).

Minimum Inhibitory Concentration (MIC)

The modified zone of inhibition method was used [Refer to Chapter 1, Materials and Methods, Zone of Inhibition, Page 21]. The acetic acid dilutions of vinegar tested on

the foodborne pathogens ranged from 0.01 to 0.5%. The vinegar samples were filtered prior to testing with 0.22 μm filters (Millex GS Filter Unit).

RESULTS

Fish and Chip (FC) Vinegar

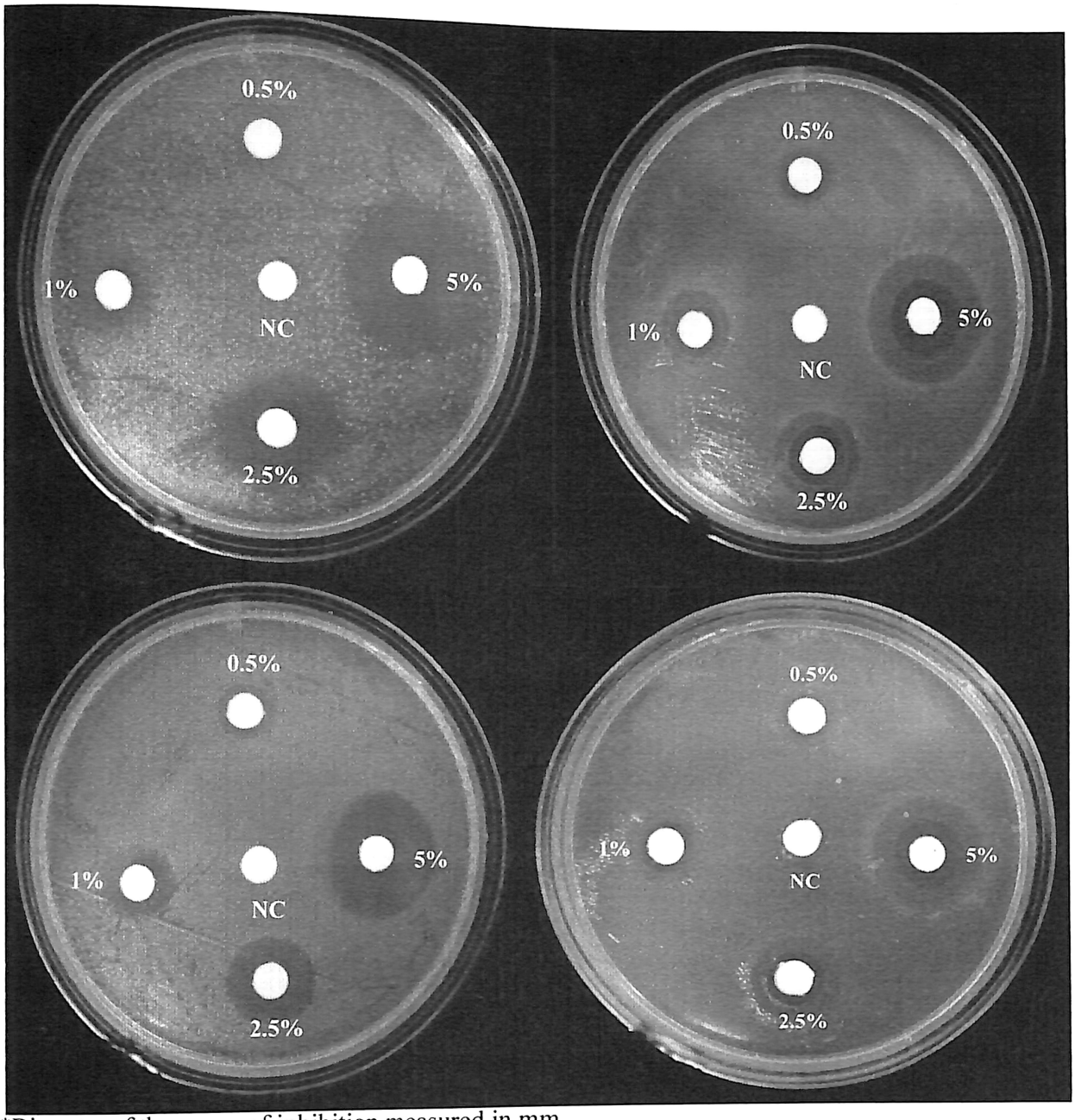
Fish and chip vinegar was effective at all acetic acid dilutions of FC tested except against *E. coli* 0111, *Salmonella* Typhimurium and *Staphylococcus aureus* (Table 2-1). FC 0.5% and 1% were not effective for *Escherichia coli* 0111 and *Salmonella* Typhimurium. *Staphylococcus aureus* was not affected at any acetic acid dilution of FC vinegar used. The largest clear zone in *E. coli* DH5α was with FC 5% acetic acid (9.82 mm) (Figure 2-1) and *Salmonella* Typhimurium had the smallest zone (0.89 mm) (Table 2-1). The *Pseudomonas* strains ranged from a zone of inhibition of 5.89 to 7.85 mm in diameter. *Staphylococcus aureus* showed no clear zones (Table 2-1). The acetic acid dilutions of FC vinegar tested in this study did not inhibit the growth of *Staphylococcus aureus*.

The Minimum Inhibitory Concentration (MIC) of FC vinegar was determined on the nine foodborne pathogens. *Escherichia coli* DH5α demonstrated the lowest minimum inhibitory concentration of FC 0.08% acetic acid. This was followed by *Pseudomonas* strains: *Pseudomonas fluorescens* with FC 0.2% acetic, *Psuedomonas putida* with FC 0.2%, and *Pseudomonas aeruginosa* with FC 0.4% acetic acid (Table 2-2). *E. coli* 0111, *Serratia marcescens,* and *Salmonella* Typhimurium gave the highest minimum inhibitory concentration of FC 2.5% acetic acid (Table 2-2).

Apple Cider (AC) Vinegar

Apple cider vinegar was effective at all dilutions tested except against AC 0.5% in *E. coli* DH5α, *E. coli* 0111, and *Salmonella* Typhimurium (Table 2-3). *Staphylococcus aureus* was not effective at any acetic acid dilution of AC vinegar used. *Serratia marcescens* resulted in the largest zone of inhibition with AC 5% with a diameter measured at 7.44 mm (Figure 2-2) and *Salmonella* Typhimurium was the smallest with a diameter measure of 4.84 mm (Table 2-3). The Pseudomonas strains ranged from a zone of inhibition of 5.19 to 6.96 mm in diameter. *Staphylococcus aureus* showed no clear zones (Table 2-3). *Staphylococcus aureus* was the only Gram positive bacterium tested in this study and the only bacterium that is resistant to the antimicrobial activity of AC vinegar. The acetic acid dilutions of AC vinegar tested in this study did not seem to inhibit the growth of *Staphylococcus aureus*.

The MIC of AC vinegar was determined on the nine foodborne pathogens. *Pseudomonas aeruginosa* demonstrated the lowest minimum inhibitory concentration of AC 0.1%. This was followed by the other *Pseudomonas* strains: *Pseudomonas putida* with AC 0.1%, *Pseudomonas fluorescens* with AC 0.2% and AC 0.3% (Table 2-4). *E. coli* strains and *Salmonella* Typhimurium resulted in the highest minimum inhibitory concentration of AC 1%. (Table 2-4).



*Diameter of the zones of inhibition measured in mm.
*Ranked from largest to smallest zone of inhibition by 5% acetic acid dilution of fish and chip vinegar.

Figure 2-1: *Foodborne pathogens with largest inhibition zone from fish and chip vinegar.* Top left: *Escherichia coli* DH5α – 9.82 mm. Top right: *Pseudomonas fluorescens* (ATCC) - 7.85 mm. Bottom left*: Pseudomonas fluorescens* (Carolina bio) - 7.07 mm. Bottom right: *Pseudomonas aeruginosa* - 6.09 mm. NC= Negative Control.

Table 2-1: *Zone of inhibition of foodborne pathogens from various acetic acid dilutions of fish and chip vinegar.*

Foodborne pathogen	Inhibition zone at various dilutions (mm)			
	0.5 %	1%	2.5%	5%[1]
1. *Escherichia coli* DH5α	0.88	3.05	7.20	9.82
2. *Pseudomonas fluorescens* (ATCC)	0.93	2.19	3.50	7.85
3. *Pseudomonas fluorescens* (Carolina bio)	1.11	1.73	3.39	7.07
4. *Pseudomonas aeruginosa*	1.37	2.68	3.52	6.09
5. *Pseudomonas putida*	2.21	3.14	4.53	5.89
6. *Escherichia coli* 0111	0	0	3.41	5.51
7. *Serratia marcescens*	0.99	2.40	3.40	5.27
8. *Salmonella* Typhimurium	0	0	0.70	0.89
9. *Staphylococcus aureus*	0	0	0	0

*Measurements were an average of two runs.
* Diameter was measured in mm.
[1]Ranked from largest to smallest zone of inhibition by 5% acetic acid dilution of fish and chip vinegar.

Table 2-2: *Minimum inhibitory concentration of fish and chip vinegar on foodborne pathogens.*

Foodborne pathogen	Minimum Inhibitory Concentration
1. *Escherichia coli* DH5α	0.08
2. *Pseudomonas fluorescens* (ATCC)	0.2
3. *Pseudomonas fluorescens* (Carolina Bio)	0.2
4. *Pseudomonas putida*	0.2
5. *Pseudomonas aeruginosa*	0.4
6. *Escherichia coli* 0111	2.5
7. *Serratia marcescens*	2.5
8. *Salmonella typhimurium*	2.5
9. *Staphylococcus aureus*	ND[1]

*MIC is ranked from low to high concentration.
[1]ND = Not Detected.

*Diameter of the zones of inhibition measured in mm.
*Ranked from largest to smallest zone of inhibition by 5% acetic acid dilution of apple cider vinegar.

Figure 2-2: *Foodborne pathogens with largest inhibition zone from apple cider vinegar.* Top left: *Serratia marcescens* - 7.44 mm. Top right: *Pseudomonas fluores*cens (ATCC) - 6.96 mm. Bottom left: *Pseudomonas fluorescens* (Carolina bio) - 6.40 mm. Bottom right: *Escherichia coli DH5α* - 6.34 mm. NC= Negative Control.

Table 2-3: *Zone of inhibition of foodborne pathogens from various acetic acid dilutions of apple cider vinegar.*

Foodborne pathogen	Inhibition zone at various dilutions (mm)			
	0.5 %	**1%**	**2.5%**	**5%**[1]
1. *Serratia marcescens*	1.92	2.74	5.15	7.44
2. *Pseudomonas fluorescens* (ATCC)	1.96	2.74	5.55	6.96
3. *Pseudomonas fluorescens* (Carolina Bio)	1.00	3.14	4.35	6.40
4. *Escherichia coli* DH5α	0	2.59	2.73	6.34
5. *Pseudomonas aeruginosa*	1.55	2.54	4.96	6.29
6. *Escherichia coli* 0111	0	2.54	3.42	5.41
7. *Pseudomonas putida*	1.10	2.09	3.56	5.19
8. *Salmonella* Typhimurium	0	1.03	1.91	4.84
9. *Staphylococcus aureus*	0	0	0	0

*Measurements were an average of two runs.
*Diameter of the zones of inhibition measured in mm.
*Ranked from largest to smallest zone of inhibition by 5% acetic acid dilution of apple cider vinegar.

Table 2-4: *Minimum inhibitory concentration of apple cider vinegar on foodborne pathogens.*

Foodborne pathogen	Minimum Inhibitory Concentration
1. *Pseudomonas aeruginosa*	0.1
2. *Pseudomonas putida*	0.1
3. *Pseudomonas fluorescens* (Carolina Bio)	0.2
4. *Pseudomonas fluorescens* (ATTC)	0.3
5. *Serratia marcescens*	0.3
6. *Escherichia coli* DH5α	1
7. *Escherichia coli* 0111	1
8. *Salmonella* Typhimurium	1
9. *Staphylococcus aureus*	ND[1]

*MIC is ranked from low to high concentration.
[1]ND = Not Detected.

DISCUSSION

The antimicrobial activity of acetic acid dilutions (0.5, 1, 2.5 and 5%) of FC and AC vinegar were tested on nine foodborne pathogens. In this study, FC vinegar exerted the largest zone of inhibition with FC 5% (pH 2.95) with a diameter measure of 9.82 mm (Figure 2-1). For AC vinegar, *Serratia marcescens* resulted in the largest AC 5% (pH of 3.25) zone of inhibition with a diameter measure of 7.44 mm (Figure 2-2). In a study by Sengun and Karapinar (2004), vinegar containing 4.03% (v/v) acetic acid resulted in a reduction of *Salmonella* Typhimurium counts inoculated in carrot samples. In a study conducted by Makino et al. (2000), chaff vinegar, made by recycling rice chaff wastes, with a pH of 2.8 to 3.3 decreased the count of *Salmonella* Typhimurium. All nine of the foodborne pathogens were less affective by FC vinegar than by AC vinegar, except for *Psuedomonas aeruginosa* and *Salmonella* Typhimurium. The zones of inhibition of FC vinegar as well as AC vinegar are presented in Tables 2-1 and 2-3, respectively.

Neither vinegar sample inhibited the growth of *Staphylococcus aureus* in this study. Gram negative bacteria have an outer membrane and gram positive bacteria lack an outer membrane. According to Todar (2009), surface structure of Gram negative bacteria may act as permeability barriers that allow selective passage of nutrients and exclusion of harmful substances such as antimicrobial agents. These results were not in agreement with Beuchat et al. (2006) who showed that salad dressings containing vinegar provide a harsh environment for *Staphylococcus* to survive due to the presence of acetic acid. Although this bacteria strain was ineffective with the zone of inhibition technique in this study, other techniques were performed in these studies resulting in vinegar exerting antimicrobial activity on *Staphylococcus aureus*.

The zone of inhibition results from the previous chapter in which acetic acid was tested on the nine foodborne pathogens, were similar to the results of the inhibitory activity of the vinegar samples, confirming that the antimicrobial activity of the two vinegars are due to acetic acid. Acetic acid and the vinegar samples did not inhibit the growth of *Staphylococcus aureus*. Acetic acid and the vinegar samples (5%) exerted antimicrobial activity on all other foodborne pathogens tested with zone of inhibition from 5% acetic acid measuring from 1.09 to 7.09 mm in diameter (Table 1-4), FC vinegar from 0.89 to 9.82 mm in diameter (Table 2-1) and AC vinegar from 4.84 to 7.44 mm in diameter (Table 2-3).

The lowest concentration that exerted antimicrobial activity was determined on the nine foodborne pathogens. Table 2-2 shows the MIC of FC vinegar on foodborne pathogens. *E. coli* DH5α showed the lowest MIC of FC 0.08% following the *Pseudomonas* strains with FC 0.2 and FC 0.4%. *E. coli* 0111, *Serratia marcescens* and *Salmonella* Typhimurium resulted with the highest minimum inhibitory concentration of FC 2.5%. This means that a concentration as low as FC 0.08% can inhibit the growth of *E. coli* DH5 and a concentration as low as FC 2.5% can inhibit the growth of *Salmonella* Typhimurium. Table 2-4 shows the MIC of AC vinegar on foodborne pathogens. *Pseudomonas aeruginosa* and *Pseudomonas putida* showed the lowest MIC at AC 0.1% following *Pseudomonas fluorescens* with AC 0.2% and AC 0.3% and *Serratia marcescens* with AC 0.3%

E. coli strains and *Salmonella* Typhimurium had the highest MIC at AC 1%. This means that a concentration as low as AC 0.1% can inhibit the growth of *Pseudomonas*

aeruginosa and *Pseudomonas putida* and a concentration as low as AC 1% can inhibit the growth of *E. coli* strains and *Salmonella* Typhimurium.

FC vinegar resulted in the lowest and highest MIC. The lowest concentration of FC vinegar that is capable of inhibiting the growth of *E. coli* was FC 0.08% and the lowest concentration of FC vinegar capable of inhibiting the growth of *Salmonella* Typhimurium was FC 2.5%. Thus, fish and chip vinegar is the most effective in inhibiting the growth of *E. coli* and the least effective in inhibiting the growth of *Salmonella* Typhimurium in this study.

CHAPTER 3

Antimicrobial Activity of Vinegar on Bacterial Species Isolated from Local and Retail Channel Catfish (*Ictalurus punctatus*)

INTRODUCTION

The antimicrobial activity of vinegar was measured on bacteria isolated from the catfish fillets using zone of inhibition and minimum inhibitory concentration tests. Domestic catfish fillets (*Ictalurus punctatus*) were used. Catfish fillets came from a local seafood market and the Delaware State University Aquaculture Pond. Bacteria derived from catfish fillets were isolated by homogenizing a fillet and 0.85% saline solution in a stomacher bag and spread plating onto Tryptic Soy Agar (TSA) plates. Plates were incubated overnight at 28°C. This was done for two cycles every 2 days until the spoilage point was reached. Bacteria with different morphologies were picked and bridge plates were made. In this study, the bacteria were tested with the vinegar samples from domestic catfish fillets. Bacteria with the largest zone of inhibition from fish and chip vinegar from both the market and pond catfish were identified through 16S rDNA sequencing. Microbial changes were evaluated and sensory evaluation was performed with different acetic acid dilutions of the two vinegars on catfish fillets.

OBJECTIVES

The objectives of this study were: (1) To measure the antimicrobial activity of different acetic acid dilutions of two vinegar samples on bacteria isolated from catfish

from a local seafood market and the Delaware State University Aquaculture Pond. (2) The bacteria derived from catfish fillets were identified by 16S rDNA to better understand which spoilage bacteria could be inhibited by vinegar to increase the quality of fishery products. (3) Microbial changes were evaluated following treatments of different acetic acid dilutions of vinegar marinades on domestic catfish fillets. (4) Sensory evaluation was performed with catfish fillets with different acetic acid dilutions of vinegar marinades to evaluate consumer sensory acceptability.

$H_0$1: Two vinegar samples do not exert antimicrobial activity on bacteria isolated from a local seafood market and the Delaware State University Aquaculture Pond.

H_A1: Two vinegar samples exert antimicrobial activity on bacteria isolated from a local seafood market and the Delaware State University Aquaculture Pond.

$H_0$2: There is a not a difference in microbial change following treatments of different acetic acid dilutions of vinegar marinades on domestic catfish fillets.

H_A2: There is a difference in microbial change following treatments of different acetic acid dilutions of vinegar marinades on domestic catfish fillets.

$H_0$3: Consumers will not accept the sensory attributes of the acetic acid dilutions of vinegar marinades on baked catfish fillets.

H_A3: Consumers will accept the sensory attributes of the acetic acid dilutions of vinegar marinades on baked catfish fillets.

MATERIALS AND METHODS

Fish and Chip (FC) Vinegar

[Refer to Chapter 1, Materials and Methods, Fish and Chip Vinegar, Page 20 and 33].

Apple Cider (AC) Vinegar

[Refer to Chapter 1, Materials and Methods, Apple Cider Vinegar, Page 20 and 34].

Zone of Inhibition

The modified zone of inhibition method was used [Refer to Chapter 1, Materials and Methods, Zone of Inhibition, Page 21]. The zone of inhibition test measured the antimicrobial activity of FC vinegar and AC vinegar on 240 bacteria isolates from catfish fillets. The vinegar samples were filtered prior to testing with 0.22 μm filters (Millex GS Filter Unit, Corrigwohill Co., Cork, Ireland).

Minimum Inhibitory Concentration (MIC)

The modified zone of inhibition method was used [Chapter 1, Materials and Methods, Zone of Inhibition, Page 21]. The acetic acid dilutions of vinegar tested on the bacteria isolated from catfish fillets from the market and pond that resulted in the largest

inhibition zone from FC vinegar and AC vinegar ranged from 0.01 to 0.5%. The vinegar samples were filtered prior to testing with 0.22 μm filter (Millex GS Filter Unit).

16S rDNA Sequencing

The ten bacteria isolated from catfish fillets from both the market and pond that showed the largest inhibition zone with 5% acetic acid dilution of FC vinegar were selected to be identified. The twenty candidates were identified through 16S rDNA sequencing. Previously prepared glycerol stocks of the twenty candidates were inoculated overnight in Tryptic Soy Broth (TSB). Each candidate was streaked on TSA plates and incubated overnight at 28°C to grow pure colonies. Once pure cultures were obtained from each candidate, plates were sealed with parafilm and delivered for 16S rDNA sequencing performed by GENEWIZ Inc. (South Plainfield, NJ). Seven hundred to one thousand base pairs of each 16S rDNA sequencing data (Refer to Appendix A, Page 97) were used to search for bacterial species using the Genbank database (www.ncbi.nlm.nih.gov/genbank). Bacterial identity was chosen based on 99% or greater matches.

Bacterial Enumeration

Domestic catfish fillets were purchased from a retail store in Dover, Delaware. Fillets were cut and 100 g were added to nine stomacher bags as follows: control, FC 0.03%, FC 0.05%, FC 0.5%, FC 5%, AC 0.03%, AC 0.05%, AC 0.5%, and AC 5%. Acetic acid dilutions of vinegar (250 ml) were poured into each of the nine stomacher bags. The stomacher bags were stored at 4°C for 15 minutes to marinate the fillets in the vinegar. Excess vinegar was drained out and 10 g of fillet and 90 ml of 0.85% saline

solution were homogenized into each of the nine stomacher bags. Ten-fold serial dilutions were performed using TSB and spread onto TSA plates and incubated for 2 days at 25°C. Total bacteria were enumerated. The nine stomacher bags were plated every 2 days until samples reached their spoilage point and the stationary phase of bacterial growth was reached.

Sensory Evaluation

Frozen catfish fillets were purchased from a retail store in Dover, Delaware and stored at 4°C overnight to thaw. Fillets were placed in zip lock bags in the following order: control, FC 0.05%, FC 0.5%, FC 5%, AC 0.05%, AC 0.5% and AC 5%. The control in this study was the same fillet used for the vinegar treated samples, but didn't contain any vinegar. Vinegar with different acetic acid dilutions were poured into each of the nine zip lock bags. The bags were stored at 4°C for 15 minutes to marinate in the vinegar. Excess vinegar was drained out and the fillets were placed on baking pans with aluminum foil to prevent sticking. Each sample was baked in separate ovens (Magic Chef, Wood Dale, Il) to avoid cross-contamination of aromas and flavors between samples. They were baked at 350°F for 10 minutes per inch of thickness or until flaky and with an opaque appearance. Fillets were cut into small cubes using a sterile kitchen knife and placed into small sampling cups. The seven samples were labeled with 3-digit random numbers. Twenty-one panelists consisting of students and faculty from Delaware State University participated in the sensory evaluation. Each panel received a sensory evaluation sheet (Refer Appendix B, Page 108), the seven samples and distilled water to cleanse their pallet after each sample. Appearance (color), flavor, aroma and texture were

evaluated using the Hedonic nine point scale; 1= Dislike extremely and 9= Like extremely.

Statistical Analysis

All sensory evaluation data were analyzed using generalized linear models generated by the SAS System ('SASApp', X64_SRV08, SAS Institute, Cary, NC). Rating was the dependent variable. The analysis was based on the Hedonic nine-point scale of attributes including appearance (color), aroma, flavor, and texture per sample (Control, FC 0.05%, FC 0.5%, FC 5%, AC 0.05%, AC 0.5% and AC 5%). A confidence interval of 95% and alpha level (α) 0.05 was used for the statistical analysis. Values of $p < 0.05$ were deemed significant.

RESULTS

Zone of Inhibition

Various acetic acid dilutions of FC vinegar and AC vinegar were tested on bacteria isolated from catfish fillets from the market and pond. The order of the bacteria sensitivity to the vinegar was ranked by 5% acetic acid of vinegar. The largest zone of inhibition of market catfish from FC vinegar was 12.30 mm in diameter (Figure 3-1) and was higher from the pond measuring at 14.98 mm in diameter (Figure 3-2). The largest zone of inhibition of market catfish from AC vinegar was 14.77 (Figure 3-3) mm and lower for pond measuring at 13.77 mm in diameter (Figure 3-4). The lowest 5% acetic acid of vinegar zone of inhibition for market catfish treated with FC vinegar measured at 4.10 mm for bacteria number 6 from cycle 2, bag 4 (Table 3-1), pond catfish from FC vinegar at 0.77 mm for bacteria number 4 from cycle 2, bag 2 (Table 3-2), market catfish

from AC vinegar at 1.86 mm in diameter for bacteria 5 from cycle 2, bag 1 (Table 3-5) and pond catfish from AC vinegar at 0.55 mm in diameter for bacteria 3 from cycle 1, bag 4 (Table 3-6). Both vinegar samples exert antimicrobial activity at all acetic acid dilutions of vinegar (0.5, 1, 2.5 and 5%) tested in market catfish (Table 3-1 and Table 3-5) but for pond catfish fillets, smaller acidity concentrations of vinegar tested in this study showed no clear zones (Table 3-2 and Table 3-6). Thus the vinegars exert higher antimicrobial activity on market catfish fillets than pond catfish fillets.

Minimum Inhibitory Concentration (MIC)

The MIC was determined on the ten bacteria isolated from both market and pond catfish fillets with the largest and smallest zones of inhibition zone by FC and AC 5%. The lowest minimum inhibitory concentration from the bacteria isolated from market catfish fillets was FC 0.05% and FC 0.02% from pond catfish fillets from FC vinegar (Table 3-3). The highest minimum inhibitory concentration was FC 0.3% for both market and pond catfish fillets from FC vinegar (Table 3-4). The lowest minimum inhibitory concentration from bacteria isolated from market catfish was AC 0.03% and AC 0.02% from pond catfish fillets from the inhibitory effect of AC vinegar (Table 3-7). The highest minimum inhibitory concentration was AC 1% for market catfish and AC 2.5% for pond catfish from AC vinegar (Table 3-8).

16S rDNA Sequencing

Bacteria with the largest zone of inhibition from FC vinegar from both the market and pond catfish were identified through 16S rDNA sequencing. These bacteria isolates were chosen to be identified because FC vinegar exerted a higher antimicrobial activity

than AC vinegar when tested on the bacteria isolated from catfish fillets. Table 3-9 shows the identification of catfish bacterial isolates from the market with the similarity percentage, length (base pairs), and Genbank accession number. Table 3-10 shows the identification of catfish bacterial isolates from the pond with the similarity percentage, length (base pairs), and Genbank accession number. Table 3-11 shows how effective FC vinegar was on the bacteria. Out of the twenty bacteria that were identified, eight were *Shewanella putrfaciens* with a zone of inhibition diameter measured at 10-15 mm. *Stenotrophomonas* and *Aeromonas* strains also exhibited a zone of inhibition diameter measured at 10-15 mm. *Enterobacteriaceae bacterium/ Rahnella aquatilis and Pseudomonas fragi* showed a zone of inhibition diameter measured at 8-10 mm. The other *Pseudomonas* strains, *Pantoea* and *Plesiomonas* showed a zone of inhibition diameter measured at 6-8 mm.

Bacterial Enumeration

Domestic catfish fillets with no vinegar treatment and treatments of 0.03, 0.05, 0.5, and 5% acetic acid dilutions of the vinegars were stored at 4°C for 20 days. Figures 3-5 and 3-6 illustrate changes in total bacteria enumerated every two days. The bacteria growth increased slightly less than the control for 0.03 and 0.05 acetic acid of vinegar and was largely reduced after 15 minutes of marinating in vinegar 5%. As the days progress, the control, 0.03 and 0.05% remained close in bacteria growth pattern and count. There was a large reduction in bacterial growth for vinegar 0.5% and then remained constant with bacteria growth. FC 5% maintained a large reduction in bacterial growth from day 0 to day 12 and no bacterial growth was recorded remaining at a constant of 0 CFU/g thereafter (Figure 3-5). AC vinegar 5% maintained a large reduction

in bacterial growth from day 0 to day 16 and remained at a constant of 1 CFU/g thereafter (Figure 3-6).

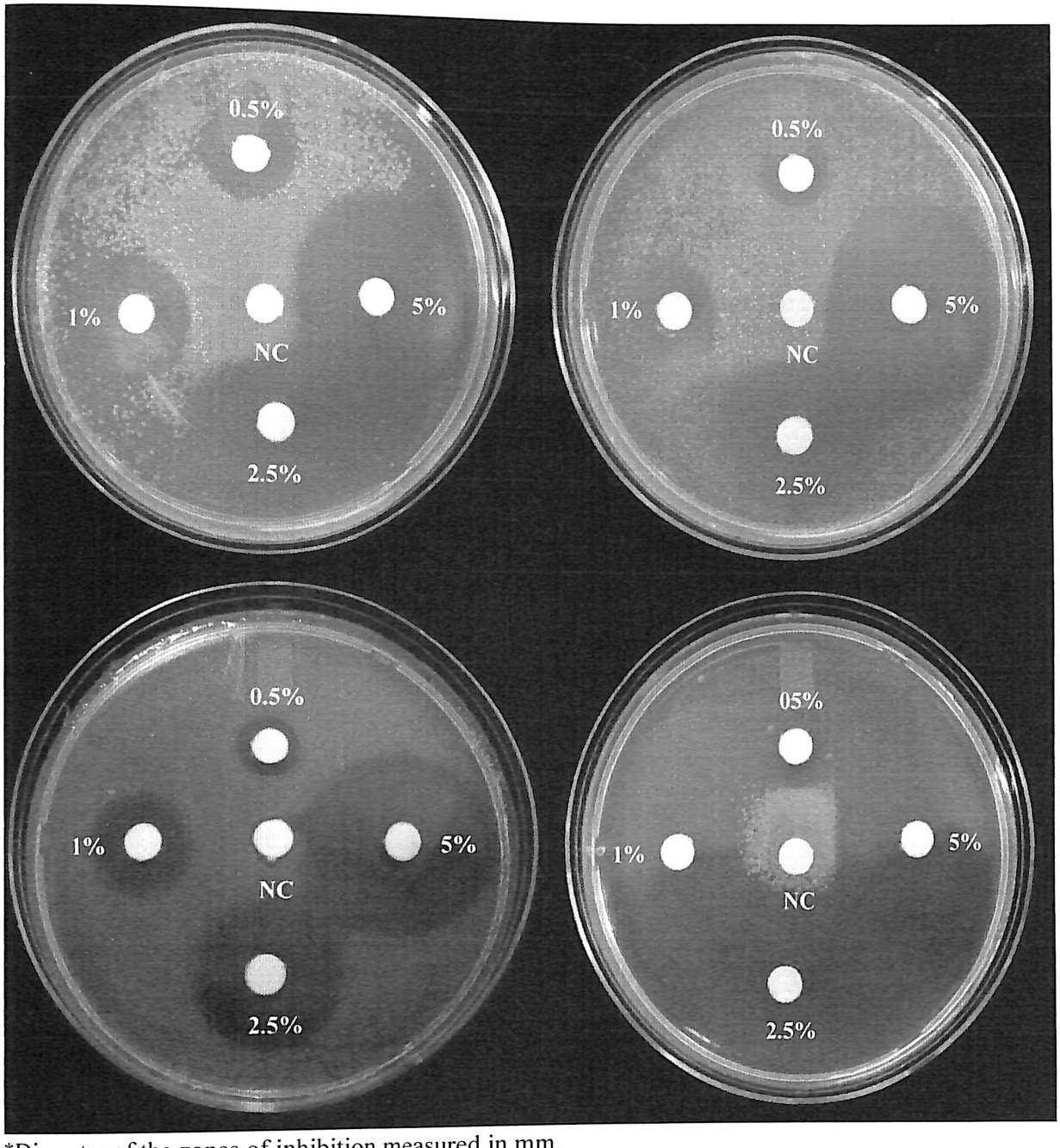
Sensory Evaluation

Twenty-one panelists evaluated four sensory attributes for the seven samples using the Hedonic nine-point scale. The attribute rating frequencies per sample were identified (Refer to Appendix C, Page 110) and the rating average for each attribute per sample were evaluated (Figures 3-7-3 to 3-13). Among the samples treated as control, FC 0.05%, FC 0.5%, FC 5%, AC 0.05%, AC 0.5% and AC 5%, the least liked from the sensory evaluation were aroma and flavor in the catfish fillets marinated with vinegar 5% (FC and AC 5%) compared to the other samples. Differences in appearance (color) and texture were also apparent with the 5% vinegar samples (FC and AC 5%) compared to the other samples. Sample FC 5% showed poorer results than AC 5% on all attributes except appearance. Panelists preferred sample AC 5% over FC 5%, however they seemed to like the appearance of sample FC 5% more.

