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Efficacy Of USDA Approved Antimicrobials during Second Processing in Reducing Salmonella and Campylobacter on Chicken Parts and Ground Chicken Frames

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By

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Efficacy of USDA approved antimicrobials during second processing in reducing *Salmonella* and *Campylobacter* on chicken parts and ground chicken frames

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The efficacy of USDA approved antimicrobials was evaluated in reducing *Salmonella* and *Campylobacter* on ground chicken frames and chicken breast fillets. Chicken frames dip treated with peracetic acid (PAA), lauric arginate (LAE), cetylpyridinium chloride (CPC) and acidified lactic acid (ALA) reduced *Salmonella* and *Campylobacter* counts in ground chicken frames without affecting meat pH and color. PAA and LAE reduced *Salmonella* by 0.9 log on d0 whereas on d1, PAA and CPC reduced by 1.4 and 0.9 log CFU/g respectively. PAA, ALA, propionic acid and LAE significantly reduced *Campylobacter* by 0.7, 1.0, 1.3, and 1.2 log CFU/g, respectively.

On chicken breast fillets, 30 s application of PAA (0.04% and 0.07% pH 6.5) reduced loosely attached *Salmonella* by 0.5 and 0.8 log CFU/ml, respectively. ALA, octanoic acid and PAA reduced loosely attached *Campylobacter* by 1 log while all treatments except propionic acid were effective against strongly attached cells on chicken breast fillets.

DEDICATION

This thesis is dedicated to my inspiring father, Mr. Alvin Moore, Sr. for his constant, unconditional love and support. My father has been a positive role model for me throughout my life. His wisdom and encouraging words has been my motivation during my graduate studies. He has always believed in my abilities and reminded me that anything is possible.

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CHAPTER I

INTRODUCTION

The Centers for Disease Control and Prevention (CDC) estimates that approximately 48 million people are infected, 128,000 are hospitalized and 3,000 die by foodborne illnesses each year in the United States (CDC, 2014). Non-typhoidal *Salmonella* and *Campylobacter* are the pathogens that cause the majority of foodborne illnesses of bacterial origin and continue to be among the current food safety concerns in the poultry industry. Collectively, these pathogens account for 20% of illnesses, 50% hospitalizations related to foodborne illnesses, and 34% of deaths associated to foodborne illnesses every year in the United States (Scallan et al., 2011). Main sources of *Salmonella* and *Campylobacter* infections involve ingestion of contaminated undercooked poultry, meats, eggs, dairy products, fish, shrimp, fresh produce and vegetables (Berger et al., 2010; Mckee, 2011; Loharikar et al., 2012; Whiley et al., 2013). The symptoms of salmonellosis (ingestion of *Salmonella* bacteria) include diarrhea, fever, and abdominal cramps, which usually occurs 12 to 72 hours after ingesting the food contaminated with *Salmonella* (CDC, 2015). *Campylobacter*iosis, infection of *Campylobacter spp.*, is the result of consuming contaminated unpasteurized dairy products and eating raw or undercooked poultry (Gillespie et al., 2002; CDC, 2014). Poultry is considered to be among the principle vehicles of these pathogens. *Salmonella* and *Campylobacter spp.* colonize in the intestinal tract of live poultry without causing

any illness to the bird (Mead, 2004). Furthermore, cross-contamination risks are increased as poultry meat is contaminated by these pathogens during the slaughtering process including distribution and handling preparation (Zhao et al., 2001).

Salmonella and *Campylobacter* contamination of poultry products continues at the retail level. According to the National Antimicrobial Resistance Monitoring System (NARMS) interim retail meat report, *Salmonella* prevalence in retail ground turkey and chicken meat was 6% and 9% in 2014 compared to the 7.5% and 8.3% in 2015, respectively. In addition, in 2011, a multistate outbreak was reported in the U.S. with 136 illnesses caused by the consumption of *S.* Heidelberg contaminated ground turkey. One of the most recent outbreaks of *Salmonella* related to comminuted poultry occurred in 2014 that resulted in over 30,000 pounds of mechanically separated chicken to be recalled for possible contamination of *S.* Heidelberg (CDC, 2014). *Salmonella* serovar Heidelberg is commonly associated with the outbreaks related to comminuted poultry products and was recovered in 8.3% and 4.3% of retail chicken and ground turkey samples at the retail level in 2015 (FDA, 2016). However, foodborne outbreaks associated to *Campylobacter* are reported more sporadically. In 2012, a multistate outbreak of *C. jejuni* was reported due to the consumption of undercooked chicken livers (CDC, 2012). The Raw Chicken Parts Baseline Study (RCPBS) of USDA, FSIS revealed that 26.3% and 24% of raw chicken parts were positive for *Salmonella* and *Campylobacter,* respectively in federally inspected poultry facilities. In addition, 83% and 20% samples were positive for *Salmonella* and *Campylobacter* in mechanically separated chicken in Not-Ready-To-Eat comminuted poultry project with 83% and 20% positive samples, respectively (USDA-FSIS, 2015). Because of the related outbreaks and

sampling projects data, the USDA, FSIS proposed new performance standards in efforts to reduce *Salmonella* and *Campylobacter* prevalence levels in poultry. According to the new performance standards, *Salmonella* positive samples should be less than 25% and 15.4% for comminuted chicken and chicken parts, respectively and *Campylobacter* positive samples should be less than 1.9% and 7.7% in comminuted chicken and chicken parts, respectively (USDA-FSIS, 2015). Poultry processors are employing various interventions such as pre-chill and post-chill antimicrobial dips and sprays to reduce *Salmonella* and *Campylobacter* during poultry processing to control contamination of carcasses and parts with these pathogens. The USDA FSIS has approved several antimicrobial treatments to control *Salmonella* and *Campylobacter* in carcasses, cut-up parts, and comminuted poultry (USDA-FSIS, 2015). Due to the continuous concern for *Salmonella* and *Campylobacter* contamination in poultry, additional food safety preventive measures are warranted to meet the new control requirements.

Chlorine, peracetic acid (PAA), cetylpyridinium chloride (CPC), and organic acids are the antimicrobials that have been commonly investigated for their use during poultry processing (Izat et al., 1989; Bauermeister et al., 2008; Nagel et al., 2013; Chen et al., 2014). Chlorine and chlorine based compounds application have been studied most extensively throughout the processing environment (Li et al., 1994, Northcutt et al., 2007; Bauermeister et al., 2008; Nagel et al., 2013). However, the antimicrobial efficacy of chlorine is decreased due to the organic matter build up in the chiller water (Tamblyn et al., 1997). Studies have reported the efficacy of peracetic acid and cetylpyridinium chloride in reducing *Salmonella* and *Campylobacter* on poultry carcasses and ground poultry (Kim and Slavik, 1996; Nagel et al., 2013; Chen et al., 2014). Organic acids,

propionic acid and acidified lactic acid, have been studied in poultry feeds and throughout the slaughtering process to control *Salmonella* and *Campylobacter* (Izat et al., 1989; Riedel et al., 2009). Octanoic acid (1.25 and 2.5 mg/ml) efficacy has been studied in reducing *Campylobacter* on chicken skin resulting in 0.5 and 1.6 log CFU reduction (Hovorkova and Skrivanova, 2015). Lauric arginate (LAE) is a GRAS compound found to be effective against pathogens such as *Salmonella, Campylobacter, Listeria monocytogenes*, *Staphylococcus aureus*, *E. coli* (Becerril et al., 2013; Sharma et al., 2013; Nair et al., 2014). However, research studies on the efficacy of the USDA approved antimicrobials to reduce *Salmonella* and *Campylobacter* in MSC is very limited.

Therefore, the main objectives of this study were: (i) to determine the efficacy of USDA approved antimicrobials in reducing *Salmonella* and *Campylobacter* on ground chicken frames when applied as a dip treatment and their effect on meat quality, and (ii) to evaluate the efficacy of antimicrobials applied as dip treatment with different contact times to reduce *Salmonella* and *Campylobacter* on chicken parts.

CHAPTER II

LITERATURE REVIEW

Poultry Industry in the United States

For centuries farming production has continuously contributed to feeding America's growing population. Farming is a chief way of life and main food supply for most countries. Farmers produce the main components for the markets to operate smoothly. Particularly, poultry production is a vital faucet of agriculture in the United States. Poultry production is an ever expanding multi-billion-dollar production in the U.S. In most recent years, poultry farming has evolved from family- owned backyard flocks to more commercial businesslike operations. Modern poultry production is more diverse ranging from different housing methods, breed selection, and nutritional improvements. It is a highly vertically integrated system which includes processing plants, grow-out farms, hatcheries, feed mills and primary breeders.

Poultry production dominates the southern state region of the United States, where Georgia is the number one broiler producing state as of 2013 (US-EPA, 2011). United States is the world's largest poultry producer (USDA-ERS, 2012). It is the second largest poultry exporter, following Brazil (US-EPA, 2011). In 2007, approximately 145,615 farms produced poultry and poultry products. Poultry production is an important component of American agriculture for which it is considered healthier food diet for most Americans.

Food Safety Concern

Every year millions of people become infected with foodborne illnesses in the United States. The Centers for Disease Control and Prevention (CDC) estimates that 48 million people become ill, 128,000 are hospitalized, and 3,000 die from foodborne illness (CDC, 2011). Foodborne illnesses cause a major economic burden. It was estimated that foodborne illnesses health-related costs $51 billion dollars annually (Scharff, 2012). *Salmonella* and *Campylobacter* are pathogens that are commonly associated with poultry and cause foodborne illnesses. In 2013, the CDC reported *Salmonella* and *Campylobacter* causing 26% and 2% of the foodborne outbreaks. Batz et al. (2011) estimated the cost of *Salmonella* and *Campylobacter* illnesses related to poultry to $712 million and $1.3 billion annually.

Salmonella is the most common bacterial foodborne pathogen causing over one million reported foodborne illnesses every year in the United States (CDC, 2012). *Salmonella* Enteritidis (32%) is reported the most common cause of foodborne illness, followed by serotypes *S.* Typhimurium (13%), *S.* Heidelberg (8%), and *S.* Newport (7%) (CDC, 2013). However, foodborne illnesses infections reported associated with *Salmonella* and *Campylobacter* increased 38% and 35% in 2013 compared to 2006-2008 (CDC, 2014). Recent *Salmonella* outbreaks related to poultry and poultry products are listed in Table 2.1

Table 2.1 *Salmonella* outbreaks related to poultry

Year	Source	Serotype	No. of Cases	No. of Deaths
2011	Turkey Burgers	*S.* Hadar	12	0
2011	Ground Turkey	*S.* Heidelberg	136	1
2012	Live Poultry	*S.* Infantis, *S.* Newport, *S.* Lille	195	2
2012	Live Poultry	*S.* Montevideo	93	1
2012	Live Poultry	*S.* Hadar	46	0
2013	Branded Chicken	*S.* Heidelberg	634	0
2013	Live Poultry	*S.* Typhimurium	356	0
2013	Live Poultry	*S.* Infantis, *S.* Lille, *S.* Newport, *S.* Mbandaka	158	0
2014	Live Poultry	*S.* Infantis, *S.*Newport, *S.* Hadar	363	0
2014	Mechanically Separated Chicken	*S.* Heidelberg	9	0
2015	Live Poultry	*S.* Enteritidis, *S.*Hadar, *S.*Indiana, *S.* Muenchen	250	0

(CDC, 2015)

Likewise, *Campylobacter* is a bacterial pathogen causing foodborne illness; although, most cases are reported sporadically. From 1997 to 2008, 262 outbreaks were reported with 9,135 illnesses, 159 hospitalizations, and 3 deaths (Taylor et al., 2013). In 2012, *Campylobacter* was the second most common infection reported by Food Net with 14.3 cases reported per 100,000 populations (CDC, 2014). Studies have identified eating and handling raw or uncooked chicken as major risk factor of *Campylobacter*iosis (Whiley et al., 2013).

Main sources of *Salmonella* and *Campylobacter* infections involve contact with contaminated raw poultry meats, eggs, milk and other dairy products, fish, shrimp and

vegetables (McKee, 2011). Among these, poultry is the principle vehicle of these pathogens. Poultry meat is frequently contaminated with *Salmonella* and *Campylobacter* spp., the organisms responsible for cases of human enteritis (Coburn et al., 2007; Whiley et al., 2013). Food contamination with these pathogens can occur at multiple steps of production, processing, distribution, retail marketing, and handling preparation (Gould et al., 2013). According to the National Antimicrobial Resistance Monitoring System (NARMS) 2014-2015 Retail Meat Interim Report a decline in *Salmonella* prevalence was found for ground turkey samples and retail chicken. The percentage of *Salmonella* in ground turkey decreased in from 19% in 2008 to 6% in 2014 and from 15% to 9% in chicken. In 2015, *Salmonella* prevalence was 7.5% and 8.3% in ground turkey and retial chicken (FDA, 2016). Williams and Oyarzabal (2012) conducted a study to determine the prevalence of *Campylobacter spp.* in retail chicken meat in Alabama from 2005 to 2011, where 41% of 755 samples were confirmed to be *Campylobacter spp.* positive. The 2012 NARMS Retail Meat Report showed the prevalence of *Campylobacter spp.* in ground turkey and retail chicken to be 1.6% and 48.8% respectively from 2002-2012.

