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Utilization of buffered vinegar to inhibit the growth of *Listeria monocytogenes* on marinated-cooked chicken breast

By

James Leland Butler

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Food Science, Nutrition, and Health Promotion
in the Department of Food Science, Nutrition, and Health Promotion

Mississippi State, Mississippi

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James Leland Butler

2017

Utilization of buffered vinegar to inhibit the growth of *Listeria monocytogenes* on marinated-cooked chicken breast

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Pages in Study: 43

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The objective of this study was to evaluate the efficacy of buffered vinegar in a marinade solution on inhibiting *Listeria monocytogenes* growth on cooked broiler breast meat. Broiler breasts were vacuum-tumbled for 30 min in a marinade consisting of dry (0%, 0.4% DV, 0.6%DV, and 0.8%) or liquid vinegar (1.5%), sodium chloride, sodium tripolyphospate, and water. The chicken breasts were then cooked to an internal temperature of 75°C. The breast meat was inoculated with *L. monocytogenes*, placed into modified atmosphere packaging, and stored at 2°C plus/minus 2 for 0-60 days. *L. monocytogenes* growth was stable on treatments for up to 30 days. However, from 35 to 60 days, the buffered vinegar treatments had fewer *L. monocytogenes* counts (P<0.05) than the control treatment. In addition, the 0.8 % DV and 1.5 % LV treatments had fewer than 2.0 log counts of *L. monocytogenes* after 60 days of storage.

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DEDICATION

I dedicate this work to my advisors and professors Dr. Taejo Kim and Dr. Wes Schilling. Through their tutelage I was able to gain a significant and proficient amount of knowledge, experience, and skill in the fields of Food Science and Microbiology. I would also like to dedicate this work to my co-worker Carlos Morris whose help in the initial step of this research greatly contributed to the success of this work. Lastly, I would like to thank my family who always motivated me and showed faith in me through this entire research process.

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I would like to sincerely thank Dr. Taejo Kim and Dr. Wes Schilling for mentoring and guiding me throughout the course of my research and studies. I would also like to express my gratitude to Dr. Juan Silva for his support during the time of my research project. Lastly, I would like to give my appreciation and gratitude to my colleagues Ms. Lurdes Siberio, Ms. Maryam Mohammadi, Mr. Carlos Morris, and Mr. Hector Portillo for their assistance throughout this work.

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CHAPTER I

INTRODUCTION

Listeria monocytogenes is a Gram-positive, ubiquitous nonspore-forming, rod shaped, facultative anaerobic, and facultative intracellular bacterium. *L. monocytogenes* is a hearty bacterium which can tolerate harsh environmental conditions. The bacterium is able to endure high salt percentages (as high as 30 percent) and can grow at pHs ranging from approximately 4.3-9.4 under otherwise ideal conditions. *L. monocytogenes* is capable of growth at a variety of temperatures ranging from 0°C to 45°C, is able to survive freezing, and can survive in products with a water activity as low as 0.92 (Romans et al., 2001). *L. monocytogenes* is an opportunistic, psychotropic foodborne pathogen that causes listeriosis. Listeriosis can cause septicemia and lead to an infection of the central nervous system with meningitis, encephalitis, or abscesses (Romans et al., 2001). Immunosuppressed individuals, children, elderly, and pregnant women are especially susceptible to listeriosis infections. The ability of *L. monocytogenes* to survive under numerous harsh conditions is a major concern in refrigerated, ready-to-eat (RTE) meat and poultry products, particularly when refrigeration is marginal (10°C or higher) (Tompkin et al., 2001).

A RTE food item is any food which does not require cooking or has been cooked prior to packaging. RTE foods, including produce, fermented products, cooked meats, and cured meat and poultry products have been associated with listeriosis-related

outbreaks. Due to the increase in foodborne illness associated with *L. monocytogenes* in RTE meat and poultry products, the USDA-FSIS has issued a zero tolerance policy and HACCP regulations directed towards the plants to address control measures for the organism in RTE products (Henning and Cutter, 2001).The widespread nature of the organism, *L. monocytogenes* contamination of RTE foods commonly occurs due to recontamination or cross-contamination of the finished product after thermal processing and prior to consumption. Cured or cooked RTE meat products; such as frankfurters and deli luncheon meat and poultry items, are commonly consumed without additional heat treatment, which can lead to the production of products that may be contaminated with *L. monocytogenes*. As an attempt to lower the frequency of microbial spoilage and recontamination, RTE food processors commonly use food additives, such as organic acids

An organic acid is an organic compound (contains carbon) with acidic properties, and the most common organic acids are carboxylic acids (contains a carboxyl group – COOH), such as acetic acid, propionic acid, and lactic acid. Organic acids are naturally occurring weak acids that are found in a variety of vegetable and animal substrates. Organic acids are naturally in fruit juices and fermented foods as the products of microbial metabolism, or they can be added to the food products during processing. Most weak organic acids are GRAS and are among the most common chemical preservatives used in food. They are frequently added to RTE meat and poultry products as antimicrobials, to inhibit or delay bacterial spoilage, increase shelf life, and contribute to the flavor and sensory qualities of RTE meat and poultry products (Lück, E. and Jager, 1997). The antimicrobial effectiveness of an organic acid is based on several

factors including pH, acid concentration, ionic strength, and the acid-adaption of the target pathogen. Organic acids have shown greater antimicrobial strength at lower pHs because the amount of undissociated acid present is higher at lower pHs. Undissociated acids are more hydrophobic, and hydrophobic organic acids better interact with the lipid material present in the microbial cell wall in order to disrupt microbial activity. Even though organic acids have a long history of being added to foods as an antimicrobial agent, there is still a need for research and investigation into the optimal concentration needed for the best antimicrobial activity in certain food items. This study was conducted to determine the inhibitory effect of different concentrations of buffered vinegar in a marinade solutions on the growth of *L. monocytogenes* on cooked broiler chicken breasts.

CHAPTER II

LITERATURE REVIEW

2.1 *Listeria monocytogenes*

2.1.1 History

Murray et al. (1926) first described *Listeria monocytogenes*, which he named *Bacterium monocytogenes* since it caused monocytosis, an increase in the number of monocytes circulating in the blood, in infected guinea pigs and rabbits. Following the deaths of infected laboratory gerbils in 1927, J. Harvey Pirie renamed the bacterium *Listeralla hepatolytica* in honor of Lord Lister, the British surgeon and pioneer of antiseptic surgery (Ryser, 1999). The similarities between the strains isolated by Murray et al. (1926) led to the bacterium being renamed *Listeriae monocytogene*; however, the generic name *Listerella* had already been used for a protozoa. Thus, Pirie proposed changing the name to *Listeria monocytogenes* in 1940. Even though the genus name was already used in botanical taxonomy for the name of an orchid and in zoology for the name of a diptera, the name *Listeria monocytogenes* was accepted for this pathogen by the governing committee (Seeliger, 1961). The epidemiology of the disease was unknown for many year because clinical isolates were rare (Seelinger, 1972). In 1929 Nyfeldt made the first confirmed isolation of the bacterium from an infected human, and the same year, Gill isolated it from sheep. (Gray and Killinger, 1966). Although clinical descriptions of *L. monocytogenes* infection in humans were published 1920's, listeriosis was not

recognized as a foodborne illness and major public health concern until 1981 after an outbreak of listeriosis in Halifax, Nova Scotia was linked to contaminated coleslaw (Sclech et al. 1983).