Minimal differences were obtained among the other samples (control, FC 0.05, FC 0.5, AC 0.05 and AC 0.5%) on all four attributes with averages ranging from 4.81 to 6.43 (Figures 3-7, 3-8, 3-9, 3-11, and 3-12). Figures 3-10 and 3-13 illustrate the averages on the Hedonic Scale of each of the four attributes for the 5% samples. The aroma average for FC 5% was 3.71 (Figure 3-10) and for AC 5%, 4.05 (Figures 3-13). The flavor average for FC 5% was 4 (Figure 3-10) and for AC 5%, 4.76 (Figure 3-13). The appearance average showed slightly higher results for FC 5% compared to AC 5%. The

appearance average for FC 5% was 5.33 (Figures 3-10) and for AC 5%, 4.81 (Figures 3-10).

The statistical analysis was based on the Hedonic nine-point scale of four attributes per seven samples (Refer to Appendix D, Page 119). Values of $p < 0.05$ were deemed significant. According to statistical analysis, there was no interaction effect based on the rating of the attributes. For example, when there was a high rating for texture for one sample, doesn't necessary indicate a high rating for aroma for that sample; the attributes didn't follow the same trend. The most significantly different sample was FC 5% ($p = 0.02$). Statistical analysis show that the attribute aroma of sample FC 5% was the most significant attribute with sample indicating the least liked of the panelists. Aroma was significantly different compared to appearance ($p = 0.04$) and texture ($p = 0.003$). Flavor of sample FC 5% and aroma and flavor of sample AC 5% was also significant among other samples and attributes ($p < 0.05$).

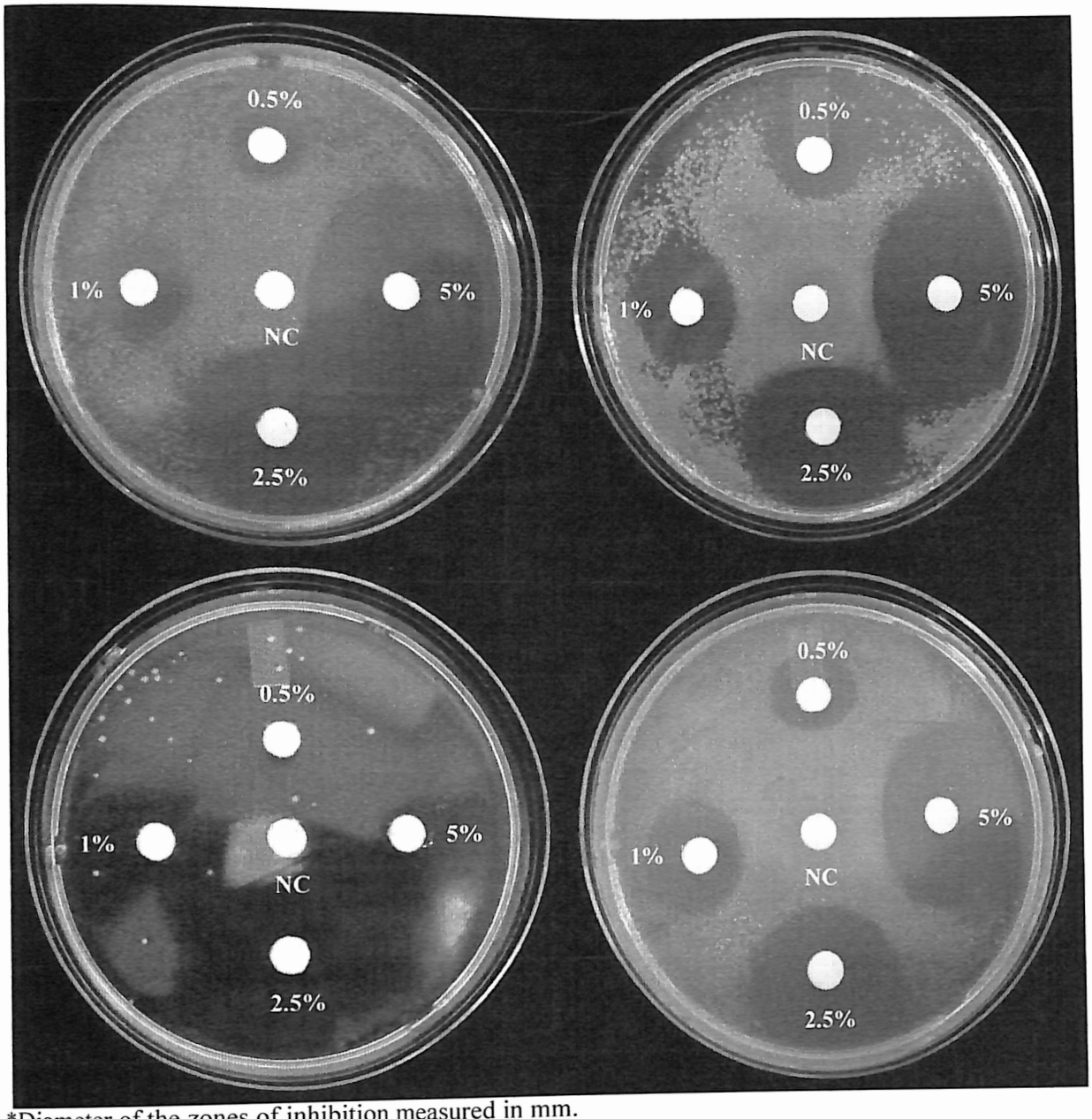


*Diameter of the zones of inhibition measured in mm.
*Ranked from largest to smallest zone of inhibition by 5% acetic acid dilution of fish and chip vinegar.

Figure 3-1: *Bacteria isolated from catfish from the market with the largest zone of inhibition from fish and chip vinegar.* Top left: Cycle 2, Bag 1, Bacteria 2 - 12.30 mm. Top right: Cycle 2, Bag 1, Bacteria 8 - 12.17 mm. Bottom left: Cycle 2, Bag 3, Bacteria 5 - 11.63 mm. Bottom right: Cycle 2, Bag 2, Bacteria 4 - 10.96 mm. NC= Negative Control.

*Diameter of the zones of inhibition measured in mm.
*Ranked from largest to smallest zone of inhibition by 5% acetic acid dilution of fish and chip vinegar.

Figure 3-2: *Bacteria isolated from catfish from the pond with the largest zone of inhibition from fish and chip vinegar.* Top left: Cycle 1, Bag 5, Bacteria 2 -14.98 mm. Top right: Cycle 1, Bag 2, Bacteria 1 - 11.98 mm. Bottom left: Cycle 1, Bag 3, Bacteria 1 - 10.39 mm. Bottom right: Cycle 1, Bag 2, Bacteria 6- 9.08 mm. NC = Negative Control.



*Diameter of the zones of inhibition measured in mm.
*Ranked from largest to smallest zone of inhibition by 5% acetic acid dilution of apple cider vinegar.

Figure 3-3: *Bacteria isolated from catfish from the market with the largest zone of inhibition from apple cider vinegar.* Top left: Cycle 2, Bag 1, Bacteria 8 - 14.77 mm. Top right: Cycle 2, Bag 1, Bacteria 2 - 13.38 mm. Bottom left: Cycle 2, Bag 3, Bacteria 4 - 12.87 mm. Bottom right: Cycle 1, Bag 1, Bacteria 2 - 11.70 mm. NC = Negative Control.

*Diameter of the zones of inhibition measured in mm.
*Ranked from largest to smallest zone of inhibition by 5% acetic acid dilution of apple cider vinegar.

Figure 3-4: *Bacteria isolated from catfish from the pond with the largest zone of inhibition from apple cider vinegar.* Top left: Cycle 1, Bag 2, Bacteria 1 -13.77 mm. Top right: Cycle 1, Bag 5, Bacteria 2 - 10.28 mm. Bottom left: Cycle 1, Bag 8, Bacteria 1 - 10.27 mm. Bottom right: Cycle 1, Bag 7, Bacteria 2 - 9.58 mm. NC = Negative Control.

Table 3-1: *Zone of inhibition of bacteria isolated from catfish from the market from various acetic acid dilutions of fish and chip vinegar.*

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 1	0.5%	1%	2.5%	5%
Bacteria 1	1.27	2.29	3.98	5.68
Bacteria 2	3.03	3.89	5.13	8.16
Bacteria 3	3.15	4.67	8.65	11.10

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 2	0.5%	1%	2.5%	5%
Bacteria 1	0.80	1.86	4.30	7.15
Bacteria 2	0.48	0.93	4.17	5.15
Bacteria 3	2.08	4.34	7.52	10.96

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 3	0.5%	1%	2.5%	5%
Bacteria 1	0.73	2.79	3.68	6.93
Bacteria 2	1.09	2.37	4.20	6.46
Bacteria 3	1.03	2.19	6.26	8.97
Bacteria 4	1.43	1.91	3.66	6.29

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 4	0.5%	1%	2.5%	5%
Bacteria 1	1.09	1.74	4.72	8.82
Bacteria 2	1.24	2.12	4.68	7.35
Bacteria 3	0.86	2.43	5.09	6.15
Bacteria 4	1.07	2.77	4.66	6.33

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 5	0.5%	1%	2.5%	5%
Bacteria 1	1.09	1.43	4.09	6.68
Bacteria 2	1.17	2.68	4.49	6.04
Bacteria 3	0.91	2.52	4.12	6.87
Bacteria 4	1.17	2.68	2.93	4.12
Bacteria 5	0.85	2.54	5.55	6.37

Cycle 1 Bag 6	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	2.72	4.91	7.24	9.78
Bacteria 2	1.13	3.35	3.80	5.95
Bacteria 3	1.83	2.78	4.78	7.04
Bacteria 4	1.07	2.11	4.65	6.25

Cycle 2 Bag 1	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.60	3.31	4.05	5.05
Bacteria 2	4.41	5.14	7.83	12.30
Bacteria 3	1.10	2.48	6.94	8.54
Bacteria 4	0.95	1.17	2.98	4.16
Bacteria 5	0.79	1.76	4.71	6.45
Bacteria 6	0.68	2.77	3.83	6.14
Bacteria 7	3.60	5.60	7.38	8.75
Bacteria 8	2.72	3.86	10.37	12.17

Cycle 2 Bag 2	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	2.30	3.77	6.32	9.53
Bacteria 2	1.04	1.89	2.89	6.48
Bacteria 3	1.15	2.61	6.12	8.14
Bacteria 4	2.44	6.95	9.87	11.55
Bacteria 5	0.87	2.33	4.30	4.99
Bacteria 6	0.71	2.67	3.51	4.25

Cycle 2 Bag 3	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.76	2.84	5.03	5.05
Bacteria 2	1.16	2.17	4.24	6.28
Bacteria 3	1.34	2.84	5.24	7.10
Bacteria 4	3.34	5.26	9.39	11.63

Cycle 2 Bag 4	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.73	0.97	3.52	4.28
Bacteria 2	0.64	1.07	3.37	4.60
Bacteria 3	0.81	0.93	2.97	6.09
Bacteria 4	0.74	2.75	3.50	5.03
Bacteria 5	1.22	1.96	3.37	4.17
Bacteria 6	0.73	2.28	2.60	4.10

Cycle 2 Bag 5	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.69	2.30	4.39	5.35
Bacteria 2	0.78	1.44	3.18	4.12
Bacteria 3	2.17	3.15	7.76	6.22
Bacteria 4	0.89	1.89	3.13	5.16

Cycle 2 Bag 6	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.76	1.04	3.75	5.32
Bacteria 2	1.03	2.99	4.81	6.97

Cycle 2 Bag 7	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.93	2.57	4.53	5.58
Bacteria 2	0.77	2.51	3.19	5.80
Bacteria 3	0.77	2.51	3.19	5.80
Bacteria 4	1.18	1.56	2.65	2.86

Table 3-2: *Zone of inhibition of bacteria isolated from catfish from the pond from various acetic acid dilutions of fish and chip vinegar.*

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 1	0.5%	1%	2.5%	5%
Bacteria 1	0.90	1.09	3.13	7.09
Bacteria 2	0.71	1.09	1.98	6.14
Bacteria 3	1.14	1.28	4.26	7.20
Bacteria 4	0.55	2.71	2.23	4.77
Bacteria 5	0.94	1.76	3.76	5.64

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 2	0.5%	1%	2.5%	5%
Bacteria 1	5.70	7.58	6.57	11.98
Bacteria 2	1.39	2.65	1.66	7.26
Bacteria 3	0	2.33	1.26	6.62
Bacteria 4	0.91	1.56	3.74	6.62
Bacteria 5	0.90	2.46	1.43	4.48
Bacteria 6	0.96	2.36	6.18	9.08

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 3	0.5%	1%	2.5%	5%
Bacteria 1	2.82	3.75	6.89	10.39
Bacteria 2	0.72	1.31	3.96	5.42
Bacteria 3	2.22	2.60	7.70	6.94

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 4	0.5%	1%	2.5%	5%
Bacteria 1	0.95	0.88	2.27	3.69
Bacteria 2	0.84	0.91	2.13	4.49
Bacteria 3	1.98	2.48	4.15	4.83
Bacteria 4	0.87	1.46	3.85	5.37

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 5	0.5%	1%	2.5%	5%
Bacteria 1	0.63	1.48	3.72	5.63
Bacteria 2	3.58	6.29	10.64	14.98
Bacteria 3	1.15	2.92	5.42	7.12

Cycle 1 Bag 6	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	1.10	2.55	4.88	5.53
Bacteria 2	0.76	1.83	4.22	5.19
Bacteria 3	0.82	1.96	3.63	5.30

Cycle 1 Bag 7	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	1.00	2.27	4.29	5.19

Cycle 1 Bag 8	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	2.44	3.71	4.87	6.03
Bacteria 2	3.11	3.00	5.11	6.93
Bacteria 3	2.77	2.88	4.53	6.42
Bacteria 4	2.69	2.24	3.99	5.16

Cycle 2 Bag 1	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.63	1.08	1.63	2.13
Bacteria 2	0	1.95	3.30	4.66
Bacteria 3	1.31	1.53	3.62	4.93

Cycle 2 Bag 2	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0	0.98	1.30	1.60
Bacteria 2	1.03	1.65	3.97	4.99
Bacteria 3	0	2.01	3.74	6.39
Bacteria 4	0	0	0.77	0.77

Cycle 2 Bag 3	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	1.05	1.73	4.13	6.44
Bacteria 2	0	2.37	2.79	6.32
Bacteria 3	3.00	3.75	4.94	6.47
Bacteria 4	0	0	3.36	5.00

	Inhibition zone at various dilutions (mm)			
Cycle 2 Bag 4	0.5%	1%	2.5%	5%
Bacteria 1	1.46	2.10	3.17	5.45
Bacteria 2	0.98	1.27	1.62	2.72

	Inhibition zone at various dilutions (mm)			
Cycle 2 Bag 5	0.5%	1%	2.5%	5%
Bacteria 1	1.53	0.64	1.28	1.33
Bacteria 2	1.20	1.20	1.00	1.81
Bacteria 3	0.89	0.89	1.23	1.24

	Inhibition zone at various dilutions (mm)			
Cycle 2 Bag 6	0.5%	1%	2.5%	5%
Bacteria 1	0.90	1.51	3.23	3.64
Bacteria 2	1.17	1.52	1.72	2.94
Bacteria 3	0.89	1.26	2.89	3.85
Bacteria 4	0.91	0.99	2.30	4.76
Bacteria 5	0.97	1.06	2.86	5.34
Bacteria 6	1.24	1.57	4.78	5.72

	Inhibition zone at various dilutions (mm)			
Cycle 2 Bag 7	0.5%	1%	2.5%	5%
Bacteria 1	0	1.53	2.37	5.46
Bacteria 2	0.95	1.39	2.69	4.31
Bacteria 3	0.86	2.01	4.27	5.75

	Inhibition zone at various dilutions (mm)			
Cycle 2 Bag 8	0.5%	1%	2.5%	5%
Bacteria 1	0	1.26	4.70	5.00
Bacteria 2	0.61	0.71	2.33	2.86
Bacteria 3	0	1.37	2.53	4.49
Bacteria 4	0	0	3.50	5.07

	Inhibition zone at various dilutions (mm)			
Cycle 2 Bag 9	0.5%	1%	2.5%	5%
Bacteria 1	0.94	1.60	2.66	5.08
Bacteria 2	1.54	1.69	2.15	3.85
Bacteria 3	0.79	1.14	3.07	4.25
Bacteria 4	1.05	0.74	1.41	2.00

Table 3-3: *Zone of inhibition of bacteria isolated from catfish from the market from various acetic acid dilutions of apple cider vinegar.*

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 1	0.5%	1%	2.5%	5%
Bacteria 1	0.87	1.45	3.86	6.01
Bacteria 2	3.20	5.86	9.06	11.70
Bacteria 3	1.02	2.81	6.87	6.87
Bacteria 4	2.02	3.03	8.12	8.12

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 2	0.5%	1%	2.5%	5%
Bacteria 1	2.24	4.18	6.46	9.57
Bacteria 2	0.83	2.80	4.44	6.24
Bacteria 3	1.30	1.95	5.84	8.12

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 3	0.5%	1%	2.5%	5%
Bacteria 1	0.75	1.03	5.01	8.97
Bacteria 2	0.92	2.75	4.08	6.40
Bacteria 3	0.86	1.18	5.13	8.32
Bacteria 4	1.26	1.63	3.84	6.01

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 4	0.5%	1%	2.5%	5%
Bacteria 1	1.31	1.79	4.63	8.87
Bacteria 2	0.69	2.09	5.03	7.31
Bacteria 3	0.80	2.21	4.07	5.98
Bacteria 4	1.00	3.52	5.11	7.24

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 5	0.5%	1%	2.5%	5%
Bacteria 1	0.90	2.41	4.50	6.27
Bacteria 2	1.86	3.78	4.60	7.16
Bacteria 3	0.67	2.22	4.69	6.97
Bacteria 4	1.02	1.61	2.98	5.54
Bacteria 5	0.77	2.04	5.61	7.55

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 6	0.5%	1%	2.5%	5%
Bacteria 1	4.57	6.79	9.43	11.22
Bacteria 2	1.24	3.47	4.15	6.29
Bacteria 3	1.13	2.97	4.93	7.03
Bacteria 4	1.10	2.05	3.84	5.22

	Inhibition zone at various dilutions (mm)			
Cycle 2 Bag 1	0.5%	1%	2.5%	5%
Bacteria 1	0.70	2.66	4.93	6.41
Bacteria 2	3.35	6.42	10.34	13.28
Bacteria 3	1.44	2.79	4.91	10.29
Bacteria 4	1.02	1.36	2.29	3.43
Bacteria 5	0.83	1.05	1.28	1.86
Bacteria 6	0.71	2.51	4.58	6.21
Bacteria 7	3.36	4.84	6.34	7.67
Bacteria 8	2.69	5.34	10.93	14.77

	Inhibition zone at various dilutions (mm)			
Cycle 2 Bag 2	0.5%	1%	2.5%	5%
Bacteria 1	3.04	4.29	6.87	8.80
Bacteria 2	0.83	1.33	2.30	4.99
Bacteria 3	0.83	1.28	7.96	8.90
Bacteria 4	3.55	5.22	6.52	8.55
Bacteria 5	0.95	1.77	4.65	6.00
Bacteria 6	1.18	2.67	4.56	6.47

	Inhibition zone at various dilutions (mm)			
Cycle 2 Bag 3	0.5%	1%	2.5%	5%
Bacteria 1	1.39	2.65	3.88	7.93
Bacteria 2	1.17	2.96	4.19	4.57
Bacteria 3	1.05	3.42	4.53	7.13
Bacteria 4	6.17	5.92	8.86	12.87

	Inhibition zone at various dilutions (mm)			
Cycle 2 Bag 4	0.5%	1%	2.5%	5%
Bacteria 1	0.79	0.88	3.26	5.51
Bacteria 2	0.78	2.19	3.88	6.97
Bacteria 3	0.66	1.21	3.17	4.85
Bacteria 4	0.87	1.84	4.84	5.88
Bacteria 5	1.47	2.17	4.27	5.25
Bacteria 6	0.82	2.63	3.05	4.33

Cycle 2 Bag 5	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.70	1.62	3.57	5.81
Bacteria 2	1.17	1.31	5.37	6.04
Bacteria 3	1.61	2.97	7.43	8.03
Bacteria 4	1.23	2.37	4.50	6.25

Cycle 2 Bag 6	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.89	2.27	4.41	8.94
Bacteria 2	1.70	3.02	5.17	8.30

Cycle 2 Bag 7	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.51	0.74	5.27	6.46
Bacteria 2	1.02	3.05	5.42	6.47
Bacteria 3	0.93	3.13	4.77	7.28
Bacteria 4	0.70	1.09	1.96	3.27

Table 3-4: *Zone of inhibition of bacteria isolated from catfish from the pond from various acetic acid dilutions of apple cider vinegar.*

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 1	0.5%	1%	2.5%	5%
Bacteria 1	0.84	0.95	2.77	5.20
Bacteria 2	0.71	1.33	3.45	6.60
Bacteria 3	1.12	1.87	5.10	7.22
Bacteria 4	0.86	0.96	4.35	4.83
Bacteria 5	0	0	0.81	0.86

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 2	0.5%	1%	2.5%	5%
Bacteria 1	5.13	4.97	10.80	13.77
Bacteria 2	1.11	1.49	4.80	6.28
Bacteria 3	1.13	0.93	3.84	4.24
Bacteria 4	0.91	0.65	3.00	3.74
Bacteria 5	0	0.56	1.90	4.22
Bacteria 6	0.30	1.23	4.65	6.67

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 3	0.5%	1%	2.5%	5%
Bacteria 1	0.62	1.44	3.86	4.84
Bacteria 2	1.71	5.26	5.26	6.41

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 4	0.5%	1%	2.5%	5%
Bacteria 1	0.94	1.54	2.49	4.55
Bacteria 2	0.94	1.61	3.49	4.24
Bacteria 3	0	0	0	0.55
Bacteria 4	0.73	0.86	3.49	5.41

	Inhibition zone at various dilutions (mm)			
Cycle 1 Bag 5	0.5%	1%	2.5%	5%
Bacteria 1	1.06	1.45	3.35	3.88
Bacteria 2	1.86	3.45	10.55	10.28
Bacteria 3	0.86	1.38	4.09	7.00

Cycle 1 Bag 6	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	1.70	2.63	6.13	6.78
Bacteria 2	1.56	4.15	5.52	7.36
Bacteria 3	1.90	3.20	3.72	5.75

Cycle 1 Bag 7	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	2.09	4.29	5.73	7.93
Bacteria 2	2.83	3.39	6.50	9.58
Bacteria 3	2.86	3.74	4.81	7.82

Cycle 1 Bag 8	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	2.77	3.59	7.03	10.27
Bacteria 2	0.90	2.58	4.10	4.62
Bacteria 3	1.64	4.03	5.76	6.74
Bacteria 4	0.91	2.78	4.80	6.17

Cycle 2 Bag 1	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.90	1.17	1.17	2.54
Bacteria 2	0.82	2.34	2.49	4.88
Bacteria 3	1.01	2.11	3.83	6.34

Cycle 2 Bag 2	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0	1.57	3.16	4.99
Bacteria 2	1.31	1.73	4.46	5.32
Bacteria 3	0.79	0.98	1.57	3.10
Bacteria 4	1.26	2.24	2.73	5.00

Cycle 2 Bag 3	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.74	1.59	2.94	5.62
Bacteria 2	1.26	2.38	3.28	5.11
Bacteria 3	0.72	1.94	4.24	5.19
Bacteria 4	0	1.93	4.37	5.93

Cycle 2 Bag 4	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	1.45	2.55	4.30	6.44
Bacteria 2	0.83	1.29	2.19	2.93

Cycle 2 Bag 5	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	1.07	1.43	2.44	3.08
Bacteria 2	0.75	0.92	1.02	1.08
Bacteria 3	0.86	1.34	1.35	2.24

Cycle 2 Bag 6	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.50	2.59	3.58	4.74
Bacteria 2	1.14	0.99	2.42	0.75
Bacteria 3	0.79	0.83	1.47	2.42
Bacteria 4	1.27	1.89	3.68	5.56
Bacteria 5	0.72	1.96	4.38	4.85
Bacteria 6	1.19	1.71	3.68	5.07

Cycle 2 Bag 7	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.54	1.06	1.19	2.42
Bacteria 2	1.17	1.85	2.21	3.68
Bacteria 3	1.18	2.02	3.88	4.86

Cycle 2 Bag 8	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	0.96	1.57	3.99	5.93
Bacteria 2	0.79	1.12	4.05	5.23
Bacteria 3	0	1.72	2.70	3.38
Bacteria 4	0.56	1.38	4.14	8.33

Cycle 2 Bag 9	Inhibition zone at various dilutions (mm)			
	0.5%	1%	2.5%	5%
Bacteria 1	1.29	2.07	3.49	5.89
Bacteria 2	0.95	1.32	1.00	1.30
Bacteria 3	1.05	0.83	4.39	5.50
Bacteria 4	0.89	1.31	1.29	2.34

Table 3-5: *Minimum inhibitory concentration of bacteria isolated from the market and pond with the largest zone of inhibition from fish and chip vinegar.*

MIC rank	Market				Pond			
	Cycle	Bag	Bacteria	MIC%	Cycle	Bag	Bacteria	MIC%
1.	2	1	2	0.05	1	5	2	0.02
2.	2	2	4	0.05	1	2	1	0.03
3.	1	6	1	0.05	1	3	1	0.03
4.	2	2	1	0.05	1	2	6	0.03
5.	2	3	4	0.06	1	2	2	0.03
6.	1	2	3	0.06	1	1	3	0.03
7.	1	1	3	0.07	1	8	2	0.03
8.	1	4	1	0.1	1	1	1	0.04
9.	2	1	7	0.1	1	3	3	0.04
10.	1	3	3	0.5	1	5	3	0.06

*Bacteria with the largest zone of inhibition (ranked by FC 5%) have the lowest minimum inhibitory concentration (lowest concentration).
*MIC is ranked from low to high concentration.

Table 3-6: *Minimum inhibitory concentration of bacteria isolated from the market and pond with the smallest zone of inhibition from fish and chip vinegar.*

MIC rank	Market				Pond			
	Cycle	Bag	Bacteria	MIC%	Cycle	Bag	Bacteria	MIC%
1.	2	7	4	0.3	2	2	4	0.3
2.	1	5	4	0.2	2	9	4	0.3
3.	2	4	4	0.2	2	4	2	0.3
4.	2	5	2	0.2	2	5	3	0.3
5.	2	1	4	0.2	2	5	1	0.2
6.	2	4	6	0.2	2	2	1	0.2
7.	2	4	1	0.2	2	5	2	0.1
8.	2	4	2	0.2	2	1	1	0.1
9.	1	1	1	0.1	2	8	2	0.09
10.	2	2	6	0.09	2	6	2	0.09

*Bacteria with the lowest zone of inhibition (ranked by FC 5%) have the highest minimum inhibitory concentration (highest concentration).
*MIC is ranked from high to low concentration.

Table 3-7: *Minimum inhibitory concentration of bacteria isolated from the market and pond with the largest zone of inhibition from apple cider vinegar.*

MIC rank	Market				Pond			
	Cycle	Bag	Bacteria	MIC%	Cycle	Bag	Bacteria	MIC%
1.	2	3	5	0.03	1	2	1	0.02
2.	2	1	2	0.04	1	5	2	0.02
3.	1	1	2	0.1	1	8	1	0.03
4.	2	1	3	0.1	1	7	1	0.03
5.	2	1	8	0.2	1	7	2	0.03
6.	1	2	1	0.2	2	8	4	0.03
7.	1	3	1	0.2	1	7	3	0.04
8.	2	2	3	0.2	1	6	2	0.06
9.	1	6	1	0.3	1	1	3	0.07
10.	2	6	1	0.3	1	5	3	0.07

*Bacteria with the largest zone of inhibition (ranked by AC 5%) have the lowest minimum inhibitory concentration (lowest concentration).
*MIC is ranked from low to high concentration.

Table 3-8: *Minimum inhibitory concentration of bacteria isolated from the market and pond with the smallest zone of inhibition from apple cider vinegar.*

MIC rank	Market				Pond			
	Cycle	Bag	Bacteria	MIC%	Cycle	Bag	Bacteria	MIC%
1.	1	6	4	1	1	4	3	2.5
2.	2	4	1	0.3	1	1	5	2.5
3.	2	4	5	0.2	2	5	2	0.3
4.	2	3	3	0.2	2	6	2	0.2
5.	2	4	6	0.2	2	10	2	0.2
6.	2	1	4	0.2	2	10	4	0.2
7.	2	1	5	0.2	2	7	1	0.2
8.	2	2	2	0.1	2	6	3	0.2
9.	2	4	3	0.1	2	1	3	0.1
10.	2	7	4	0.1	2	5	3	0.1

*Bacteria with the smallest zone of inhibition (ranked by AC 5%) have the highest minimum inhibitory concentration (highest concentration).
*MIC is ranked from high to low concentration.