Salmonella

Salmonella is gram-negative bacteria in the Enterobacteriaceae family. There are two species of the Genus, *S. enterica* and *S. bongori.* There are over 2500 serotypes of the *S. enterica* species and less than 50 of the *S. bongori* species. *Salmonella* species grow optimally at temperatures between 35-42°C; temperatures of the human body and chicken are well within range of optimal growth for *Salmonella* (Russell, 2012). *Salmonella* spp. are normally found in the intestines of poultry. *Salmonella* convert carbohydrates to by-products such as acid and gas, while solely using citrate as their

carbon source (Russell, 2012). *Salmonella* possess three major antigens which are used to categorize them into different serotypes; the H or flagellar antigen, the O antigen or somatic antigen, and the Vi or capsular antigen (Russell, 2012). The surface of *Salmonella* is covered with lipopolysaccharide (LPS) endotoxin which is a characteristic of gram-negative bacteria, with the O antigen being the outermost portion of the LPS (Russell, 2012; CDC, 2014).

As mentioned earlier, *Salmonella* is found in the intestinal tract of animals, including birds. The bacteria normally transmitted to people by consuming contaminated food or direct contact with young animals (Hoelzer et al., 2011). The possible sources of the infection are contaminated foods of meat origin, such as raw poultry meat, milk, fish, shrimp and vegetables (Acheson and Hohmann, 2001; Berger et al., 2010; McKee, 2011). There are two main *Salmonella* serotypes that are associated with human illnesses, *S.* Typhimurium and *S.* Enteritidis (CDC, 2008). There are several of the *S.* enterica serovars that actively invade the mucosal surface of the intestine and has the ability to spread to deeper tissues of humans, including the spleen, liver, and bone marrow (Parkhill et al., 2001).

Typhoidal *Salmonella* involves serovars Typhi, Paratyphi A, B, or C. Typhoidal *Salmonella* is the causative agent of enteric fever, also referred to as typhoid or paratyphoid fever depending on the serovar (Gal-Mor et al., 2014). Unlike non-typhoidal *Salmonella*, Typhoidal *Salmonella* is a more common in developing countries with an estimated 27 million cases and more than 200,000 fatalities annually (Buckle et al., 2012; Gal-Mor et al., 2014). The incubation period of typhoidal *Salmonella* is 14 days with symptoms such as, diarrhea, abdominal pain, chills, and headaches that may last up to 3

weeks (Gal-Mor et al., 2014). Non-typhoidal *Salmonella* infections occur worldwide and affects people of all ages and is estimated to cause 94 million cases of gastroenteritis and 155,000 deaths annually (Majowicz et al., 2010; Eguale et al., 2015). Non-typhoidal infections are self-limiting lasting less than 10 days. Symptoms such as acute gastroenteritis, watery diarrhea, vomiting, abdominal pain and fever may onset as early as 12 to 36 hours (Foley and Lynne, 2008; Gal-Mor et al., 2014). Mead et al. (1999) estimated that 95% of cases of salmonellosis are foodborne in origin. The majority of foodborne salmonellosis infection likely result from improper food handling or cross-contamination (Finstad, 2012).

Upon completion of intestinal colonization, *Salmonella* enter enterocytes, M cells and dendritic cells (DCs) in the tissues of the intestine. The submucosa can be internalized by macrophages and immediately disseminate through the blood stream accumulating in the lymph nodes and eventually reaching the spleen (Salcedo et al., 2001). To be fully pathogenic, *Salmonella* must possess a variety of attributes called virulence factors. These factors include the ability to invade cells, obtain a lipopolysaccharide coat, conduct intracellular replications, and secrete toxins (Giannella, 1996). Invasion of the *Salmonella* occurs by inducing the enterocyte membrane to go through "ruffling" and thereby stimulate pinocytosis of the organisms (Ibarra and Steele-Mortimer, 2009). Phagocytes such as macrophages utilize phagocytic uptake to efficiently identify and suppress bacterial pathogens. The Type III secretion system (T3SS1) is used by *Salmonella* to invade cells, both phagocytic and non-phagocytic (Ibarra and Steele-Mortimer, 2009). Phagocytosis is an innate immune response developed to sample different pathogens, T3SS1-mediated invasion by *Salmonella* is a

highly specific process that depends on the tightly regulated expression of a number of bacterial factors (Ibarra and Steele-Mortimer, 2009).

Campylobacter

Campylobacter is a curved shaped, gram-negative bacteria of the family Campylobacteraceae. *Campylobacter* spp. are highly motile in the presence of moisture and grow optimally at temperatures between 37- 42°C. *Campylobacter* species are microaerophilic, preferring an environment containing 5% oxygen, 10% carbon dioxide, and 85% nitrogen (Kenner eta al. 2004). There are 17 species and 6 sub-species comprised of the *Campylobacter* genus, in which *C. jejuni* and *C. coli* are the main species to cause human illnesses (Gillespie et al., 2002). Other *Campylobacter* species known to cause foodborne illnesses in humans include *C. lari*, *C. fetus*, and *C. upsaliensis* (Patrick et al., 2013; Whiley et al., 2013).

Campylobacter spp. have been identified as the leading cause of human bacterial gastroenteritis in countries such as United States, Europe, Canada, and Australia (Altekruse et al., 1999; Gillespie et al., 2002; Whiley et al., 2013; CDC, 2014). The infection of *Campylobacter*, Campylobacteriosis, is caused by the exposure to contaminated fresh produce, unpasteurized dairy products and eating or handling raw or undercooked poultry meat products (Gillespie et al., 2002; Whiley et al., 2013). Although, *Campylobacter* is considered a commensal organism of avian species, since poultry can host the bacteria in the intestinal tract without becoming ill (Altekruse et al., 1999). Other modes of transmission for *Campylobacter* infection includes contact with contaminated poultry, livestock animals, and young pets (McCarthy et al., 2007). Reported symptoms include diarrhea, which may be bloody, cramping, abdominal pain,

and fever that may occur within two to five days after being exposed to the organism (CDC, 2014). Campylobacteriosis is most likely to occur in young children, elderly, and immunocompromised individuals (Allos, 2001; Gillespie et al., 2002; CDC, 2014). However, *Campylobacter* infections are reported sporadically; deaths are rare and the incidence of an infection is much higher in HIV-infected patients than general population (Altekruse et al., 1999; Gillespie et al., 2002).

Guillain- Barré Syndrome (GBS) is one of the most important post-infection complication of a *Campylobacter* infection. GBS is the most common cause of flaccid paralysis, characterized as an autoimmune disorder of the peripheral nervous system (Nachamkin et al., 1998). Altekruse et al. (1999) estimated one case of GBS occurs for every 1000 cases of Campylobacteriosis. Some patients may be left disabled while other may die due to respiratory failure. Neurological symptoms of GBS develop one to three weeks after diarrheal illness and humoral immunopathogenic mechanisms are likely involved; however, many GBS associated *Campylobacter* infections are asymptomatic (Allos, 2001). *Campylobacter* is believed to cause GBS through a mechanism called molecular mimicry; the ganglioside-like epitopes in the lipopolysaccharide moiety that stimulate the production of autoantibodies to react with the peripheral nerves (Allos et al., 1998). Campylobacteriosis is also associated with Reiter Syndrome (Altekruse et al., 1999). Reiter's Syndrome is a reactive arthropathy or joint pain and swelling caused by an infection in body, generally the intestines, genitals, or urinary tract (Pope et al., 2007).

There are not any strict recommendations for treatment of Campylobacteriosis; however, maintenance of fluid intake and electrolyte balance is the basis of therapy for people with the infection (Altekruse et al., 1999; Allos, 2001). Antibiotic use for

treatment is not commonly used since the infection is usually self-limited and does not require further care. In the past, if antibiotic therapy was needed fluoroquinolones were the drug of choice; however, *Campylobacter* and other pathogens such as *Salmonella* and *Shigella* have been found to be fluoroquinolones resistant (Allos, 2001).

Once infected, the *Campylobacter* bacterium colonize in the mucus layer covering the intestinal cells and adhere to the epithelial cells causing damage (Wooldridge and Ketley, 1997). There are several processes involved with the internalization of *Campylobacter*, protein phosphorylation is very important in complete internalization (Wooldridge and Ketley, 1997). Adherence and invasiveness are correlated with the internalization of *Campylobacter*, invasion is also thought to stimulate inflammation as invasion of *Campylobacter* is required for induction of many markers of inflammation such as cytokine interleukin 8 (Van Vliet and Ketley, 2001). Other proposed virulence traits include polysaccharide capsule, secreted proteins, type 6 secretion (T6SS) effector molecules, apoptosis-inducing proteins, and bacterial adhesion and invasion promoting factors (FlaC, PEB1, CapA) (Bouwman et al., 2014). In addition, gram-negative bacteria outer membrane contains lipo-oligosaccharide (LOS) and lipopolysaccharide (LPS) components; both are important virulence factors involved in serum resistance, endotoxicity and adhesion (Van Vliet and Ketley, 2001).

Regulatory Standards for Control of *Salmonella* and *Campylobacter*

Poultry and poultry products are regulated by the United States Department of Agriculture Food Safety and Inspection Service (USDA, FSIS). In 1996, the USDA-FSIS implemented the Pathogen Reduction- Hazard Analysis and Critical Control Point (PR-HACCP) system for raw poultry meat and products. PR-HACCP mandates processing

facilities to implement HACCP programs to help improve the quality and wholesomeness of the products. Performance standards are then applied to the processing facilities which then implement sampling procedures to monitor the number of *Salmonella* positive samples (FSIS, 1998). Since then, performance standards have been proposed for *Salmonella* and *Campylobacter* that processing facilities must meet. Currently, one of the main goals of the FSIS is to establish performance standards that reduce *Salmonella* and *Campylobacter* by 25% and 33%, respectively.

According to the Raw Chicken Parts Baseline Study (RCPBS) conducted by FSIS, 26.3% of the samples tested were positive for *Salmonella* and 21.4% of the samples tested were *Campylobacter* positive in federally inspected establishments (USDA-FSIS, 2012). In addition, a not-ready-to-eat comminuted poultry sampling project (June 2013- January 2014) conducted by USDA-FSIS revealed high levels of *Salmonella* and *Campylobacter* contamination. There were more *Salmonella* positive samples than *Campylobacter* samples for mechanically separated chicken with 83% and 20% of the samples being positive, respectively (USDA-FSIS, 2015). From this project the testing sample size increased from 25 g to 325 g of comminuted product. Samples from this project included all comminuted products: non-breaded or battered, ground, and mechanically separated kind (USDA-FSIS, 2015).

Therefore, USDA-FSIS proposed new performance standards for *Salmonella* and *Campylobacter* in raw chicken parts and NRTE comminuted poultry due to the high incidences of these particular pathogens, in the Federal Register Notice in 2015 (USDA-FSIS, 2015). The USDA FSIS newly proposed performance standards for *Salmonella* and *Campylobacter* in comminuted chicken and chicken parts are presented in Table 2.2.

Table 2.2 USDA-FSIS New Performance Standards for Comminuted Poultry and Chicken Parts

Product	Maximum % Positive		Performance Standards	
	Salmonella	*Campylobacter*	*Salmonella*	*Campylobacter*
Comminuted Chicken	25.0	1.9	13 of 52	1 of 52
Chicken Parts	15.4	7.7	8 of 52	4 of 52
Comminuted Turkey	13.5	1.9	7 of 52	1 of 52

(USDA-FSIS, 2015)

Poultry processors in the United States have been employing several antimicrobial interventions at pre-chill, chilling, and post-chill levels for reducing pathogens during commercial processing. The USDA-FSIS has approved various antimicrobials such as peracetic acid (PAA), cetylpyridinium chloride (CPC), chlorine, organic acids and several other antimicrobials that can be applied as spray or dips to control *Salmonella* and *Campylobacter* in poultry products (USDA, 2015). The application of antimicrobials can provide additional approaches to the already established interventions used throughout the slaughter process.