2.1.2 Genus, Taxonomy, and Morphology

Listeria monocytogenes (L. monocytogenes) is a species of pathogenic bacteria in the genus *Listeria*. Named after British surgeon and antiseptic pioneer Lord Lister, the genus *Listeria* consists of 6 species: *L. innocua, L. grayi, L. L. ivanovii, L. seeligeri, and L. welshimeri.* In recent years, many new *L. monocytogenes* species have been proposed; however, these new species are not widely adopted (Hitchins, et al., 2016). *L. monocytogenes* and *L. ivanovii* are pathogenic in mice and other animals, but *L. monocytogenes* is the only species that is pathogenic to humans.

L. monocytogenes is a Gram-positive, non-spore-forming, rod-shaped, facultative anaerobic bacterium. Microscopically*, L. monocytogenes* appears as a small, short (0.4-0.5 x 0.5-2.0 μm) single cell or arranged in short chains with parallel sides and blunt ends (Wagner and McLauchlin, 2008). On nutrient agar, colonies are 0.2-0.8 mm in diameter (up to 5 mm in diameter after 48 h incubation), smooth, punctiform, blueish gray to black, translucent, and slightly raised with a fine surface texture after 48 h of incubation at 37°C (Gray, 1957; Mckellar, 1994).

L. monocytogenes is psychrotrophic (able to grow at low temperature) and is capable of growth at temperatures ranging from (0-45°C). However, growth has been observed at temperatures as low as -1.5°C and survival has been documented at temperatures below freezing (Lawley, 2013). The pH range for *L. monocytogenes* growth is approximately 4.3-9.4 (Lawley, 2013). *L. monocytogenes* is capable of growth

in environment of up to 10% sodium chloride (NaCl), and can survive in concentrations of 20-30% salt (Lado and Yousef, 2007). The optimum water activity for growth is >0.97, with 0.92 as a minimum requirement for growth. *L. monocytogenes* requires glucose and glutamine as primary sources of carbon and nitrogen when cultured in a chemically defined media (Premaratne et al., 1991). *L. monocytogenes* has the ability to form biofilms, which allow them to attach to solid surfaces where they proliferate and become extremely difficult to remove through the use of sanitizers and disinfectants (Doijad et al., 2015; Carpentier and Cerf, 2011).

L. monocytogenes is oxidase-negative, catalase-positive, expresses β-hemolysin, and exhibits tumbling motility (Todar, 2008). It is motile via flagella at room temperature (20-30°C), but usually not at body temperature (37°C). At 37° C, it can move within the eukaryotic cells by explosive polymerization of actin filaments (Gründling et al., 2004). Since hemolysis is a major virulence factor of *L. monocytogenes*, hemolytic activity on blood agar has been used to identify and differentiate *L. monocytogenes* from other *Listeria* species. However, it is not absolutely definitive since other *Listeria spp*. can express β-hemolysis (Ryser and Marth, 1999; Todar, 2008).

L. monocytogenes has 13 serotypes, each characterized by a distinct somatic (O) and flagellar (H) antigenic pattern. Serotypes 1/2a, 1/2b, and 4b account for up to 95% of *L. monocytogenes* isolates from human illness (Kathariou, 2002). Serotype 1/2a accounts for : >50% of the isolates recovered from foods and the environment, while most major outbreaks of human listeriosis have been caused by serotype 4b strains (Gilbreth et. al, 2005; Lukinmaa, 2003; Mead et al., 2006; Revazishvili et al, 2004). *L. monocytogenes*

consists of at least three major phylogenetic lineages ((I, II, and III), with lineages I and II harboring the serotypes most commonly associated with human clinical cases, including serotype 1/2a (lineage II) and serotypes 1/2b and 4b (lineage I) (Nadon et al., 2001; Wiedman, 2002; Wiedman et al, 1997).

2.1.3 Disease and Pathogenesis

Listeria monocytogenes is the causative agent for the infection Listeriosis (Cossart, 2001). Listeriosis is generally caused by the consumption of contaminated food, and is one of the most severe forms of foodborne infection with a mortality of rate of approximately 15-20% (Scallen et. al, 2011, CDC, 2016). The Center for Disease Control and Prevention estimates that approximately 1600 listeriosis cases, resulting in 260 deaths, occur annually in the United States (CDC, 2016). *L. monocytogenes* causes two types of listeriosis, a noninvasive, gastrointestinal form and a more severe invasive form that spreads beyond the intestinal tract, with invasive listeriosis accounting for all reported cases (Allerberger and Wagner, 2010; CDC, 2013). Many cases go undiagnosed due to the long incubation period (3-70 days) and the self-limiting symptoms of noninvasive listeriosis (Mardis et al., 2012). Most healthy adults and children are likely to experience only mild to moderate flu-like symptoms and gastroenteritis lasting only 2 to 3 days (FDA, 2012; NIH, 2009; Ooi and Lorber, 2005; Mardis et. al., 2012). Invasive listeriosis predominantly affects high-rick groups with suppressed cell-mediated immunities such as pregnant women and their fetus/newborn, adults over the age of 65, and other immunocompromised people (Allerberger, and Wagner, 2010; Arslan et. al, 2015; CDC, 2016). Cell-mediated immunity (innate immunity) is the body's defense against *L. monocytogenes* and other intracellular pathogens. Individuals

whose T cell-mediated immunity is weakened are more susceptible to the severe effects of listeriosis (i.e. meningitis, brain abscesses, or rhombencephalitis) (Schuppler and Loessner, 2010). Pregnant women have very naturally weakened cell-mediated immunity, which is believed to be a way to protect the fetus from rejection (Lorber and Bennet, 2000; Silver, 1998). In addition, fetuses and newborns have very immature immune systems, which make them extremely susceptible to listeriosis infection (Silver, 1998). Pregnant women usually experience mild symptoms like fever, fatigue, and muscle ache, but the infection can be passed to the fetus resulting in stillbirths, preterm labor, or severe illness or death in the newborn (CDC, 2013; Posfay-Barbe et al., 2009). More than half of all listeriosis cases occur in people older than 65 since their immune system and organs aren't able to fight listeriosis infections and have other chronic conditions that require medications that weaken the immune system (CDC, 2016; Marler, 2013; Pinner et al., 1992). People with weakened immune systems due to underlying medical conditions and the treatments for those conditions are also more likely to get listeriosis (Schuchat et al., 1991). In elderly and immunocompromised people, septicemia and meningitis are the most common complications of listeriosis (Bartolussi, 2008; CDC, 2016; Marler 2013) Noninvasive listeriosis is commonly asymptomatic, and invasive listeriosis can be treated with antibiotics (Lorber and Bennett, 2000).