Table 3-9: *Identification of bacterial isolates from market catfish based on 16S rDNA sequencing.*

Catfish Isolate			Bacterial Identification 16s rDNA Sequencing Result	*Similarity (%)	Length (Base Pairs)	GenBank Accession Number
Cycle	Bag	Bacteria				
2	1	2	*Shewanella putrefaciens*	100	840	AB681550.1
2	1	7	*Shewanella putrefaciens*	100	840	AB681550.1
2	3	4	*Shewanella putrefaciens*	100	770	FJ375179.1
2	2	4	*Shewanella putrefaciens*	99	764	AB681550.1
1	1	2	*Aeromonas sobria*	100	840	JN55613.1
1	2	3	*Aeromonas sobria*	99	839	JN55613.1
1	6	1	*Shewanella putrefaciens*	100	818	NR 044863.1
2	2	1	*Shewanella putrefaciens*	100	840	AB681550.1
1	3	3	*Enterobacteriaceae bacterium/*	100	840	HQ824881.1
			Rahnella aquatilis	99	839	DQ440548.1
1	4	1	*Pseudomonas fragi*	100	827	AB685609.1

*Similarity of 16S rDNA region between catfish isolate samples and closest relative found in GenBank database .

Table 3-10: *Identification of bacterial isolates from pond catfish based on 16S rDNA sequencing.*

Catfish Isolate			Bacterial Identification 16s rDNA Sequencing Result	*Similiarity (%)	Length (Base Pairs)	GenBank Accession Number
Cycle	Bag	Bacteria				
1	5	2	*Shewanella putrefaciens*	99	837	AB208055.1
1	2	1	*Stenotrophomonas maltophila/*	100	910	HM007572.1
			Stenotrophomonas rhizophia	100	910	HQ831393.1
1	3	1	*Shewanella putrefaciens*	99	769	AB208055.1
1	2	6	*Aeromonas hydrophila/*	100	840	HE681732.1
			Aeromonas veronii	100	840	JQ301790.1
1	2	2	*Pantoea agglomerans*	99	766	FJ357834.1
1	1	3	*Plesiomonas shigelloisdes*	99	839	HM007572.1
1	5	3	*Pseudomonas fluorescens/*	100	840	AB681956.1
			Pseudomonas mandelii	100	840	JN638055.1
1	1	1	*Plesiomonas shigelloides*	100	770	FJ375179.1
1	3	3	*Pantoea agglomerans*	99	697	AJ233423.1

*Similarity of 16S rDNA region between catfish isolate samples and closest relative found in GenBank database.

Table 3-11: *Identification of bacterial isolates from market and pond based on 16S rDNA sequencing with zone of inhibition by fish and chip vinegar with 5% acetic acid.*

Zone of inhibition with FC 5% (mm)	Bacterial identification	Number of bacteria
10-15	*Shewanella putrefaciens*	8
	Stenotrophomonas maltophila/Stenotrophomonas rhizophia	1
	Aeromonas sobria	2
8-10	*Aeromonas hydrophila/ Aeromonas veronii*	1
	Enterobacteriaceae bacterium/Rahnella aquatilis	1
6-8	*Pseudomonas fragi*	1
	Pantoea agglomerans	2
	Plesiomonas shigelloides	2
	Pseudomonas fluorescens/Pseudomonas mandelii	1
	Pseudomonas putida/Pseudomonas veronii	1

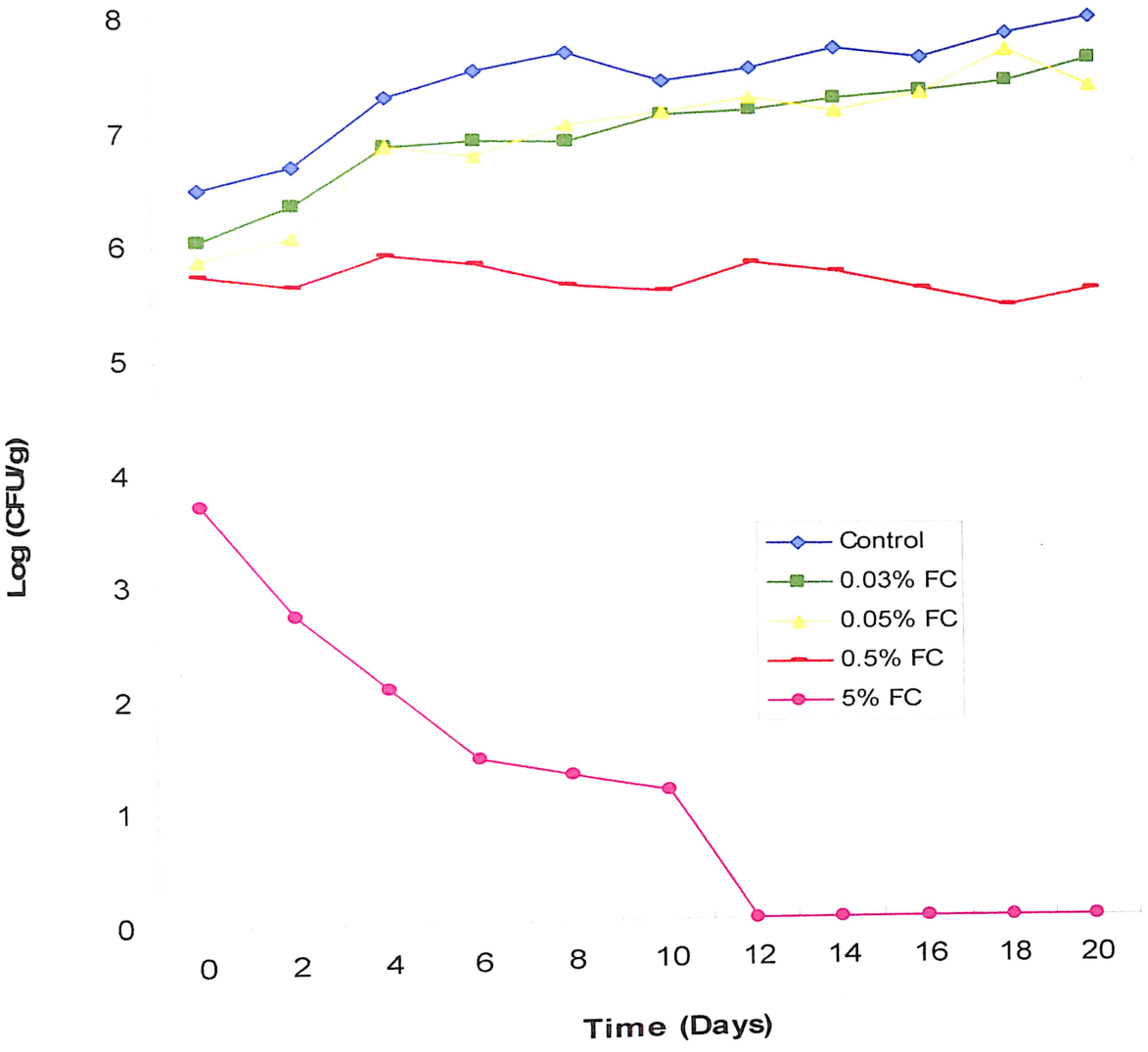


Figure 3-5: *Changes in total bacterial count from catfish fillet with control with no vinegar and treatments of 0.03, 0.05, 0.5 and 5% acetic acid of fish and chip vinegar during storage at 4°C for 20 days.*

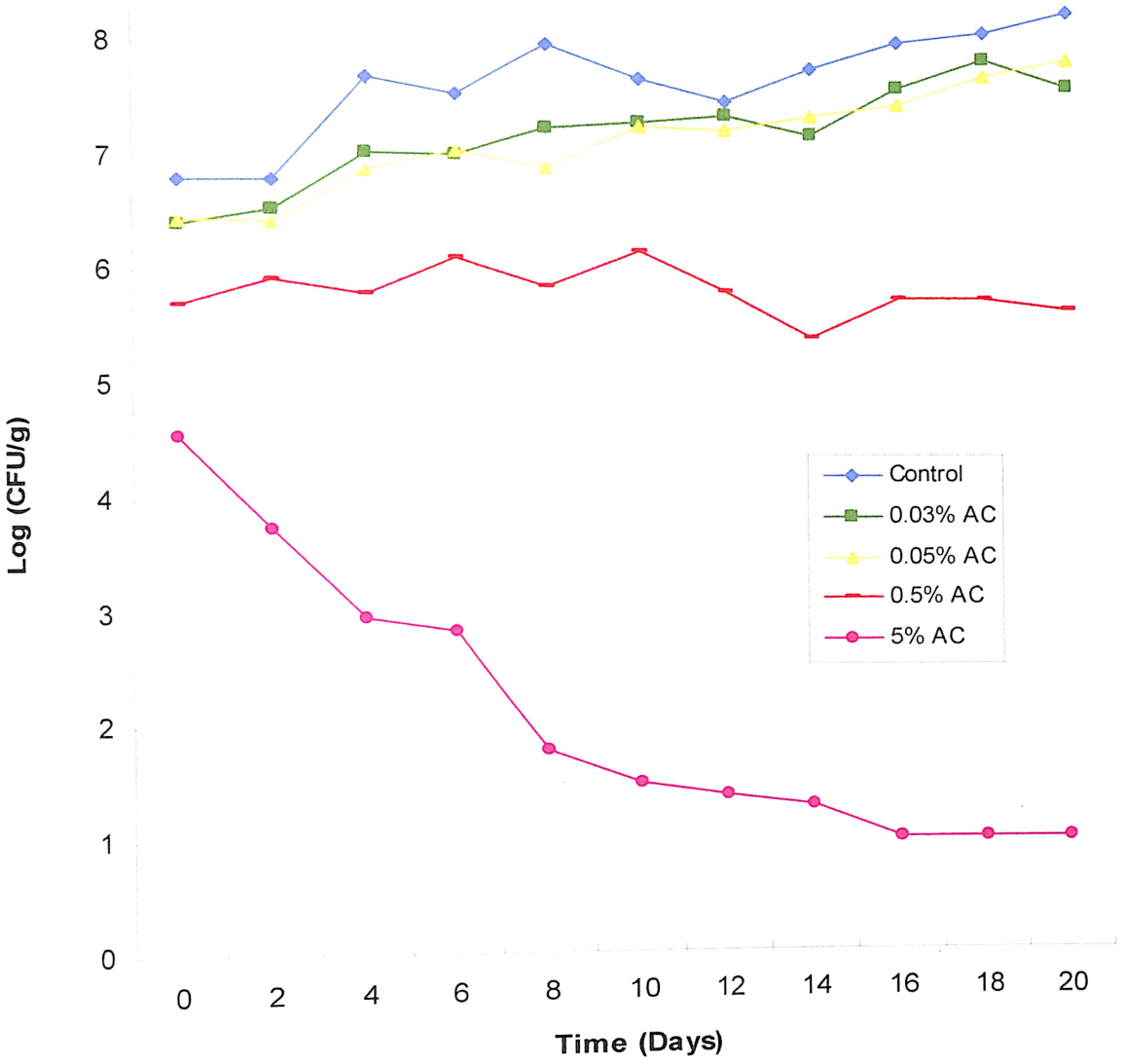
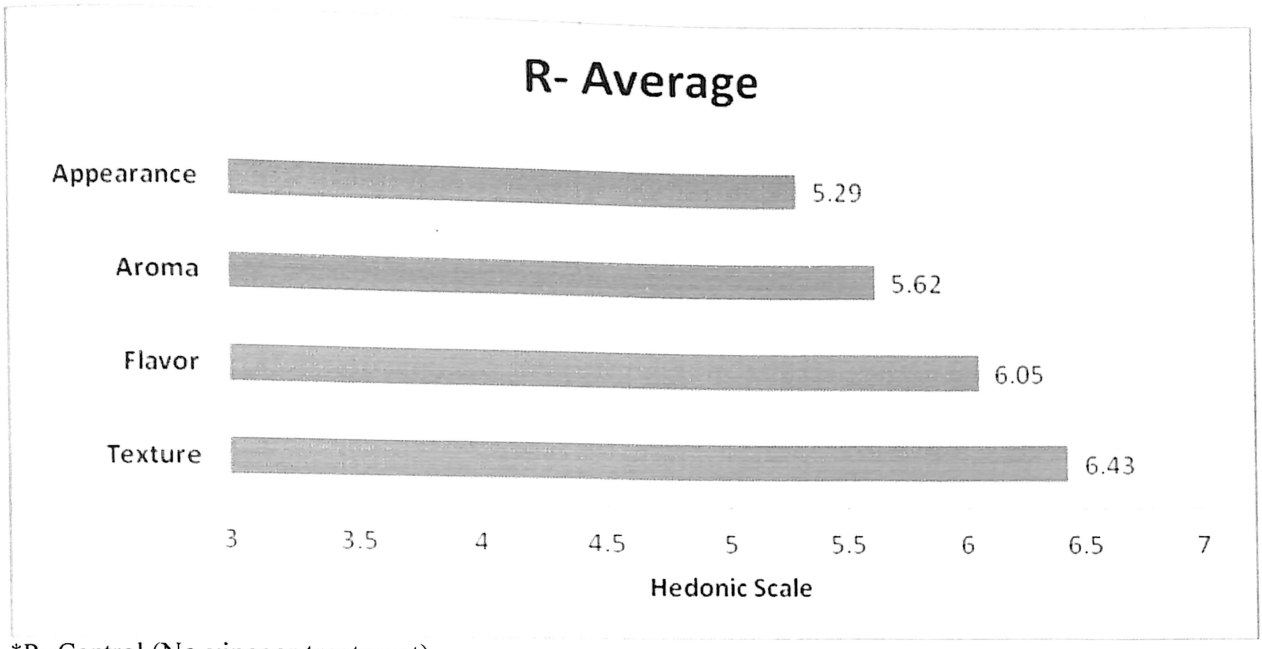


Figure 3-6: *Changes in total bacterial count from catfish fillet with control with no vinegar and treatments of 0.03, 0.05, 0.5 and 5% acetic acid of apple cider vinegar during storage at 4°C for 20 days.*



*R=Control (No vinegar treatment)

Figure 3-7: *Sensory attributes rating average for catfish with no treatment.*

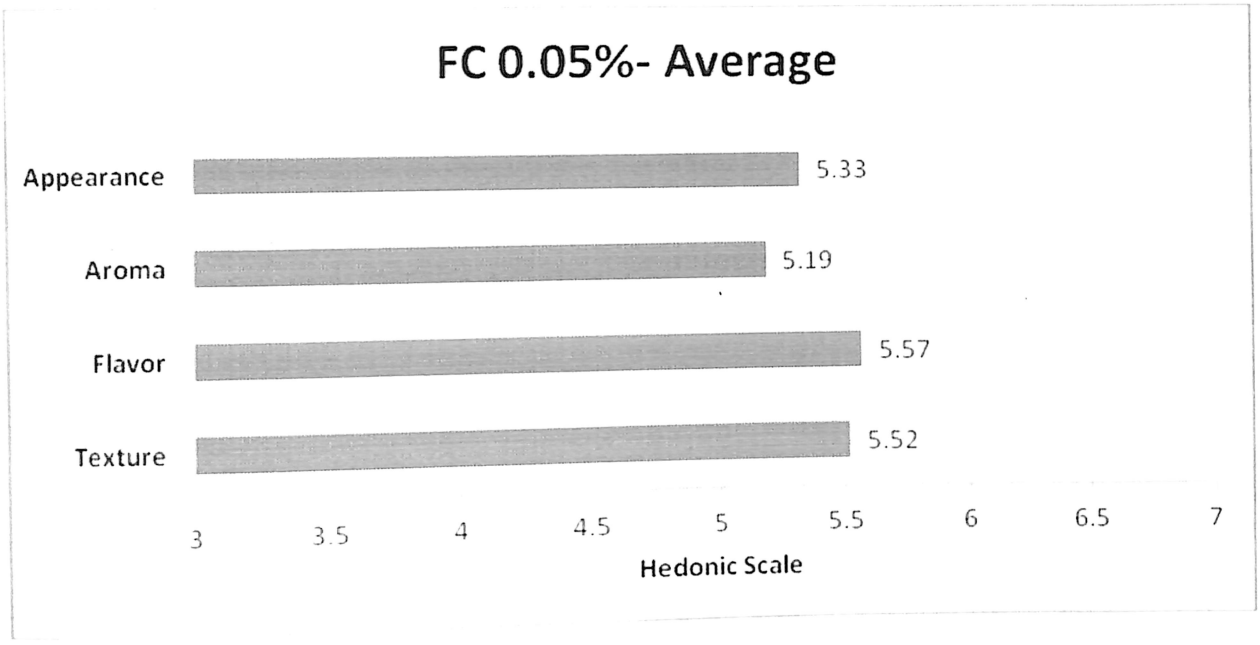


Figure 3-8: *Sensory attributes rating average for catfish treated with fish and chip vinegar with 0.05% acetic acid.*

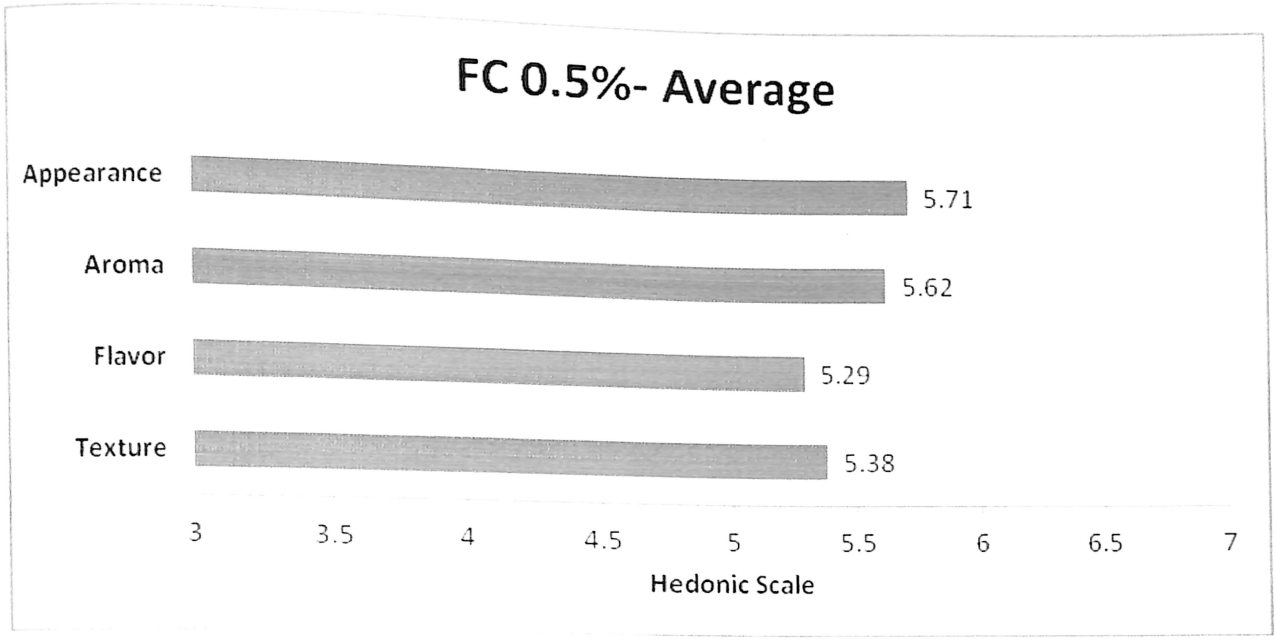


Figure 3-9: *Sensory attributes rating average for catfish treated with fish and chip vinegar with 0.5% acetic acid.*

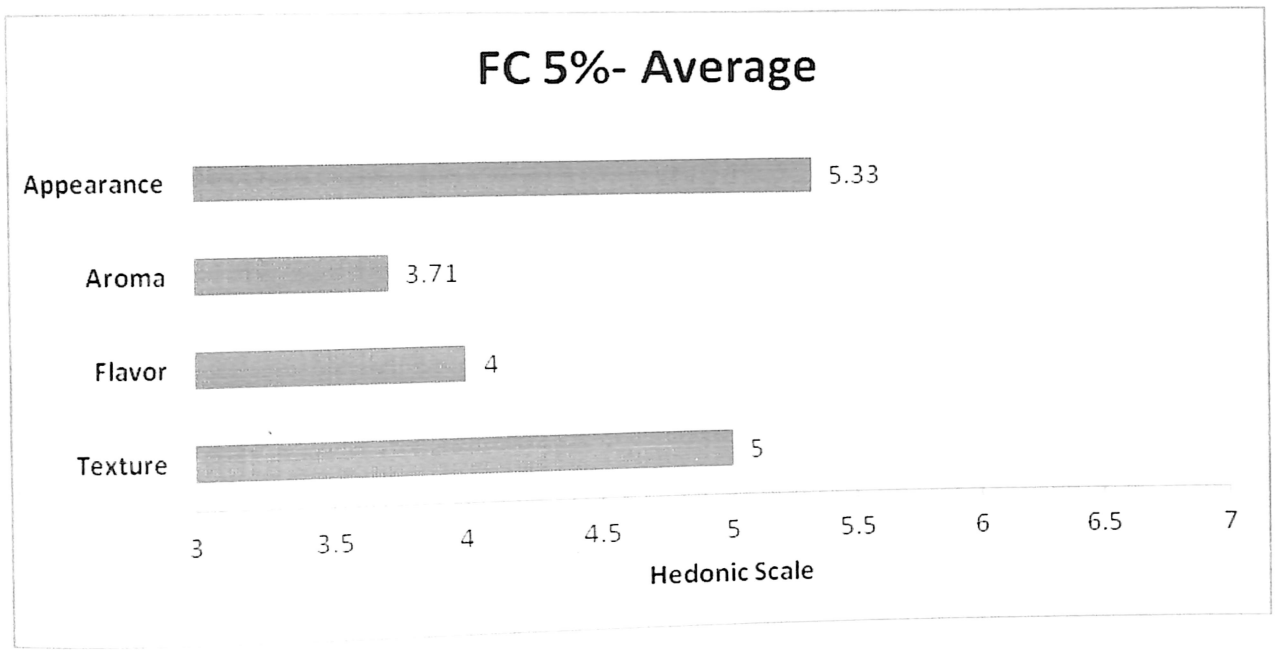


Figure 3-10: *Sensory attributes rating average for catfish treated with fish and chip vinegar with 5% acetic acid.*

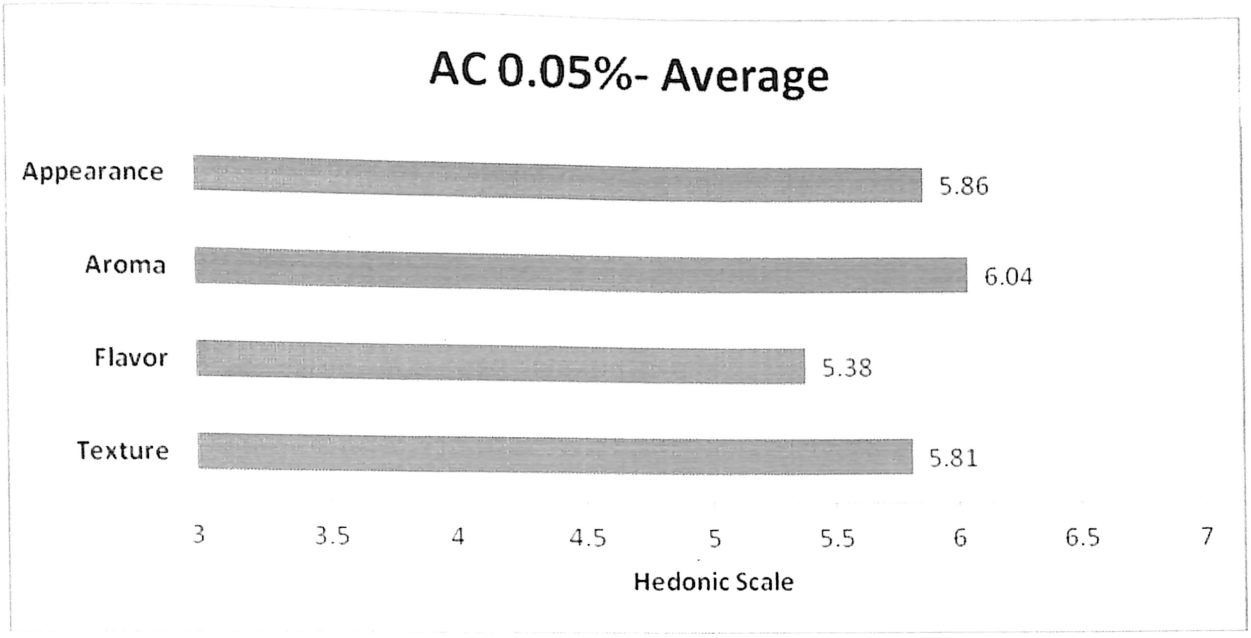


Figure 3-11: *Sensory attributes rating average for catfish treated with apple cider vinegar with 0.05% acetic acid.*

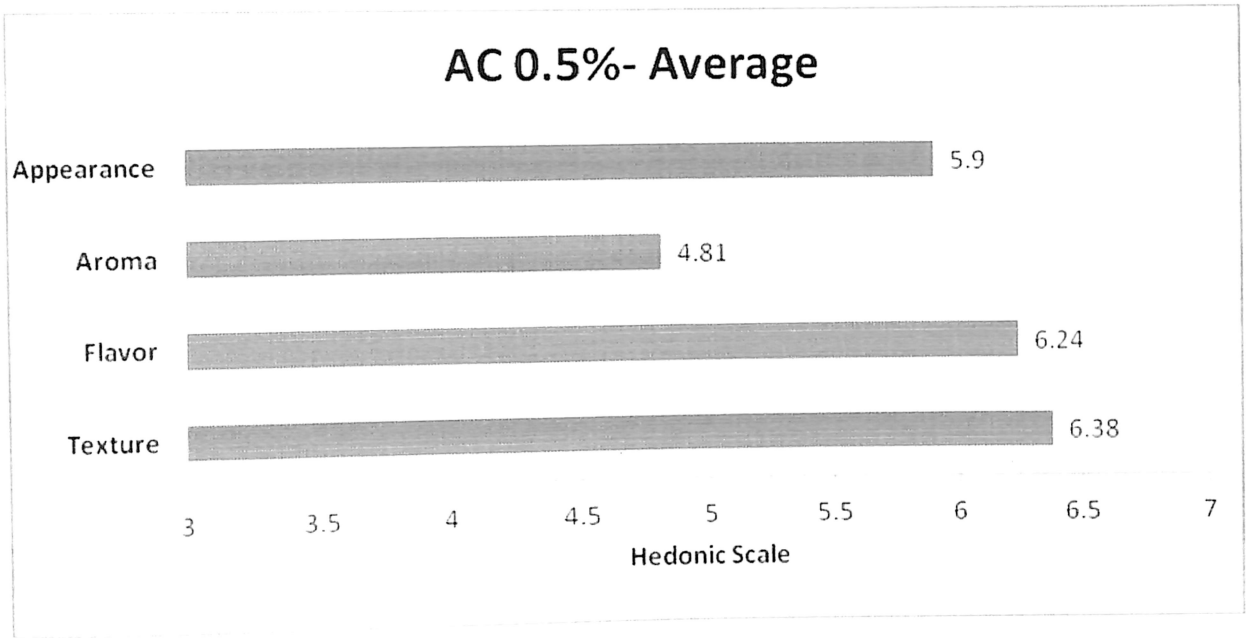


Figure 3-12: *Sensory attributes rating average for catfish treated with apple cider vinegar with 0.5% acetic acid.*

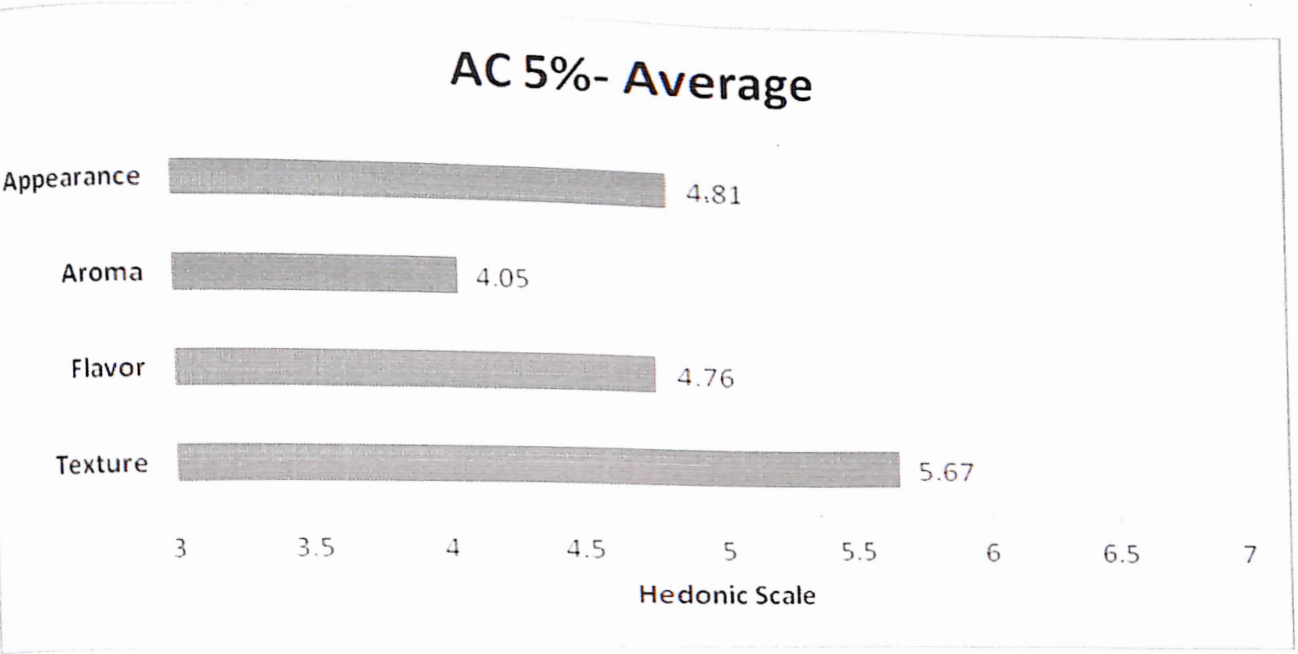


Figure 3-13: *Sensory attributes rating average for catfish treated with apple cider vinegar with 5% acetic acid.*

DISCUSSION

Various acetic acid dilutions of FC vinegar and AC vinegar were tested on bacteria isolated from market and pond catfish fillets. The zones of inhibition were ranked according to 5% acetic acid of vinegar. The antimicrobial activity of FC vinegar with a pH of 2.95 on bacteria isolated from pond catfish fillets showed slightly higher results than AC vinegar with a pH of 3.25 but the largest zone of inhibition for market catfish from FC vinegar was smaller for AC vinegar. Both vinegar samples exerted antimicrobial activity at all acetic acid dilutions of vinegar (0.5, 1, 2.5 and 5%) tested in market catfish (Table 3-1 and Table 3-5) but for pond catfish fillets, lower acetic acid dilutions of vinegar showed zones of inhibition (Table 3-2 and Table 3-6). The culture system of many freshwater fish depends on water sources such as lakes and reservoirs. Consequently, flooding could reach into ponds where fish are commercially raised for human consumption (Suhalim, 2007). Thus outdoor fish ponds are continually exposed to contamination and pathogens. Therefore, in this study, bacteria isolated from market catfish fillets may be treated previously or stored in ice that would minimize the bacterial growth compared to the bacteria isolated from pond catfish fillets which harbor more bacterial species.