Post-Harvest Control of *Salmonella* and *Campylobacter* during poultry processing

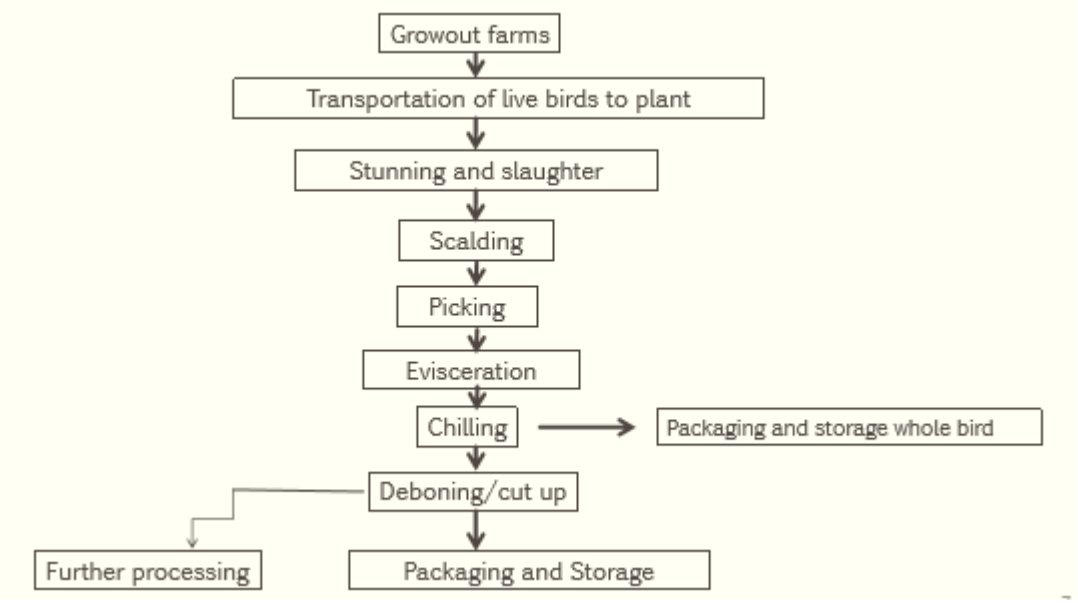

Figure 2.1 Flow diagram of poultry processing steps

Poultry processing is a multifaceted operation in the food industry**.** Processing involves several highly automated phases where many points of cross-contamination exists if birds enter the plant as *Salmonella* and *Campylobacter* positive. Scalding, picking, evisceration, and chilling are among the most common points where cross-contamination can occur. *Salmonella* and *Campylobacter* colonize the intestinal tract of the bird and can then cause contamination of poultry meat (Byrd and McKee, 2005; McKee, 2011). Picking and evisceration are steps with higher microbial load compared to other steps of processing (Izat et al., 1990). To alleviate the points of contamination during processing, multiple chemical antimicrobials are used during scalding, the chilling, and post chill applications (McKee, 2012). However, poultry carcasses are not

the only vehicles of transmission. *Campylobacter* spp. may be transported throughout the processing plant by personnel moving from one area to another (Whiley et al., 2013).

Scalding tanks are used during processing to make the feathers easier to remove; hot water bath denature protein structures that hold the feathers in place (Sam, 2001). Blood, litter, fecal matter, and bacteria that may be present on the body enter in the water when the carcasses enter the scalding tanks (Notermans et al., 1977). Soft scalding requires 53° C for 120 s and does not cause any damage to outer skin layer. Hard scalding requires 62 to 64 °C for 45 s and the outer skin layer is lost (USDA-FSIS, 2010). There are hundreds of carcasses in the scalding tanks at once allowing the microbial load to increase (Cason and Hinton Jr., 2006). To minimize cross-contamination, multi-stage scalding tanks are used to reduce pathogens such as *Salmonella, Campylobacter,* and other microorganisms (Cason and Hinton Jr., 2006). Some companies have incorporated a bird brush and wash prior to picking to eliminate contamination before scalding (Russell, 2012). Scalding systems are generally designed to use a countercurrent mechanism. The opposing water flow is important to wash the bird removing organic matter from the carcass (Russell, 2012). Because the risks of cross-contamination exist during scalding, antimicrobial interventions can be added to reduce the microbial load. Many studies have shown the use of antimicrobials such as chlorine, propionic acid, and trisodium phosphate use in scalding tanks (Humphrey et al., 1981; Tambyln et al., 1997). One study conducted by Tambyln et al. (1997) showed a 2 log reduction of *S.* Typhimurium when a 5% acetic acid solution is used in the scalding tank water. The implementation of additives to adjust the pH in the scald tanks can also reduce the bacterial load. The addition of sodium hydroxide to obtain an alkaline pH was reported to

reduce the organic matter and microbial population in the scald tanks (Humphrey et al., 1981).

The picking machines are designed to remove the feathers loosened during scalding. The pickers have two rows of finger-like projectors directed at various areas of the carcass moving at rapid speeds. Picking is another point of processing where cross contamination is an issue. In one study Izat et al. (1988) reported bacterial counts increased by approximately 10 to 100- fold during picking and increased even more during the evisceration process. Berrang et al. (2001) reported that *Campylobacter* species contamination after picking is related to the fecal material released from the vent during picking. Antimicrobial sprays can reduce *Salmonella* and *Campylobacter* during this step. Berrang et al. (2011) reported chloride dioxide as an effective antimicrobial spray during de-feathering.

Evisceration is a more complex step and involves highly automated steps. Evisceration is the process of removing all the edible and inedible contents from the carcass (Sams, 2001). A reduction in *Campylobacter* spp. and *Salmonella* spp. has been associated with the use of commercial antimicrobial technologies during the processing of broiler chickens (Oyarzabal, 2005). There are many steps involved in evisceration and the equipment must be maintained, and multiple antimicrobials are generally used during this phase. The USDA-FSIS has a zero tolerance policy of fecal material on carcass entering the chiller (Russell, 2012). During this complicated step, damage to the internal organs and crop allows a pathway for *Salmonella, Campylobacter,* and other bacteria to be transferred onto the bird's body. Inside outside bird washers (IOBW) are used to remove any fecal material present on the carcass before it enters the chilling tanks.

Several studies have shown how practical bird washing sprays are to reduce *Salmonella* (Xiong et al., 1998; Yang et al., 1998).

In the United States, immersion chilling is the method of choice. Chilling is a critical step in poultry processing and can be a source of carcass contamination by the organic material in the water. However, studies have reported that *Salmonella* and *Campylobacter* levels were reduced after chilling (Izat et al., 1988). Several antimicrobials are approved for use in the chiller tanks by the USDA, FSIS. Currently approved antimicrobials include, acidified sodium chlorite, bromine, chlorine dioxide, cetylpyridinium chloride (CPC), organic acids (lactic, citric, propionic), and peracetic acid (PAA) (McKee, 2011; Bauermeister et al., 2008). Post chill antimicrobial dips have been effective method in reducing *Salmonella* and *Campylobacter* on carcasses (Nagel et al., 2013). According to a survey conducted by Mckee (2011), PAA was reported to be the most commonly used antimicrobial for pre and post- chill applications. Several studies have investigated the use of antimicrobials as post chill dips and sprays (Bauermeister et al., 2008; Nagel et al., 2013; Smith et al., 2015). Slavik et al. (1994) reported 10% trisodium phosphate during a 15 s post chill dip reduced *Campylobacter* on chicken carcasses by 1.5 log CFU on d1 of storage. Smith et al. (2015) reported 200 ppm PAA during a post-chill dip reduced *C. jejuni* by 1.4 log CFU/ ml. In fact, some antimicrobials may have different effects on meat quality, and must be applied at permissible levels as per USDA regulation.

Commonly used Antimicrobials during Poultry Processing

Chlorine

Chlorine is the most commonly used antimicrobial in chiller applications, equipment sanitation, and other stages throughout the poultry processing plant in the United States to reduce microbial contamination (Lillard, 1990; Northcutt et al., 2005; Bauermeister et al., 2008; McKee 2011; Nagel et al., 2013). It is approved by the USDA, FSIS with levels not exceeding 50 ppm (USDA-FSIS, 2011). The main disinfecting property of chlorine is due to hypochlorous acid when added to water. A slightly acidic pH (6.5 or below) is ideal for the active antimicrobial form of chlorine (Lilliad, 1979; Byrd and Mckee, 2005). Traditionally, chlorine has been used during poultry processing because it is inexpensive and easy to use (White, 1998). Chlorine has been reported to be effective against *Salmonella* at levels as high as 100 and 400 ppm on broilers; but this is not acceptable due to the current regulations (Tamblyn and Conner, 1997; Sams, 2000). Northcutt et al. (2007) reported a 15 s chlorine wash at 50 ppm reduced *Salmonella* and *Campylobacter* by 1.6 and 2.4 log CFU/ml, respectively. In the same study, a 50 ppm chlorinated spray significantly reduced *Salmonella* population on broiler carcasses. *S.* Typhimurium has been reduced by 50 ppm chlorinated chiller water with a 50- minute dwell time (Yang et al., 2001). The antimicrobial efficacy of chlorine is altered by many factors including the initial organic load, contact time, water temperature and pH (Northcutt et al., 2005; Bauermeister et al., 2008). Organic matter in the water and an increased pH negatively influences chlorine by decreasing its ability to act upon pathogens (Lillard, 1979; Northcutt and Lacy, 2000). Improper use of chlorine at higher concentrations may be a health hazard to employees due to the strong odors produced by

chlorine gas. High concentrations can also cause discoloration of carcass and be corrosive to the metal equipment (Northcutt and Lacy, 2000; Sams, 2001; Northcutt et al., 2007).

Cetylpyridinium Chloride (CPC)

Cetylpyridinium chloride (CPC) is a USDA approved antimicrobial agent for use on raw poultry parts and poultry carcasses during processing with concentrations not exceeding 0.8% of the final weight of product (USDA-FSIS, 2011). CPC is a water soluble, non-volatile, quaternary ammonium compound. Due to the USDA regulations, poultry carcasses and cut up parts required undergo a rinse with potable water after immersion treatment with CPC. Its main mode of disinfection involves the interaction of basic cetylpyridinium ions with acids of the bacteria causing inhibition of bacterial metabolism (McDonnell and Russell, 1999; Conner et al., 2001). CPC is effective as spray or post chill application to reduce pathogenic bacteria without altering the product quality (Bunic and Sofos, 2012).

Several studies have investigated the effects of CPC on poultry products (Yang et al., 1998; Waldroup et al., 2010; Chen et al., 2014). Kim and Slavik (1996) revealed that 0.1% CPC on chicken skins was effective against *S.* Typhimurium which resulted in a 1.7 log reduction. Cutter et al. (1997) reported the application of 0.1% of CPC reduced *S.* Typhimurium by 2.5 log reduction of. By the same application method, 0.5% CPC demonstrated a 1.47 log reduction of *Salmonella* on beef trimmings (Pohlman et al., 2002). Conner et al. (2001) reported CPC to be effective without causing any negative sensory effects on the carcass. Chicken skin exposed to 0.5% CPC for 1 min resulted in a 4.2 log reduction CFU of *C. jejuni* (Riedel et al., 2009). Waldroup et al. (2010) reported that the post-chill application of 0.1% and 0.5% CPC on raw poultry yielded a 2-3 log

reduction of *Campylobacter spp.* In a recent study, the use of 0.35 and 0.6% CPC resulted in 0.8 log CFU/g reduction of *Salmonella* and *Campylobacter* in ground chicken without causing any negative effects on meat quality (Chen et al., 2014).

Organic Acids

Organic acids are inexpensive, easy to manage, and have been proven to be effective in reducing pathogens. Organic acids have been labeled by the FDA as generally recognized as safe (GRAS) for poultry (Mani-Lopez et al., 2012). However, some studies revealed that organic acids could have negative meat quality effects on flavor and color of meat products if used at high concentrations (Bauermeister et al., 2008). Several organic acids have been approved by the USDA, FSIS as processing aid to reduce pathogens on meat and poultry products (USDA-FSIS, 2011). The commonly used organic acids during poultry processing include: acetic acid, lactic acid, propionic acid, formic acid, and citric acid (Izat et al., 1990; Zhao and Doyle, 2006; Bauermeister et al., 2008; Mani-Lopez et al., 2012). It is believed that using a combination of organic acids is a better approach to eliminate the negative effects while increasing their efficacy. Conner et al. (2001) reported application of organic acid combinations and surfactants in the processing areas effectively reduced *Salmonella* spp., *C. jejuni* and *L. monocytogenes* on broiler carcasses. The application of organic acids use has been investigated in chiller water, scalding tanks, and post- chill decontamination tanks in poultry (Tamblyn et al., 1997).

Acidified lactic acid is a low-pH acid blend, specifically lactic acid, which is permitted up to 5%, antimicrobial treatment (USDA-FSIS, 2015c). Lactic acid is produced by a lactic acid starter culture bacteria and it functions as a natural

antimicrobial (Alakomi et al., 2000). Lactic acid is able to inhibit the growth of many types of food spoilage bacteria, including gram-negative species of the families Enterobacteriaceae and Pseudomonadaceae (Doores, 1993). Lactic acid antimicrobial activity is caused by the penetration of the cytoplasmic membrane, which alters the pH of the cells and disrupts the proton motive force (Alakomi et al., 2000). Conner et al. (2001) reported a 1-2% lactic acid spray post-inoculation did not reduce *C.jejuni* significantly in 24 hrs; but 2% lactic acid 10 min post-inoculation did show a reduction within 24 hrs.