Several factors have key synergistic roles in the pathogenicity of *L. monocytogenes*, including entry into the host cell lysis of phagocytic vacuoles, intracellular movement in the cytoplasmic environment, and cell-to-cell spread in the host (Cabanes et al., 2002). Listeriosis, which often results from ingestion of

contaminated foods containing *L monocytogenes*, has the ability to cross the intestinal, blood-brain, and fetoplacental barriers (Mardis et al., 2012). The noninvasive form of listeriosis is believed to remain in the intestinal tract for one or more of the following reasons: the lack of necessary virulence factor, insufficient amount of *L. monocytogenes* cells ingested and/or and the host's immune response is able to eradicate the bacterium before invasion (Stearns, 2015; Ooi and Lorber, 2005; Ryser and Marth, 2007). However, in invasive listeriosis, *L. monocytogenes* cells, assisted by several virulence factors, are able to attach to host cells (Internalin A and B), translocate past the intestinal membrane into the cytosol (LLO and Hly), and spread cell-to-cell (ActA) throughout the bloodstream, central nervous system, to the fetus, and other parts of the body (Schubert et al. 2002; Berche et al., 1988; Auerbuch et al., 2004;Mardis et al, 2012; Wing and Gregory, 2002).

2.1.4 Sources and Outbreaks

Listeria monocytogenes (*L. monocytogenes*) is ubiquitous in nature and the environment. It is widely present in soil, water, sewage, vegetation, feed, farms, and can persist in food. The bacterium easily comes into contact with farm animals, as it has been found to be present in grazing areas, stale water, and poorly prepared animal feed. *L. monocytogenes* can be isolated from humans, domesticated animals, fishery products, raw agriculture, and livestock and food processing environments. It tolerates high salt and pH, grows under low-oxygen conditions and low refrigeration temperatures. Given that *L. monocytogenes* is present in nearly every environment, numerous opportunities for contamination exist during the food production process. Food products, the environment, equipment, employees, customers, and vendors are all potential sources of contamination.

Raw items such as meats, poultry, seafood, and some fruits and vegetables may carry *L. monocytogenes*. Although processing methods such as heat or chemical treatments can destroy *L. monocytogenes*, processed foods may be frequently contaminated due to inadequate thermal-processing or post-processing cross-contamination. Potential sources of contamination of foods in food service and retail operations include food products, environment, equipment, employees, customers, and vendors. Contamination can also occur after purchase and before consumption at the household or other establishment.

L. monocytogenes was recognized as a foodborne pathogen in 1981, when it was linked to consumption of contaminated coleslaw in Canada (Schlech et al. 1983). Since then, listeriosis has been linked to a variety of food products such as cheese milk, processed meat, and fresh produce including celery, sprouts, and cantaloupe (Cartwright et. al, 2013; Norton and Braden, 2007; CDC, 2014). In 1983, the first recorded outbreak of listeriosis in the United States was epidemiologically linked to the consumption of a specific brand of pasteurized, whole and 2%, milk in Massachusetts (Fleming et al., 1985). The outbreak resulted in 49 illnesses with 14 deaths in Massachusetts. Since 1983, dairy products have been linked to several notable listeriosis outbreaks. In 1985, *a L. monocytogenes* outbreak linked to Mexican style soft cheeses caused 142 illnesses, resulting in at least 28 to 52 deaths, depending on the report. One of the most published outbreaks of listeriosis in the United States was linked to Blue Bell Ice Cream. The Blue Bell outbreak lasted from 2010 to 2015 and resulted in 10 illnesses, 3 deaths, in four states and an $850,000 fine plus an estimated loss in the hundreds of millions of dollars for Blue Bell. The number of *L. monocytogenes* outbreaks linked to fresh produce and reported to the United States Centers for Disease Control and Prevention (CDC) has

increased since 1990 (Bean and Griffin, 1990; CDC, 2011; CDC, 2015). In 2011, cantaloupe was linked to one of the deadliest outbreaks of listeriosis in the United States history, causing 147 illnesses and 30 deaths in 28 states. Meat products, especially RTE items, have been implicated in several listeriosis outbreaks. The first reported listeriosis outbreak from processed meat (turkey frankfurter) occurred in the US in 1988, with numerous reported outbreaks due to the consumption of processed meat products, thereafter (CDC, 2014). In 1998, a multistate outbreak of listeriosis in which frankfurters manufactured by Bil Mar Foods, were implicated, resulted in 101 hospitalizations, 15 deaths, and 6 stillbirths or miscarriages. This led to the recall of 35 million pounds of frankfurters and deli meats (CDC, 2016). In 2000, a multistate outbreak of listeriosis was associated with the consumption of sliced deli meat that was manufactured by Cargill Turkey Products, Inc. This resulted in 29 cases with 4 deaths and 3 miscarriages, and recalls of processed turkey and chicken deli meats (CDC, 2016). In 2002, a multistate outbreak of listeriosis was linked to the consumption of sliced turkey deli meat that was manufactured by Pilgrim's Pride Foods. This outbreak resulted in 54 cases with 8 deaths, 3 fetal deaths, and the recall of 27.4 million pounds of ready-to-eat turkey and chicken products (CDC, 2016). Due to several recalls and outbreaks involving *L. monocytogenes* in RTE meat and poultry products in 1998 and 1999, USDA-FSIS issued an interim final rule (*Listeria* Rule) for the control of *L. monocytogenes* in RTE meat and poultry products (USDA-FSIS, 2003).

2.1.5 Prevention and Regulations

The emergence of *L. monocytogenes* outbreaks and recalls in processed meat and poultry products began during the 1980's, which led to the implementation of several

protective protocols and regulations pertaining to the control of *L. monocytogenes* in the food industry (CDC, 2011; USDA-FSIS, 2003). Recent outbreaks that were associated with RTE meat and poultry products prompted the US Department of Agriculture-Food Safety and Inspection Service to prompt processors to implement measures in their hazard analysis critical control points (HACCP) plans to control and prevent the proliferation of *Listeria* on RTE meat and poultry products that are exposed to the environment after cooking (USDA-FSIS, 2002). In 1987, USDA-FSIS developed a monitoring and verification program for *L. monocytogenes* in meat products, including luncheon meat, beef jerky, sausages, cooked/uncured poultry, salads and spreads, and various meat products (USDA-FSIS, 2014). As a result of several major listeriosis outbreaks in 1998, 1999, 2000, and 2002, USDA-FSIS issued stringent rules and regulations, called the *Listeria* Rule, that were designed to prevent *L. monocytogenes* contamination of RTE meat and poultry products (USDA-FSIS, 2012) Under the *Listeria* Rule, the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) placed a zero tolerance (no detectable level permitted) on *L. monocytogenes* in post-lethality exposed RTE meat and poultry products (USDA-FSIS, 2012). This policy means that detection of *L. monocytogenes* in a RTE meat and poultry product or on a food-contact surface renders the product adulterated under the Federal Meat Inspection Act of the Poultry Products Inspection Act [21 USC 453 9 9 (g) or 601 (m)] (USDA-FSIS, 2003). The *Listeria* Rule also states that *L. monocytogenes* is a hazard in post-lethality exposed products and must be controlled (USDA-FSIS, 2003). In 2003, the *Listeria* Rule established three alternatives that can be implemented to control *L. monocytogenes* contamination of post-lethality exposed RTE products (USDA-FSIS,