The MIC was determined on the ten bacteria isolated from both market and pond catfish fillets with the largest and smallest zones of inhibition zone with 5% acetic acid dilution of vinegar. FC vinegar resulted in the lowest MIC and AC vinegar resulted in the highest MIC. Thus FC vinegar is the most effective in inhibiting the growth of bacteria from market catfish fillets compared to the inhibitory effect on bacteria isolated from pond catfish fillets and the inhibitory effect of AC vinegar on bacteria isolated from

market and pond catfish fillets. AC vinegar was the least effective on bacteria isolated from pond catfish compared to market catfish and the inhibitory effect of FC vinegar on bacteria isolated from market and pond catfish fillets. The lowest concentration of FC vinegar that is capable of inhibiting the growth of bacteria isolated from market catfish fillets is 0.05% acetic acid of vinegar (Table 3-3). The lowest concentration of AC vinegar that is capable of inhibiting the growth of bacteria isolated from pond catfish is 2.5% acetic acid (Table 3-8).

Bacteria with the largest zone of inhibition from FC vinegar from both the market and pond catfish were identified through 16S rDNA sequencing. A greater variety of bacterial species were identified from pond catfish fillets than market catfish fillets. This is in agreement with Suhalim (2007) who states that ponds where fish are commercially raised for human consumption are continually exposed to contamination and human pathogens due to the flooding of lakes and reservoirs. Out of the twenty bacteria that were identified, eight were *Shewanella putrefaciens* with a zone of inhibition measured at 10-15 mm in diameter (Table 3-11). According to Ruiter (1971), since acetate is the end product during the TMA-dependent respiration of specific spoilage bacteria such as *Shewanella* species, adding acetate to fish tissue can inhibit the TMAO-reduction by bacterial trimethylamine oxide reductases. *Stenotrophomonas* and *Aeromonas* strains also exhibited a zone of inhibition measured at 10-15 mm in diameter. *Enterobacteriaceae bacterium/Rahnella aquatilis* and *Pseudomonas fragi* showed a zone of inhibition measured at 8-10 mm in diameter. The other *Pseudomonas* strains, *Pantoea* and *Plesiomonas* showed a zone of inhibition measured at 6-8 mm in diameter.

Domestic catfish fillets with no vinegar treatment and treatments of 0.03, 0.05, 0.5%, and 5% acetic acid of FC vinegar and AC vinegar were stored at 4°C for 20 days. Changes in total bacteria were enumerated every two days. The bacterial growth increased slightly less than the control for vinegar 0.03 and 0.05 acetic acid of vinegar and was largely reduced after 15 minutes of marinating in 5% acetic acid of the vinegar samples. There was a large reduction in bacterial growth for vinegar 0.5% and then remained constant in bacteria growth with little variation. These results were similar to a study by Bal'a and Marshall (1998) who evaluated the microbiological changes of catfish fillets following treatment of dipping 2% acetic acid. He concluded that little microbial proliferation was observed. The shelf life of modified atmosphere packaged fillets can be prolonged by spraying with 10% acetic acid/acetate with a pH of 5.6 (Debevere and Boskou, 1996). FC 5% maintained a large reduction in bacterial growth from day 0 to day 12 and remained at a constant of 0 CFU/g thereafter. AC 5% maintained a large reduction in bacteria growth from day 0 to day 16 and remained at a constant of 1 CFU/g thereafter. Therefore, the addition of FC 0.5 and 5% with a pH of 2.95 prolongs the shelf life of domestic catfish fillets more than AC 0.5 and 5% with a pH of 3.25.

Sensory evaluation was performed on baked catfish fillets marinated with FC vinegar and AC vinegar. The control was the catfish fillet without vinegar. Students and faculty of Delaware State University in Dover, Delaware evaluated the control and compared it to the different acetic acid dilutions of the vinegar (FC 0.05%, FC 0.5%, FC 5%, AC 0.05%, AC 0.5% and AC 5%). Appearance (color), aroma, flavor, and texture were evaluated using the nine-point Hedonic Scale. Results showed that the 5% samples (FC 5% and AC 5%) were the least liked of the samples overall. Panelists preferred

sample AC 5% over FC 5% in all attributes but appearance. This may be because a visible color change was observed on the fillet with FC 5% because of the vinegar's darker color which seemed to be preferred to panelists than AC vinegar which is lighter in color. Sample FC 5% was the only marinade sample that resulted in a color change on the baked catfish fillet. The aroma and the flavor showed the lowest results in the 5% marinade samples because of the strong aroma and flavor of the acid. The results also showed that the fillet with FC 5% marinade had a stronger aroma and flavor than AC 5%. This may be due to the fact that FC vinegar contains 100% natural malt which has a rich, distinctive aroma and flavor and the aroma and flavor of apple cider contributes to that of apple cider vinegar which the panelists seemed to prefer over the FC malt vinegar. Results showed that the flavor for both samples (FC 5% and AC 5%) was "disliked slightly" and the aroma for AC 5% was also "disliked slightly". However, the panelists rating for aroma was the lowest for FC 5%. Panelists "moderately disliked" FC 5% marinade on the baked catfish fillet. Its strong aroma and flavor restricts the amount that can be used to treat catfish. One study used organic acids to prolong the shelf life of fresh salmon (Schirmer et al., 2009). Salmon samples treated with acetic acid marinades showed significant acid odor and flavor.

Results showed that the control was similar to the rest of the treated samples FC 0.05, FC 0.5, AC 0.05 and AC 0.5%). There were minimal sensory differences among these samples. Panelists "dislike slightly", "neither like nor dislike" or "like slightly" the fillet without vinegar and the fillets with 0.05 and 0.5% acetic acid of vinegar marinade on the fillet. Among all the samples in the sensory evaluation, texture was the most consistent in rating. Panelists "neither like nor dislike" or "like slightly" the texture of the

fillets without vinegar and with vinegar. According to Pons-Sanchez-Cascado et al. (2005), marinating involves changes in textural properties of fish. In their study, anchovies (*Engraulis encrasicholus*) were immersed in a vinegar solution (20% acetic acid v/v) and vacuum-packed (Pons-Sanchez-Cascado et al., 2005). However, in a study of Schirmer et al. (2009), with organic acids on fresh salmon, no significant differences were found for hardness.

The statistical analysis was based on the Hedonic nine-point scale of attributes including appearance (color), aroma, flavor, and texture per sample (Control, FC 0.05, FC 0.5, FC 5, AC 0.05, AC 0.5, and AC 5%). Values of $p < 0.05$ were deemed significant. The most significant sample was FC 5% ($p = 0.02$). Panelists liked this sample the least. Statistical analysis showed that the attribute aroma of sample FC 5% was the most significant attribute with sample indicating that aroma from sample FC 5% was the least liked from the panelists. Aroma was significantly different compared to appearance ($p = 0.04$) and texture ($p = 0.003$). Flavor of sample FC 5% and aroma and flavor of AC 5% was also significant among other samples and attributes ($p < 0.05$).

CONCLUSIONS

The purpose of this study was to evaluate the antimicrobial activity of vinegar on nine foodborne pathogens and various bacterial species isolated from channel catfish fillets. Vinegar applied as an antimicrobial agent could reduce illnesses caused by foodborne pathogens and in this study was shown to be effective in reducing spoilage bacteria. The antimicrobial activity of different acetic acid dilutions of vinegar was assessed based on zone of inhibition testing and minimum inhibitory concentration. The results indicate that fish and chip vinegar with a pH of 2.95 is a stronger antimicrobial agent than apple cider vinegar with a pH of 3.25 on foodborne pathogens and bacteria isolated from catfish fillets. Acetic acid 5% of fish and chip vinegar exerted the highest antimicrobial activity on *E. coli* DH5α (9.82 mm) and *Pseudomonas fluorescens* (7.85 mm). This was the only study that evaluated the inhibitory effect of acetic acid from vinegar on bacteria isolated from catfish. Selected bacteria species from catfish fillets were identified by 16S rDNA sequencing and fish and chip vinegar was the most effective on *Shewanella putrifaciens* isolated from catfish fillets from the market and pond. The vinegar samples were more effective on bacteria isolated from catfish fillets than the specific foodborne pathogens. Vinegars were more effective on bacteria isolated from market catfish fillets than pond catfish fillets.

Microbial changes were determined following treatments with different acetic acid dilutions of the vinegar samples. Acetic acid 5% of vinegar maintained large reduction in bacterial growth while the control, 0.03, and 0.05% fish and chip vinegar and apple cider vinegar remained close in bacteria growth pattern and count. Vinegar 0.5% resulted in a large reduction and then remained constant in bacteria growth. The same

acetic acid dilutions of vinegar were used on baked catfish involved in a sensory evaluation where the aroma and flavor of 5% acetic acid of vinegar was the least liked of the panelists. Therefore, 5% acetic acid of vinegar would be appropriate in reducing bacteria but an acetic acid dilution of vinegar less than 5% appeals more to consumers in terms of aroma and flavor. The most suitable acetic acid dilution of vinegar on catfish fillets would be 0.5% for prolonging shelf life and appealing to consumers. Organic acids, such as the acetic acid in vinegar, are recognized as safe antimicrobial agents and diluted to 1 to 3% generally doesn't affect desirable sensory properties (Kotula and Thelappurate, 1994; Smulders and Greer, 1998). According to Bal'a and Marshall (1998), organic acid treatments may be the most applicable to the catfish industry because major investment is not needed and there is easy application. The vinegar products can be applied as natural antimicrobial agents that can increase the safety, shelf life, and quality of fishery and other food products.

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Appendix A

16S rDNA Sequencing Data

Cycle 2, Bag 1, Bacteria 2 (market)

TGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAACATTCTG
ATTTGCGATTACTAGCGATTCCGACTTCACGGAGTCGAGTTGCAGACTCCGAT
CCGGACTACGACGCGCTTTTTGGGATTCGCTCACTATCGCTAGCTTGCAGCCC
TCTGTACGCGCCATTGTAGCACGTGTGTAGCCCTGGCCGTAAGGGCCATGAT
GACTTGACGTCATCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCCTTGA
GTTCCCACCATTACGTGCTGGCAACAAAGGACAGGGGTTGCGCTCGTTGCGG
GACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTG
TGTTCTGATTCCCGAAGGCACTCCCGTATCTCTACAGGATTCCAGACATGTCA
AGGCCAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCG
CTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCC
CCAGGCGGTCGATTTAACGCGTTAGCTCCGGAAGCCACGTCTCAAGGACACA
GCCTCCAAATCGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGT
TTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCT
TCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAAT
TCTACCCCCCTCTACAAGACTCTAGCTGAACAGTTTTAAATGCAATTCCCAGG
TTGAGCCCGGGGCTTTCACATCTAACTTATCCAACCGCCTGCGTGCGCTTT

Cycle 1, Bag 2, Bacteria 3 (market)

GGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAACATTC
TGATTTGCGATTACTAGCGATTCCGACTTCACGGAGTCGAGTTGCAGACTCCG
ATCCGGACTACGACGCGCTTTTTGGGATTCGCTCACTATCGCTAGCTTGCAGC
CCTCTGTACGCGCCATTGTAGCACGTGTGTAGCCCTGGCCGTAAGGGCCATG
ATGACTTGACGTCATCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCCTT
GAGTTCCCACCATTACGTGCTGGCAACAAAGGACAGGGGTTGCGCTCGTTGC
GGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACC
TGTGTTCTGATTCCCGAAGGCACTCCCGTATCTCTACAGGATTCCAGACATGT
CAAGGCCAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCAC
CGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACT
CCCCAGGCGGTCGATTTAACGCGTTAGCTCCGGAAGCCACGTCTCAAGGACA
CAGCCTCCAAATCGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCT
GTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGC
CTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGA
ATTCTACCCCCCTCTACAAGACTCTANCTGAACAGTTTTAAATGCAATTCCCA
GGTTGAGCCCGGGGCTTTCACATCTAACTTATCCAACCGCCTGCGTGCGCT

Cycle 1, Bag 3, Bacteria 3 (market)

GTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTAGCATTCT
GATCTACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAA
TCCGGACTACGACATACTTTATGAGGTCCGCTTGCTCTCGCGAGTTTGCTTCT
CTTTGTATATGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGAT
GACTTGACGTCATCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCTTTGA
GTTCCCACCATTACGTGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGG
GACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTG
TCTCACGGTTCCCGAAGGCACTAAGCTATCTCTAGCGAATTCCGTGGATGTCA
AGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCG
CTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCC
CCAGGCGGTCGACTTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACA
ACCTCCAAGTCGACATCGTTTACAGCGTGGACTACCAGGGTATCTAATCCTGT
TTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCT
TCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAAT
TCTACCCCCCTCTACAAGACTCTAGCTTGCCAGTTTCAAATGCAGTTCCCAAG
TTAAGCTCGGGGATTTCACATCTGACTTAACAAACCGCCTGCGTGCGCTT

Cycle 1, Bag 4, Bacteria 1 (market)

GTGAGTAATACCTAGGAATCTGCCTGATAGTGGGGGATAACGTTCGGAAACG
GACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCT
TGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCT
CACCAAGGCTACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGA
ACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGAC
AATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGG
ATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTTACCTAATACGTGACTG
TCTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGC
GGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGC
GTAGGTGGTTTGTTAAGTTGAATGTGAAATCCCCGGGCTCAACCTGGGAACT
GCATCCAAAACTGGCAAGCTAGAGTATGGTAGAGGGTAGTGGAATTTCCTGT
GTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGAC
TACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGG
ATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGA
GTCTTGAACTCTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAG
TACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCG
GNGGAGCATGT

Cycle 1, Bag 6, Bacteria 1 (market)

CGGCGGACGGGTGAGTAATGCCTAGGGATCTGCCCAGTCGAGGGGGATAAC
AGTTGGAAACGACTGCTAATACCGCATACGCCCTACGGGGGAAAGGAGGGG
ACCTTCGGGCCTTCCGCGATTGGATGAACCTAGGTGGGATTAGCTAGTTGGTG
AGGTAATGGCTCACCAAGGCGACGATCCCTAGCTGTTCTGAGAGGATGATCA
GCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGG
GGAATATTGCACAATGGGGGAAACCCTGATGCAGCCATGCCGCGTGTGTGAA
GAAGGCCTTCGGGTTGTAAAGCACTTTCAGTAGGGAGGAAAGGGTGTAGCTT
AATACGCTATATCTGTGACGTTACCTACAGAAGAAGGACCGGCTAACTCCGT
GCCAGCAGCCGCGGTAATACGGAGGGTCCGAGCGTTAATCGGAATTACTGGG
CGTAAAGCGTGCGCAGGCGGTTTGTTAAGCGAGATGTGAAAGCCCTGGGCTC
AACCTAGGAATAGCATTTCGAACTGGCGAACTAGAGTCTTGTAGAGGGGGGT
AGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTG
GCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCATGCACGAAAGCGTGGG
GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCTACT
CGGAGTTTGGTGTCTTGAACACTGGGCTCTCAAGCTAACGCATTAAGTAGAC
CGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGANGGGGGC
CCGCACAAGCGG

Cycle 2, Bag 1, Bacteria 2 (market)

TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCATT
CTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCC
AATCCGGACTACGACGAGCTTTGTGAGATTAGCTCCACCTCGCGGCTTTGCAA
CCCTCTGTACTCGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATG
ATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCCTA
GAGTTCCCACCATTACGTGCTGGCAAATAAGGATAGGGGTTGCGCTCGTTGC
GGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACC
TGTCTCACGGTTCCCGAAGGCACTAAGCTATCTCTAGCGAATTCCGTGGATGT
CAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCAC
CGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACT
CCCCAGGCGGTCTACTTAATGCGTTAGCTTGAGAGCCCAGTGTTCAAGACAC
CAAACTCCGAGTAGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCC
TGTTTGCTCCCCACGCTTTCGTGCATGAGCGTCAGTCTTTGTCCAGGGGGCCG
CCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGG
AATTCTACCCCCCTCTACAAGACTCTAGTTCGCCAGTTCGAAATGCTATTCCT
AGGTTGAGCCCAGGGCTTTCACATCTCGCTTAACAAACCGCCTGCGCACGC

Cycle 2, Bag 1, Bacteria 7 (market)

ATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCAT
TCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTC
CAATCCGGACTACGACGAGCTTTGTGAGATTAGCTCCACCTCGCGGCTTTGCA
ACCCTCTGTACTCGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCAT
GATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCCT
AGAGTTCCCACCATTACGTGCTGGCAAATAAGGATAGGGGTTGCGCTCGTTG
CGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCAC
CTGTCTCACGGTTCCCGAAGGCACTAAGCTATCTCTAGCGAATTCCGTGGATG
TCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCAC
CGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACT
CCCCAGGCGGTCTACTTAATGCGTTAGCTTGAGAGCCCAGTGTTCAAGACAC
CAAACTCCGAGTAGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCC
TGTTTGCTCCCCACGCTTTCGTGCATGAGCGTCAGTCTTTGTCCAGGGGGCCG
CCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGG
AATTCTACCCCCCTCTACAAGACTCTAGTTCGCCAGTTCGAAATGCTATTCCT
AGGTTGAGCCCAGGGCTTTCACATCTCGCTTAACAAACCGCCTGCGCACG

Cycle 2, Bag 2, Bacteria 1 (market)

TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCATT
CTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCC
AATCCGGACTACGACGAGCTTTGTGAGATTAGCTCCACCTCGCGGCTTTGCAA
CCCTCTGTACTCGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATG
ATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCCTA
GAGTTCCCACCATTACGTGCTGGCAAATAAGGATAGGGGTTGCGCTCGTTGC
GGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACC
TGTCTCACGGTTCCCGAAGGCACTAAGCTATCTCTAGCGAATTCCGTGGATGT
CAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCAC
CGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACT
CCCCAGGCGGTCTACTTAATGCGTTAGCTTGAGAGCCCAGTGTTCAAGACAC
CAAACTCCGAGTAGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCC
TGTTTGCTCCCCACGCTTTCGTGCATGAGCGTCAGTCTTTGTCCAGGGGGCCG
CCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGG
AATTCTACCCCCCTCTACAAGACTCTAGTTCGCCAGTTCGAAATGCTATTCCT
AGGTTGAGCCCAGGGCTTTCACATCTCGCTTAACAAACCGCCTGCGCACGC

Cycle 2, Bag 2, Bacteria 4 (market)

CCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGC
ATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGAC
TCCAATCCGGACTACGACGAGCTTTGTGAGATTAGCTCCACCTCGCGGCTTTG
CAACCCTCTGTACTCGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCC
ATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCC
CTAGAGTTCCCACCATTACGTGCTGGCAAATAAGGATAGGGGTTGCGCTCGT
TGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGC
ACCTGTCTCACGGTTCCCGAAGGCACTAAGCTATCTCTAGCGAATTCCGTGGA
TGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCC
ACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTA
CTCCCCAGGCGGTCTACTTAATGCGTTAGCTTGAGAGCCCAGTGTTCAAGACA
CCAAACTCCGAGTAGACATCGTTTACGGCGTGNACTACCNNGGTATCTAATC
CTGTTTGCTCCCCACGCTTTCGTGCATGAGCGTCAGTCTTTGTCCNGGGGCCN
CCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGG
AATTCTACCCCCCTCTACANACTCTAGTTCGC

Cycle 2, Bag 3, Bacteria 4 (market)

TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTAC
GACGAGCTTTGTGAGATTAGCTCCACCTCGCGGCTTTGCAACCCTCTGTACTC
GCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGT
CGTCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCCTAGAGTTCCCACCA
TTACGTGCTGGCAAATAAGGATAGGGGTTGCGCTCGTTGCGGGACTTAACCC
AACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACGGTT
CCCGAAGGCACTAAGCTATCTCTAGCGAATTCCGTGGATGTCAAGAGTAGGT
AAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGG
CCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTC
TACTTAATGCGTTAGCTTGAGAGCCCAGTGTTCAAGACACCAAACTCCGAGT
AGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCA
CGCTTTCGTGCATGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGG
TATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCCCC
TCTACAAGACTCTAGTTTCGCCAGTTCGAAATGCTATTCCTAGGTTGAGCCCA
GGGCTTTCACATCTCGCTTAACAAACCGCCTGCGCACGCTTTACGCCCAGTAA
TTCCGATTAACGCTCGGACCCTCCGTATTACCGCGGCTGCTGGCACGNA

Cycle 1, Bag 1, Bacteria 1 (pond)

TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATT
CTGATCCGCGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCC
AATCCGGACTACGACATACTTTATGGGATTCGCTCACTATCGCTAGCTTGCCG
CCCTTTGTATATGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATG
ATGACTTGACGTCATCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCTTT
GGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCT
GTCTCAGAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCTGGATGTC
AAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACC
GCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTC
CCCAGGCGGTCGATTTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCAC
AACCTCCAAATCGACATCGTTTACAGCGTGGACTACCAGGGTATCTAATCCTG
TTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCC
TTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAA
TTCTACCCCCCTCTACAAGACTCTAGCTTGCCAGTTTCAAATGCAGTTCCCAA
GTTGAGCTCGGGGANTTCACATCTGACTTAACAAACCGCCTGCGTGCGCTTTA
CGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGG
CACGGANTTAGCCGGTGCTTCTTCTGCGANNAACGTCANTGCNACTANGTAT
TNNNANNGACCCNNCCTCCCCNNNTGAAAGNGNTTTNNNACCCGANGGCNT
NN

Cycle 1, Bag 1, Bacteria 3 (pond)

GGACGGGTGAGTAATGCCTGGGGATCTGCCCGATAGAGGGGGATAACTACG
GGAAACTGTAGCTAATACCGCATAACGTCTACGGACCAAAGTGGGGGCTCTT
CGGACCTCATGCTATCGGATGAACCCAGGTGGGATTAGCTAGTTGGTGAGGT
AATGGCTCACCAAGGCGACGATCTCTAACTGGTTTGAGAGAATGACCAGTCA
CACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAA
TATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAG
GCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGGTCACTAGTTAATA
CCTAGTGGCATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCA
GCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTA
AAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGAGCTCAACT
TGGGAACTGCATTTGAAACTGGCAAGCTAGAGTCTTGTAGAGGGGGGTAGAA
TTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGA
AGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGC
AAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGA
GGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCT
GGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCA
CAAGCGGTGGA

Cycle 1, Bag 2, Bacteria 1 (pond)

GCGGACGGGTGAGGAATACATCGGAATCTACCTTTTCGTGGGGGATAACGTA
GGGAAACTTACGCTAATACCGCATACGACCTTCGGGTGAAAGCAGGGGACCT
TCGGGCCTTGCGCGGATAGATGAGCCGATGTCGGATTAGCTAGTTGGCGGGG
TAAAGGCCCACCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCC
ACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAA
GGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAAAGCAGTCGGCTAAT
ACCCGGTTGTTCTGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCC
AGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGT
AAAGCGTGCGTAGGTGGTTGTTTAAGTCTGTTGTGAAAGCCCTGGGCTCAAC
CTGGGAATTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGGA
ATTCCCGGTGTAGCAGTGAAATGCGTAGAGATCGGGAGGAACATCCATGGCG
AAGGCAGCTACCTGGACCAACACTGACACTGAGGCACGAAAGCGTGGGGAG
CAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGA
TGTTGGGTGCAATTTGGCACGCAGTATCGAAGCTAACGCGTTAAGTTCGCCG
CCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCC
GCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTAC
CTGGTCTTGACATGTCGAGAACTTTCCAG

Cycle 1, Bag 2, Bacteria 2 (pond)

GGACGGGTGAGTAATGTCTGGGGATCTGCCCGATAGAGGGGGATAACCACTG
GAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTT
CGGGCCTCTCACTATCGGATGAACCCAGATGGGATTAGCTAGTAGGCGGGGT
AATGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCA
CACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAA
TATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAG
GCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATGGGGTTAATA
ACCTCGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCA
GCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTA
AAGCGCACGCAGGCGGTCTGTTAAGTCAGATGTGAAATCCCCGGGCTTAACC
TGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCTTGTANAGGGGGGTAGAA
TTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGA
ANGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGC
AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGA
GGNTGTTCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTC

Cycle 1, Bag 2, Bacteria 6 (pond)

GGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAACATTC
TGATTTGCGATTACTAGCGATTCCGACTTCACGGAGTCGAGTTGCAGACTCCG
ATCCGGACTACGACGCGCTTTTTGGGATTCGCTCACTATCGCTAGCTTGCAGC
CCTCTGTACGCGCCATTGTAGCACGTGTGTAGCCCTGGCCGTAAGGGCCATG
ATGACTTGACGTCATCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCCTT
GAGTTCCCACCATTACGTGCTGGCAACAAAGGACAGGGGTTGCGCTCGTTGC
GGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACC
TGTGTTCTGATTCCCGAAGGCACTCCCGTATCTCTACAGGATTCCAGACATGT
CAAGGCCAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCAC
CGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACT
CCCCAGGCGGTCGATTTAACGCGTTAGCTCCGGAAGCCACGTCTCAAGGACA
CAGCCTCCAAATCGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCT
GTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGC
CTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGA
ATTCTACCCCCCTCTACAAGACTCTAGCTGGACAGTTTTAAATGCAATTCCCA
GGTTGAGCCCGGGGCTTTCACATCTAACTTATCCAACCGCCTGCGTGCGCT

Cycle 1, Bag 3, Bacteria 1 (pond)

GCGGACGGGTGAGTAATGCCTAGGGATCTGCCCAGTCGAGGGGGATAACAGT
TGGAAACGACTGCTAATACCGCATACGCCCTACGGGGGAAAGGAGGGGACC
TTCGGGCCTTCCGCGATTGGATGAACCTAGGTGGGATTAGCTAGTTGGTGAG
GTAATGGCTCACCAAGGCGACGATCCCTAGCTGTTCTGAGAGGATGATCAGC
CACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGG
AATATTGCACAATGGGGGAAACCCTGATGCAGCCATGCCGCGTGTGTGAAGA
AGGCCTTCGGGTTGTAAAGCACTTTCAGTAGGGAGGAAAGGGTGAGTCTTAA
TACGGCTCATCTGTGACGTTACCTACAGAAGAAGGACCGGCTAACTCCGTGC
CAGCAGCCGCGGTAATACGGAGGGTCCGAGCGTTAATCGGAATTACTGGGCG
TAAAGCGTGCGCAGGCGGTTTGTTAAGCGAGATGTGAAAGCCCCGGGCTCAA
CCTGGGAATTGCATTTCGAACTGGCGAACTAGAGTCTTGTAGAGGGGGGTAG
AATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGC
GAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGCACGAAAGCGTGGGGA
GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCTACTCG
GAGTTTGGTGTCTTGAACACTGGGCTCTCAAGCTAACGCATTAAG

Cycle 1, Bag 3, Bacteria 3 (pond)

GGACGGGTGAGTAATGTCTGGGGATCTGCCCGATAGAGGGGGATAACCACTG
GAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTT
CGGGCCTCTCACTATCGGATGAACCCAGATGGGATTAGCTAGTAGGCGGGGT
AATGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCA
CACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAA
TATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAG
GCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATGGGGTTAATA
ACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCA
GCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTA
AAGCGCACGCAGGCGGTCTGTTAAGTCAGATGTGAAATCCCCGGGCTTAACC
TGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCTTGTANAGGGGGGTAGAA
TTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGA
ANGCGGCCCCCTGGACAAAGACTGACGCTCANGTGCGAAAGCGTGGGGAGC
AAACAGGATTAGATACCCTGGTAGTCC

Cycle 1, Bag 5, Bacteria 2 (pond)

GCGGACGGGTGAGTAATGCCTAGGGATCTGCCCAGTCGAGGGGGATAACAGT
TGGAAACGACTGCTAATACCGCATACGCCCTACGGGGGAAAGGAGGGGACC
TTCGGGCCTTCCGCGATTGGATGAACCTAGGTGGGATTAGCTAGTTGGTGAG
GTAATGGCTCACCAAGGCGACGATCCCTAGCTGTTCTGAGAGGATGATCAGC
CACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGG
AATATTGCACAATGGGGGAAACCCTGATGCAGCCATGCCGCGTGTGTGAAGA
AGGCCTTCGGGTTGTAAAGCACTTTCAGTAGGGAGGAAAGGGTGAGTCTTAA
TACGGCTCATCTGTGACGTTACCTACAGAAGAAGGACCGGCTAACTCCGTGC
CAGCAGCCGCGGTAATACGGAGGGTCCGAGCGTTAATCGGAATTACTGGGCG
TAAAGCGTGCGCAGGCGGTTTGTTAAGCGAGATGTGAAAGCCCCGGGCTCAA
CCTGGGAATTGCATTTCGAACTGGCGAACTAGAGTCTTGTAGAGGGGGGTAG
AATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGC
GAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGCACGAAAGCGTGGGGA
GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCTACTCG
GAGTTTGGTGTCTTGAACACTGGGCTCTCAAGCTAACGCATTAAGTAGACCG
CCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCG
CACAAGCGGNG

Cycle 1, Bag 5, Bacteria 3 (pond)

ATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGACAT
TCTGATTCGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTG
CGATCCGGACTACGATCGGTTTTCTGGGATTAGCTCCACCTCGCGGCTTGGCA
ACCCTCTGTACCGACCATTGTAGCACGTGTGTAGCCCAGGCCGTAAGGGCCA
TGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCCT
TAGAGTGCCCACCATTACGTGCTGGTAACTAAGGACAAGGGTTGCGCTCGTT
ACGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCA
CCTGTCTCAATGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCATTGGAT
GTCAAGGCCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCA
CCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTAC
TCCCCAGGCGGTCAACTTAATGCGTTAGCTGCGCCACTAAGAGCTCAAGGCT
CCCAACGGCTAGTTGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCC
TGTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTATCAGTCCAGGTGGTCG
CCTTCGCCACTGGTGTTCCTTCCTATATCTACGCATTTCACCGCTACACAGGA
AATTCCACCACCCTCTACCATACTCTAGCTTGTCAGTTTTGAATGCAGTTCCC
AGGTTGAGCCCGGGGATTTCACATCCAACTTAACAAACCACCTACGCGCG

Cycle 1, Bag 8, Bacteria 2 (pond)

GGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGACATTC
TGATTCGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCG
ATCCGGACTACGATCGGTTTTCTGGGATTAGCTCCACCTCGCGGCTTGGCAAC
CCTCTGTACCGACCATTGTAGCACGTGTGTAGCCCAGGCCGTAAGGGCCATG
ATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCCTTA
GAGTGCCCACCATTACGTGCTGGTAACTAAGGACAAGGGTTGCGCTCGTTAC
GGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACC
TGTCTCAATGTTCCCGAAGGCACCAATCTATCTCTNGAAAGTTCATTGGATGT
CAAGGCCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACC
GCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTC
CCCAGGCGGTCAACTTAATGCGTTAGCTGCGCCACTAAGAGCTCAAGGCTCC
CAACGGCTAGTTGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTG
TTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTATCAGTCCAGGTGGTCGCC
TTCGCCACTGGTGTTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAA
TTCCACCACCCTCTACCATACTCTAGTCAGTCAGTTTTGAATGCAGTTCCCAG
GTTGAGCCCGGGGATTTCACATCCAACTTAACTAACCACCTACGCGCGCT

Appendix B

Sensory Evaluation Sheet

Name_____ Date_____

Baked Catfish Evaluation

1. Please rinse your mouth with water before starting and in between tasting samples.
2. **Any person who smokes or quit recently cannot be a panel.**

You have been given a reference sample marked "R" (control) and six unknown samples labeled with a 3-digit number. Rate the following attributes according to the Hedonic Scale provided below:

1 – Dislike extremely
2 – Dislike very much
3 – Dislike moderately
4 – Dislike slightly
5 – Neither like nor dislike
6 – Like slightly
7 – Like moderately
8 – Like very much
9 – Like extremely

Attributes	R	Sample #____	Sample #____	Sample #____	Sample #____	Sample #____	Sample #____
Appearance (color)							
Aroma							
Flavor							
Texture							

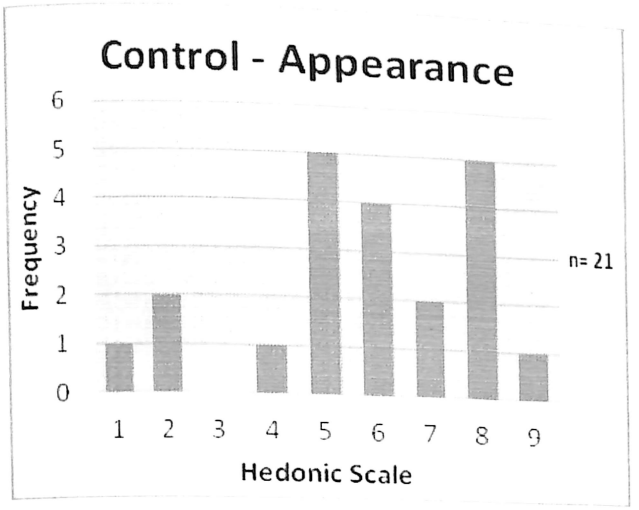
Comments:_____

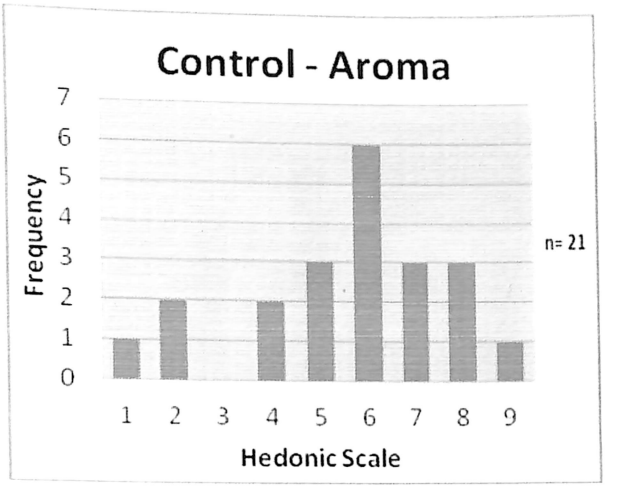
_____

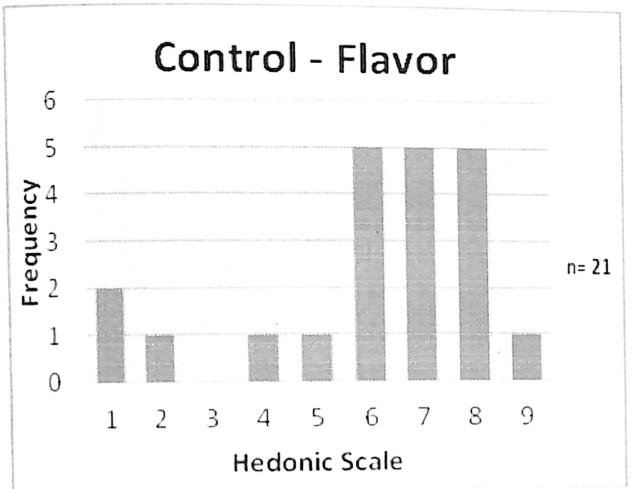
Thank you!

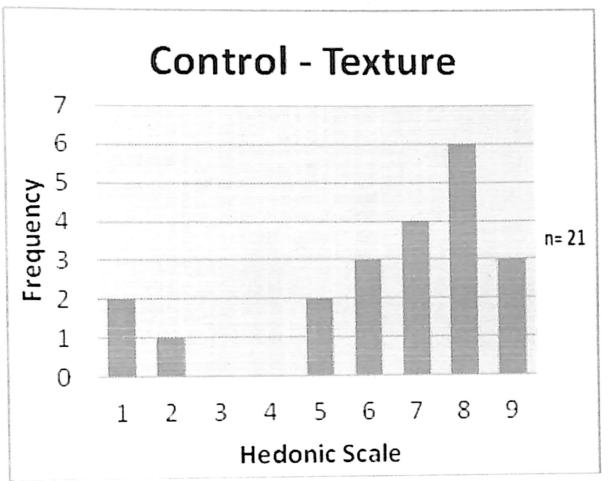
Appendix C

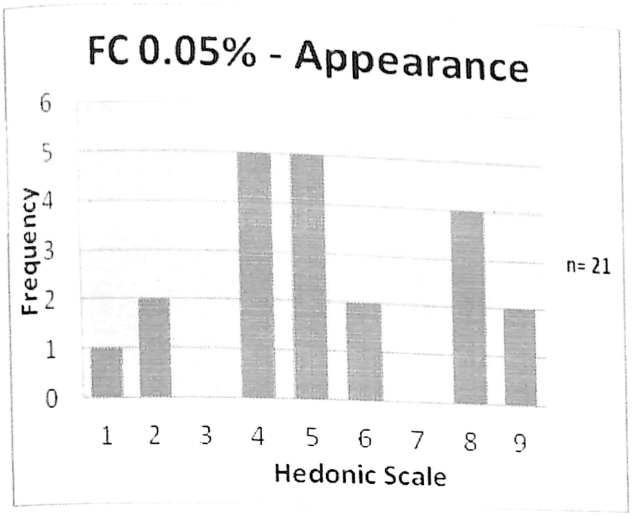
Histograms of sensory evaluation attribute rating frequencies

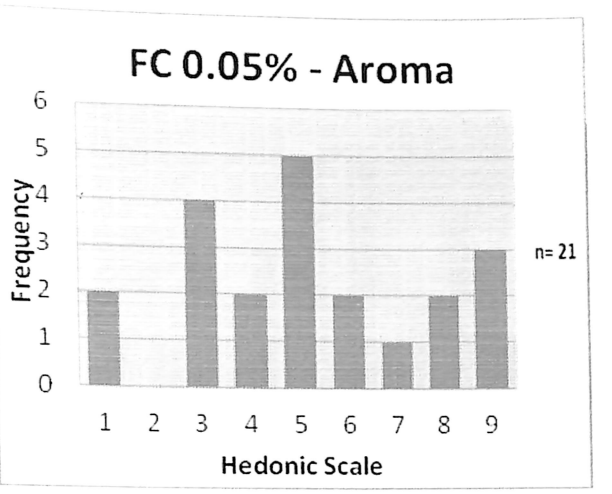

Control - Appearance
Frequency
0
1
2
3
4
5
6
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21

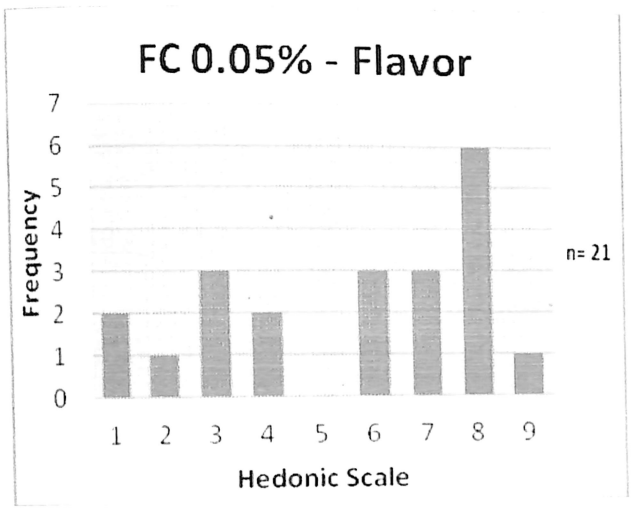

Control - Aroma
Frequency
0
1
2
3
4
5
6
7
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21

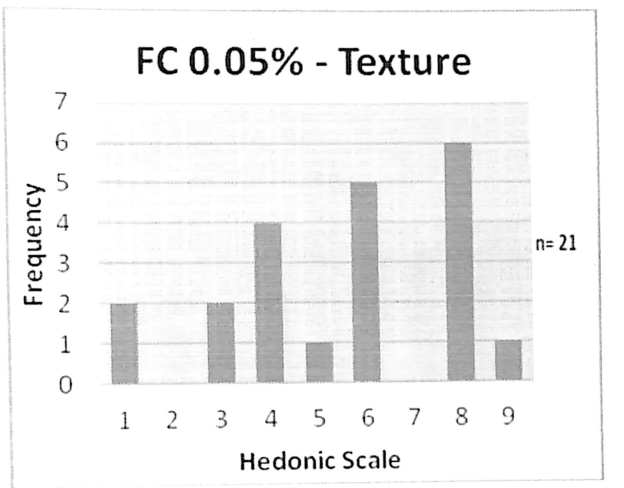

Control - Flavor
Frequency
0
1
2
3
4
5
6
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21

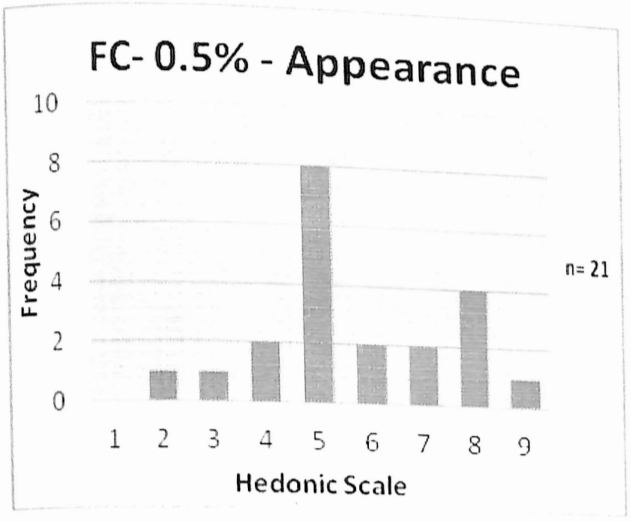

Control - Texture
Frequency
0
1
2
3
4
5
6
7
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21

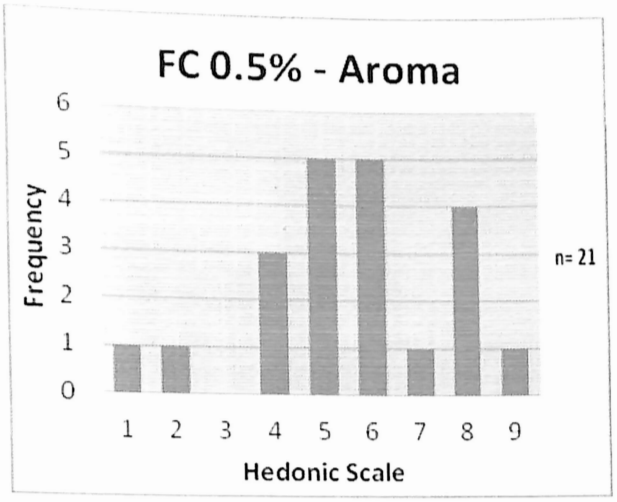

FC 0.05% - Appearance
Frequency
0
1
2
3
4
5
6
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21

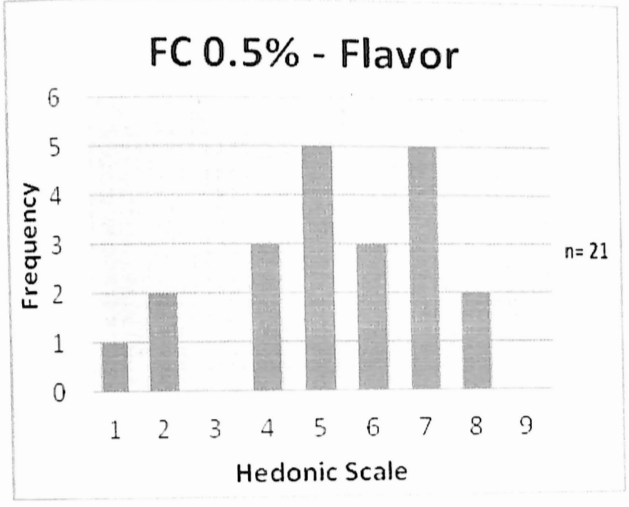

FC 0.05% - Aroma
Frequency
0
1
2
3
4
5
6
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21

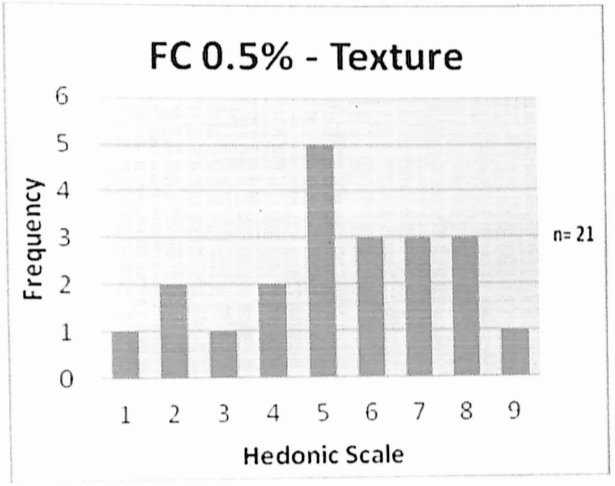

FC 0.05% - Flavor
Frequency
0
1
2
3
4
5
6
7
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21

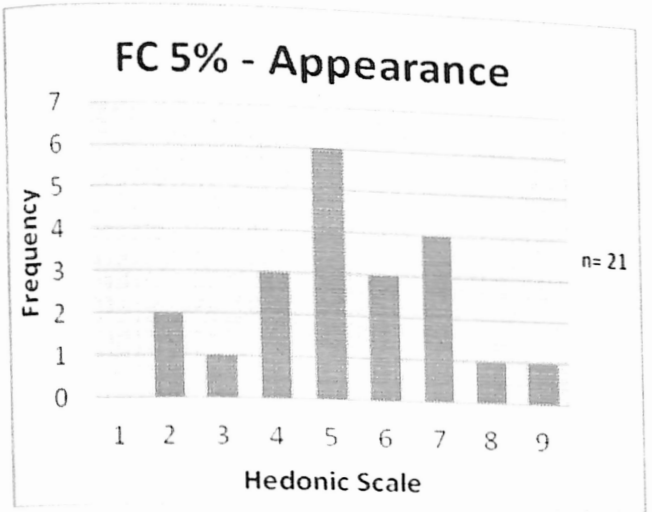

FC 0.05% - Texture
Frequency
0
1
2
3
4
5
6
7
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21

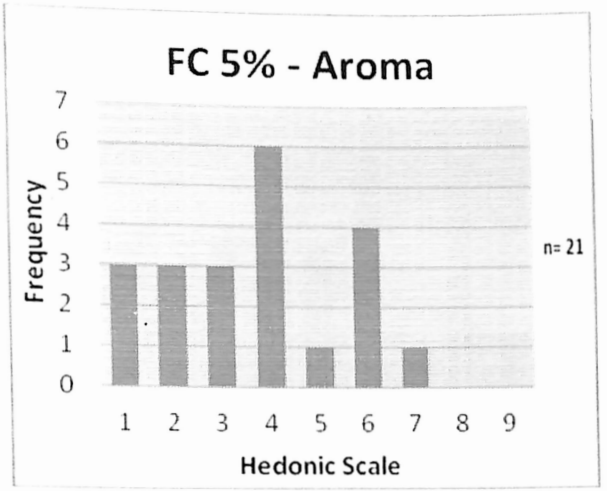

FC- 0.5% - Appearance
Frequency
Hedonic Scale
n= 21

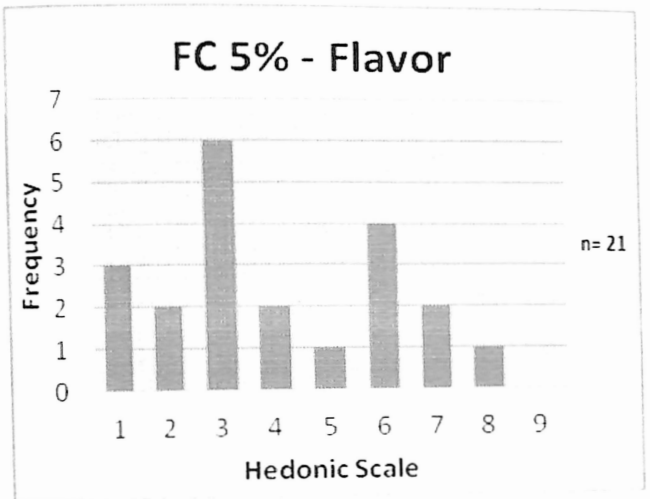

FC 0.5% - Aroma
Frequency
Hedonic Scale
n= 21

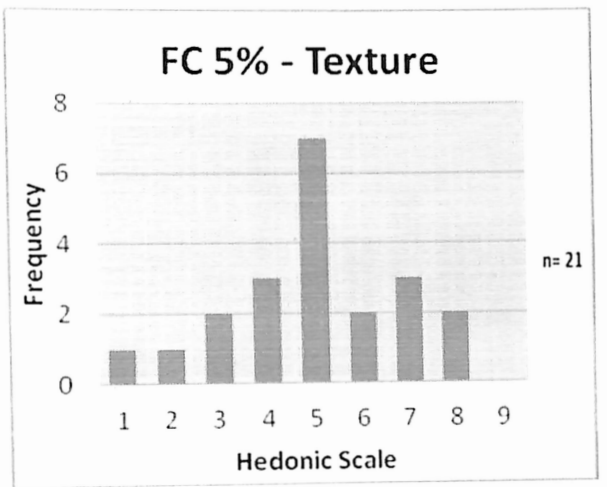

FC 0.5% - Flavor
Frequency
Hedonic Scale
n= 21

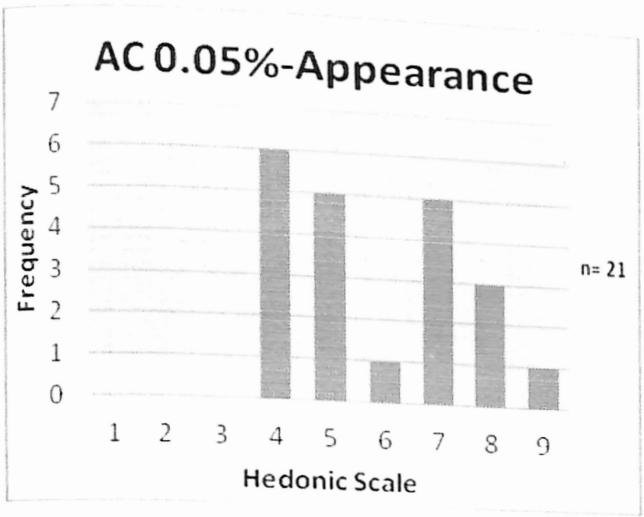

FC 0.5% - Texture
Frequency
Hedonic Scale
n= 21

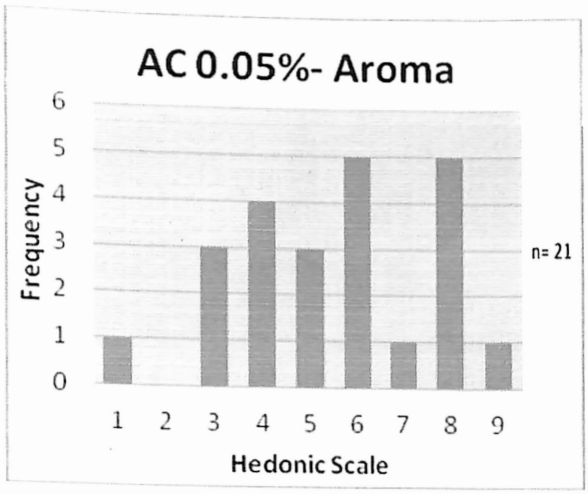

FC 5% - Appearance
Frequency
0
1
2
3
4
5
6
7
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21

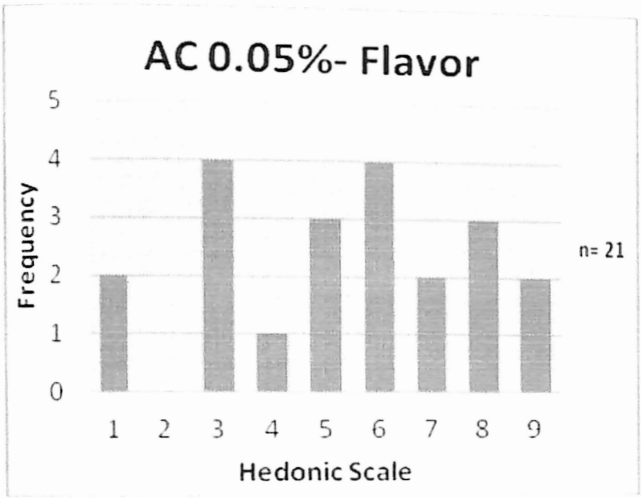

FC 5% - Aroma
Frequency
0
1
2
3
4
5
6
7
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21

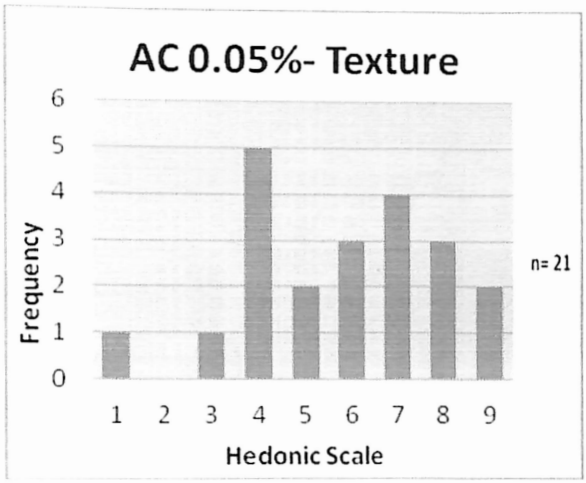

FC 5% - Flavor
Frequency
0
1
2
3
4
5
6
7
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21

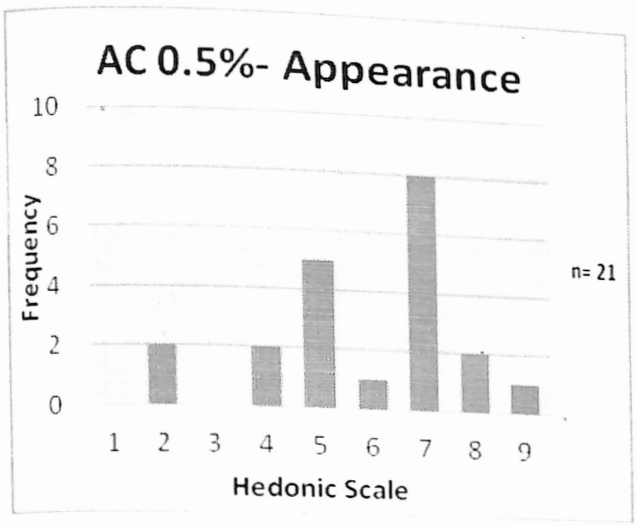

FC 5% - Texture
Frequency
0
2
4
6
8
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21


AC 0.05%-Appearance
Frequency
0
1
2
3
4
5
6
7
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21


AC 0.05%- Aroma
Frequency
0
1
2
3
4
5
6
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21


AC 0.05%- Flavor
Frequency
0
1
2
3
4
5
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21


AC 0.05%- Texture
Frequency
0
1
2
3
4
5
6
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21


AC 0.5%- Appearance
Frequency
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8
6
4
2
0
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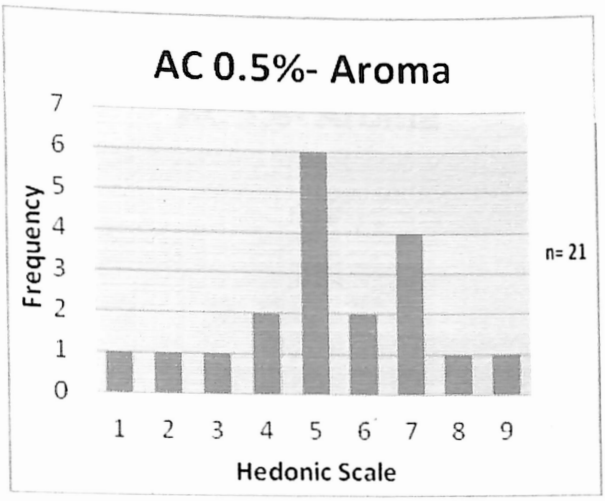
Hedonic Scale
n= 21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Frequency
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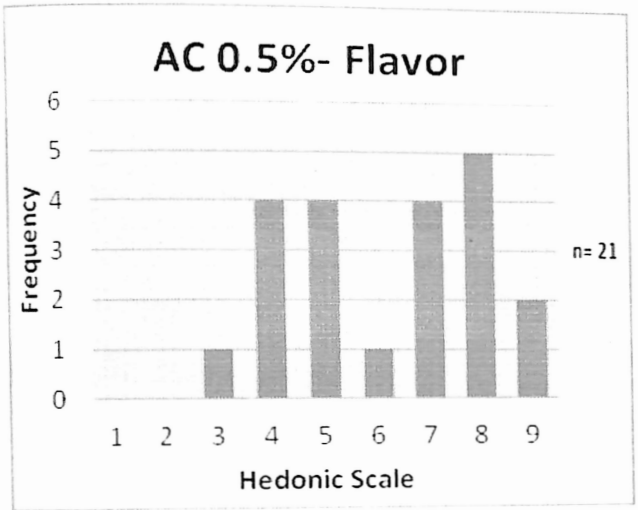
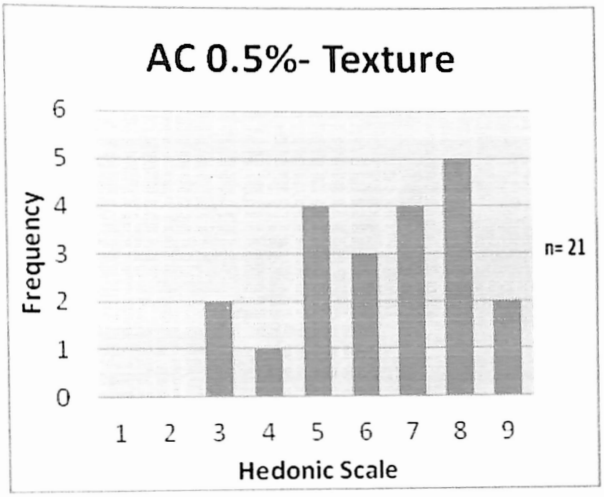
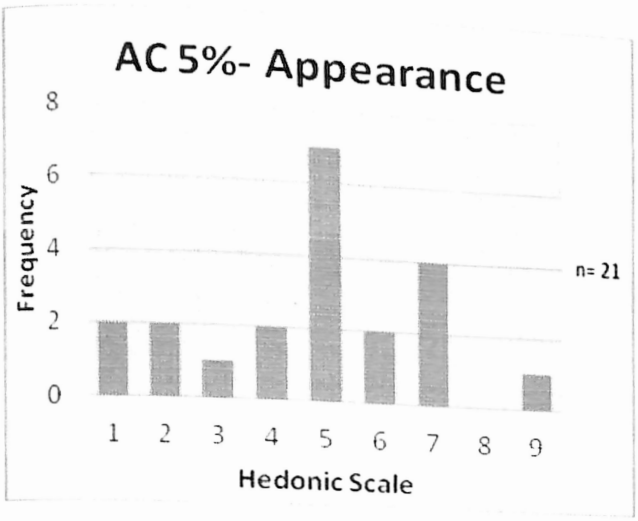
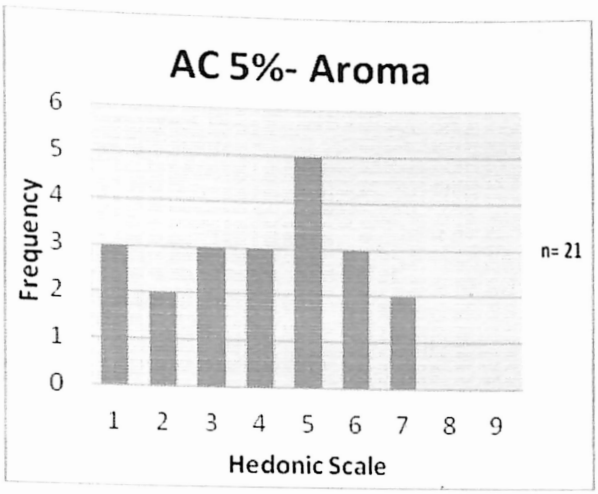
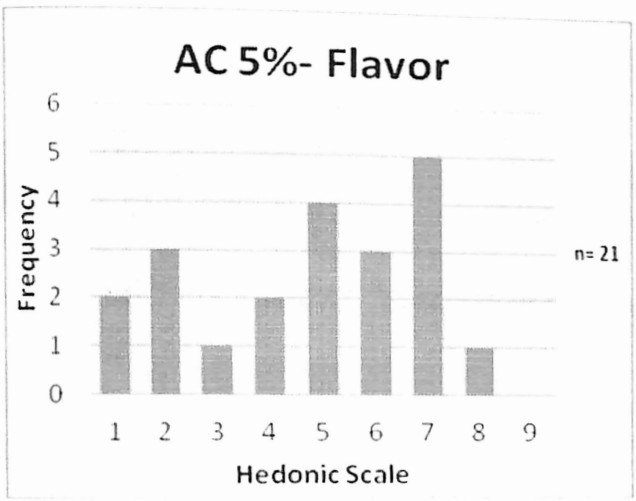
6
5
4
3
2
1
0
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21