Acetic acid is the main component of vinegars and is used for its flavoring abilities in a variety of products (Mani-Lopez et al., 2012). *Campylobacter jejuni* and *Salmonella* are reduced by 0.5 to 1.5 logs by a 0.1% acetic acid level at 50°C in the poultry scalding tanks (Okrend et al., 1986). Conner et al. (2001) reported that acetic acid and lactic acid were both effective at reducing *S.* Typhimurium. Zhao and Doyle (2006) demonstrated that a 2% acetic acid solution reduced *C. jejuni* on chicken wings by 1.4 log CFU/g when using a 45 s dip. Acetic acid was used in a study inside the chillers at a 0.6% concentration which reduced *Salmonella* on the chicken carcasses by 87, 80, and 53% respectively (Russell, 2012). Other studies have observed that acetic acid used alone on products resulted in a brownish yellowish discoloration on meat products; however, flavor was not effected (Mani-Lopez et al., 2012; Russell, 2012).

Citric acid is a naturally occurring antimicrobial used in fresh and processed meats in the food industry. It is a hydroxyl tricarboxylic acid and has GRAS status (Mani-Lopez et al., 2012). Metal chelation is the mode of action for citric acid which inhibits cell growth (Miller et al., 1993). A study by Mani-Lopez and others demonstrated that 4% citric acid was effective against *S.* Typhimurium resulting in a 1.9 log reduction

(Mani-Lopez et al., 2012). Citric acid at 3% does not have any negative effects on color or any other sensory characteristics; however, at higher concentrations it is believed to have negative impacts on meat quality (Laury et al., 2009).

Propionic acid is a natural antimicrobial agent and the maximum allowed level is 0.5% of the final formulation (USDA-FSIS, 2015b). Compared to other organic acids, propionic acid treatments were reported to cause a bleaching effect on broiler carcasses (Bilgili et al., 1998). Dubal et al. (2004) reported propionic acid and acetic acid antimicrobial activity as a spray treatment on the inhibition of *S.* Typhimurium on sheep meat. In another study, 0.2% propionic acid organic acid mixture dip treatment reduced *S.* Typhimurium by 1.3 log CFU on chicken skin (Meconi et al., 2013). Gonzalez-Fandos et al. (2015) reported a 1.6 log reduction of *C. jejuni* with a 2% propionic acid dip on chicken legs. There are several studies that show the use of propionic acid in vitro; but very few are available that study the effect on chicken.

Octanoic Acid

Octanoic acid, also known as caprylic acid, is a natural antimicrobial found in coconut oil and milk (Venkitanarayanan et al., 2013). This is a food grade chemical approved by the FDA and USDA, FSIS for use on various RTE meat and poultry products with levels not exceeding 400 ppm by weight of the finished product (USDA-FSIS, 2016). Skrivanovae et al. (2015) reported that 1.25 and 2.5 mg/ml caprylic acid reduced *S.* Enteritidis on chicken skin as surface treatment. Caprylic acid has been used in several studies to reduce *Salmonella* levels in intestinal tracts by the inclusion of broiler diets. Vasudendvan et al. (2005) showed the effectiveness of 0.7% caprylic acid supplementation in broiler diets. The caprylic acid dietary supplementation significantly

reduced *S.* Enteritidis in the ceca, intestine, and crop of birds. Caprylic acid is also reported to be effective against *Campylobacter*; surface treatment with 1.25 and 2.5 mg/ml caprylic acid reduced *C. jejuni* by 0.5 and 1.6 log CFU on chicken skin (Hovorkova and Skrivanova, 2015).

Lauric Arginate (LAE)

Lauric Arginate (LAE) is a cationic surfactant and acts by altering the cytoplasmic membranes of a cell but is dependent on the type of cell structure (Rodríguez et al., 2004). LAE is a GRAS antimicrobial approved for use in raw chicken parts and comminuted poultry products at a maximum level of 200 ppm by weight of the final product (USDA-FSIS, 2015b). LAE was reported to be effective against pathogens such as *Salmonella*, *Campylobacter*, *L. monocytogenes, Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *E. coli* (Becerril et al., 2013; Ma et al., 2013; Sharma et al., 2013; Nair et al., 2014).

LAE antimicrobial efficacy has been studied in various food types such as milk, ham, chicken breasts, queso cheese, and on broiler carcasses (Soni et al., 2010; Benil et al., 2011; Ma et al., 2013; Nair et al., 2014). Higueras et al. (2013) evaluated a chitosan film wrap with 1and 5% of LAE solutions to determine the reduction of gram-negative bacteria. The film wraps (1% LAE) resulted in ~ 2 log reduction; and 5% LAE film wraps resulted in 2.5 log CFU. Stopforth et al. (2010) studied the antimicrobial activity of LAE as a surface treatment on *L. monocytogenes* inoculated cured ham. LAE (0.07%) resulting in 1 log reduction. LAE as surface treatment level at 400 ppm has been reported effective against *C. jejuni* in chicken breasts fillets reducing counts by 1.5 log CFU/g on

d 7 (Nair et al., 2014). In addition, Sukumaran et al., (2015) showed 200 ppm LAE reduced *Salmonella* by 0.8 log CFU/g on chicken breasts.

Peracetic Acid (PAA)

Peracetic acid (PAA) is an USDA approved antimicrobial agent for use during poultry processing. It is an equilibrium combination of acetic acid and hydrogen peroxide and contain very strong oxidizing properties (Bauermeister et al., 2008). PAA has been proven to be effective in reducing *Salmonella* and *Campylobacter* in chilling and post chill decontamination tanks (Bauermeister et al., 2008; Nagel et al., 2013; Chen et al., 2014; Smith et al., 2015). PAA is regulated by the USDA-FSIS and is not to exceed 2000 ppm (USDA, 2011).

The post-chill application of 1000 ppm PAA on chicken carcasses was found to be effective against *Salmonella* and *Campylobacter* with no negative effects on meat quality (Nagel et al., 2013). PAA resulted in a 2 log reduction of *S.* Typhimurium and *C. jejuni* on broiler carcasses. In another study, PAA as a spray application reduced *S.* Typhimurium and *E. coli* on beef carcasses and broiler carcasses (King et al., 2005; Bauermeister et al., 2008). Chen et al. (2014) revealed PAA levels of 0.07% and 0.1% reduced *Campylobacter* and *Salmonella* of approximately 1.3 log and 1.5 logs with in ground chicken, respectively. Following this further, samples treated with PAA showed extended shelf-life throughout 7 days of storage compared with chlorine and other antimicrobials. Smith et al. (2015) investigated PAA at 100 and 200 ppm as a spray and immersion dip application. Immersion dip of PAA at 200 ppm significantly reduced *C. jejuni* on broiler carcasses by 1.4 log CFU. The 100 ppm and 200 ppm concentrations significantly differed in their ability to reduce *C. jejuni,* with the greater reductions

achieved by 200 ppm. Similarly, the spray application of 200 ppm PAA effectively reduced *C. jejuni* compared to the 25 and 50 ppm sodium hypochlorite treatments (Smith et al., 2015).

The use of the aforementioned antimicrobials has been investigated on reducing *Salmonella* and *Campylobacter* on poultry carcasses. However, studies are needed to evaluate their application, during second processing, after the primary chiller to carcass deboning since very few studies have investigated antimicrobial use in MSC and chicken parts. In the current study, the main objective was to evaluate the USDA approved antimicrobials (chlorine, peracetic acid, cetylpyridinium chloride, octanoic acid, lauric arginate, and organic acids) in reducing *Salmonella* and *Campylobacter* in raw comminuted chicken and chicken cut-up parts to help poultry processors meet the new performance standards raw comminuted chicken and chicken parts.

CHAPTER III

EVALUATION OF USDA APPROVED ANTIMICROBIALS ON THE REDUCTION OF *SALMONELLA* AND *CAMPYLOBACTER* IN GROUND CHICKEN FRAMES AND THEIR EFFECT ON MEAT QUALITY

Abstract

The main objective of this study was to examine the efficacy of USDA approved antimicrobials in reducing *Salmonella* Heidelberg and *Campylobacter jejuni* and to determine the effect of treatments on total aerobic counts and meat color in ground chicken frames. Six antimicrobial compounds (0.1% peracetic acid (PAA), 0.6% cetylpyridinium chloride (CPC), 0.005% sodium hypochlorite (NaOCl), 1.5% acidified lactic acid, 0.3% propionic acid, and 0.1% lauric arginate; LAE) applied as a dip treatment were evaluated. Fresh chicken frames were inoculated with a Nalidixic Acid resistant strain of *S.* Heidelberg and *C. jejuni* (ATCC 33291) to achieve a final inoculum level of ca. 3 log CFU/g in the final product. Frames were dipped for 10 s in each antimicrobial solution and each treatment was replicated on three frames. Three separate replications were conducted for this experiment. Frames were blended and ground samples similar to mechanically separated chicken (MSC) were obtained and stored at 4°C for 24 hours. Samples were analyzed after d0 (2 hr) and d1 (24 hr) to determine reduction in counts of *S.* Heidelberg and *C. jejuni* in MSC. Color measurement, ph value and aerobic plate counts (APC) of ground meat samples for all treatments were

conducted to evaluate the treatment effects on these parameters. PAA and LAE treatments had the highest reductions on *Salmonella* counts ($P \leq 0.05$), both treatments resulting in 0.9 log CFU/g reduction as compared to control. 0.1% PAA and 0.5% CPC, reduced *Salmonella* counts by 1.4 and 0.9 log CFU/g, respectively, on D1. 0.1% PAA, 0.3% propionic acid, 1.5% acidified lactic acid, and 0.1% LAE resulted in 1 log CFU/g reduction of *C. jejuni* as compared to control and other treatments. There was no significant difference ($P \geq 0.05$) among the treatments in their ability to reduce *C. jejuni* on d1. The treatments had no effect on total aerobic counts ($P \geq 0.05$). PAA and LAE can reduce *S.* Heidelberg and *C. jejuni* in ground frames.

Introduction

Salmonella and *Campylobacter* continues to be a major concern in the poultry industry since poultry and poultry products are among the common sources of these pathogens (McKee 2012; CDC 2014). Furthermore, the USDA-FSIS conducted a Not-Ready-to- Eat (NRTE) Comminuted Poultry Sampling project that revealed high levels of *Salmonella* (82.9%) and *Campylobacter* (19.7%) contamination in mechanically separated chicken (USDA-FSIS, 2013). One recent outbreak, in 2014, linked to *Salmonella* Heidelberg resulted in over 30,000 pounds of mechanically separated chicken to be recalled (CDC, 2014).

Because of the high prevalence of *Salmonella* and *Campylobacter* in comminuted chicken, the USDA-FSIS introduced new pathogen reduction performance standards to control *Salmonella* and *Campylobacter* contamination in comminuted poultry products (USDA, 2015a). In accordance to the guidelines, the maximum limit for *Salmonella* positive samples is 25% (13 of 52 samples) and 1.9% (1 of 52 samples) for

Campylobacter positive samples of NRTE comminuted chicken (USDA, 2015). It is critical for comminuted poultry processors to identify and employ successful interventions strategies to prevent and reduce the contamination risks of these pathogenic agents during the flow of production. The USDA-FSIS has approved various antimicrobial agents to control *Salmonella* and *Campylobacter* in raw poultry products that can be applied as spray or dip treatments (USDA, 2015b). Traditionally, chlorine, usually in the form of sodium hypochlorite, and chlorine-based compounds are commonly applied as antimicrobial treatments during poultry processing in the United States (Bauermeister et al., 2008; Buncic and Sofos, 2012). The increased levels of organic matter and pH decrease the efficacy of chlorine as an antimicrobial agent (Lillard, 1979; Sams, 2000; Bauermeister et al., 2008).

Organic acids are evolving in the poultry industry as the new and improved way to treat raw poultry during processing. Organic acids are inexpensive, easy to manage, and have been proven to be effective in reducing pathogens. The antimicrobial efficacy of organic acids is dependent on factors such as contact time, temperature, and concentration (Dickson and Anderson, 1992). Organic acids are generally recognized as safe (GRAS), and the USDA-FSIS has also approved the use of various organic acids during poultry processing (USDA, 2015b). Conner et al. (2001) reported that application of organic acid combinations and surfactants in the processing areas effectively reduced *Salmonella* spp. and *C. jejuni* on broiler carcasses while eliminating cross-contamination. The application of organic acids has been reported (Tamblyn et al., 1997) in the various stages of poultry processing such as chilling water, post-chill tanks, and scalding tanks. Acidified lactic acid is a low-pH acid blend, specifically lactic acid permitted up to 5%,

antimicrobial treatment (USDA-FSIS, 2015c). Lactic acid antimicrobial activity is by penetrating the cytoplasmic membrane, altering the pH and disrupting the proton motive force (Alakomi et al., 2000). Propionic acid is an organic acid antimicrobial agent and the maximum allowed level is 0.5% of the final formulation (USDA-FSIS, 2015b). Organic acids have been reported to negatively affect the flavor and color when applied at higher concentrations (Blankenship et al., 1990; Bauermeister et al., 2008). Bilgili et al. (1998) reported 0.5% - 6% propionic acid treatment caused a bleaching effect on broiler skin resulting in a lighter color when compared to the other acid treatments. Organic acids have been combined with other antimicrobials to avoid the negative effects caused at higher concentrations while maintaining the efficacy.