2003; USDA-FSIS 2012). The three strategies for controlling *L. monocytogenes* in a processing environment are: Alternative 1 - apply both a post-lethality treatment and an antimicrobial agent or process to suppress the growth of *L. monocytogenes*, Alternative 2 –apply either a post-lethality treatment or antimicrobial agent to control the growth of *L. monocytogenes*, Alternative 3 -use of sanitation control measures to prevent recontamination after processing (USDA-FSIS, 2012).

2.2 Ready-to-Eat Foods

2.2.1 Introduction

The Food Safety Inspection Service (FSIS) defines a Ready-to-Eat (RTE) meat or poultry product as a food product that is in a form that is edible and safe without additional preparation, but may receive additional preparation for palatability, aesthetic, gastronomic or culinary purposes (9 CFR Part 430) (USDA-FSIS, 2012). These food products are commonly labeled as convenient, ready, and instant and include food items such as pastries, meat products, poultry products, milk and milk products (Charles & Kerr, 1988). RTE products are not required to bear safe-handling instructions that are required for non-RTE products as stated in 9 CFR 317.2 (USDA-FSIS, 2012). RTE products fall under the label of high risk foods and are of particular concern for contamination with *L. monocytogenes* because they may support growth during refrigerated storage, are often consumed without further cooking, and cross-contamination may occur (USDA-FSIS, 2014). Listeriosis outbreaks associated with RTE meat and poultry products prompted the US Department of Agriculture-Food Safety and Inspection Service to encourage the addition of antimicrobial agents to control *L. monocytogenes* in RTE meat products (USDA-FSIS, 2003).

2.2.2 *Listeria monocytogenes* and Ready-to-Eat Foods

Listeria monocytogenes presents a significant concern as a number of widespread outbreaks that involve processed meats have been reported in the past decade (CDC, 2016; Olsen et al., 2005; Gottlieb et al., 2006). Epidemiological studies, product testing, and risk assessment models have shown that the most prevalent sources of listeriosis are from RTE foods, such as, delicatessen meat and poultry, dairy products, frankfurters, fish and seafood products, and fresh produce (Rocourt and Cossart, 1997; USDA-FSIS, 2003; EFSA, 2007; Lianou and Sofos, 2007). The contamination by *L. monocytogenes* of cured and non-cured RTE cooked meat and poultry is a major safety concern for RTE cooked meat products since (1) RTE cooked meats have extended shelf-lives and consumed without further heating, (2) *L. monocytogenes* can proliferate to a threatening level during refrigerated storage because of its ability to grow in the presence of curing salt at refrigerated temperature, and (3) its resistance to relatively high acid concentrations (Lou and Yousef, 1999; Lemaitre et al., 1998).

Contaminated dairy products have been implicated in several listeriosis outbreaks (Becker et al., 1988). In 1983, an outbreak of Listeriosis in Massachusetts that involved pasteurized milk, resulted in 14 deaths. In 1985, a large listeriosis epidemic (34% mortality rate) occurred in Los Angeles County due to the consumption of soft, "Mexican-Style" cheese. This brought listeriosis to the forefront of public health and regulatory concern when 142 recognized cases were reported and confirmed over an 8 month period (Linnan et al., 1988). Other listeriosis outbreaks caused by the consumption of contaminated dairy products, especially soft cheeses and unpasteurized or pasteurized

milk, have been reported and confirmed in Switzerland, France, Denmark, Canada, and at least 33 states.

Deli meats, poultry products, and frankfurters have been the cause of several outbreaks in the U.S. and internationally (Pinner, R.W., et al., 1992). The first case of laboratory-confirmed association of meat and poultry products with invasive listeriosis occurred in 1988, when a case was linked to contaminated turkey frankfurters (Ryser and Marth, 2007). One of the largest listeriosis outbreaks in the United States occurred in 1998-1999 due to the consumption of contaminated frankfurters and deli meats that were linked to 101 cases and 21 deaths (CDC, 2014). Deli-turkey meat was implicated as the cause of a multistate listeriosis outbreak with 29 cases and 4 deaths reported to the CDC (CDC, 2014). In 2002, another listeriosis outbreak resulting in 54 infections and 11 deaths was linked to the consumption of RTE turkey deli meat (Gottlieb et al., 2006).

Smoked and cured seafood products have also been associated with listeriosis outbreaks (Brett et al., 1998; Ericsson et al., 1997; Mietinen et al., 1999). For instance, smoked mussels have been linked to *Listeria* outbreaks in Australia and New Zealand, cold smoked rainbow trout has been linked to an outbreak in Sweden, smoked salmon has been linked to sporadic cases in Australia, and smoked cod roe has been linked to sporadic cases in Denmark (Brett et al. 1998; Ericsson et al., 1997; Ryser, 1999). In contrast, raw seafood has a low predicted relative risk of causing listeriosis in the U.S (FDA, 2012).

Vegetables have the potential to become contaminated with *Listeria* in the growing environment from the soil or contact with animal manure (Thigell, Kendall, and Bunding, 2012). Produce-associated *Listeria* outbreaks have been linked to several types

of vegetables and fruits including cabbage, celery, and lettuce. In 2011, whole cantaloupes were linked to one of the largest and most deadly outbreaks of listeriosis in the U.S., in which 146 persons were infected and 30 deaths were reported (CDC, 2011)

2.2.3 Antimicrobials in Ready-to-Eat Foods

An antimicrobial agent is defined as a substance that effectively reduces or eliminates microorganisms or suppresses growth to no more than 2 log units throughout the shelf life of the product (USDA-FSIS, 2003). Antimicrobial agents are substances in or added to a RTE product that has the effect of reducing or eliminating a microorganism, or that has the effect of suppressing or limiting growth in the product throughout the shelf life of the product (FSIS, 2003). The purpose of antimicrobial agents is to reduce or eliminate microbial activity in two different manners: stasis or cidal mechanisms. Agents that inhibit microbial growth are bacteriostatic and bactericidal agents destroy or kill microbes. Many chemicals used for sterilization and disinfections are cidal. However, food preservatives must not be toxic to the consumer and therefore tend to be static agents (Beverly, 2004). As a result, organic acids (lactic, acetic, citric, benzoic, propionic, and sorbic) and their salts (sodium diacetate, sodium lactate, potassium sorbate, and potassium benzoate) are used as antimicrobials to inhibit the proliferation of *L. monocytogenes* and other foodborne pathogens (Zhang et al., 2007).