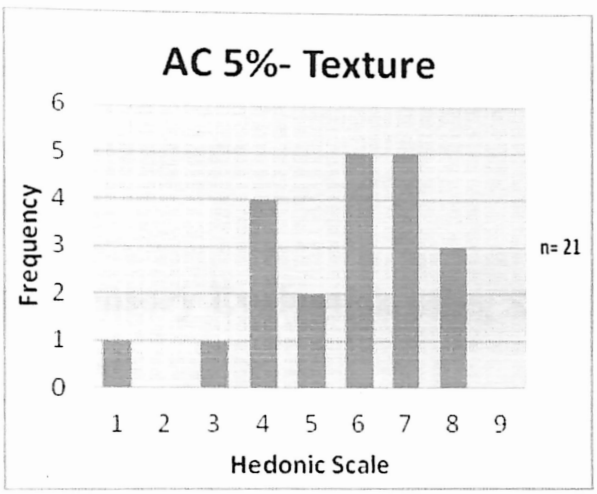

AC 0.5%- Flavor
Frequency
6
5
4
3
2
1
0
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21


AC 0.5%- Texture
Frequency
6
5
4
3
2
1
0
1 2 3 4 5 6 7 8 9
Hedonic Scale
n= 21


AC 5%- Appearance
Frequency
Hedonic Scale
n= 21


AC 5%- Aroma
Frequency
Hedonic Scale
n= 21


AC 5%- Flavor
Frequency
Hedonic Scale
n= 21


AC 5%- Texture
Frequency
Hedonic Scale
n= 21

Appendix D

Generalized Linear Models Analysis of Sensory Evaluation using SAS

Generalized Linear Models Analysis

The GENMOD Procedure

Highlighted parts indicates significance ($p < 0.05$)

Model Information	
Data Set	WORK.SORTTEMPTABLESORTED
Distribution	Normal
Link Function	Identity
Dependent Variable	Rating

Number of Observations Read	588
Number of Observations Used	588

Class Level Information

Class	Levels	Values
Sample	7	AC 0.05% AC 0.5% AC 5% FC 0.05% FC 0.5% FC 5% R (Control)
Attribute	4	Appearance Aroma Flavor Texture

Parameter Information

Parameter	Effect	Sample	Attribute
Prm1	Intercept		
Prm2	Sample	AC 0.05%	
Prm3	Sample	AC 0.5%	
Prm4	Sample	AC 5%	
Prm5	Sample	FC 0.05%	
Prm6	Sample	FC 0.5%	
Prm7	Sample	FC 5%	
Prm8	Sample	R (Control)	
Prm9	Attribute		Appearance
Prm10	Attribute		Aroma
Prm11	Attribute		Flavor

Prm12	Attribute		Texture
Prm13	Sample*Attribute	AC 0.05%	Appearance
Prm14	Sample*Attribute	AC 0.05%	Aroma
Prm15	Sample*Attribute	AC 0.05%	Flavor
Prm16	Sample*Attribute	AC 0.05%	Texture
Prm17	Sample*Attribute	AC 0.5%	Appearance
Prm18	Sample*Attribute	AC 0.5%	Aroma
Prm19	Sample*Attribute	AC 0.5%	Flavor
Prm20	Sample*Attribute	AC 0.5%	Texture
Prm21	Sample*Attribute	AC 5%	Appearance
Prm22	Sample*Attribute	AC 5%	Aroma
Prm23	Sample*Attribute	AC 5%	Flavor
Prm24	Sample*Attribute	AC 5%	Texture
Prm25	Sample*Attribute	FC 0.05%	Appearance
Prm26	Sample*Attribute	FC 0.05%	Aroma
Prm27	Sample*Attribute	FC 0.05%	Flavor
Prm28	Sample*Attribute	FC 0.05%	Texture
Prm29	Sample*Attribute	FC 0.5%	Appearance
Prm30	Sample*Attribute	FC 0.5%	Aroma
Prm31	Sample*Attribute	FC 0.5%	Flavor
Prm32	Sample*Attribute	FC 0.5%	Texture
Prm33	Sample*Attribute	FC 5%	Appearance
Prm34	Sample*Attribute	FC 5%	Aroma
Prm35	Sample*Attribute	FC 5%	Flavor
Prm36	Sample*Attribute	FC 5%	Texture
Prm37	Sample*Attribute	R (Control)	Appearance
Prm38	Sample*Attribute	R (Control)	Aroma
Prm39	Sample*Attribute	R (Control)	Flavor
Prm40	Sample*Attribute	R (Control)	Texture

Criteria For Assessing Goodness Of Fit			
Criterion	DF	Value	Value/DF
Deviance	560	2438.6667	4.3548
Scaled Deviance	560	588.0000	1.0500
Pearson Chi-Square	560	2438.6667	4.3548
Scaled Pearson X2	560	588.0000	1.0500
Log Likelihood		-1252.5449	
Full Log Likelihood		-1252.5449	
AIC (smaller is better)		2563.0898	
AICC (smaller is better)		2566.2081	
BIC (smaller is better)		2690.0149	

Algorithm converged.

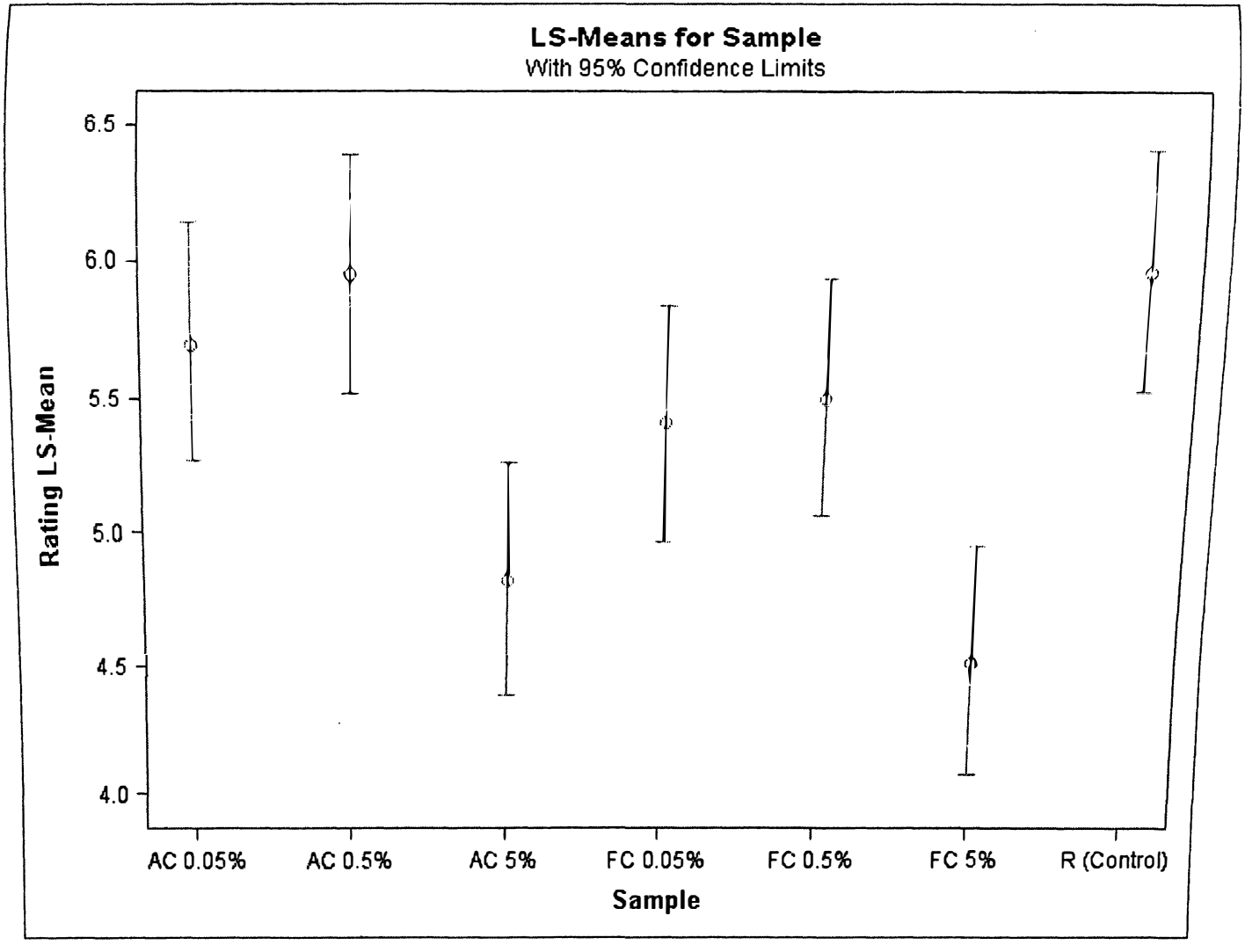
Analysis Of Maximum Likelihood Parameter Estimates									
Parameter			DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Intercept			1	6.4286	0.4444	5.5576	7.2996	209.25	<.0001
Sample	AC 0.05%		1	-0.6190	0.6285	-1.8508	0.6128	0.97	0.3246
Sample	AC 0.5%		1	-0.0476	0.6285	-1.2794	1.1842	0.01	0.9396
Sample	AC 5%		1	-0.7619	0.6285	-1.9937	0.4699	1.47	0.2254
Sample	FC 0.05%		1	-0.9048	0.6285	-2.1366	0.3270	2.07	0.1500
Sample	FC 0.5%		1	-1.0476	0.6285	-2.2794	0.1842	2.78	0.0955
Sample	FC 5%		1	-1.4286	0.6285	-2.6604	-0.1968	5.17	0.0230
Sample	R (Control)		0	0.0000	0.0000	0.0000	0.0000	.	.
Attribute	Appearance		1	-0.6667	0.6285	-1.8985	0.5651	1.13	0.2888
Attribute	Aroma		1	-0.8095	0.6285	-2.0413	0.4223	1.66	0.1977
Attribute	Flavor		1	-0.3810	0.6285	-1.6128	0.8508	0.37	0.5444
Attribute	Texture		0	0.0000	0.0000	0.0000	0.0000	.	.
Sample*Attribute	AC 0.05%	Appearance	1	0.7143	0.8888	-1.0277	2.4563	0.65	0.4216
Sample*Attribute	AC 0.05%	Aroma	1	0.7619	0.8888	-0.9801	2.5039	0.73	0.3913

Sample*AttributeAC 0.05%	Flavor	1	-0.0476	0.8888	-1.7897	1.6944	0.00	0.9573
Sample*AttributeAC 0.05%	Texture	0	0.0000	0.0000	0.0000	0.0000	.	.
Sample*AttributeAC 0.5%	Appearance	1	0.1905	0.8888	-1.5516	1.9325	0.05	0.8303
Sample*AttributeAC 0.5%	Aroma	1	-0.2857	0.8888	-2.0277	1.4563	0.10	0.7479
Sample*AttributeAC 0.5%	Flavor	1	0.2381	0.8888	-1.5039	1.9801	0.07	0.7888
Sample*AttributeAC 0.5%	Texture	0	0.0000	0.0000	0.0000	0.0000	.	.
Sample*AttributeAC 5%	Appearance	1	-0.1905	0.8888	-1.9325	1.5516	0.05	0.8303
Sample*AttributeAC 5%	Aroma	1	-0.8095	0.8888	-2.5516	0.9325	0.83	0.3624
Sample*AttributeAC 5%	Flavor	1	-0.5238	0.8888	-2.2658	1.2182	0.35	0.5556
Sample*AttributeAC 5%	Texture	0	0.0000	0.0000	0.0000	0.0000	.	.
Sample*AttributeFC 0.05%	Appearance	1	0.4762	0.8888	-1.2658	2.2182	0.29	0.5921
Sample*AttributeFC 0.05%	Aroma	1	0.4762	0.8888	-1.2658	2.2182	0.29	0.5921
Sample*AttributeFC 0.05%	Flavor	1	0.4286	0.8888	-1.3135	2.1706	0.23	0.6297
Sample*AttributeFC 0.05%	Texture	0	0.0000	0.0000	0.0000	0.0000	.	.
Sample*AttributeFC 0.5%	Appearance	1	1.0000	0.8888	-0.7420	2.7420	1.27	0.2605
Sample*AttributeFC 0.5%	Aroma	1	1.0476	0.8888	-0.6944	2.7897	1.39	0.2385
Sample*AttributeFC 0.5%	Flavor	1	0.2857	0.8888	-1.4563	2.0277	0.10	0.7479
Sample*AttributeFC 0.5%	Texture	0	0.0000	0.0000	0.0000	0.0000	.	.
Sample*AttributeFC 5%	Appearance	1	1.0000	0.8888	-0.7420	2.7420	1.27	0.2605
Sample*AttributeFC 5%	Aroma	1	-0.4762	0.8888	-2.2182	1.2658	0.29	0.5921
Sample*AttributeFC 5%	Flavor	1	-0.6190	0.8888	-2.3611	1.1230	0.49	0.4861
Sample*AttributeFC 5%	Texture	0	0.0000	0.0000	0.0000	0.0000	.	.
Sample*AttributeR (Control)	Appearance	0	0.0000	0.0000	0.0000	0.0000	.	.
Sample*AttributeR (Control)	Aroma	0	0.0000	0.0000	0.0000	0.0000	.	.
Sample*AttributeR (Control)	Flavor	0	0.0000	0.0000	0.0000	0.0000	.	.
Sample*AttributeR (Control)	Texture	0	0.0000	0.0000	0.0000	0.0000	.	.
Scale		1	2.0365	0.0594	1.9234	2.1563		

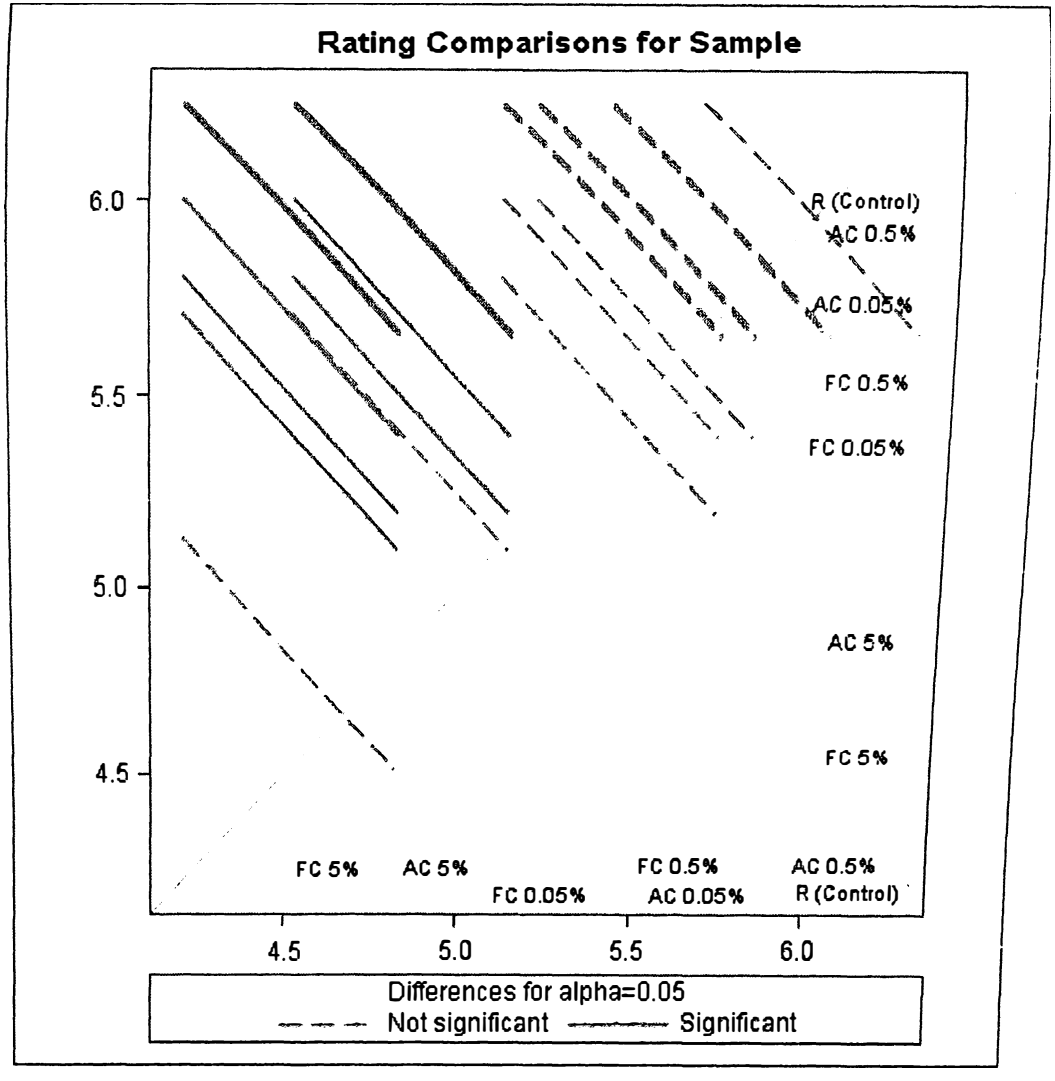
Note: The scale parameter was estimated by maximum likelihood.

LR Statistics For Type 3 Analysis			
Source	DF	Chi-Square	Pr > ChiSq
Sample	6	36.29	<.0001
Attribute	3	9.59	0.0224
Sample*Attribute	18	13.36	0.7697

Sample Least Squares Means							
Sample	Estimate	Standard Error	z Value	Pr > |z|	Alpha	Lower	Upper
AC 0.05%	5.7024	0.2222	25.66	<.0001	0.05	5.2669	6.1379
AC 0.5%	5.9524	0.2222	26.79	<.0001	0.05	5.5169	6.3879
AC 5%	4.8214	0.2222	21.70	<.0001	0.05	4.3859	5.2569
FC 0.05%	5.4048	0.2222	24.32	<.0001	0.05	4.9693	5.8403
FC 0.5%	5.5000	0.2222	24.75	<.0001	0.05	5.0645	5.9355
FC 5%	4.5119	0.2222	20.31	<.0001	0.05	4.0764	4.9474
R (Control)	5.9643	0.2222	26.84	<.0001	0.05	5.5288	6.3998

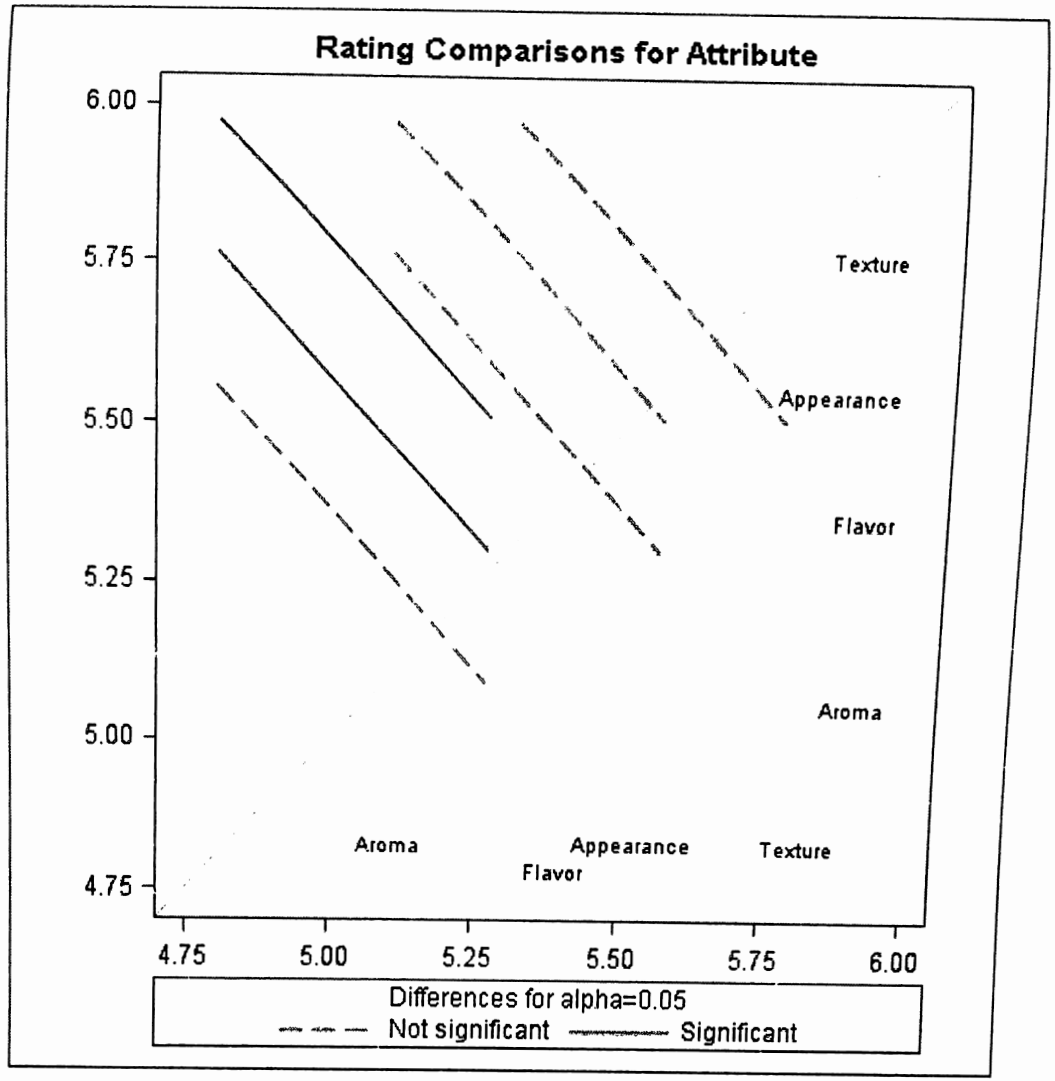

LS-Means for Sample
With 95% Confidence Limits
Rating LS-Mean
6.5
6.0
5.5
5.0
4.5
4.0
AC 0.05%
AC 0.5%
AC 5%
FC 0.05%
FC 0.5%
FC 5%
R (Control)
Sample

Differences of Sample Least Squares Means								
Sample	_Sample	Estimate	Standard Error	z Value	Pr > |z|	Alpha	Lower	Upper
AC 0.05%	AC 0.5%	-0.2500	0.3142	-0.80	0.4263	0.05	-0.8659	0.3659
AC 0.05%	AC 5%	0.8810	0.3142	2.80	0.0051	0.05	0.2651	1.4969
AC 0.05%	FC 0.05%	0.2976	0.3142	0.95	0.3436	0.05	-0.3183	0.9135
AC 0.05%	FC 0.5%	0.2024	0.3142	0.64	0.5196	0.05	-0.4135	0.8183
AC 0.05%	FC 5%	1.1905	0.3142	3.79	0.0002	0.05	0.5746	1.8064
AC 0.05%	R (Control)	-0.2619	0.3142	-0.83	0.4046	0.05	-0.8778	0.3540
AC 0.5%	AC 5%	1.1310	0.3142	3.60	0.0003	0.05	0.5151	1.7469
AC 0.5%	FC 0.05%	0.5476	0.3142	1.74	0.0814	0.05	-0.06828	1.1635
AC 0.5%	FC 0.5%	0.4524	0.3142	1.44	0.1500	0.05	-0.1635	1.0683
AC 0.5%	FC 5%	1.4405	0.3142	4.58	<.0001	0.05	0.8246	2.0564
AC 0.5%	R (Control)	-0.01190	0.3142	-0.04	0.9698	0.05	-0.6278	0.6040
AC 5%	FC 0.05%	-0.5833	0.3142	-1.86	0.0634	0.05	-1.1992	0.03257
AC 5%	FC 0.5%	-0.6786	0.3142	-2.16	0.0308	0.05	-1.2945	-0.06267
AC 5%	FC 5%	0.3095	0.3142	0.98	0.3246	0.05	-0.3064	0.9254
AC 5%	R (Control)	-1.1429	0.3142	-3.64	0.0003	0.05	-1.7588	-0.5270
FC 0.05%	FC 0.5%	-0.09524	0.3142	-0.30	0.7618	0.05	-0.7111	0.5207
FC 0.05%	FC 5%	0.8929	0.3142	2.84	0.0045	0.05	0.2770	1.5088
FC 0.05%	R (Control)	-0.5595	0.3142	-1.78	0.0750	0.05	-1.1754	0.05638
FC 0.5%	FC 5%	0.9881	0.3142	3.14	0.0017	0.05	0.3722	1.6040
FC 0.5%	R (Control)	-0.4643	0.3142	-1.48	0.1395	0.05	-1.0802	0.1516
FC 5%	R (Control)	-1.4524	0.3142	-4.62	<.0001	0.05	-2.0683	-0.8365

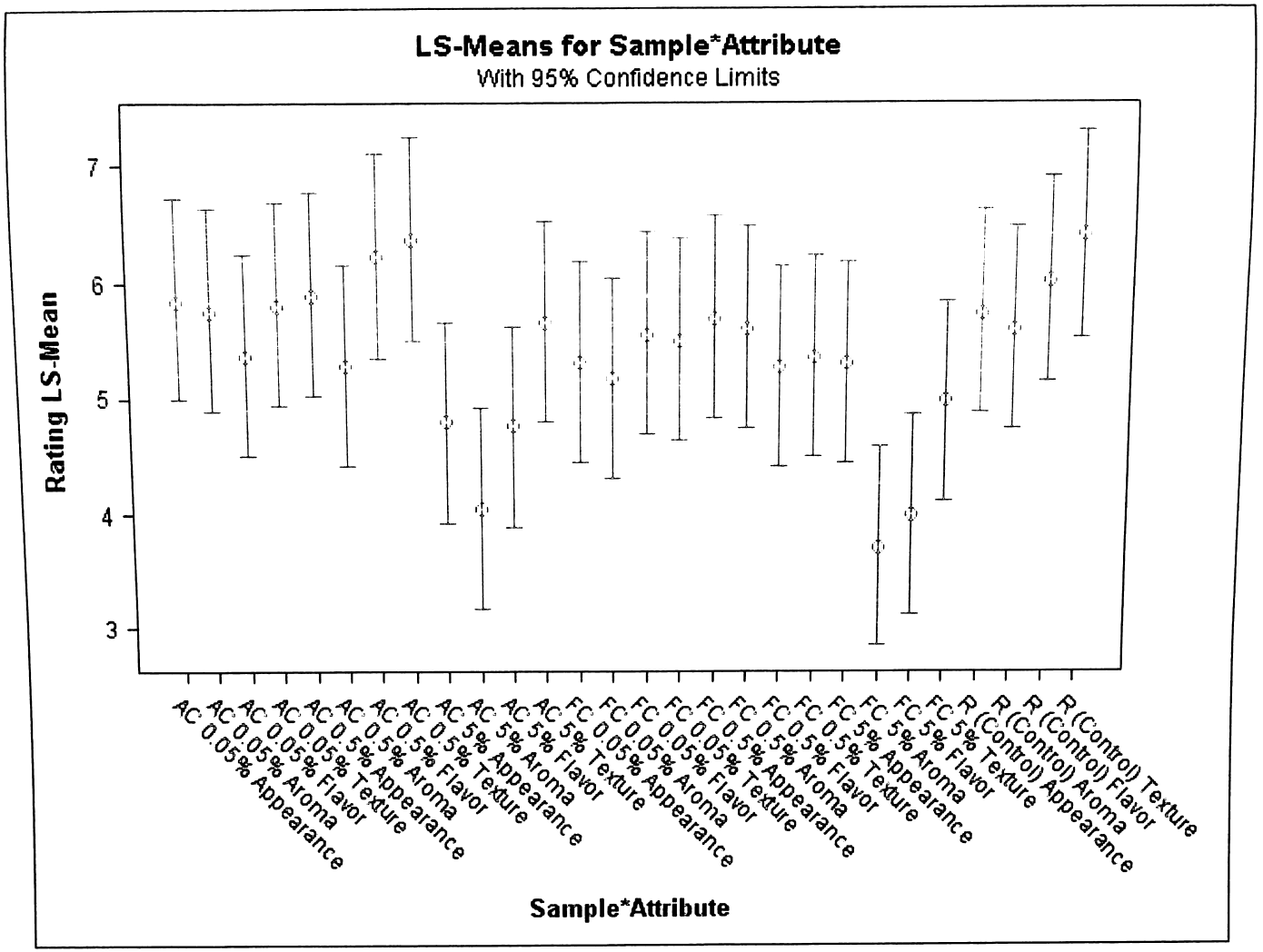

Rating Comparisons for Sample
6.0
5.5
5.0
4.5
R (Control)
AC 0.5%
AC 0.05%
FC 0.5%
FC 0.05%
AC 5%
FC 5%
FC 5%
AC 5%
FC 0.05%
FC 0.5%
AC 0.05%
AC 0.5%
R (Control)
4.5
5.0
5.5
6.0
Differences for alpha=0.05
Not significant
Significant

Attribute Least Squares Means							
Attribute	Estimate	Standard Error	z Value	Pr > |z|	Alpha	Lower	Upper
Appearance	5.5306	0.1680	32.93	<.0001	0.05	5.2014	5.8598
Aroma	5.0340	0.1680	29.97	<.0001	0.05	4.7048	5.3632
Flavor	5.3265	0.1680	31.71	<.0001	0.05	4.9973	5.6557
Texture	5.7415	0.1680	34.18	<.0001	0.05	5.4123	6.0707

Differences of Attribute Least Squares Means								
Attribute	_Attribute	Estimate	Standard Error	z Value	Pr > |z|	Alpha	Lower	Upper
Appearance	Aroma	0.4966	0.2375	2.09	0.0366	0.05	0.03102	0.9622
Appearance	Flavor	0.2041	0.2375	0.86	0.3903	0.05	-0.2615	0.6697
Appearance	Texture	-0.2109	0.2375	-0.89	0.3747	0.05	-0.6765	0.2547
Aroma	Flavor	-0.2925	0.2375	-1.23	0.2182	0.05	-0.7581	0.1731
Aroma	Texture	-0.7075	0.2375	-2.98	0.0029	0.05	-1.1731	-0.2419
Flavor	Texture	-0.4150	0.2375	-1.75	0.0807	0.05	-0.8805	0.05061


Rating Comparisons for Attribute
6.00
5.75
5.50
5.25
5.00
4.75
Texture
Appearance
Flavor
Aroma
Aroma
Appearance
Flavor
Texture
4.75
5.00
5.25
5.50
5.75
6.00
Differences for alpha=0.05
Not significant
Significant