Peracetic acid (PAA) is an equilibrium mixture of acetic acid and hydrogen peroxide in water (Baldry and Fraser, 1998; Bauermeister et al., 2008). PAA has both acidic and oxidizing properties and the maximum allowable level is 2000 ppm in poultry carcasses and cut-up parts (USDA, 2014). Nagel et al. (2013) found PAA to be effective against *Salmonella* and *Campylobacter* on poultry carcasses when applied as a 20 s post-chill dip without altering the quality of the final product. This study revealed a 2 log and 2.1 log reduction of *Salmonella* Typhimurium on inoculated broiler carcasses for post chill tank dips with 0.04% and 0.1% PAA. Bauermeister et al. (2008) reported that PAA at levels as low as 25 ppm and found them to be effective against *Salmonella;* however, increased level at 200 ppm resulted in higher reductions of *Salmonella* Typhimurium and resulted in a 1.5 log reduction of *Campylobacter jejuni.* In another study, Chen et al. (2014) revealed a reduction of *Campylobacter* and *Salmonella* of approximately 1.3 and 1.5 log CFU with PAA at levels of 0.07% and 0.1% on ground chicken, respectively.

Chantarapanont et al. (2004) reported 1.0 log reduction of *Campylobacter spp.* on chicken skin using a 0.01% PAA solution with a 15 min contact time. Several studies have investigated PAA as an immersion treatment on broiler carcasses (Bauermeister et al., 2008; Bunic and Sofos, 2012).

Cetylpyridinium chloride (CPC) is approved at the maximum acceptable level of 0.8% by weight of the final product. It is a water soluble, non-volatile, quaternary ammonium compound and had been found to be effective against various pathogenic bacteria without negatively affecting the product quality (USDA-FSIS, 2010; Buncic and Sofos, 2012). Yang et al. (1998) reported that the application of 0.5% CPC reduced *Salmonella* on chicken carcasses by 3.6 log CFU per carcass. Kim and Slavik (1996) revealed that 0.1% CPC application on chicken skins was effective against *S.* Typhimurium resulting in a 1.7 log reduction. Conner et al. (2001) reported CPC to be effective without causing any negative sensory effects on the carcass.

Lauric Arginate (LAE) is a broad spectrum antimicrobial, cationic surfactant and acts by altering the cytoplasmic membranes of the cell (Rodríguez et al., 2004). LAE is a GRAS antimicrobial, approved for use in raw poultry parts and comminuted poultry products at the maximum level of 200 ppm by weight of the final product (USDA-FSIS, 2015b). LAE has been found to be effective against microorganisms like *Salmonella* enterica, *Listeria monocytogenes, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli* in a variety of foods such as milk and chicken breasts (Becerril et al., 2013; Ma et al., 2013; Sharma et al., 2013; Nair et al., 2014).

Several studies have been published on the effect of aforementioned antimicrobials on poultry carcasses, poultry products and on chicken skin. However,

there is lack of research information on the efficacy of antimicrobials (sodium hypochlorite, acidified lactic acid, propionic acid, PAA, CPC, and LAE) against *Salmonella* and *Campylobacter* in raw mechanically separated chicken (MSC).

The main objective of the current study is to evaluate the efficacy of various USDA approved antimicrobials on the reduction of *Salmonella* and *Campylobacter* in ground chicken frames, and to observe the treatments effect on aerobic microorganisms, meat pH and color.

Materials and Methods

Salmonella Inoculum Preparation

Nalidixic acid-resistant *S.* Heidelberg strain (NA19) was used in this study to test the efficacy of various antimicrobials in reducing *Salmonella* on ground chicken frames. One loop full of frozen *S.* Heidelberg culture was streaked onto tryptic soy agar (TSA) and xylose lysine Tergitol 4 (XLT4; BD Difco, Franklin Lakes, NJ) agar containing nalidixic acid (60µl/ml; Sigma-Aldrich, St. Louis, MO) and incubated at 37 °C for 48 hours. Broth cultures were prepared by inoculating 10 ml of tryptic soy broth (TSB; 60µl/ml) with a single colony from TSA and incubated at 37 °C for 18- 20 hours. Broth cultures were centrifuged at 5500 rpm for 10 min at 4 °C and the pellet was suspended in 0.1% sterile peptone water. A stock culture of 10^8 CFU/ml was prepared. Serial ten-fold dilutions were performed to prepare a final working solution containing approximately10^6 CFU/ml.

Campylobacter Inoculum Preparation

One loop full of frozen *C. jejuni* (ATCC 33291) was streaked on campy-cefex agar (Acumedia Manufacturers, Inc., Lansing, MI, USA). Broth cultures were prepared by inoculating 10 ml of Bolton's broth with fresh colonies from the Campy-cefex agar and incubated at 42 °C for 24 hours under microaerophilic growth conditions. Gas packs (BD Gas Pak™ EZ Gas Generating System) were used in each anaerobe jar (Mart Anaerobic Jars; Anoxomat) to maintain microaerophilic conditions providing approximately 5% O_2, 10% CO_2, and 85% N_2. The broth culture was centrifuged for 10 min at 4290 rpm at 4 °C and the pellet was suspended in 0.1% peptone water. A stock culture of 10^6 cells/ml was prepared as described for *Salmonella*.

Antimicrobial Treatments

Chlorine, in the form of sodium hypochlorite, containing 7.85% available chlorine was used (Clorox Company, Oakland, USA). The concentration of chlorine was validated using the Aquachek Water Quality Test Strips (Aquacheck, Elkhart, IN, USA). Cetylpyridinium chloride (CPC; Cecure®) consisting of 40% CPC in propylene glycol and water was procured from Safe Foods Corporation (SafeFoods Corporation, Little Rock, AR, USA). Lauric arginate (LAE) also known as Lauramide arginine ethyl ester, containing a 15% concentration of LAE was obtained from Vedeqsa (Vedeqsa, Inc., New York, NY, USA). Peracetic acid (PAA; Peragonn™) was obtained from SafeFoods Corporation and contained 15-20% peracetic acid, 6% hydrogen peroxide, and 30% acetic acid. Propionic acid solution was purchased from Sigma-Aldrich (St. Louis, MO, USA) containing a 99.5% solution. Acidified lactic acid was procured from Synergy Technologies (Shreveport, LA, USA) with a lactic acid concentration of 0.5%. The

concentration of CPC and PAA was confirmed using titration test kits obtained from the manufacturer before each experiment. The pH values of each treatment solution was recorded before performing the experiment.

The average pH of 0.005% chlorine solution was adjusted to 6.5 using 0.1N hypochloric acid solution (Fisher Scientific, Pittsburg, PA, USA). The average pH of 0.3% propionic acid and 0.1% PAA was 3.5 and 2.8; average pH of 0.6% CPC, 0.1% LAE and 1.5% acidified lactic acid was 6.7, 6.2, and 2.3, respectively.

Chicken Frames Challenge Study

A total of 63 chicken frames were used for the experiment (3 chicken frames per treatment x 7 treatments x 3 replications). For each replication, chicken frames were obtained from a local processing facility, transported on ice and stored at 4 °C the day prior to the experiment. On the day of the experiment,chicken frames were transferred to sterile whirl-Pak bags (Nasco, Fort Atkinson, WI, USA) and spot inoculated with 2 ml of nalidixic-acid resistant *S.* Heidelberg and *C. jejuni* culture to achieve ca. 3-log CFU/g recovery level in the final product. The frames were stored undisturbed in the biological safety cabinet for 30 minutes to allow proper attachment of bacterial cells to frames before dipping frames in the treatment solution. The frames were dip treated in sterile 5.5-quart stainless steel bowls (Norpro, WA, USA). Each chicken frame was completely submerged for 10 seconds using a separate bowl. Six dip treatments consisting of 0.005% chlorine, 0.1 % PAA, 0.6% CPC, 1.5% acidified lactic acid, 0.1% LAE, and 0.3% propionic acid were formulated to a final dip volume of 3 L using sterile DI water. Positive and negative controls were included. *Salmonella* and *Campylobacter* inoculated frames dipped in sterile DI water were used as the positive control. Non-inoculated

frames were used to detect the presence of background *Campylobacter spp.* on the chicken frames.

After treatment, chicken frames were blended, using a separate sterile blender top (WARING Commercial, Stamford, CT; Model: 7011S) for each frame, to obtain a ground product similar to MSC. Then, the blended product was aseptically transferred to sterile whirl-Pak bags and hand massaged to ensure proper distribution throughout the sample.

Sampling and Enumeration of *Salmonella* and *Campylobacter*

Four individual 25-g samples were weighed and placed into sterile whirl-Pak bags and then stored at 4 °C. The microbiological analysis for the recovery of *S.* Heidelberg and *C. jejuni* counts was conducted after 2 hours (d0) and 24 hours (d1) of refrigerated storage. Samples were prepared by aseptically adding 225 ml of sterile 0.1% peptone water and hand massaging for 2 minutes to suspend the cells into the solution. Direct plating method was used for enumeration of *Salmonella* and *Campylobacter*. After hand massaging, a volume of 250 µl of the homogenate was plated onto four XLT4 agar plates (BD Difco, Franklin Lakes, NJ) agar plates containing Nalidixic Acid (60µl/ml) and spread using a sterile spreader rod. Plates were incubated aerobically at 37 °C for 48 hours.

Plating methods for *C. jejuni* cell enumeration on campy-cefex agar (Acumedia Manufacturers, Inc., Lansing, MI, USA) were similar to *Salmonella* procedure. Plates were stored in anaerobe jars containing gas packs providing microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2) for 48 hours. Results for *Salmonella and Campylobacter*

counts were converted to log CFU/g of blended product. Each treatment had duplicate samples and the entire experiment was replicated three times.

Meat pH Determination

The meat pH was measured using an Accumet pH meter (AB15 Accumet Basic, Fisher Scientific, Pittsburg, PA, USA) on each day of analysis. The pH probe was inserted in the blended meat sample; two pH readings were averaged and recorded.

Color Analysis and Aerobic Plate Count

Fifteen non-inoculated chicken frames (1 chicken frames x 5 treatments x 3 replications) were included in the study and randomly assigned to the treatments. Antimicrobials deemed effective in reducing *Salmonella* and *Campylobacter* were selected as treatments to determine their efficacy in reducing aerobic microflora and effect on color of ground meat samples. Four antimicrobial treatments (0.1% PAA, 0.6% CPC, 1.5% acidified lactic acid, and 0.3% propionic acid) were prepared in 3L of sterile DI water. Sterile DI water was used as treatment as control treatment.

One non-inoculated chicken frames were used for each treatment and dip treated for10 s by submerging in each antimicrobial solution. A separate bowl (Norpro, WA, USA) was used for each frame. Each frame was blended in a separate sterile blender top (WARING Commercial, Stamford, CT; Model: 7011S) to obtain product similar to MSC.

Color measurements of blended product after the antimicrobial treatments were recorded. Two 25 g samples were weighed from each treatment. Each sample was flattened to make uniform square patties on tin foil lined pans. The color value of each ground meat sample was obtained by placing the probe of the Chroma Meter (CR-400,

Konica Minolta Sensing, INC, Ramsey, NJ, USA) directly onto the meat surface before storing the sample at 4°C. The Chroma meter was calibrated prior the start of each replication. Color readings were recorded as L*, a*, and b* representing lightness, redness, and yellowness of the samples, respectively. Two color value readings were recorded per sample.

After 2 hrs of storage a 4°C, samples were homogenized in 225 ml of sterile 0.1% peptone water by hand massaging for 2 mins. Serial (10-fold) dilutions were prepared using sterile 0.1% peptone water. APC enumeration was achieved by the pour plate method as described in the USDA-FSIS Laboratory Guidebook by pipetting one milliliter of the appropriate dilution to a sterile petri dish and adding approximately 25 ml of molten plate count agar. Plates were settled 30 mins undisturbed to allow hardening of the media. Plates were incubated aerobically at 37 ºC for 48 hours before being counted.

Statistical Analysis

All experiments were replicated three times. *Salmonella, Campylobacter,* and aerobic plate count were converted to log CFU/g of the blended meat sample. Analysis of variance (ANOVA) in the General Linear Model (GLM) of SAS v. 9.4 (SAS Institute, Cary, NC, USA) was used to analyze the data. Means were separated by Fisher's Least Significant Difference (LSD) test with the level of significance considered at $P \leq 0.05$.