2.3 Organic Acids and Salts of Organic Acids

2.3.1 Introduction

Organic acids are usually the fermentative by-product of ubiquitous organisms such as lactic acid bacteria (LAB), which produces the antimicrobial lactic acid (Casey et al., 2002). Organic acids can either be naturally present in the food or can be added

directly or indirectly to the products. One of most common organic acids is carboxylic acid, in which the carboxyl group (-COOH) dissociates into a proton and conjugate base with acidic properties. Other common organic acids include alcohols (-OH), thiols (-SH), enols, and phenol groups. Organic acids are generally weak acids due to the fact that they only partially ionize or dissociate in neutral aqueous solutions. This means that weak acids do not easily give up their protons (H+) in solution. Organic acids exist in 2 basic forms, which include pure acid or buffered acids. Lactic acid, propionic acid, acetic acid, citric acid, and benzoic acid are pure acids, and the calcium and sodium salts of propionic, acetic, citric, and benzoic acids are buffered organic acids. These buffered organic acids are safer to handle and less caustic to machinery (Brul and Coote, 1999). Some of the organic acids used in foods (lactic, acetic, propionic, citric, and benzoic acid) are weak acids mainly due to their solubility in water, flavor, and low toxicity (ICMSF, 1980).

Organic acids that are commonly found in foods differ considerably in structure and in their inhibitory effects on various bacteria. Lactic acid contributes to several biochemical processes, as well as in the production of dairy products such as cheese and yogurt. Acetic or ethanoic acid (CH_3–COOH; log P – 0.319), a monocarboxylic acid, is produced naturally during spoilage of plants and in animal tissue and is also a product of ethanol oxidation by the bacterium *Acetobacter*, Gluconobacter and other heterofermentative strains of lactic acid bacteria (LAB) (Doores, 2003; Leo et al., 1971). Vinegar, a 5% acetic acid solution, is known for its strong sensory characteristics, and thus acetic acid is self-limiting in food applications.

When neutralized, monobasic acids can produce sodium, potassium, and calcium salts. These salts are equivalent to an acid that has been neutralized with a strong base, and constitutes a buffer solution. Potassium lactate is commonly used as an antimicrobial in meat in poultry products. Sodium lactate is the sodium salt of lactic acid and is produced by fermentation of a sugar source, and then, by neutralizing the resulting lactic acid to create a compound with the chemical formula $NaC_3H_5O_3$. Sodium diacetate is the acid salt of acetic acid, which is formed upon half-neutralization of acetic acid followed by the evaporation of the solution (Linstrom and Mallard, 2014).

2.3.2 Mode of Action

In food products, organic acids exist in a pH-dependent equilibrium between the undissociated and dissociated state (Brul and Coote, 1999). The antimicrobial mechanism of organic acids and organic acid salts is based on the increase in proton concentration thereby lowering the external pH of the cell (Dubal et al., 2004). The effectiveness of an antimicrobial of an antimicrobial agent depends on several factors, including its hydrophobicity, the type of food, the type of acidulate used, the number of microorganisms present, and the dissociation constant (pka), which is the pH at which 50% of the acid is dissociated (Cherrington et al., 1991). The mode of action of organic acids on bacteria is that uncharged, undissociated organic acids, which exists in the majority only at a pH below the pKa of the compound, can penetrate the bacteria cell wall and plasma membrane and disrupt the normal physiology of certain types of pH-sensitive bacteria or fungal cells (Theron and Lues, 2007; Samelis et al., 2001). Upon passive diffusion of the organic acids in the bacteria, where the pH is near or slightly above neutrality, the acids will dissociate, resulting in the release of charged anions and

protons that could not have crossed the plasma membrane on their own. This in turn lowers the internal pH of the bacteria, which impairs or stops the growth of bacteria (Quintavalla and Vicini, 2002). Other mechanisms that have been proposed for the inhibition of microbial growth by organic acids and their salts include membrane disruption, stress on intracellular pH homeostasis, decreased water activity (a_w), and the inhibitory effects of the lactate ion (Ricke, 2003; Koos, 1992; Houtsma et al., 1996). Organic acids inhibit both bactericidal and bacteriostatic properties, while the salts of lactic and sorbic acid appear to act primarily bacteriostatically. However, mixtures of acids and salts could exert a wider antimicrobial activity than a single organic acid or salt (Smulders, 1995).

2.3.3 Uses in Ready-to-Eat Meat Products

Spraying or dipping of cured meat products in post-processing anti-microbial solutions, such as organic acids, alone or in combination with other compounds prior to packaging helped control *L. monocytogenes* (Samelis et al., 2005). Organic acid spray washes are commonly used in the early steps of beef carcass processing. Acetic and lactic acids are reported as the most effective carcass decontamination rinses (Berry and Cutter, 2000).

Acetic or ethanoic acid (CH3 –COOH; log P – 0.319), a monocarboxylic acid and one of the oldest chemicals known to humanity, is produced naturally during spoilage of plants and in animal tissue and is also a product of ethanol oxidation by the bacterium Acetobacter, Gluconobacter and other heterofermentative strains of lactic acid bacteria (LAB) (Doores, 2003; Leo et al., 1971). Vinegar, a 5% acetic acid solution, is known for its strong sensory characteristics, and thus acetic acid is self-limiting in food applications

(Tarte, 2009). As an antimicrobial agent, acetic acid (2-5% solutions) is used to decontaminate food animal carcasses and potassium and sodium/diacetate can be in combination with sodium/potassium lactate can be added in combination with sodium/potassium lactate to processed meat and poultry products to control *L. monocytogenes* (Shelef and Addala, 1994). Lactic acid is produced by lactic acid bacteria, plays a role in several biochemical processes, as well as in the production of dairy products such as cheese and yogurt. Sodium lactate has been used for years in the meat industry because of its ability to enhance flavor, lengthen shelf-life and improve the microbiological safety of products (De La Zerda, 2006). Lactic acid and its salts (lactates) sodium lactate and potassium lactate are widely used as antimicrobials in food products, in particular, meat and poultry such as ham, cooked beef, frankfurters, and sausages (Mbandi and Shelef, 2001). Sodium lactate (CH3H5O3Na, pKa 3.86), an antimicrobial agent approved for use in meat products at up to 4.8% by weight of the total formulation, and sodium diacetate are generally recognized as safe (GRAS) ingredients and have antimicrobial effects against *L. monocytogenes*, improve shelf life, and can be used as a partial replacements for sodium chloride (NaCl) (Glass et al., 2002; Mbandi and Shelef 2001; Samelis et al., 2002; Stekelenburg, 2003: Angersbach, 1971; Houtsma et al., 1996, USDA-FSIS, 2003). Salts of acetic and lactic acids have been successfully used to delay the spoilage of products such as fresh pork sausage and beef and poultry products (Sundaram, 2000)