Sample*Attribute Least Squares Means								
Sample	Attribute	Estimate	Standard Error	z Value	Pr > |z|	Alpha	Lower	Upper
AC 0.05%	Appearance	5.8571	0.4444	13.18	<.0001	0.05	4.9861	6.7282
AC 0.05%	Aroma	5.7619	0.4444	12.97	<.0001	0.05	4.8909	6.6329
AC 0.05%	Flavor	5.3810	0.4444	12.11	<.0001	0.05	4.5099	6.2520
AC 0.05%	Texture	5.8095	0.4444	13.07	<.0001	0.05	4.9385	6.6805
AC 0.5%	Appearance	5.9048	0.4444	13.29	<.0001	0.05	5.0337	6.7758
AC 0.5%	Aroma	5.2857	0.4444	11.89	<.0001	0.05	4.4147	6.1567
AC 0.5%	Flavor	6.2381	0.4444	14.04	<.0001	0.05	5.3671	7.1091
AC 0.5%	Texture	6.3810	0.4444	14.36	<.0001	0.05	5.5099	7.2520
AC 5%	Appearance	4.8095	0.4444	10.82	<.0001	0.05	3.9385	5.6805
AC 5%	Aroma	4.0476	0.4444	9.11	<.0001	0.05	3.1766	4.9186
AC 5%	Flavor	4.7619	0.4444	10.72	<.0001	0.05	3.8909	5.6329
AC 5%	Texture	5.6667	0.4444	12.75	<.0001	0.05	4.7957	6.5377
FC 0.05%	Appearance	5.3333	0.4444	12.00	<.0001	0.05	4.4623	6.2043
FC 0.05%	Aroma	5.1905	0.4444	11.68	<.0001	0.05	4.3195	6.0615
FC 0.05%	Flavor	5.5714	0.4444	12.54	<.0001	0.05	4.7004	6.4424
FC 0.05%	Texture	5.5238	0.4444	12.43	<.0001	0.05	4.6528	6.3948
FC 0.5%	Appearance	5.7143	0.4444	12.86	<.0001	0.05	4.8433	6.5853
FC 0.5%	Aroma	5.6190	0.4444	12.64	<.0001	0.05	4.7480	6.4901
FC 0.5%	Flavor	5.2857	0.4444	11.89	<.0001	0.05	4.4147	6.1567
FC 0.5%	Texture	5.3810	0.4444	12.11	<.0001	0.05	4.5099	6.2520
FC 5%	Appearance	5.3333	0.4444	12.00	<.0001	0.05	4.4623	6.2043
FC 5%	Aroma	3.7143	0.4444	8.36	<.0001	0.05	2.8433	4.5853
FC 5%	Flavor	4.0000	0.4444	9.00	<.0001	0.05	3.1290	4.8710
FC 5%	Texture	5.0000	0.4444	11.25	<.0001	0.05	4.1290	5.8710
R (Control)	Appearance	5.7619	0.4444	12.97	<.0001	0.05	4.8909	6.6329
R (Control)	Aroma	5.6190	0.4444	12.64	<.0001	0.05	4.7480	6.4901
R (Control)	Flavor	6.0476	0.4444	13.61	<.0001	0.05	5.1766	6.9186
R (Control)	Texture	6.4286	0.4444	14.47	<.0001	0.05	5.5576	7.2996


LS-Means for Sample*Attribute
With 95% Confidence Limits
Rating LS-Mean
7
6
5
4
3
AC 0.05% Appearance
AC 0.05% Aroma
AC 0.05% Flavor
AC 0.05% Texture
AC 0.5% Appearance
AC 0.5% Aroma
AC 0.5% Flavor
AC 0.5% Texture
AC 5% Appearance
AC 5% Aroma
AC 5% Flavor
AC 5% Texture
FC 0.05% Appearance
FC 0.05% Aroma
FC 0.05% Flavor
FC 0.05% Texture
FC 0.5% Appearance
FC 0.5% Aroma
FC 0.5% Flavor
FC 0.5% Texture
FC 5% Appearance
FC 5% Aroma
FC 5% Flavor
FC 5% Texture
R (Control) Appearance
R (Control) Aroma
R (Control) Flavor
R (Control) Texture
Sample*Attribute

Differences of Sample*Attribute Least Squares Means										
Sample	Attribute	_Sample	_Attribute	Estimate	Standard Error	z Value	Pr > |z|	Alpha	Lower	Upper
AC 0.05%	Appearance	AC 0.05%	Aroma	0.09524	0.6285	0.15	0.8796	0.05	-1.1366	1.3270
AC 0.05%	Appearance	AC 0.05%	Flavor	0.4762	0.6285	0.76	0.4486	0.05	-0.7556	1.7080
AC 0.05%	Appearance	AC 0.05%	Texture	0.04762	0.6285	0.08	0.9396	0.05	-1.1842	1.2794
AC 0.05%	Appearance	AC 0.5%	Appearance	-0.04762	0.6285	-0.08	0.9396	0.05	-1.2794	1.1842
AC 0.05%	Appearance	AC 0.5%	Aroma	0.5714	0.6285	0.91	0.3632	0.05	-0.6604	1.8032
AC 0.05%	Appearance	AC 0.5%	Flavor	-0.3810	0.6285	-0.61	0.5444	0.05	-1.6128	0.8508
AC 0.05%	Appearance	AC 0.5%	Texture	-0.5238	0.6285	-0.83	0.4046	0.05	-1.7556	0.7080
AC 0.05%	Appearance	AC 5%	Appearance	1.0476	0.6285	1.67	0.0955	0.05	-0.1842	2.2794
AC 0.05%	Appearance	AC 5%	Aroma	1.8095	0.6285	2.88	0.0040	0.05	0.5777	3.0413
AC 0.05%	Appearance	AC 5%	Flavor	1.0952	0.6285	1.74	0.0814	0.05	-0.1366	2.3270
AC 0.05%	Appearance	AC 5%	Texture	0.1905	0.6285	0.30	0.7618	0.05	-1.0413	1.4223
AC 0.05%	Appearance	FC 0.05%	Appearance	0.5238	0.6285	0.83	0.4046	0.05	-0.7080	1.7556
AC 0.05%	Appearance	FC 0.05%	Aroma	0.6667	0.6285	1.06	0.2888	0.05	-0.5651	1.8985
AC 0.05%	Appearance	FC 0.05%	Flavor	0.2857	0.6285	0.45	0.6494	0.05	-0.9461	1.5175
AC 0.05%	Appearance	FC 0.05%	Texture	0.3333	0.6285	0.53	0.5958	0.05	-0.8985	1.5651
AC 0.05%	Appearance	FC 0.5%	Appearance	0.1429	0.6285	0.23	0.8202	0.05	-1.0889	1.3747
AC 0.05%	Appearance	FC 0.5%	Aroma	0.2381	0.6285	0.38	0.7048	0.05	-0.9937	1.4699
AC 0.05%	Appearance	FC 0.5%	Flavor	0.5714	0.6285	0.91	0.3632	0.05	-0.6604	1.8032
AC 0.05%	Appearance	FC 0.5%	Texture	0.4762	0.6285	0.76	0.4486	0.05	-0.7556	1.7080
AC 0.05%	Appearance	FC 5%	Appearance	0.5238	0.6285	0.83	0.4046	0.05	-0.7080	1.7556
AC 0.05%	Appearance	FC 5%	Aroma	2.1429	0.6285	3.41	0.0007	0.05	0.9111	3.3747
AC 0.05%	Appearance	FC 5%	Flavor	1.8571	0.6285	2.95	0.0031	0.05	0.6253	3.0889
AC 0.05%	Appearance	FC 5%	Texture	0.8571	0.6285	1.36	0.1726	0.05	-0.3747	2.0889
AC 0.05%	Appearance	R (Control)	Appearance	0.09524	0.6285	0.15	0.8796	0.05	-1.1366	1.3270
AC 0.05%	Appearance	R (Control)	Aroma	0.2381	0.6285	0.38	0.7048	0.05	-0.9937	1.4699
AC 0.05%	Appearance	R (Control)	Flavor	-0.1905	0.6285	-0.30	0.7618	0.05	-1.4223	1.0413
AC 0.05%	Appearance	R (Control)	Texture	-0.5714	0.6285	-0.91	0.3632	0.05	-1.8032	0.6604
AC 0.05%	Aroma	AC 0.05%	Flavor	0.3810	0.6285	0.61	0.5444	0.05	-0.8508	1.6128

AC 0.05%	Aroma	AC 0.05%	Texture	-0.04762	0.6285	-0.08	0.9396	0.05	-1.2794	1.1842
AC 0.05%	Aroma	AC 0.5%	Appearance	-0.1429	0.6285	-0.23	0.8202	0.05	-1.3747	1.0889
AC 0.05%	Aroma	AC 0.5%	Aroma	0.4762	0.6285	0.76	0.4486	0.05	-0.7556	1.7080
AC 0.05%	Aroma	AC 0.5%	Flavor	-0.4762	0.6285	-0.76	0.4486	0.05	-1.7080	0.7556
AC 0.05%	Aroma	AC 0.5%	Texture	-0.6190	0.6285	-0.98	0.3246	0.05	-1.8508	0.6128
AC 0.05%	Aroma	AC 5%	Appearance	0.9524	0.6285	1.52	0.1297	0.05	-0.2794	2.1842
AC 0.05%	Aroma	AC 5%	Aroma	1.7143	0.6285	2.73	0.0064	0.05	0.4825	2.9461
AC 0.05%	Aroma	AC 5%	Flavor	1.0000	0.6285	1.59	0.1116	0.05	-0.2318	2.2318
AC 0.05%	Aroma	AC 5%	Texture	0.09524	0.6285	0.15	0.8796	0.05	-1.1366	1.3270
AC 0.05%	Aroma	FC 0.05%	Appearance	0.4286	0.6285	0.68	0.4953	0.05	-0.8032	1.6604
AC 0.05%	Aroma	FC 0.05%	Aroma	0.5714	0.6285	0.91	0.3632	0.05	-0.6604	1.8032
AC 0.05%	Aroma	FC 0.05%	Flavor	0.1905	0.6285	0.30	0.7618	0.05	-1.0413	1.4223
AC 0.05%	Aroma	FC 0.05%	Texture	0.2381	0.6285	0.38	0.7048	0.05	-0.9937	1.4699
AC 0.05%	Aroma	FC 0.5%	Appearance	0.04762	0.6285	0.08	0.9396	0.05	-1.1842	1.2794
AC 0.05%	Aroma	FC 0.5%	Aroma	0.1429	0.6285	0.23	0.8202	0.05	-1.0889	1.3747
AC 0.05%	Aroma	FC 0.5%	Flavor	0.4762	0.6285	0.76	0.4486	0.05	-0.7556	1.7080
AC 0.05%	Aroma	FC 0.5%	Texture	0.3810	0.6285	0.61	0.5444	0.05	-0.8508	1.6128
AC 0.05%	Aroma	FC 5%	Appearance	0.4286	0.6285	0.68	0.4953	0.05	-0.8032	1.6604
AC 0.05%	Aroma	FC 5%	Aroma	2.0476	0.6285	3.26	0.0011	0.05	0.8158	3.2794
AC 0.05%	Aroma	FC 5%	Flavor	1.7619	0.6285	2.80	0.0051	0.05	0.5301	2.9937
AC 0.05%	Aroma	FC 5%	Texture	0.7619	0.6285	1.21	0.2254	0.05	-0.4699	1.9937
AC 0.05%	Aroma	R (Control)	Appearance	2.22E-16	0.6285	0.00	1.0000	0.05	-1.2318	1.2318
AC 0.05%	Aroma	R (Control)	Aroma	0.1429	0.6285	0.23	0.8202	0.05	-1.0889	1.3747
AC 0.05%	Aroma	R (Control)	Flavor	-0.2857	0.6285	-0.45	0.6494	0.05	-1.5175	0.9461
AC 0.05%	Aroma	R (Control)	Texture	-0.6667	0.6285	-1.06	0.2888	0.05	-1.8985	0.5651
AC 0.05%	Flavor	AC 0.05%	Texture	-0.4286	0.6285	-0.68	0.4953	0.05	-1.6604	0.8032
AC 0.05%	Flavor	AC 0.5%	Appearance	-0.5238	0.6285	-0.83	0.4046	0.05	-1.7556	0.7080
AC 0.05%	Flavor	AC 0.5%	Aroma	0.09524	0.6285	0.15	0.8796	0.05	-1.1366	1.3270
AC 0.05%	Flavor	AC 0.5%	Flavor	-0.8571	0.6285	-1.36	0.1726	0.05	-2.0889	0.3747
AC 0.05%	Flavor	AC 0.5%	Texture	-1.0000	0.6285	-1.59	0.1116	0.05	-2.2318	0.2318

AC 0.05%	Flavor	AC 5%	Appearance	0.5714	0.6285	0.91	0.3632	0.05	-0.6604	1.8032
AC 0.05%	Flavor	AC 5%	Aroma	1.3333	0.6285	2.12	0.0339	0.05	0.1015	2.5651
AC 0.05%	Flavor	AC 5%	Flavor	0.6190	0.6285	0.98	0.3246	0.05	-0.6128	1.8508
AC 0.05%	Flavor	AC 5%	Texture	-0.2857	0.6285	-0.45	0.6494	0.05	-1.5175	0.9461
AC 0.05%	Flavor	FC 0.05%	Appearance	0.04762	0.6285	0.08	0.9396	0.05	-1.1842	1.2794
AC 0.05%	Flavor	FC 0.05%	Aroma	0.1905	0.6285	0.30	0.7618	0.05	-1.0413	1.4223
AC 0.05%	Flavor	FC 0.05%	Flavor	-0.1905	0.6285	-0.30	0.7618	0.05	-1.4223	1.0413
AC 0.05%	Flavor	FC 0.05%	Texture	-0.1429	0.6285	-0.23	0.8202	0.05	-1.3747	1.0889
AC 0.05%	Flavor	FC 0.5%	Appearance	-0.3333	0.6285	-0.53	0.5958	0.05	-1.5651	0.8985
AC 0.05%	Flavor	FC 0.5%	Aroma	-0.2381	0.6285	-0.38	0.7048	0.05	-1.4699	0.9937
AC 0.05%	Flavor	FC 0.5%	Flavor	0.09524	0.6285	0.15	0.8796	0.05	-1.1366	1.3270
AC 0.05%	Flavor	FC 0.5%	Texture	1.67E-16	0.6285	0.00	1.0000	0.05	-1.2318	1.2318
AC 0.05%	Flavor	FC 5%	Appearance	0.04762	0.6285	0.08	0.9396	0.05	-1.1842	1.2794
AC 0.05%	Flavor	FC 5%	Aroma	1.6667	0.6285	2.65	0.0080	0.05	0.4349	2.8985
AC 0.05%	Flavor	FC 5%	Flavor	1.3810	0.6285	2.20	0.0280	0.05	0.1492	2.6128
AC 0.05%	Flavor	FC 5%	Texture	0.3810	0.6285	0.61	0.5444	0.05	-0.8508	1.6128
AC 0.05%	Flavor	R (Control)	Appearance	-0.3810	0.6285	-0.61	0.5444	0.05	-1.6128	0.8508
AC 0.05%	Flavor	R (Control)	Aroma	-0.2381	0.6285	-0.38	0.7048	0.05	-1.4699	0.9937
AC 0.05%	Flavor	R (Control)	Flavor	-0.6667	0.6285	-1.06	0.2888	0.05	-1.8985	0.5651
AC 0.05%	Flavor	R (Control)	Texture	-1.0476	0.6285	-1.67	0.0955	0.05	-2.2794	0.1842
AC 0.05%	Texture	AC 0.5%	Appearance	-0.09524	0.6285	-0.15	0.8796	0.05	-1.3270	1.1366
AC 0.05%	Texture	AC 0.5%	Aroma	0.5238	0.6285	0.83	0.4046	0.05	-0.7080	1.7556
AC 0.05%	Texture	AC 0.5%	Flavor	-0.4286	0.6285	-0.68	0.4953	0.05	-1.6604	0.8032
AC 0.05%	Texture	AC 0.5%	Texture	-0.5714	0.6285	-0.91	0.3632	0.05	-1.8032	0.6604
AC 0.05%	Texture	AC 5%	Appearance	1.0000	0.6285	1.59	0.1116	0.05	-0.2318	2.2318
AC 0.05%	Texture	AC 5%	Aroma	1.7619	0.6285	2.80	0.0051	0.05	0.5301	2.9937
AC 0.05%	Texture	AC 5%	Flavor	1.0476	0.6285	1.67	0.0955	0.05	-0.1842	2.2794
AC 0.05%	Texture	AC 5%	Texture	0.1429	0.6285	0.23	0.8202	0.05	-1.0889	1.3747
AC 0.05%	Texture	FC 0.05%	Appearance	0.4762	0.6285	0.76	0.4486	0.05	-0.7556	1.7080
AC 0.05%	Texture	FC 0.05%	Aroma	0.6190	0.6285	0.98	0.3246	0.05	-0.6128	1.8508

AC 0.05%	Texture	FC 0.05%	Flavor	0.2381	0.6285	0.38	0.7048	0.05	-0.9937	1.4699
AC 0.05%	Texture	FC 0.05%	Texture	0.2857	0.6285	0.45	0.6494	0.05	-0.9461	1.5175
AC 0.05%	Texture	FC 0.5%	Appearance	0.09524	0.6285	0.15	0.8796	0.05	-1.1366	1.3270
AC 0.05%	Texture	FC 0.5%	Aroma	0.1905	0.6285	0.30	0.7618	0.05	-1.0413	1.4223
AC 0.05%	Texture	FC 0.5%	Flavor	0.5238	0.6285	0.83	0.4046	0.05	-0.7080	1.7556
AC 0.05%	Texture	FC 0.5%	Texture	0.4286	0.6285	0.68	0.4953	0.05	-0.8032	1.6604
AC 0.05%	Texture	FC 5%	Appearance	0.4762	0.6285	0.76	0.4486	0.05	-0.7556	1.7080
AC 0.05%	Texture	FC 5%	Aroma	2.0952	0.6285	3.33	0.0009	0.05	0.8634	3.3270
AC 0.05%	Texture	FC 5%	Flavor	1.8095	0.6285	2.88	0.0040	0.05	0.5777	3.0413
AC 0.05%	Texture	FC 5%	Texture	0.8095	0.6285	1.29	0.1977	0.05	-0.4223	2.0413
AC 0.05%	Texture	R (Control)	Appearance	0.04762	0.6285	0.08	0.9396	0.05	-1.1842	1.2794
AC 0.05%	Texture	R (Control)	Aroma	0.1905	0.6285	0.30	0.7618	0.05	-1.0413	1.4223
AC 0.05%	Texture	R (Control)	Flavor	-0.2381	0.6285	-0.38	0.7048	0.05	-1.4699	0.9937
AC 0.05%	Texture	R (Control)	Texture	-0.6190	0.6285	-0.98	0.3246	0.05	-1.8508	0.6128
AC 0.5%	Appearance	AC 0.5%	Aroma	0.6190	0.6285	0.98	0.3246	0.05	-0.6128	1.8508
AC 0.5%	Appearance	AC 0.5%	Flavor	-0.3333	0.6285	-0.53	0.5958	0.05	-1.5651	0.8985
AC 0.5%	Appearance	AC 0.5%	Texture	-0.4762	0.6285	-0.76	0.4486	0.05	-1.7080	0.7556
AC 0.5%	Appearance	AC 5%	Appearance	1.0952	0.6285	1.74	0.0814	0.05	-0.1366	2.3270
AC 0.5%	Appearance	AC 5%	Aroma	1.8571	0.6285	2.95	0.0031	0.05	0.6253	3.0889
AC 0.5%	Appearance	AC 5%	Flavor	1.1429	0.6285	1.82	0.0690	0.05	-0.08895	2.3747
AC 0.5%	Appearance	AC 5%	Texture	0.2381	0.6285	0.38	0.7048	0.05	-0.9937	1.4699
AC 0.5%	Appearance	FC 0.05%	Appearance	0.5714	0.6285	0.91	0.3632	0.05	-0.6604	1.8032
AC 0.5%	Appearance	FC 0.05%	Aroma	0.7143	0.6285	1.14	0.2557	0.05	-0.5175	1.9461
AC 0.5%	Appearance	FC 0.05%	Flavor	0.3333	0.6285	0.53	0.5958	0.05	-0.8985	1.5651
AC 0.5%	Appearance	FC 0.05%	Texture	0.3810	0.6285	0.61	0.5444	0.05	-0.8508	1.6128
AC 0.5%	Appearance	FC 0.5%	Appearance	0.1905	0.6285	0.30	0.7618	0.05	-1.0413	1.4223
AC 0.5%	Appearance	FC 0.5%	Aroma	0.2857	0.6285	0.45	0.6494	0.05	-0.9461	1.5175
AC 0.5%	Appearance	FC 0.5%	Flavor	0.6190	0.6285	0.98	0.3246	0.05	-0.6128	1.8508
AC 0.5%	Appearance	FC 0.5%	Texture	0.5238	0.6285	0.83	0.4046	0.05	-0.7080	1.7556
AC 0.5%	Appearance	FC 5%	Appearance	0.5714	0.6285	0.91	0.3632	0.05	-0.6604	1.8032

AC 0.5%	Appearance	FC 5%	Aroma	2.1905	0.6285	3.49	0.0005	0.05	0.9587	3.4223
AC 0.5%	Appearance	FC 5%	Flavor	1.9048	0.6285	3.03	0.0024	0.05	0.6730	3.1366
AC 0.5%	Appearance	FC 5%	Texture	0.9048	0.6285	1.44	0.1500	0.05	-0.3270	2.1366
AC 0.5%	Appearance	R (Control)	Appearance	0.1429	0.6285	0.23	0.8202	0.05	-1.0889	1.3747
AC 0.5%	Appearance	R (Control)	Aroma	0.2857	0.6285	0.45	0.6494	0.05	-0.9461	1.5175
AC 0.5%	Appearance	R (Control)	Flavor	-0.1429	0.6285	-0.23	0.8202	0.05	-1.3747	1.0889
AC 0.5%	Appearance	R (Control)	Texture	-0.5238	0.6285	-0.83	0.4046	0.05	-1.7556	0.7080
AC 0.5%	Aroma	AC 0.5%	Flavor	-0.9524	0.6285	-1.52	0.1297	0.05	-2.1842	0.2794
AC 0.5%	Aroma	AC 0.5%	Texture	-1.0952	0.6285	-1.74	0.0814	0.05	-2.3270	0.1366
AC 0.5%	Aroma	AC 5%	Appearance	0.4762	0.6285	0.76	0.4486	0.05	-0.7556	1.7080
AC 0.5%	Aroma	AC 5%	Aroma	1.2381	0.6285	1.97	0.0488	0.05	0.006293	2.4699
AC 0.5%	Aroma	AC 5%	Flavor	0.5238	0.6285	0.83	0.4046	0.05	-0.7080	1.7556
AC 0.5%	Aroma	AC 5%	Texture	-0.3810	0.6285	-0.61	0.5444	0.05	-1.6128	0.8508
AC 0.5%	Aroma	FC 0.05%	Appearance	-0.04762	0.6285	-0.08	0.9396	0.05	-1.2794	1.1842
AC 0.5%	Aroma	FC 0.05%	Aroma	0.09524	0.6285	0.15	0.8796	0.05	-1.1366	1.3270
AC 0.5%	Aroma	FC 0.05%	Flavor	-0.2857	0.6285	-0.45	0.6494	0.05	-1.5175	0.9461
AC 0.5%	Aroma	FC 0.05%	Texture	-0.2381	0.6285	-0.38	0.7048	0.05	-1.4699	0.9937
AC 0.5%	Aroma	FC 0.5%	Appearance	-0.4286	0.6285	-0.68	0.4953	0.05	-1.6604	0.8032
AC 0.5%	Aroma	FC 0.5%	Aroma	-0.3333	0.6285	-0.53	0.5958	0.05	-1.5651	0.8985
AC 0.5%	Aroma	FC 0.5%	Flavor	1.11E-16	0.6285	0.00	1.0000	0.05	-1.2318	1.2318
AC 0.5%	Aroma	FC 0.5%	Texture	-0.09524	0.6285	-0.15	0.8796	0.05	-1.3270	1.1366
AC 0.5%	Aroma	FC 5%	Appearance	-0.04762	0.6285	-0.08	0.9396	0.05	-1.2794	1.1842
AC 0.5%	Aroma	FC 5%	Aroma	1.5714	0.6285	2.50	0.0124	0.05	0.3396	2.8032
AC 0.5%	Aroma	FC 5%	Flavor	1.2857	0.6285	2.05	0.0408	0.05	0.05391	2.5175
AC 0.5%	Aroma	FC 5%	Texture	0.2857	0.6285	0.45	0.6494	0.05	-0.9461	1.5175
AC 0.5%	Aroma	R (Control)	Appearance	-0.4762	0.6285	-0.76	0.4486	0.05	-1.7080	0.7556
AC 0.5%	Aroma	R (Control)	Aroma	-0.3333	0.6285	-0.53	0.5958	0.05	-1.5651	0.8985
AC 0.5%	Aroma	R (Control)	Flavor	-0.7619	0.6285	-1.21	0.2254	0.05	-1.9937	0.4699
AC 0.5%	Aroma	R (Control)	Texture	-1.1429	0.6285	-1.82	0.0690	0.05	-2.3747	0.08895
AC 0.5%	Flavor	AC 0.5%	Texture	-0.1429	0.6285	-0.23	0.8202	0.05	-1.3747	1.0889

AC 0.5%	Flavor	AC 5%	Appearance	1.4286	0.6285	2.27	0.0230	0.05	0.1968	2.6604
AC 0.5%	Flavor	AC 5%	Aroma	2.1905	0.6285	3.49	0.0005	0.05	0.9587	3.4223
AC 0.5%	Flavor	AC 5%	Flavor	1.4762	0.6285	2.35	0.0188	0.05	0.2444	2.7080
AC 0.5%	Flavor	AC 5%	Texture	0.5714	0.6285	0.91	0.3632	0.05	-0.6604	1.8032
AC 0.5%	Flavor	FC 0.05%	Appearance	0.9048	0.6285	1.44	0.1500	0.05	-0.3270	2.1366
AC 0.5%	Flavor	FC 0.05%	Aroma	1.0476	0.6285	1.67	0.0955	0.05	-0.1842	2.2794
AC 0.5%	Flavor	FC 0.05%	Flavor	0.6667	0.6285	1.06	0.2888	0.05	-0.5651	1.8985
AC 0.5%	Flavor	FC 0.05%	Texture	0.7143	0.6285	1.14	0.2557	0.05	-0.5175	1.9461
AC 0.5%	Flavor	FC 0.5%	Appearance	0.5238	0.6285	0.83	0.4046	0.05	-0.7080	1.7556
AC 0.5%	Flavor	FC 0.5%	Aroma	0.6190	0.6285	0.98	0.3246	0.05	-0.6128	1.8508
AC 0.5%	Flavor	FC 0.5%	Flavor	0.9524	0.6285	1.52	0.1297	0.05	-0.2794	2.1842
AC 0.5%	Flavor	FC 0.5%	Texture	0.8571	0.6285	1.36	0.1726	0.05	-0.3747	2.0889
AC 0.5%	Flavor	FC 5%	Appearance	0.9048	0.6285	1.44	0.1500	0.05	-0.3270	2.1366
AC 0.5%	Flavor	FC 5%	Aroma	2.5238	0.6285	4.02	<.0001	0.05	1.2920	3.7556
AC 0.5%	Flavor	FC 5%	Flavor	2.2381	0.6285	3.56	0.0004	0.05	1.0063	3.4699
AC 0.5%	Flavor	FC 5%	Texture	1.2381	0.6285	1.97	0.0488	0.05	0.006293	2.4699
AC 0.5%	Flavor	R (Control)	Appearance	0.4762	0.6285	0.76	0.4486	0.05	-0.7556	1.7080
AC 0.5%	Flavor	R (Control)	Aroma	0.6190	0.6285	0.98	0.3246	0.05	-0.6128	1.8508
AC 0.5%	Flavor	R (Control)	Flavor	0.1905	0.6285	0.30	0.7618	0.05	-1.0413	1.4223
AC 0.5%	Flavor	R (Control)	Texture	-0.1905	0.6285	-0.30	0.7618	0.05	-1.4223	1.0413
AC 0.5%	Texture	AC 5%	Appearance	1.5714	0.6285	2.50	0.0124	0.05	0.3396	2.8032
AC 0.5%	Texture	AC 5%	Aroma	2.3333	0.6285	3.71	0.0002	0.05	1.1015	3.5651
AC 0.5%	Texture	AC 5%	Flavor	1.6190	0.6285	2.58	0.0100	0.05	0.3872	2.8508
AC 0.5%	Texture	AC 5%	Texture	0.7143	0.6285	1.14	0.2557	0.05	-0.5175	1.9461
AC 0.5%	Texture	FC 0.05%	Appearance	1.0476	0.6285	1.67	0.0955	0.05	-0.1842	2.2794
AC 0.5%	Texture	FC 0.05%	Aroma	1.1905	0.6285	1.89	0.0582	0.05	-0.04133	2.4223
AC 0.5%	Texture	FC 0.05%	Flavor	0.8095	0.6285	1.29	0.1977	0.05	-0.4223	2.0413
AC 0.5%	Texture	FC 0.05%	Texture	0.8571	0.6285	1.36	0.1726	0.05	-0.3747	2.0889
AC 0.5%	Texture	FC 0.5%	Appearance	0.6667	0.6285	1.06	0.2888	0.05	-0.5651	1.8985
AC 0.5%	Texture	FC 0.5%	Aroma	0.7619	0.6285	1.21	0.2254	0.05	-0.4699	1.9937