Results and Discussion

Antimicrobial efficacy of treatments on *Salmonella* and *Campylobacter*

The data on the antimicrobial efficacy of various treatments in reducing *Salmonella* and *Campylobacter* in MSC has been presented in Fig. 3.1 and 3.2. Overall, PAA, LAE and acidified lactic acid treatments were effective ($P \leq 0.05$) in reducing *S.* Heidelberg in ground chicken frames. The PAA treatment was effective on both days of storage resulting in 0.9 and 1.4 log CFU/g reduction of *Salmonella* on d0 and d1, respectively. Propionic acid (0.3%), acidified lactic acid (1.5%), and CPC (0.5%) treatments also achieved a 0.5 log reduction of *Salmonella* on d0. However, this reduction was statistically non-significant as compared to the control. Treatment with 0.005% sodium hypochlorite was the least effective resulting in a non-significant 0.4 log reduction on both days of storage with no differences observed compared to treatment with water. There were no significant differences observed among the CPC, chlorine, propionic acid, and acidified lactic acid treatments on their ability to reduce the *S.* Heidelberg when compared to control; although, the counts for all treatments were lower than control for both days. On d1, 0.5% CPC treatment resulted in a 0.9 log CFU/g reduction of *Salmonella* with no differences compared to PAA, LAE, or the other treatments. Acidified lactic acid and LAE treatments were effective and reduced *Salmonella* by 0.7 log on d1 (**Fig. 3.1**).

Propionic acid, acidified lactic acid, and LAE treatments reduced *C. jejuni* by 1 log CFU/g on d0, but these reductions were not statistically significantly different from the control. PAA treatment achieved a 0.7 reduction on d0 which was not different from abovementioned treatments. CPC and sodium hypochlorite treatments were not effective

($P \geq 0.05$) in reducing *C. jejuni* and were similar to control on both days. The reductions of *C. jejuni* on d1 ranged from 0.1 log to 1.2 log CFU/g. Propionic acid and acidified lactic acid treatments significantly reduced resulting in 1 log CFU/g reduction of *C. jejuni* ($P \leq 0.05$). On d1, 0.1% PAA treatment was effective and achieved a 0.8 log reduction of *C. jejuni* in ground chicken frames as compared to the control ($P \leq 0.05$). (**Fig. 3.2**).

Traditionally, chlorine treatments at varying ranges have been applied during the various poultry processing steps and found to be effective against *Salmonella* and *Campylobacter.* However, chlorine (0.005%) was the least effective against *S.* Heidelberg and *C. jejuni* in ground chicken frames in the current study. The short contact time and the organic matter present may have resulted in ineffective bactericidal activity of chlorine (Sams, 2001; Bauermeister et al., 2008). The antimicrobial efficacy chorine (0.004%**)** when applied as dip with 20s contact was reported ineffective on poultry carcasses (Nagel et al., 2013). In another study, chlorine (0.003%) was found least effective in ground chicken with 23 s dip (Chen et al., 2014). Acidified lactic acid and propionic acid reduced *Salmonella* and *Campylobacter* in this study; however, the PAA treatment had the greatest log reductions on both days of analysis. The contact time, temperature, and concentration are among the common factors affecting the disinfecting ability of organic acids (Dickson and Anderson, 1992). This result may also be to the antimicrobial efficacy PAA is not easily influenced by the amount of organic matter present in the processing environment (Brinez et al., 2006). Menconi et al. (2013) reported that an antimicrobial mixture of propionic acid and acetic acid was effective in reducing *S.* Typhimurium on chicken skin when applied as a 30 s dip at two concentrations, 0.4% and 0.8%, resulting in a 1.4 and 1.7 log CFU/g reduction,

respectively. In the current study, 0.3% propionic acid dip with a shorter contact time of 10 s achieved 1.3 log CFU/g reduction of *C. jejuni* in ground chicken frames.

Several studies have reported CPC and PAA as immersion tank treatments in reducing pathogenic bacteria in raw poultry. Nagel et al. (2013) reported that application of 0.04% and 0.1% PAA as post-chill immersion treatment (20 s contact time) on *Salmonella* inoculated broiler carcasses achieved 2 to 2.1 log reduction of *Salmonella* with no differences observed between the two treatment levels of PAA to reduce *Salmonella.* In the same study, 0.04% and 0.1% PAA yielded 1.9 and 2.0 log reduction of *Campylobacter spp.*, respectively. Chen et al. (2014) reported the effectiveness of CPC (0.35% and 0.6%) and PAA (0.07% and 0.1%) in reducing *Salmonella* and *Campylobacter* raw in ground chicken. Both CPC concentrations resulted in a 0.8 log CFU/g reduction of *Salmonella* and *Campylobacter.* The PAA treatments had the greatest reduction of *Salmonella* (~ 1.5 log CFU/g) and *Campylobacter* (~ 1.3 log CFU/g). There were no significant differences observed between the different concentrations for CPC and PAA treatments.

LAE (0.1% immersion treatment) in the current study significantly reduced *S.* Heidelberg and *C. jejuni.* However, greater reductions of *C. jejuni* (~1.2 log CFU/g) were observed on both days of analysis. In a previous study, Sharma et al. (2013) reported LAE to be ineffective against *Salmonella* in ground chicken at a concentration of 200 ppm (by weight of ground chicken) when applied as a surface treatment. In the current study, a 0.1% LAE dip treatment reduced *Salmonella* by 0.9 log and 0.7 log on day 0 and day 1, respectively. Nair et al. (2014) reported that a post-chill application of LAE at levels of 200 and 400 ppm reduced *C. jejuni* by more than 1 log CFU/g in fresh

chicken breasts fillets after 7 days of storage. In the present study, LAE achieved more than 1 log CFU/g reduction of *C. jeuni* in ground chicken frames after 24 hours of storage.

The main objective of this study was to identify various USDA approved antimicrobials that would be effective in reducing *Salmonella* and *Campylobacter* in MSC. The findings from this study suggest that LAE and PAA, at concentrations tested in the study can reduce *Salmonella* and *Campylobacter.* The information from this research would provide beneficial information to the poultry processors to reduce *Salmonella* and *Campylobacter* contamination in raw comminuted chicken, particularly MSC.

APC and Color Measurements

Non-inoculated chicken frames were treated with 0.1% PAA, 0.6 % CPC, 0.3% propionic acid, and 1.5% acidified lactic acid were analyzed for APC after 2 hours of refrigerated storage. Total APC for the chicken frames treated with sterile DI water (control) was ca. 3.0 log CFU/g (**Table 3.2**). There were no treatment effects ($P \geq 0.05$) on the reduction of aerobic microorganisms as compared to control with any treatment. In addition, there was no differences among treatments in reduction of APC. Chen et al. (2014) reported the application of 0.07% and 0.1% PAA treatments had lower APC levels than 0.6% CPC in ground chicken. However, there were no significant differences observed among the treatments in their inability to reduce the total APC.

Color measurements were recorded to determine any treatment effect on lightness (L*), redness (a*), and yellowness (b*) of final ground product. Overall, there were no differences ($P \geq 0.05$) observed for the treatments in terms of lightness, redness

and yellowness when compared to control frames treated with water (**Table 3.3**). Blankenship et al. (1990) reported the application of organic acids can negatively affect the color and flavor of the meat. Moreover, CPC, acidified lactic acid, and propionic acid redness values were significantly higher than the PAA. The a* values for PAA treated samples were slightly decreased but not statistically different ($P \geq 0.05$) compared to CPC, acidified lactic acid, and control (**Table 3.3**). Bauermeister et al. (2008) reported lower a* values with PAA treatments up to the 200 ppm in broiler carcasses chilled for 2h (Chen et al., 2014) is due the oxidizing property of the antimicrobial; but not negatively affecting the sensory attributes.

Meat pH

Meat pH is an important factor for determining final product quality. Meat pH affects the functional properties of muscle proteins. In this experiment, the mean pH value for the control samples (frames treated with DI water) was 6.6 (**Table 3.4**). Sharma et al. (2013) reported no significant differences among ground chicken samples treated with and without LAE (200 and 400 ppm). Similar findings were obtained with LAE treatment and the control in this study ($P \geq 0.05$). Chlorine, CPC, and PAA treated samples had significantly ($P \leq 0.05$) higher pH values than the LAE, propionic acid, and acidified lactic acid treated samples (**Table 3.4**). In addition, acidified lactic acid's mean pH values were significantly lower than all the other treatments and control ($P \leq 0.05$).

Conclusion

The findings from this study indicate that PAA, LAE, CPC and acidified lactic acid, are effective in reducing *S.* Heidelberg and *C. jejuni* in ground chicken frames. Although, 0.1% PAA and LAE had the highest reductions of *Salmonella* in ground frames. In addition, PAA, acidified lactic acid, propionic acid and LAE were effective against *Campylobacter*. Moreover, the meat pH and color were not negatively altered. Thus, their application, concentrations and contact time can improve the safety levels of *Salmonella* and *Campylobacter* without causing negative effects on meat quality.

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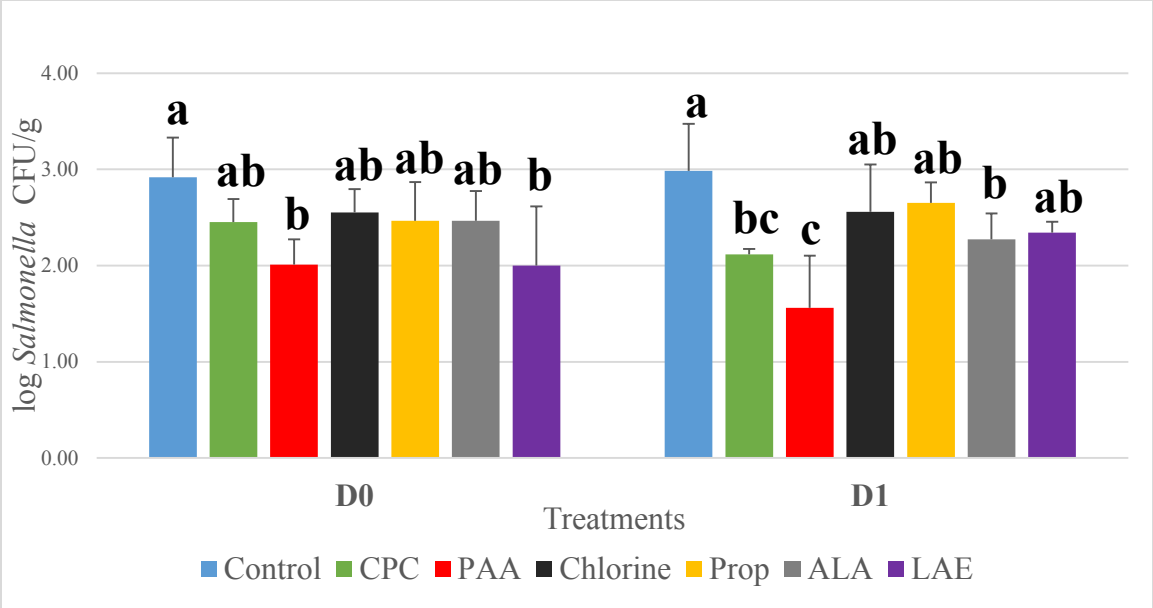

Figure 3.1 Effects of antimicrobial dip treatments in reducing *Salmonella* Heidelberg in ground chicken frames

Treatments with different superscripts indicates significant differences ($P \leq 0.05$)
Control- Sterile DI water
CPC- Cetylpyridinium chloride
PAA-Peracetic acid
Prop- Propionic acid
ALA-Acidified lactic acid
LAE-Lauric arginate

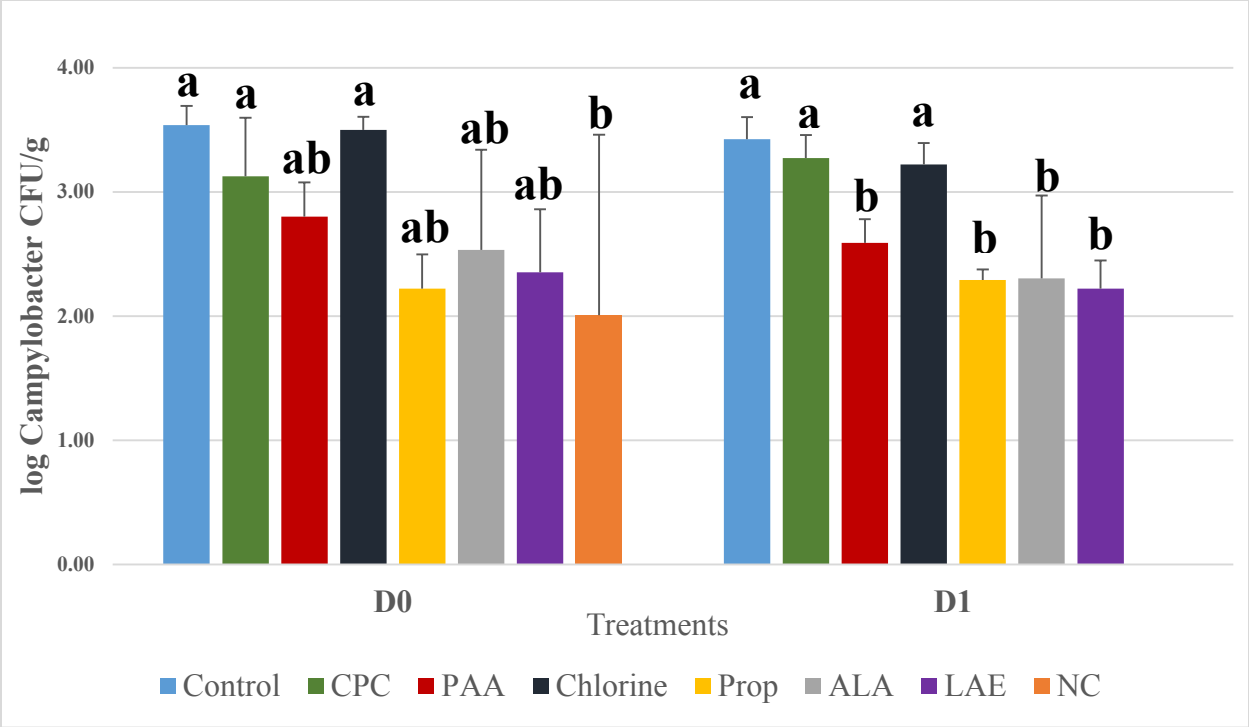

Figure 3.2 Effects of antimicrobial dip treatments in reducing *Campylobacter jejuni* in ground chicken frames