Sorbic acid (C_5H_7-COOH) and sorbate, including the free acid and salt forms, are commonly used to preserve food products and are effective at controlling many gram-positive and gram-negative bacteria and fungi (Stopforth, Sofos, and Bousta, 2005). In

one study, the addition of sorbate to a broth system did not affect growth, but did interfere with the ability of *L. monocytogenes* to secrete the toxin listeriolysin O (Mckellar, 1993). Of the sorbate salts, potassium sorbate is used more often than calcium or sodium counterparts since it is more soluble in water (Sofos, 1989). Furmaric acid (HOOC-$[CH]_2$-COOH) is a short chain fatty (carboxylic) acid, which may be used to stabilize cured meat color, is effective at preventing bacterial and mold growth and has been more effective as a preservative when used in combination with sorbates (Beuchat, 1998; Doores, 2003; Podolak, Zayas, Kastner, & Fung, 1996). Citric acid is one of the most commonly used food additives in the food industry. It is used as a flavoring and preservative, and the buffering properties of citrates are used to control pH and flavor. Propionic acid is a naturally occurring carboxylic acid that inhibits the growth of mold and some bacteria at concentrations between 0.1 and 1% by weight. It is used as a preservative in food for human consumption, and as a preservative in baked goods when combined with sodium and calcium salts. Benzoic acid is a naturally occurring substance that is found in plants, and benzoic salts are used as inhibitors of mold

CHAPTER III

ACETIC ACID CONCENTRATION AFFECTS THE GROWTH OF *LISTERIA MONOCYTOGENES* ON RTE-CHICKEN BREAST

3.1 Materials and Methods

3.1.1 Sample Preparation and Marination

Broiler breast meat was obtained from 3.2 kg broiler chickens at a commercial chicken processing plant within 24 h postmortem. For each treatment within each of three replications, 6.8 kg of broiler breasts (approximately 350g/breast) were marinated with a brine solution that was formulated for a target pick-up of 12-15% over initial weight. The brine solution contained water, 0.4% sodium tripolyphosphate (STPnew, ICL Performance Products, St. Louis, MO) on a finished product basis (FPB), 1.0% sodium chloride (NaCl) FPB (salt, Culinox 999, Morton Salt, NY), and either 0% dry vinegar (DV), 0.4% DV, 0.6% DV, 0.8% DV, or 1.5% liquid vinegar (LV) based on FPB. Each 6.8 kg treatment of boiler breast was placed in a BIRO Vacuum Tumbler (VTS-44, BIRO Manufacturing, Marblehead, OH) (825mm in length and 393.7 in width with twin 9.1kg drums) and was vacuum tumbled (20 mm Hg) at 2-4°C for 30 min to allow the marinade solution to adequately adhere the broiler breast.. After tumbling, the treatments were weighed to determine marinade pick-up. Treatments were then separately cooked in a Hobart Steam Oven (Troy, OH) at 177°C to a final internal temperature of 77°C. The

cooked broiler breasts were then stored in vacuum packages (Model 75840157 Clarity Vacuum Pouches Koch Supplies Inc, Kansas City, MO) for 18 h at 2-4°C.

3.1.2 Solution Pick-up

Solution pick-up was determined by calculating the difference in weight of the broiler breast prior to marination and the weight of the broiler breast after marination and vacuum tumbling. The solution pick-up was reported as a percentage and was calculated as follows:

$$\%\ \text{Solution pick-up} = (\text{marinated weight} - \text{raw weight}) / \text{raw weight} \times 100 \quad (3.1)$$

3.1.3 Cooking Loss

Each treatment was weighed prior to cooking and then reweighed after cooking to a final internal temperature of 77°C. The temperature was measured by inserting a Taylor TruTemp thermometer (3519N Oak Brook, IL) into the thickest portion of the broiler breast muscle. The cooked chicken was cooled to ambient temperature (20°C) and reweighed. Cooking loss was determined using the pre-cooked initial weight and final cooked weight and was reported as a percentage and calculated using the equation below.

$$\%\ \text{cook loss} = (\text{raw weight} - \text{cooked weight}) / \text{raw weight} \times 100$$

3.1.4 pH Measurement

The instrumental pH measurements of the broiler breast samples were taken 24 h after cooking using an Accument pH meter (model Accument AP61, Fisher Scientific, Pittsburg, PA) with an attached meat penetration probe (penetration tip 05998-20, Cole Palmer, Vernon Hills, IL). The penetration probe was inserted 2.5 cm into the broiler

breast muscle from top to bottom allowing the pH to stabilize. For each replication, two broiler breasts from each treatment (n=6) were analyzed for pH.

3.1.5 Proximate Analysis

Broiler breast meat (n=3) samples within each treatment and replication were used to determine moisture, protein, and fat percentage using a near-infrared spectrometer (Food Scan Lab Analyzer model 78800, Foss Analytical, Eden Prairie, MN) that is AOAC approved (AOAC, 2007). Fresh samples were ground (Fisher Scientific Laboratory Homogenizer 500//08451666, Fisher Scientific LLC, Pittsburg, PA) through a 3-mm grinder plate. Ground samples were packed tightly in a sample cup prior to analysis.

3.1.6 *Listeria monocytogenes* Inoculation

Three verified strains of *Listeria monocytogenes* (ATCC 19115, ATCC 7644, ATCC 19144, American Type Culture Collection, Manassas, VA) were individually cultured in tryptic soy broth (Becton Dickinson) prior to inoculation, and 0.1 to 1.0 mL of each *L. monocytogenes* culture was homogenized in 1L of 0.1% peptone water (Oxoid Basingstoke, Hampshire, England) to form a *L. monocytogenes* solution. The marinated and cooked broiler breasts were aseptically inoculated on both sides of the muscle with 1 mL of the *L. monocytogenes* mixture. Individual broiler breasts were packaged (Turbovac 320-ST-S, Inject Star of the Americas Inc., Brookfield, CT) in modified atmosphere (95% O_2, 5% CO_2) in 15.2 × 20.3 cm, 3-mil vacuum pouches (75001815, Rebel Butcher Supply Co. Inc., Flowood, MS) pouch and then massaged for one min. Initial inoculation level on each sample was 2-4 log CFU/g with a target of 3 log CFU/g. The packages were

then filled with 100 % carbon dioxide. The Modified Atmosphere Packaged (MAP) broiler breasts were stored at 2°C±2°C for up to 60 d and evaluated every 5 d for *L. monocytogenes* and total plate counts.

3.1.7 Measurement of *Listeria monocytogenes* growth

A 25-g portion of each breast (2 breasts per replication at each time point) was aseptically cut and placed into a stomaching bag and homogenized with 225 mL of 0.1% sterilized peptone water solution for 1 min in a Stomacher (Seward 400 Circulator, Seward Limited, Worthing, West Sussex, United Kingdom). Dilutions were made by serially placing 1 mL of the homogenate into dilution tubes with 9 mL of 0.1% sterilized peptone water solution. A 0.1 mL aliquot of the diluted samples was spread onto sterile plates of Difco Oxford Medium Base (BD, Sparks, MD, US) that was supplemented with the antibiotic Polymyxin. The inoculated plates were placed into a 35°C incubator for 48 h, and then examined for microbial growth. The *L. monocytogenes* colonies were manually counted and recorded as log CFU/g.