AC 0.5%	Texture	FC 0.5%	Flavor	1.0952	0.6285	1.74	0.0814	0.05	-0.1366	2.3270
AC 0.5%	Texture	FC 0.5%	Texture	1.0000	0.6285	1.59	0.1116	0.05	-0.2318	2.2318
AC 0.5%	Texture	FC 5%	Appearance	1.0476	0.6285	1.67	0.0955	0.05	-0.1842	2.2794
AC 0.5%	Texture	FC 5%	Aroma	2.6667	0.6285	4.24	<.0001	0.05	1.4349	3.8985
AC 0.5%	Texture	FC 5%	Flavor	2.3810	0.6285	3.79	0.0002	0.05	1.1492	3.6128
AC 0.5%	Texture	FC 5%	Texture	1.3810	0.6285	2.20	0.0280	0.05	0.1492	2.6128
AC 0.5%	Texture	R (Control)	Appearance	0.6190	0.6285	0.98	0.3246	0.05	-0.6128	1.8508
AC 0.5%	Texture	R (Control)	Aroma	0.7619	0.6285	1.21	0.2254	0.05	-0.4699	1.9937
AC 0.5%	Texture	R (Control)	Flavor	0.3333	0.6285	0.53	0.5958	0.05	-0.8985	1.5651
AC 0.5%	Texture	R (Control)	Texture	-0.04762	0.6285	-0.08	0.9396	0.05	-1.2794	1.1842
AC 5%	Appearance	AC 5%	Aroma	0.7619	0.6285	1.21	0.2254	0.05	-0.4699	1.9937
AC 5%	Appearance	AC 5%	Flavor	0.04762	0.6285	0.08	0.9396	0.05	-1.1842	1.2794
AC 5%	Appearance	AC 5%	Texture	-0.8571	0.6285	-1.36	0.1726	0.05	-2.0889	0.3747
AC 5%	Appearance	FC 0.05%	Appearance	-0.5238	0.6285	-0.83	0.4046	0.05	-1.7556	0.7080
AC 5%	Appearance	FC 0.05%	Aroma	-0.3810	0.6285	-0.61	0.5444	0.05	-1.6128	0.8508
AC 5%	Appearance	FC 0.05%	Flavor	-0.7619	0.6285	-1.21	0.2254	0.05	-1.9937	0.4699
AC 5%	Appearance	FC 0.05%	Texture	-0.7143	0.6285	-1.14	0.2557	0.05	-1.9461	0.5175
AC 5%	Appearance	FC 0.5%	Appearance	-0.9048	0.6285	-1.44	0.1500	0.05	-2.1366	0.3270
AC 5%	Appearance	FC 0.5%	Aroma	-0.8095	0.6285	-1.29	0.1977	0.05	-2.0413	0.4223
AC 5%	Appearance	FC 0.5%	Flavor	-0.4762	0.6285	-0.76	0.4486	0.05	-1.7080	0.7556
AC 5%	Appearance	FC 0.5%	Texture	-0.5714	0.6285	-0.91	0.3632	0.05	-1.8032	0.6604
AC 5%	Appearance	FC 5%	Appearance	-0.5238	0.6285	-0.83	0.4046	0.05	-1.7556	0.7080
AC 5%	Appearance	FC 5%	Aroma	1.0952	0.6285	1.74	0.0814	0.05	-0.1366	2.3270
AC 5%	Appearance	FC 5%	Flavor	0.8095	0.6285	1.29	0.1977	0.05	-0.4223	2.0413
AC 5%	Appearance	FC 5%	Texture	-0.1905	0.6285	-0.30	0.7618	0.05	-1.4223	1.0413
AC 5%	Appearance	R (Control)	Appearance	-0.9524	0.6285	-1.52	0.1297	0.05	-2.1842	0.2794
AC 5%	Appearance	R (Control)	Aroma	-0.8095	0.6285	-1.29	0.1977	0.05	-2.0413	0.4223
AC 5%	Appearance	R (Control)	Flavor	-1.2381	0.6285	-1.97	0.0488	0.05	-2.4699	-0.00629
AC 5%	Appearance	R (Control)	Texture	-1.6190	0.6285	-2.58	0.0100	0.05	-2.8508	-0.3872

AC 5%	Aroma	AC 5%	Flavor	-0.7143	0.6285	-1.14	0.2557	0.05	-1.9461	0.5175
AC 5%	Aroma	AC 5%	Texture	-1.6190	0.6285	-2.58	0.0100	0.05	-2.8508	-0.3872
AC 5%	Aroma	FC 0.05%	Appearance	-1.2857	0.6285	-2.05	0.0408	0.05	-2.5175	0.05391
AC 5%	Aroma	FC 0.05%	Aroma	-1.1429	0.6285	-1.82	0.0690	0.05	-2.3747	0.08895
AC 5%	Aroma	FC 0.05%	Flavor	-1.5238	0.6285	-2.42	0.0153	0.05	-2.7556	-0.2920
AC 5%	Aroma	FC 0.05%	Texture	-1.4762	0.6285	-2.35	0.0188	0.05	-2.7080	-0.2444
AC 5%	Aroma	FC 0.5%	Appearance	-1.6667	0.6285	-2.65	0.0080	0.05	-2.8985	-0.4349
AC 5%	Aroma	FC 0.5%	Aroma	-1.5714	0.6285	-2.50	0.0124	0.05	-2.8032	-0.3396
AC 5%	Aroma	FC 0.5%	Flavor	-1.2381	0.6285	-1.97	0.0488	0.05	-2.4699	0.00629
AC 5%	Aroma	FC 0.5%	Texture	-1.3333	0.6285	-2.12	0.0339	0.05	-2.5651	-0.1015
AC 5%	Aroma	FC 5%	Appearance	-1.2857	0.6285	-2.05	0.0408	0.05	-2.5175	0.05391
AC 5%	Aroma	FC 5%	Aroma	0.3333	0.6285	0.53	0.5958	0.05	-0.8985	1.5651
AC 5%	Aroma	FC 5%	Flavor	0.04762	0.6285	0.08	0.9396	0.05	-1.1842	1.2794
AC 5%	Aroma	FC 5%	Texture	-0.9524	0.6285	-1.52	0.1297	0.05	-2.1842	0.2794
AC 5%	Aroma	R (Control)	Appearance	-1.7143	0.6285	-2.73	0.0064	0.05	-2.9461	-0.4825
AC 5%	Aroma	R (Control)	Aroma	-1.5714	0.6285	-2.50	0.0124	0.05	-2.8032	-0.3396
AC 5%	Aroma	R (Control)	Flavor	-2.0000	0.6285	-3.18	0.0015	0.05	-3.2318	-0.7682
AC 5%	Aroma	R (Control)	Texture	-2.3810	0.6285	-3.79	0.0002	0.05	-3.6128	-1.1492
AC 5%	Flavor	AC 5%	Texture	-0.9048	0.6285	-1.44	0.1500	0.05	-2.1366	0.3270
AC 5%	Flavor	FC 0.05%	Appearance	-0.5714	0.6285	-0.91	0.3632	0.05	-1.8032	0.6604
AC 5%	Flavor	FC 0.05%	Aroma	-0.4286	0.6285	-0.68	0.4953	0.05	-1.6604	0.8032
AC 5%	Flavor	FC 0.05%	Flavor	-0.8095	0.6285	-1.29	0.1977	0.05	-2.0413	0.4223
AC 5%	Flavor	FC 0.05%	Texture	-0.7619	0.6285	-1.21	0.2254	0.05	-1.9937	0.4699
AC 5%	Flavor	FC 0.5%	Appearance	-0.9524	0.6285	-1.52	0.1297	0.05	-2.1842	0.2794
AC 5%	Flavor	FC 0.5%	Aroma	-0.8571	0.6285	-1.36	0.1726	0.05	-2.0889	0.3747
AC 5%	Flavor	FC 0.5%	Flavor	-0.5238	0.6285	-0.83	0.4046	0.05	-1.7556	0.7080
AC 5%	Flavor	FC 0.5%	Texture	-0.6190	0.6285	-0.98	0.3246	0.05	-1.8508	0.6128

AC 5%	Flavor	FC 5%	Appearance	-0.5714	0.6285	-0.91	0.3632	0.05	-1.8032	0.6604
AC 5%	Flavor	FC 5%	Aroma	1.0476	0.6285	1.67	0.0955	0.05	-0.1842	2.2794
AC 5%	Flavor	FC 5%	Flavor	0.7619	0.6285	1.21	0.2254	0.05	-0.4699	1.9937
AC 5%	Flavor	FC 5%	Texture	-0.2381	0.6285	-0.38	0.7048	0.05	-1.4699	0.9937
AC 5%	Flavor	R (Control)	Appearance	-1.0000	0.6285	-1.59	0.1116	0.05	-2.2318	0.2318
AC 5%	Flavor	R (Control)	Aroma	-0.8571	0.6285	-1.36	0.1726	0.05	-2.0889	0.3747
AC 5%	Flavor	R (Control)	Flavor	-1.2857	0.6285	-2.05	0.0408	0.05	-2.5175	-0.05391
AC 5%	Flavor	R (Control)	Texture	-1.6667	0.6285	-2.65	0.0080	0.05	-2.8985	-0.4349
AC 5%	Texture	FC 0.05%	Appearance	0.3333	0.6285	0.53	0.5958	0.05	-0.8985	1.5651
AC 5%	Texture	FC 0.05%	Aroma	0.4762	0.6285	0.76	0.4486	0.05	-0.7556	1.7080
AC 5%	Texture	FC 0.05%	Flavor	0.09524	0.6285	0.15	0.8796	0.05	-1.1366	1.3270
AC 5%	Texture	FC 0.05%	Texture	0.1429	0.6285	0.23	0.8202	0.05	-1.0889	1.3747
AC 5%	Texture	FC 0.5%	Appearance	-0.04762	0.6285	-0.08	0.9396	0.05	-1.2794	1.1842
AC 5%	Texture	FC 0.5%	Aroma	0.04762	0.6285	0.08	0.9396	0.05	-1.1842	1.2794
AC 5%	Texture	FC 0.5%	Flavor	0.3810	0.6285	0.61	0.5444	0.05	-0.8508	1.6128
AC 5%	Texture	FC 0.5%	Texture	0.2857	0.6285	0.45	0.6494	0.05	-0.9461	1.5175
AC 5%	Texture	FC 5%	Appearance	0.3333	0.6285	0.53	0.5958	0.05	-0.8985	1.5651
AC 5%	Texture	FC 5%	Aroma	1.9524	0.6285	3.11	0.0019	0.05	0.7206	3.1842
AC 5%	Texture	FC 5%	Flavor	1.6667	0.6285	2.65	0.0080	0.05	0.4349	2.8985
AC 5%	Texture	FC 5%	Texture	0.6667	0.6285	1.06	0.2888	0.05	-0.5651	1.8985
AC 5%	Texture	R (Control)	Appearance	-0.09524	0.6285	-0.15	0.8796	0.05	-1.3270	1.1366
AC 5%	Texture	R (Control)	Aroma	0.04762	0.6285	0.08	0.9396	0.05	-1.1842	1.2794
AC 5%	Texture	R (Control)	Flavor	-0.3810	0.6285	-0.61	0.5444	0.05	-1.6128	0.8508
AC 5%	Texture	R (Control)	Texture	-0.7619	0.6285	-1.21	0.2254	0.05	-1.9937	0.4699
FC 0.05%	Appearance	FC 0.05%	Aroma	0.1429	0.6285	0.23	0.8202	0.05	-1.0889	1.3747
FC 0.05%	Appearance	FC 0.05%	Flavor	-0.2381	0.6285	-0.38	0.7048	0.05	-1.4699	0.9937
FC 0.05%	Appearance	FC 0.05%	Texture	-0.1905	0.6285	-0.30	0.7618	0.05	-1.4223	1.0413
FC 0.05%	Appearance	FC 0.5%	Appearance	-0.3810	0.6285	-0.61	0.5444	0.05	-1.6128	0.8508
FC 0.05%	Appearance	FC 0.5%	Aroma	-0.2857	0.6285	-0.45	0.6494	0.05	-1.5175	0.9461

FC 0.05%	Appearance	FC 0.5%	Flavor	0.04762	0.6285	0.08	0.9396	0.05	-1.1842	1.2794
FC 0.05%	Appearance	FC 0.5%	Texture	-0.04762	0.6285	-0.08	0.9396	0.05	-1.2794	1.1842
FC 0.05%	Appearance	FC 5%	Appearance	-222E-18	0.6285	-0.00	1.0000	0.05	-1.2318	1.2318
FC 0.05%	Appearance	FC 5%	Aroma	1.6190	0.6285	2.58	0.0100	0.05	0.3872	2.8508
FC 0.05%	Appearance	FC 5%	Flavor	1.3333	0.6285	2.12	0.0339	0.05	0.1015	2.5651
FC 0.05%	Appearance	FC 5%	Texture	0.3333	0.6285	0.53	0.5958	0.05	-0.8985	1.5651
FC 0.05%	Appearance	R (Control)	Appearance	-0.4286	0.6285	-0.68	0.4953	0.05	-1.6604	0.8032
FC 0.05%	Appearance	R (Control)	Aroma	-0.2857	0.6285	-0.45	0.6494	0.05	-1.5175	0.9461
FC 0.05%	Appearance	R (Control)	Flavor	-0.7143	0.6285	-1.14	0.2557	0.05	-1.9461	0.5175
FC 0.05%	Appearance	R (Control)	Texture	-1.0952	0.6285	-1.74	0.0814	0.05	-2.3270	0.1366
FC 0.05%	Aroma	FC 0.05%	Flavor	-0.3810	0.6285	-0.61	0.5444	0.05	-1.6128	0.8508
FC 0.05%	Aroma	FC 0.05%	Texture	-0.3333	0.6285	-0.53	0.5958	0.05	-1.5651	0.8985
FC 0.05%	Aroma	FC 0.5%	Appearance	-0.5238	0.6285	-0.83	0.4046	0.05	-1.7556	0.7080
FC 0.05%	Aroma	FC 0.5%	Aroma	-0.4286	0.6285	-0.68	0.4953	0.05	-1.6604	0.8032
FC 0.05%	Aroma	FC 0.5%	Flavor	-0.09524	0.6285	-0.15	0.8796	0.05	-1.3270	1.1366
FC 0.05%	Aroma	FC 0.5%	Texture	-0.1905	0.6285	-0.30	0.7618	0.05	-1.4223	1.0413
FC 0.05%	Aroma	FC 5%	Appearance	-0.1429	0.6285	-0.23	0.8202	0.05	-1.3747	1.0889
FC 0.05%	Aroma	FC 5%	Aroma	1.4762	0.6285	2.35	0.0188	0.05	0.2444	2.7080
FC 0.05%	Aroma	FC 5%	Flavor	1.1905	0.6285	1.89	0.0582	0.05	-0.04133	2.4223
FC 0.05%	Aroma	FC 5%	Texture	0.1905	0.6285	0.30	0.7618	0.05	-1.0413	1.4223
FC 0.05%	Aroma	R (Control)	Appearance	-0.5714	0.6285	-0.91	0.3632	0.05	-1.8032	0.6604
FC 0.05%	Aroma	R (Control)	Aroma	-0.4286	0.6285	-0.68	0.4953	0.05	-1.6604	0.8032
FC 0.05%	Aroma	R (Control)	Flavor	-0.8571	0.6285	-1.36	0.1726	0.05	-2.0889	0.3747
FC 0.05%	Aroma	R (Control)	Texture	-1.2381	0.6285	-1.97	0.0488	0.05	-2.4699	-0.00629
FC 0.05%	Flavor	FC 0.05%	Texture	0.04762	0.6285	0.08	0.9396	0.05	-1.1842	1.2794
FC 0.05%	Flavor	FC 0.5%	Appearance	-0.1429	0.6285	-0.23	0.8202	0.05	-1.3747	1.0889
FC 0.05%	Flavor	FC 0.5%	Aroma	-0.04762	0.6285	-0.08	0.9396	0.05	-1.2794	1.1842
FC 0.05%	Flavor	FC 0.5%	Flavor	0.2857	0.6285	0.45	0.6494	0.05	-0.9461	1.5175
FC 0.05%	Flavor	FC 0.5%	Texture	0.1905	0.6285	0.30	0.7618	0.05	-1.0413	1.4223

FC 0.05%	Flavor	FC 5%	Appearance	0.2381	0.6285	0.38	0.7048	0.05	-0.9937	1.4699
FC 0.05%	Flavor	FC 5%	Aroma	1.8571	0.6285	2.95	0.0031	0.05	0.6253	3.0889
FC 0.05%	Flavor	FC 5%	Flavor	1.5714	0.6285	2.50	0.0124	0.05	0.3396	2.8032
FC 0.05%	Flavor	FC 5%	Texture	0.5714	0.6285	0.91	0.3632	0.05	-0.6604	1.8032
FC 0.05%	Flavor	R (Control)	Appearance	-0.1905	0.6285	-0.30	0.7618	0.05	-1.4223	1.0413
FC 0.05%	Flavor	R (Control)	Aroma	-0.04762	0.6285	-0.08	0.9396	0.05	-1.2794	1.1842
FC 0.05%	Flavor	R (Control)	Flavor	-0.4762	0.6285	-0.76	0.4486	0.05	-1.7080	0.7556
FC 0.05%	Flavor	R (Control)	Texture	-0.8571	0.6285	-1.36	0.1726	0.05	-2.0889	0.3747
FC 0.05%	Texture	FC 0.5%	Appearance	-0.1905	0.6285	-0.30	0.7618	0.05	-1.4223	1.0413
FC 0.05%	Texture	FC 0.5%	Aroma	-0.09524	0.6285	-0.15	0.8796	0.05	-1.3270	1.1366
FC 0.05%	Texture	FC 0.5%	Flavor	0.2381	0.6285	0.38	0.7048	0.05	-0.9937	1.4699
FC 0.05%	Texture	FC 0.5%	Texture	0.1429	0.6285	0.23	0.8202	0.05	-1.0889	1.3747
FC 0.05%	Texture	FC 5%	Appearance	0.1905	0.6285	0.30	0.7618	0.05	-1.0413	1.4223
FC 0.05%	Texture	FC 5%	Aroma	1.8095	0.6285	2.88	0.0040	0.05	0.5777	3.0413
FC 0.05%	Texture	FC 5%	Flavor	1.5238	0.6285	2.42	0.0153	0.05	0.2920	2.7556
FC 0.05%	Texture	FC 5%	Texture	0.5238	0.6285	0.83	0.4046	0.05	-0.7080	1.7556
FC 0.05%	Texture	R (Control)	Appearance	-0.2381	0.6285	-0.38	0.7048	0.05	-1.4699	0.9937
FC 0.05%	Texture	R (Control)	Aroma	-0.09524	0.6285	-0.15	0.8796	0.05	-1.3270	1.1366
FC 0.05%	Texture	R (Control)	Flavor	-0.5238	0.6285	-0.83	0.4046	0.05	-1.7556	0.7080
FC 0.05%	Texture	R (Control)	Texture	-0.9048	0.6285	-1.44	0.1500	0.05	-2.1366	0.3270
FC 0.5%	Appearance	FC 0.5%	Aroma	0.09524	0.6285	0.15	0.8796	0.05	-1.1366	1.3270
FC 0.5%	Appearance	FC 0.5%	Flavor	0.4286	0.6285	0.68	0.4953	0.05	-0.8032	1.6604
FC 0.5%	Appearance	FC 0.5%	Texture	0.3333	0.6285	0.53	0.5958	0.05	-0.8985	1.5651
FC 0.5%	Appearance	FC 5%	Appearance	0.3810	0.6285	0.61	0.5444	0.05	-0.8508	1.6128
FC 0.5%	Appearance	FC 5%	Aroma	2.0000	0.6285	3.18	0.0015	0.05	0.7682	3.2318
FC 0.5%	Appearance	FC 5%	Flavor	1.7143	0.6285	2.73	0.0064	0.05	0.4825	2.9461
FC 0.5%	Appearance	FC 5%	Texture	0.7143	0.6285	1.14	0.2557	0.05	-0.5175	1.9461
FC 0.5%	Appearance	R (Control)	Appearance	-0.04762	0.6285	-0.08	0.9396	0.05	-1.2794	1.1842
FC 0.5%	Appearance	R (Control)	Aroma	0.09524	0.6285	0.15	0.8796	0.05	-1.1366	1.3270
FC 0.5%	Appearance	R (Control)	Flavor	-0.3333	0.6285	-0.53	0.5958	0.05	-1.5651	0.8985

FC 0.5%	Appearance	R (Control)	Texture	-0.7143	0.6285	-1.14	0.2557	0.05	-1.9461	0.5175
FC 0.5%	Aroma	FC 0.5%	Flavor	0.3333	0.6285	0.53	0.5958	0.05	-0.8985	1.5651
FC 0.5%	Aroma	FC 0.5%	Texture	0.2381	0.6285	0.38	0.7048	0.05	-0.9937	1.4699
FC 0.5%	Aroma	FC 5%	Appearance	0.2857	0.6285	0.45	0.6494	0.05	-0.9461	1.5175
FC 0.5%	Aroma	FC 5%	Aroma	1.9048	0.6285	3.03	0.0024	0.05	0.6730	3.1366
FC 0.5%	Aroma	FC 5%	Flavor	1.6190	0.6285	2.58	0.0100	0.05	0.3872	2.8508
FC 0.5%	Aroma	FC 5%	Texture	0.6190	0.6285	0.98	0.3246	0.05	-0.6128	1.8508
FC 0.5%	Aroma	R (Control)	Appearance	-0.1429	0.6285	-0.23	0.8202	0.05	-1.3747	1.0889
FC 0.5%	Aroma	R (Control)	Aroma	-666E-18	0.6285	-0.00	1.0000	0.05	-1.2318	1.2318
FC 0.5%	Aroma	R (Control)	Flavor	-0.4286	0.6285	-0.68	0.4953	0.05	-1.6604	0.8032
FC 0.5%	Aroma	R (Control)	Texture	-0.8095	0.6285	-1.29	0.1977	0.05	-2.0413	0.4223
FC 0.5%	Flavor	FC 0.5%	Texture	-0.09524	0.6285	-0.15	0.8796	0.05	-1.3270	1.1366
FC 0.5%	Flavor	FC 5%	Appearance	-0.04762	0.6285	-0.08	0.9396	0.05	-1.2794	1.1842
FC 0.5%	Flavor	FC 5%	Aroma	1.5714	0.6285	2.50	0.0124	0.05	0.3396	2.8032
FC 0.5%	Flavor	FC 5%	Flavor	1.2857	0.6285	2.05	0.0408	0.05	0.05391	2.5175
FC 0.5%	Flavor	FC 5%	Texture	0.2857	0.6285	0.45	0.6494	0.05	-0.9461	1.5175
FC 0.5%	Flavor	R (Control)	Appearance	-0.4762	0.6285	-0.76	0.4486	0.05	-1.7080	0.7556
FC 0.5%	Flavor	R (Control)	Aroma	-0.3333	0.6285	-0.53	0.5958	0.05	-1.5651	0.8985
FC 0.5%	Flavor	R (Control)	Flavor	-0.7619	0.6285	-1.21	0.2254	0.05	-1.9937	0.4699
FC 0.5%	Flavor	R (Control)	Texture	-1.1429	0.6285	-1.82	0.0690	0.05	-2.3747	0.08895
FC 0.5%	Texture	FC 5%	Appearance	0.04762	0.6285	0.08	0.9396	0.05	-1.1842	1.2794
FC 0.5%	Texture	FC 5%	Aroma	1.6667	0.6285	2.65	0.0080	0.05	0.4349	2.8985
FC 0.5%	Texture	FC 5%	Flavor	1.3810	0.6285	2.20	0.0280	0.05	0.1492	2.6128
FC 0.5%	Texture	FC 5%	Texture	0.3810	0.6285	0.61	0.5444	0.05	-0.8508	1.6128
FC 0.5%	Texture	R (Control)	Appearance	-0.3810	0.6285	-0.61	0.5444	0.05	-1.6128	0.8508
FC 0.5%	Texture	R (Control)	Aroma	-0.2381	0.6285	-0.38	0.7048	0.05	-1.4699	0.9937
FC 0.5%	Texture	R (Control)	Flavor	-0.6667	0.6285	-1.06	0.2888	0.05	-1.8985	0.5651
FC 0.5%	Texture	R (Control)	Texture	-1.0476	0.6285	-1.67	0.0955	0.05	-2.2794	0.1842
FC 5%	Appearance	FC 5%	Aroma	1.6190	0.6285	2.58	0.0100	0.05	0.3872	2.8508
FC 5%	Appearance	FC 5%	Flavor	1.3333	0.6285	2.12	0.0339	0.05	0.1015	2.5651

FC 5%	Appearance	FC 5%	Texture	0.3333	0.6285	0.53	0.5958	0.05	-0.8985	1.5651
FC 5%	Appearance	R (Control)	Appearance	-0.4286	0.6285	-0.68	0.4953	0.05	-1.6604	0.8032
FC 5%	Appearance	R (Control)	Aroma	-0.2857	0.6285	-0.45	0.6494	0.05	-1.5175	0.9461
FC 5%	Appearance	R (Control)	Flavor	-0.7143	0.6285	-1.14	0.2557	0.05	-1.9461	0.5175
FC 5%	Appearance	R (Control)	Texture	-1.0952	0.6285	-1.74	0.0814	0.05	-2.3270	0.1366
FC 5%	Aroma	FC 5%	Flavor	-0.2857	0.6285	-0.45	0.6494	0.05	-1.5175	0.9461
FC 5%	Aroma	FC 5%	Texture	-1.2857	0.6285	-2.05	0.0408	0.05	-2.5175	-0.05391
FC 5%	Aroma	R (Control)	Appearance	-2.0476	0.6285	-3.26	0.0011	0.05	-3.2794	-0.8158
FC 5%	Aroma	R (Control)	Aroma	-1.9048	0.6285	-3.03	0.0024	0.05	-3.1366	-0.6730
FC 5%	Aroma	R (Control)	Flavor	-2.3333	0.6285	-3.71	0.0002	0.05	-3.5651	-1.1015
FC 5%	Aroma	R (Control)	Texture	-2.7143	0.6285	-4.32	<.0001	0.05	-3.9461	-1.4825
FC 5%	Flavor	FC 5%	Texture	-1.0000	0.6285	-1.59	0.1116	0.05	-2.2318	0.2318
FC 5%	Flavor	R (Control)	Appearance	-1.7619	0.6285	-2.80	0.0051	0.05	-2.9937	-0.5301
FC 5%	Flavor	R (Control)	Aroma	-1.6190	0.6285	-2.58	0.0100	0.05	-2.8508	-0.3872
FC 5%	Flavor	R (Control)	Flavor	-2.0476	0.6285	-3.26	0.0011	0.05	-3.2794	-0.8158
FC 5%	Flavor	R (Control)	Texture	-2.4286	0.6285	-3.86	0.0001	0.05	-3.6604	-1.1968
FC 5%	Texture	R (Control)	Appearance	-0.7619	0.6285	-1.21	0.2254	0.05	-1.9937	0.4699
FC 5%	Texture	R (Control)	Aroma	-0.6190	0.6285	-0.98	0.3246	0.05	-1.8508	0.6128
FC 5%	Texture	R (Control)	Flavor	-1.0476	0.6285	-1.67	0.0955	0.05	-2.2794	0.1842
FC 5%	Texture	R (Control)	Texture	-1.4286	0.6285	-2.27	0.0230	0.05	-2.6604	-0.1968
R (Control)	Appearance	R (Control)	Aroma	0.1429	0.6285	0.23	0.8202	0.05	-1.0889	1.3747
R (Control)	Appearance	R (Control)	Flavor	-0.2857	0.6285	-0.45	0.6494	0.05	-1.5175	0.9461
R (Control)	Appearance	R (Control)	Texture	-0.6667	0.6285	-1.06	0.2888	0.05	-1.8985	0.5651
R (Control)	Aroma	R (Control)	Flavor	-0.4286	0.6285	-0.68	0.4953	0.05	-1.6604	0.8032
R (Control)	Aroma	R (Control)	Texture	-0.8095	0.6285	-1.29	0.1977	0.05	-2.0413	0.4223
R (Control)	Flavor	R (Control)	Texture	-0.3810	0.6285	-0.61	0.5444	0.05	-1.6128	0.8508

TALAYSHA LINGHAM

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EDUCATION

M.S. Food Science, concentration Food Microbiology, May 2012
Delaware State University, Dover, DE

B.S. Food & Nutritional Science, May 2010
Delaware State University, Dover, DE

EXPERIENCE

Food Science, concentration Food Microbiology Master Degree Program
Delaware State University, Dover DE 2010 - 2012

- Advisor: Dr. Jung-lim Lee
- Thesis Title: Antimicrobial Activity of Vinegar on Foodborne Pathogens and Bacteria Species Isolated from Catfish (*Ictalurus Punctatus*)

Research Procedures Performed:

- Basic Microbiology lab skills
- Zone of Inhibition and Minimum inhibitory concentration testing
- Isolation of bacteria from fish fillets
- Bacterial counts- Microbial changes overtime
- Sensory Evaluation

Research Assistant of Human Ecology Dept.
Delaware State University, Dover, DE April 2009 - April 2010

- Analyzed and reported research data on high school student Healthy Eating and Physical Activity Survey.
- Assisted with experiment on quality of cookies and muffins with flaxseed added.

Shadow Registered Dietician
Delaware Veterans Home, Milford, DE March - April 2010

- Looked at patient's charts filling out nutrition assessment sheets.
- Care Plan- problem, goal and approach

Server
Salvation Army Soup Kitchen, Dover, DE October 2009

- Volunteered on the Soup Kitchen line as a server.
- Observed the appearance and the nutritional status of the community being served.
- Learned the nutritional quality of the food being served to the community.

Cook/Server
Delaware State University Village Cafe, Dover, DE
- Shadowed the executive chef at the University. August 2009
- Assisted in preparing food.
- Developed and presented an entrée and served it to the students.

EFNEP Kids Cooking Camp Aid
Delaware State University, Dover DE
- Supervised and assisted children ages nine to fourteen on meal and snack preparation. August 2009
- Introduced children ages nine to fourteen to MyPyramid for Kids.

Educator
Drop A Tot Center, Dover DE
- Educated children ages six to ten on the Food Pyramid. July - August 2009

ACTIVITIES & HONORS

Greater Philadelphia Region LSAMP Bridge to Doctorate Program
National Society of Collegiate Scholars (NSCS)
American Dietetic Association
FAN (Food and Nutrition) Club – Treasurer
Delaware State University Marching Band/Pep Band/Concert Band- Section leader
Dean's List (4 semesters)

CERTIFICATIONS

Certified Food Safety Manager
Lab Safety Certified

PRESENTATIONS

The Study of the Antimicrobial Activity from Vinegar on Bacteria Species. Delaware State University Graduate Symposium. April 2012. Poster Presentation- 2nd Place.

The Study of the Antimicrobial Activity from Vinegar on Bacteria Species. Emerging Researchers National Conference. February 2012. Poster Presentation.

The Study of the Antimicrobial Activity from Vinegar on Foodborne Pathogen and Bacteria Species Isolated from Catfish. Delaware State University College of Agriculture and Related Sciences Research Day. November 2011. Poster Presentation.

The Study of the Antimicrobial Activity from Vinegar on Foodborne Pathogen and Bacteria Species Isolated from Catfish. Bridge to Doctorate Annual Research Symposium. October 2011. Oral Presentation.

Study of Antimicrobial Activity from Plant Leaves on Bacterial Species. USDA-ARS Beltsville Area Research Symposium. February 2011. Poster Presentation.

Food Nutrition Label Use among High School Students in Delaware. Delaware State University Honors Day. March 2010. Oral Presentation.

RELEVANT GRADUATE COURSEWORK

Food Microbiology
Fundamentals of Food Science
Molecular and Biotechnology Application in Food Science
Research Problems in Food Science
Food Toxicology
Advanced Food Safety
ADV Statistics Tech for Data Analysis

RELEVANT UNDERGRADUATE COURSEWORK

Introduction to Nutrition
Advanced Nutrition
Fundamentals of Microbiology
Principles and Analysis of Food Preparation
Human Nutrition Assessment
Nutrition Through Life Cycle
Sanitation and Safety
Introduction to Food Science
Community Nutrition
Institutional Food Service
Medical Nutrition Therapy I and II
Nutrition Education and Counseling
Nutritional Biochemistry
Microbial Physiology and Ecology

REFERENCES

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