Treatments with different superscripts indicates significant differences ($P \leq 0.05$)
Control- Sterile DI water
CPC- Cetylpyridinium chloride
PAA-Peracetic acid
Prop- Propionic acid
ALA-Acidified lactic acid
LAE-Lauric arginate

Table 3.2 Effect of antimicrobial dip treatment in reducing aerobic bacteria in ground chicken frames

Treatment	**Aerobic Plate Count** (log CFU/g)
Control	3.0 ± 0.3[a]
0.3%Propionic acid	3.2 ± 0.6[a]
1.5% Acidified lactic acid	3.1 ± 0.7[a]
0.1% Peracetic acid (PAA)	2.8 ± 0.4[a]
0.5% Cetylpyridinium chloride (CPC)	2.8 ± 0.6[a]

Mean ± SD Values within the same column with the same letter are the same ($P \leq 0.05$)

Table 3.3 Color analysis of raw chicken patties treated with various antimicrobials

Treatment	**L* va**	**a* value**	**b* value**
Control	60.73	15.71 ± 1.26	12.50 ± 1.36
0.1% PAA	61.21	12.42 ± 2.24	12.71 ± 0.89
0.5% CPC	63.96	13.71 ± 1.42	12.47 ± 1.57
0.3% Propionic acid	67.05	18.16 ± 3.22	13.75 ± 1.86
1.5% Acidified lactic acid	64.42	14.52 ± 1.13	13.45 ± 0.24

Mean ± SD Values within the same column with the same letter are the same ($P \leq 0.05$)

Table 3.4 Effect of various antimicrobial treatments on pH of ground chicken frames

Treatment	**Mean pH ± SD**
Control	6.67 ± 0.29[ab]
0.3% Propionic acid	6.37 ± 0.04[c]
1.5 % Acidified lactic acid	6.06 ± 0.11[d]
0.1% PAA	6.79 ± 0.16[a]
0.5% CPC	6.89 ± 0.13[a]
0.1% LAE	6.52 ± 0.09[bc]
0.005% Chlorine	6.93 ± 0.13[a]

[a-d] Means within column lacking a common superscript differ ($P \leq 0.05$).
PAA: Peracetic acid
CPC: Cetylpyridinium chloride
LAE: Lauric arginate

CHAPTER IV

EVALUATION OF USDA APRPOVED ANTIMICROBIALS IN REDUCTION OF SALMONELLA AND *CAMPYLOBACTER* ON RAW CHICKEN BREASTS FILLETS

Abstract

The objective of this study was to evaluate the effectiveness of USDA approved antimicrobials as a post-chill dip treatment in reducing *Salmonella* and *Campylobacter* on raw skinless and boneless chicken breasts fillets. Seven treatments in chilled water (4°C), 0.04%,0.07%, and 0.07% (pH adjusted to 6.5) peracetic acid (PAA), 0.5% cetylpyridinium chloride (CPC), 1.5% acidified lactic acid, 0.3% propionic acid, and 0.5% octanoic acid, were evaluated. Fresh boneless, skinless chicken breast fillet samples (100 g per sample) were inoculated with nalidixic acid resistant *Salmonella* Heidelberg (10^8 CFU/ml) and *Campylobacter jejuni* (10^8 CFU/ml; ATCC 33291). The inoculated samples were dipped in sterile glass beakers for a contact time of 20 s or 30 s and stored at 4°C. Loosely attached bacteria (in rinsate) and strongly attached bacteria (attached on meat sample after rinsing) were determined by direct plating. Both CPC (0.5%) and PAA (0.07%) treatments achieved a 1 log CFU/ml reduction of loosely attached *C. jejuni* with 20 s dip of chicken breast fillets (P <0.05). No differences were reported between the treatments to reduce the loosely attached *Salmonella* on the chicken breast fillets, although PAA (0.07%) showed a 1.2 log CFU/ml reduction of loosely attached

Salmonella (P >0.05). Similarly, no antimicrobial effect was observed for the strongly attached *Salmonella* and *Campylobacter* on the chicken breast fillets. Nonsignificant reductions were reported with 0.07% PAA of 0.7 and 0.5 log CFU/ml for strongly attached *Salmonella* and *Campylobacter*, respectively (P >0.05). For the 30 s application, PAA (0.04% and 0.07%) treatments decreased loosely attached *S.* Heidelberg (P<0.05) by 0.5 and 0.8 log CFU/ml, respectively. Acidified lactic acid (1.5%), octanoic acid (0.5%), and all PAA treatments (0.04%, 0.07%, 0.07-6.5) reduced loosely attached *C. jejuni* (P <0.05). In addition, acidified lactic acid, octanoic acid, CPC and PAA concentrations (0.04%, 0.07% and 0.07% -6.5) treatments reduced strongly attached *C. jejuni* (P < 0.05). Based on these findings, PAA (0.07%) effectively reduced both loosely and strongly attached *Salmonella* and *Campylobacter* on chicken parts during 30 s post chill dip.

Introduction

It is estimated that 48 million people become ill from foodborne illnesses each year in the United States (CDC, 2011). Non- typhoidal *Salmonella* and *Campylobacter spp.* continue to be major concerns for the poultry industry since these pathogens are commonly associated with raw poultry meat and parts (Altekruse et al., 1999; Batz et al., 2011; Mckee, 2012; CDC, 2014). Overall, non-typhoidal *Salmonella* and *Campylobacter spp.* are estimated to cause 20% of the foodborne illnesses, 50% of the hospitalizations, and 34% of the fatalities annually in the United States (CDC, 2011; Scallan et al., 2011). Batz et al. (2011) estimated the cost of *Salmonella* and *Campylobacter* illnesses related to poultry to be approximately $712 million and $1.3 billion annually, respectively.

The prevalence of *Salmonella* and *Campylobacter* in raw chicken parts sampled from federally inspected poultry processing facilities was, 26.3% and 24% in raw chicken parts, respectively (USDA-FSIS, 2013). In addition, poultry meat per capita consumption is estimated to increase 109 pounds in 2016 as compared to 100 pounds in 2014 (USDA-FSIS, 2016). The increased consumption of poultry meat and products escalates the potential risks of consuming undercooked *Salmonella* and *Campylobacter* contaminated poultry. In 2015, USDA-FSIS proposed new performance standards for these pathogens in raw chicken parts which entail a maximum amount of *Salmonella* percent positive being 15.4% and maximum amount of *Campylobacter* to be 7.7% (USDA-FSIS, 2015). Since the high contamination level of *Salmonella* and *Campylobacter* in raw chicken parts and the more stringent regulations, poultry processors must identify and employ strategies to reduce these pathogens and alleviate the burden on public health.

The U.S. poultry processors employ USDA approved antimicrobial treatments during various steps of poultry processing such as chiller interventions, post-chill dips and sprays. Post-chill antimicrobial applications have been proven to be an effective measure to reduce pathogens such as *Salmonella* and *Campylobacter* on poultry carcasses and parts (Nagel et al., 2013; Chen et al., 2014; Smith et al., 2015). The approved and commonly used antimicrobials during post-chill applications include peracetic acid (PAA), cetylpyridinium chloride (CPC), chlorine, organic acids, and trisodium phosphate (Slavik et al., 1994, Mckee, 2011; USDA-FSIS, 2015). Chorine, in the form of sodium hypochlorite, has been traditionally used in the chiller at pH levels lower than 7.0 (Lillard, 1979; Bauermeister et al., 2008). The maximum permissible limit of chlorine in

the chiller water is 50 ppm (USDA-FSIS, 2015). PAA is allowed in post-chill immersion dips or sprays of poultry carcasses and parts up to 2000 ppm (USDA-FSIS, 2015). Studies have shown that PAA is effective against *Salmonella* and *Campylobacter* on chicken carcasses, ground chicken, and chicken skin during chilling and post-chill decontamination (Bauermeister et al., 2008; Nagel et al., 2013, Smith et al., 2015 Sukumaran et al., 2015). Smith et al. (2015) reported that the application of 200 ppm PAA achieved a1.4 log CFU/ml reduction of *C. jejuni* on broiler carcasses. Other studies have also reported the effectiveness of PAA against *Salmonella* and *Campylobacter* at varying concentrations (0.02%, 0.04%, and 0.1%) without altering the quality of the product (Bauermeister et al., 2008; Nagel et al., 2013; Chen et al., 2014). CPC is permitted for use to control *Salmonella* and *Campylobacter.* Due to USDA-FSIS regulations, poultry carcasses and cut up parts are required to undergo a rinse with potable water after a CPC immersion treatment. Waldroup et al. (2010) suggested 0.1% and 0.5% CPC post-chill application will yield a 2-3 log reduction of *Campylobacter spp.* on poultry carcasses. Moreover, Zhang et al. (2013) showed 0.6% CPC significantly reduced *S.* Typhimurium and *C. jejuni* on chicken parts by 3.5 log and 5.0 log CFU/ml in a 23 s dip, respectively. Organic acids (lactic acid, acetic acid, citric acid, propionic acid) are generally recognized as safe (GRAS), easy to manage, and inexpensive antimicrobials that have been investigated for their effectiveness to reduce pathogens in poultry applications (Conner et al., 2001; Mani-Lopez et al., 2012). Acidified lactic acid is USDA approved antimicrobial that can be used to treat poultry carcasses, cut parts, and trimmings in post-chill applications (USDA-FSIS, 2015). Propionic acid antimicrobial activity is greater at lower pH and is proposed to be more effective in reducing

Salmonella in meat and poultry products compared to other organic acids like acetic acid and lactic acid (Mani-Lopez et al, 2012; Gonzalez-Fandos et al., 2015). In one study, Tamblyn and Conner (1997) reported 2% propionic acid achieved a 1.2 log reduction of *Salmonella* on chicken skin. Additionally, Gonzalez-Fandos et al. (2015) tested an immersion dip of chicken legs in 2% propionic acid for 5 min which resulted in a 1.62 log reduction of *Campylobacter jejuni.* Octanoic acid, a natural antimicrobial found in coconut oil and milk is approved for use during processing on poultry carcasses and cut-up parts up to 400 ppm by weight of the final product (USDA-FSIS, 2016). Research is limited on the efficacy of octanoic acid in poultry meat and products. However, octanoic acid (1.25 and 2.5 mg/ml) was found effective against *C. jejuni* resulting in 0.5 and 1.6 log reduction on chicken skin when applied as a surface treatment (Hovorkova and Skrivanova, 2015).

The purpose of the study was to determine the effectiveness of antimicrobial interventions peracetic acid (PAA; 0.04%, 0.07%, and 0.07% (pH adjusted to 6.5), cetylpyridinium chloride, (CPC; 0.5%), propionic acid (0.3%), acidified lactic acid (1.5%), and octanoic acid (0.5%) to aid poultry processors meet the new stringent regulations of poultry cut up parts proposed by the USDA-FSIS.

Materials and Method

Salmonella Inoculum Preparation

One loop full of frozen nalidixic acid resistant *S.* Heidelberg was streaked onto tryptic agar (TSA) and XLT4 (BD Difco, Franklin Lakes, NJ) agar containing nalidixic acid (60µl/ml; Sigma-Aldrich, St. Louis, MO) and incubated at 37 °C for 48 hours. Broth cultures were prepared by inoculating 10 ml of tryptic soy broth (TSB; 60µl/ml) with a

single colony from TSA plates and incubated at 37 °C for 18- 20 hours. Broth cultures were centrifuged at 5500 rpm for 10 min at 4 °C and the pellet was suspended in 0.1% sterile peptone water. A stock culture of ~10^{8-9} CFU/ml of *S.* Heidelberg was prepared. A final working solution was prepared by making one serial dilution.