3.1.8 Acetic Acid Concentration

High Performance Liquid Chromatography (HPLC) was utilized to determine the acetic acid concentration that was present in broiler breast meat from the 0%, 0.4% DV, 0.6% DV, 0.8% DV, and 1.5% LV treatments on Day 0 and Day 45 of the experiment. A standard curve for acetic acid was produced by using HPLC measurements and concentrations of 0.05%, 0.1%, 0.2%, and 0.4% HPLC-grade glacial acetic acid. One broiler breast from each of the five treatments was randomly selected from samples that were stored for Day 0 and Day 45 for each of the 3 replications to quantify acetic acid

concentration. A 10-g sample of the randomly selected broiler breast was homogenized with 50 mL of 0.1 N sulfuric acid (H2SO4). The homogenate was centrifuged (Sovall Lynx 400, Thermo Scientific, Asheville, NC) for 15 min at 18,000 RPM at 4C. One mL of the centrifuged samples were placed into amber glass screw top autosampler (Thermo Fisher Scientific Inc., Waltham, MA USA) vials using a 20 mL general purpose disposable Medi-Pak Performance sterile Luer lock tip syringe (102-S20C, Mckesson, Richmond, VA USA). The vials were individually analyzed using an Agilent High Performance Liquid Chromatograph (HPLC) (Agilent 1100, Agilent, Santa Clara, CA USA) with an Ion Exclusion Column (300mm x 7.8 Ion Aminex HPX – 87H Ion Exclusion Column, Bio-Rad, Hercules, CA USA). This hydrogen-form, 300 x 7.8 mm column performs most analyses with sensitivity to the nanogram level when a 20 minute program is used. Samples were analyzed at a flow rate of 0.6mL/min, 2°C, and 88 bar for 20 min. Once HPLC measurements were complete, the acetic acid concentration in each broiler breast sample was calculated and verified using the standard curve previously designed for acetic acid (0.05%, 0.1%, 0.2%, and 0.4%) and the resulting concentrations were recorded as a percentage. Bio-Rad, Hercules, CA USA).

3.1.9 Statistical Analysis

A two-way factorial design within a randomized complete block design with 3 replications was used to determine if differences existed ($P<0.05$) among acetic acid treatments with respect to treatment, time, and treatment*time interaction (SAS Verizon 9.4, Cary, NC, USA) for *Listeria monocytogenes* counts. A randomized complete block design was utilized to determine if differences existed ($P<0.05$) among vinegar treatments with respect to marination pick-up, cooking loss, pH, proximate analysis, and acetic acid

concentration. Tukey's Honestly Significant Difference (HSD) test was utilized to separate treatment means (P<0.05) when differences occurred among treatments (SAS Version 9.4, Cary, NC, USA).

3.2 Results and Discussion

3.2.1 Solution Pick-up, Cooking Loss, Yields, Proximate analysis, and Acetic Acid Concentration

No differences (P>0.05) existed between treatments with respect to marinade pick-up, yield based on green weight, pH, percentage moisture, and percentage fat (Table 3.1). This is similar to research reported by Badvela et al. (2016), which indicated that the use of buffered vinegar did not impact yields, pH, and proximate composition of uncured deli turkey. In addition, Theron and Yues (2007) reported that buffered forms of organic acids have an advantage over pure forms since it does not significantly change the pH of the food. If the pH of the chicken is not changed, it is not likely that yields would be affected either. The acetic acid concentration found in the control treatment was not different (P>0.05) from the acetic acid concentration in the 0.4% treatment. This is not logical since the 0.4 % DV treatment should have a greater concentration than the control treatment. This may have occurred due to variability in the method. The acetic acid concentration in the 0.6 % and 0. 8 % DV treatments had more acetic acid than the control treatments, and the 1.5 % LV treatment had a greater acetic acid concentration than all other treatments (Table 3.1). Chicken breasts from the control treatment (0%) and 0.6 % DV treatment had less cooking loss (P<0.05) than chicken breasts from the 0.4 % DV and 1.5 % LV treatments. No other differences in cooking loss existed (P>0.05) among treatments (Table 3.1). Chicken breast from the 0.4% DV and 0.6% DV yielded

breast meat with a greater concentration of protein (P<0.05) than broiler breast meat from the control treatment. This may be partially due to the control treatment having the highest numerical fat percentage and the lowest numerical cooking loss percentage as well as slight variability among raw chicken breast.

3.2.2 Microbial Analysis

The average initial concentration of *L. monocytogenes* inoculated chicken breasts was 2.5 log colony units (CFU/g) for all 5 treatments, and there were no differences between treatments at day 0 (P>0.05). No differences (P<0.05) existed in CFU/g among treatments from 5 to 30 days of storage, with approximately 2 log CFU/ g for all treatments. After 35 d of storage, all vinegar treatments remained bacteriostatic, with approximately 2 log CFU/g growth and no differences (P>0.05) among treatments. However, the control treatment had 3.5 log CFU/g growth, which was greater (P<0.05) than all other treatments with the exception of the 0.6 % DV treatment. Similarly, Lavieri et al. (2014) demonstrated that buffered vinegar treatments demonstrate a bacteriostatic effect, but not a bactericidal effect, when applied to contaminated cured frankfurters. After 40 days of storage, the control treatment grew 4.5 log CFU/g *L. monocytogenes*, which was greater (P<0.05) than the counts for all vinegar treatments, which remained at approximately 2.0 log CFU/g. This trend continued from 40 to 60 days of storage, in which the control chicken grew between 4.4 and 5.4 logs CFU/g *Listeria monocytogenes*, which was greater (P>0.05) than the counts on the chicken breasts with any of the vinegar concentrations. This result is comparable to results reported by Porto-Fett et al. (2014), which indicated that buffered vinegar was more effective (P<0.05) at inhibiting the growth of *L. monocytogenes* on the surface of uncured turkey breast when comparted

with samples that were not formulated with BV during extended storage. The only other statistical difference occurred after 55 days of storage in which the 0.8 % DV treatment had fewer counts ($P<0.05$) than the 0.4% and 0.6 % DV treatments. After 60 days of storage, there were no differences ($P>0.05$) in counts between vinegar treatments. However, the 0.8 % DV and 1.5 % LV treatments yielded chicken breasts with than 2.0 logs CFU/g, which indicates these treatments may be the best options for long-term prevention of *L. monocytogenes* growth. These results are similar to those of Badvela et al. (2016), which demonstrated that concentrations between 0.5 and 0.9 % of dry vinegar were bacteriostatic towards *Listeria monocytogenes* that was inoculated onto uncured turkey breast at 3 log CFU/g. However, 0.4 % dry vinegar treatment was not effective at controlling *Listeria* growth (Badvela et al., 2016).These results are also in agreement with results reported by McDonnell et al. (2013) and Gonzalez-Fandos and Herrera (2014). These researchers reported that the antimicrobial effect of vinegar and other natural or clean-label antimicrobials is impacted by the concentration that is included in the product.

The total plate count (TPC) for the control and treated broiler chicken breasts was less than 2.0 log (CFU)/g in replication 1, which indicates that the samples were not spoiled, and that inhibition of L. monocytogenes was likely due to the antimicrobial treatment, rather than to interference from competitive microflora. This is logical since Glass et al., (2013) previously reported that the population of microorganisms on cured deli-style turkey treated with organic acids and salts of organic acids were not significant enough to interfere with *L. monocytogenes* growth. In addition, Desai et al. found that the addition of buffered vinegar to chicken retail cuts increased the shelf life of the chicken

by up to 16 days without negatively effecting quality and sensory properties (Desai et al., 2014). Since the TPC counts were low and there was no indication of spoilage throughout Rep 1, TPC was not evaluated in subsequent replications

3.3 Conclusion

All vinegar treatments (0.4% DV, 0.6% DV, 0.8% DV, and 1.5% DV) inhibited *L. monocytogenes* growth on cooked broiler breast when compared to the control treatment (0%) when stored between 35 and 60 days at 2 C. Therefore, the addition of buffered vinegar (~5% acetic acid) to a marinade solution with salt and phosphate was effective at inhibiting *L. monocytogenes* growth for 60 days of refrigerated storage (2°C±2°C), which was approximately 30 days longer than the control treatment. After 50 days of storage, *L. monocytogenes* counts remained at 2-3 logs in the 0.4% DV and 0.6% DV treatments. In comparison, the 0.8 % DV and 1.5 % LV treatments remained stable at 2 logs of growth through 60 days of storage. In addition, there was no indication of microbial growth or spoilage based on the average TPC of 2 log CFU/g or less found in Rep 1. Future research could include the evaluation of the sensory characteristics and the consumer acceptability of the marinated broiler breasts to determine if acetic acid concentration effects sensory attributes and consumer acceptability.

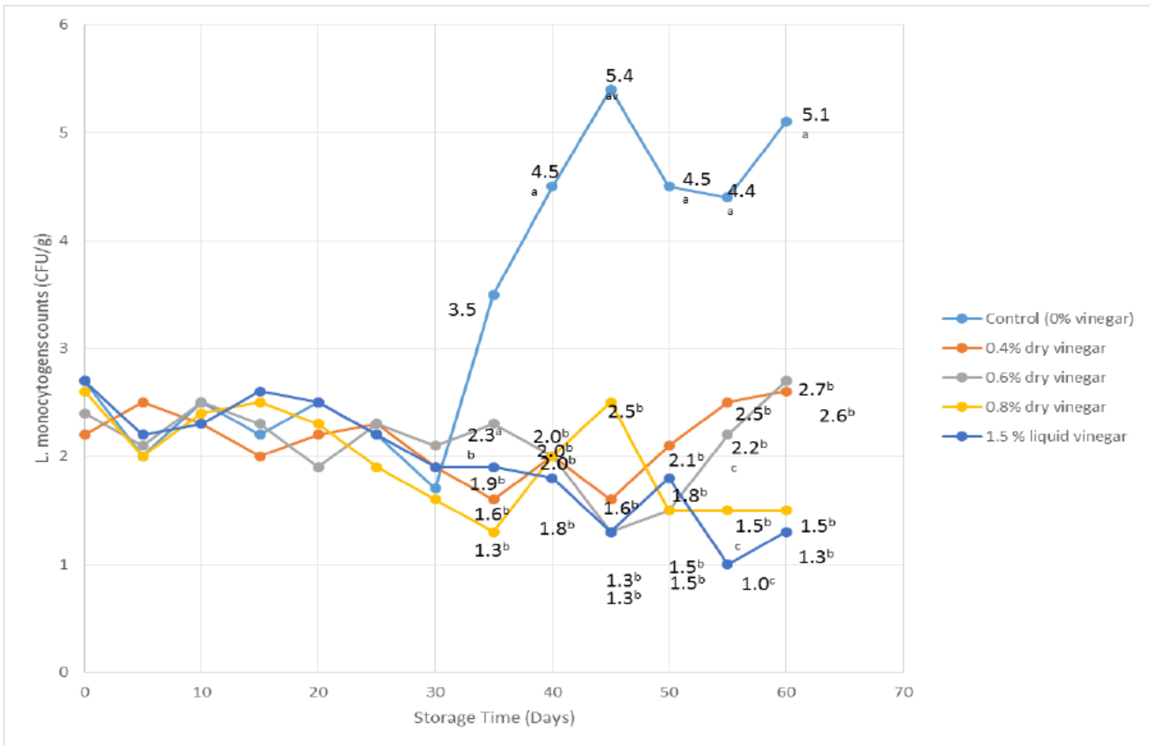

Figure 3.1 Mean *Listeria monocytogenes* Counts (log CFU/g) of cooked chicken breasts over storage time (0-60 days)

[a, b] Means within each day with different superscripts differ (P<0.05).
[A, B, C, D] Means within treatment over time with different superscripts differ (P<0.05).

Table 3.1 Marinade Pick-Up, Cooking Loss, Yields, pH, proximate composition, and Acetic Acid Concentration of marinated chicken breast (1.0 % salt and 0.4 % sodium tripolyphosphate (STP) that is vacuum tumbled with a 15 % brine containing water, salt, STP and Dry vinegar (0 %, 0.4 %, 0.6 %, 0.8 % DV) or Liquid Vinegar (1.5% LV)).

Treatment	Solution Pick-Up (%)	Cooking Loss (%)	Yield based on green weight (%)	pH	Moisture (%)	Protein (%)	Fat (%)	Acetic Acid Conc. (mg/g)
Control	11.7	29.4^{b}	78.8	6.20	68.0	28.8^{b}	3.1	0.6^{c}
0.4 % DV	11.8	33.2^{a}	74.9	6.24	67.7	31.3^{a}	2.5	2.1^{bc}
0.6 % DV	12.3	29.9^{b}	78.8	6.25	68.2	30.9^{a}	2.3	4.0^{ab}
0.8 % DV	12.5	31.7^{ab}	76.8	6.20	68.4	30.1^{ab}	2.2	3.4^{b}
1.5 % LV	12.2	32.6^{a}	75.7	6.18	67.8	29.9^{ab}	2.3	5.6^{a}
P-value	0.154	0.014	0.151	0.744	0.540	0.007	0.51	0.001
SEM	0.78	0.80	1.50	0.048	1.03	0.44	0.51	0.66

^{ab} Means within each column with different superscripts significantly differ ($P<0.05$) between treatments within each column

DV= Dry vinegar; LV=Liquid vinegar

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