Campylobacter jejuni Inoculum Preparation

Fresh colonies of *C. jejuni* (ATCC 33291) from campy cefex agar plates incubated for 48 hrs at 42° C in a Anaero-Jar with Campy EZ Gas Packs providing microaerophilic conditions (5% 0_2, 10% CO_2 and 85% N_2) were used to inoculate 10ml of Bolton's broth. The broth culture was incubated at 42° C for 24 hrs under the same conditions. Cultures were centrifuged at 4290 rpm for 10 min at 4° C. The pellet was resuspened in sterile 0.1% peptone water to prepare a stock culture of ~10^{8-9} CFU/ml of *C. jejuni.* Stock culture was serial diluted once to prepare final working solution.

Antimicrobial Treatments

Seven chilled treatments containing 0.04% peracetic acid, 0.07% peracetic acid, 0.07% peracetic (pH adjusted to 6.5 pH), 0.5% cetylpyridinium chloride, 0.3% propionic acid, 1.5% acidified lactic acid, and 0.5% octanoic acid were prepared using sterile DI water in sterile glass beakers to make a final volume of 500 ml. Peracetic acid (PAA; Peragonn ™) was procured from SafeFoods Corporation (Little Rock, AR, USA) that contained 15-17% peracetic acid, 6% hydrogen peroxide, and 30% acetic acid. The averaged pH of 0.04%, 0.07% and 0.07% (6.5 pH) PAA was 3.1, 2.8, and 6.6, respectively. The pH was adjusted to a final pH of 6.5 with 2M NaOH for the third PAA treatment. Cetylpyridinium chloride (CPC; Cecure; SafeFoods Corporation) was obtained

containing 40% CPC in propylene glycol and water. The average pH of the treatment solution was 5.4. Propionic acid solution was purchased from Fisher Scientific (Pittsburg, PA, USA) containing 99.5% solution. Acidified lactic acid, containing 0.5% lactic acid, low pH blend was obtained from Synergy Technologies (Shreveport, LA, USA). The pH of 0.3% propionic acid and 1.5% acidified lactic acid was 3.2 and 1.3, respectively. Octanoic acid consisting of 99.5% was obtained from Sigma-Aldrich (St. Louis, MO, USA) with an average pH of 3.5. Sterile DI water was used as a control. The concentration of PAA and CPC was confirmed using test kits obtained from the manufacturer before each replication. All treatment solutions were maintained at 4°C.

Challenge Study

Boneless skinless chicken breast fillets were weighed into 100g samples before the start of experiment. The surface of the chicken breast meat was inoculated with 100µl of nalidixic acid resistant *Salmonella* Heidelberg (~10^8 log CFU/ml) and *Campylobacter jejuni* (ATCC 33291, ~10^7 log CFU/ml). The inoculum was spread evenly on the surface of the meat piece using a sterile spreader rod. The chicken breast samples were stored undisturbed for 30 min under the biosafety hood to allow proper attachment of the bacterial cells before antimicrobial treatments. Chicken breast samples then were dipped for either 20 s or 30 s in individual sterile glass beakers. The CPC samples were dipped and rinsed with sterile DI water on the front and back surface of the meat piece to meet the USDA requirements for CPC use. After treatment chicken breasts were aseptically placed in sterile whirl-pak bags and stored for 2 hrs at 4°C.

Enumeration of *Salmonella* and *Campylobacter*

After a 2 hour holding period, 100 ml of buffered peptone water (BPW) was aseptically poured into each bag and rinsed for 1 min by shaking the bag vigorously. The rinsate was removed and poured into sterile screw-top plastic jars to enumerate the loosely attached cells. Serial dilutions were performed using sterile 0.1% peptone water. Direct plating was used for enumeration of *S.* Heidelberg and *C. jejuni* by adding 100 µl of each dilution onto XLT4 containing nalidixic acid (60 µl/ml) and campy cefex agar plates using a spreader rod. After removing the rinsate, 200 ml of 0.1% peptone water was aseptically poured into the bag containing the 100 g chicken breast sample and a meat homogenate was prepared by hand massaging the sample for 2 mins to loosen the remaining bacteria cells from the meat piece. This procedure was used to detect the strongly attached cells. After hand massaging, 250 µl of the homogenate was plated onto four XLT4 and campy cefex agar plates. XLT4 plates were aerobically incubated at 37°C for 48 hrs, whereas campy cefex agar plates were incubated at 42°C in Anaero-Jars for 48 hrs which contained Campy EZ Gas packs providing the proper gas mixture as mentioned earlier. *Salmonella* and *Campylobacter* counts were converted to log CFU/ml of the rinsate and CFU/g of the meat. Each treatment included duplicate samples and the whole experiment was replicated three times. All beakers and screw-top plastic jar were sterilized before the start of each replication.

Statistical Analysis

All experiments were replicated three times. *Salmonella* and *Campylobacter* counts were converted to log CFU/ml and log CFU/g of the meat sample. Analysis of variance (ANOVA) in the General Linear Model (GLM) of SAS v. 9.4 (SAS Institute,

Cary, NC, USA) was used to analyze the data. Means were separated by Fisher's Least Significant Difference (LSD) test with the level of significance considered at $P \leq 0.05$.

Results and Discussion

The antimicrobial efficacy of 0.04% PAA, 0.07% PAA, 0.07% PAA (pH adjusted to 6.5 pH), 0.5% CPC, 0.3% propionic acid, 1.5% acidified lactic acid and 0.5% octanoic acid in reducing *S.* Heidelberg and *C. jejuni* on chicken breast fillets is presented in tables 4.1 and 4.2. CPC (0.5%) and PAA (0.07%) treatments with 20 s contact time resulted in 1 log reduction of *C. jejuni* as compared to the water treatment (Table 4.1). However, CPC and PAA treatments were not significantly different from the octanoic acid and acidified lactic acid treatments in their ability to reduce loosely attached *C. jejuni* (P >0.05). PAA (0.07%) achieved a 1.2 log CFU/ml reduction of loosely attached *Salmonella* cells but was not significantly different from the control and other treatments in the 20 s application ($P > 0.05$). There were no differences among the 0.07% PAA, 0.5% CPC, 0.5% octanoic acid, 0.3% propionic acid, and 1.5% acidified lactic acid antimicrobial treatment to reduce *S.* Heidelberg and *C. jejuni* strongly attached cells on the meat piece after a 20 s contact time ($P > 0.05$).

PAA treatments (0.04%, 0.07%, and 0.07% -pH 6.5) with a 30 s dip time significantly reduced loosely attached *Salmonella* on chicken breast fillets (Table 4.2) as compared to the water treatment ($P< 0.05$). However, greater reductions were observed with the 0.04% and 0.07% (pH 6.5) PAA treatments resulting in 0.8 and 0.5 log CFU/ml reduction of loosely attached *S.* Heidelberg cells, respectively. No other treatments including CPC (0.5%), acidified lactic acid (1.5%), octanoic acid (0.5%), and propionic acid (0.3%) were effective in reducing *S.* Heidelberg in the rinsate. There was no

difference in the reduction of loosely attached *S.* Heidelberg (P >0.05) between the adjusted pH and non-adjusted pH treatments. PAA treatment (0.07%) was effective in reducing strongly attached *S.* Heidelberg as compared to the control treatment of water (P< 0.05) resulting in a 1.1 log CFU/g on chicken breast fillet. PAA 0.07% (pH 6.5) also achieved a 0.6 log reduction but was not different from treatment with water and other treatments (P >0.05). No other treatment was effective in reducing *S.* Heidelberg cells strongly attached to chicken breasts fillet samples after washing off the rinsate (P >0.05).

C. jejuni was more sensitive to various antimicrobial treatments in the 30 s dip study as compared to *Salmonella.* Loosely attached *C. jejuni* was significantly reduced by more than 1 log CFU/ml following treatment with acidified lactic acid (1.5%), octanoic acid (0.5%), and all PAA concentrations (0.04%, 0.07%, 0.07% (pH 6.5)) on chicken breast fillets (P <0.05). The greatest reductions of loosely attached *C. jejuni* were achieved with PAA treatments (0.07% (pH 6.5), 0.04% and 0.07%) with 1.8, 1.8, and 1.6 log CFU/ml, respectively. There were no significant differences observed among the various PAA concentrations (P>0.05) tested to reduce *C. jejuni* in rinsate. Acidified lactic acid and octanoic acid showed 1 and 1.2 log CFU/ml reduction of loosely attached *C. jejuni.*

Strongly attached *C. jejuni* (cells that remained attached to the breast fillet surface after 1 min rinsing with buffered peptone water) was reduced by all treatments compared to the control. The data in table 4.2 reveals PAA treatments (0.04%, 0.07%, 0.07% (6.5) reduced strongly attached *C. jejuni* on chicken parts by 1.2, 1.7, and 1.5 log CFU/g, respectively (P< 0.05). However, there was no difference between the PAA treatments. In an earlier study, no differences were reported for different concentrations of PAA (0.04%

and 0.1%) to reduce *Salmonella* and *Campylobacter* on poultry carcasses during post-chill dip application (Nagel et al., 2013). However, Nagel et al. (2013) reported 2.0 and 2.1 log CFU/ml reductions of *S.* Typhimurium with 0.04% and 0.1% PAA treatments on poultry carcasses in a 20 s post-chill dip. Both octanoic acid (0.5%) and CPC (0.5%) treatments achieved 0.7 log CFU/ml reductions of *C. jeuni* on the breast fillet sample as compared to control ($P < 0.05$). Propionic acid only slightly decreased the level of strongly attached *C. jejuni* with counts being similar to those of the water treatment.

Previous research has reported the use of CPC and PAA as effective antimicrobial treatments in reducing *Salmonella* and *Campylobacter* on chicken carcasses and parts during post-chill applications (Bauermeister et al., 2008; Nagel et al, 2013; Chen et al., 2014; Smith et al., 2015). *S.* Heidelberg was selected because of its association with the reported outbreaks related to poultry. In this study, chicken breast fillets were first rinsed to detect loosely attached bacteria and homogenized for enumeration of the strongly attached cells of *Salmonella* and *Campylobacter* as compared to enumeration of these pathogens only in the rinsate as conducted in earlier studies. In this study, PAA treatments with adjusted pH were also evaluated since poultry processors apply PAA in chiller water with a pH near neutral. Research information is lacking on the use of propionic acid, acidified lactic acid, octanoic acid, and PAA (pH 6.5) antimicrobial activity to reduce *Salmonella* and *Campylobacter* on poultry meat and products. In addition, Chen et al. (2014) achieved 0.8 log CFU/ml reductions of *Salmonella* and *Campylobacter* with 0.6% CPC during a 23 s post-chill dip. In this study, 0.5% CPC achieved a 1.1 log CFU/ml reduction of *C. jejuni* but had no effect on *Salmonella* after 20 s dip treatment. Nagel et al. (2013) showed a 1.9 log CFU/ml reduction of *C. jejuni* in the

rinsate with 0.04% PAA. Similarly, 0.04% PAA achieved 1.8 log CFU/ml reduction in the rinsate in this study. In another study, Hovorkova and Skrivanova (2015) reported that octanoic acid (1.25 mg/ml) surface treatment reduced *C. jejuni* by 0.5 log on chicken skin. Propionic acid (2%) achieved a 1.6 log reduction of *C. jejuni* on chicken legs dipped for 5 min (Gonzalez-Fandos et al., 2015). However, propionic acid was reported to cause discoloration on meat products compared to other organic acids such as lactic and citric acid (Grilli et al., 2013).

Overall, the results from this study indicate CPC (0.5%) was more effective in reducing the loosely attached *C. jejuni* with the 20 s contact time as compared to the 30 s contact time. In addition, the 20 s PAA (0.07%) dip showed reductions of the loosely and strongly attached *Salmonella*; although they were nonsignificant when compared to the 20 s water treatments. Moreover, PAA treatments (0.04% and 0.07%) were only effective in reducing the loosely attached *S.* Heidelberg. Similarly, loosely attached *C. jejuni* was also reduced significantly by the acidified lactic acid (1.5%), octanoic acid (0.5%), and all PAA concentrations with 30 s dip on chicken breast fillets. PAA (0.07%) treatment and a 30 s contact time was found to be more effective against *Salmonella* strongly attached to chicken parts. All treatments in this study were effective in reducing strongly attached *Campylobacter,* except propionic acid, on chicken breast fillets during a 30 s post-chill dip application.

The findings from the current study indicate that, PAA (0.07%) treatment as the post-chill dip is an effective intervention strategy to reduce both *Salmonella* and *Campylobacter* on chicken breast fillets. However, lower concentrations of PAA (0.04%) can reduce loosely attached *Salmonella* in the rinsate. Antimicrobials, other than PAA,

such as acidified lactic acid and octanoic acid can be applied in post chill tanks with 30 s contact times to reduce *Campylobacter* on chicken breast fillets. Further studies are needed to determine the effect of these antimicrobials on organoleptic properties of poultry products.

Table 4.1 Antimicrobial efficacy on reduction of *S.* Heidelberg and *C. jejuni* with 20 s post-chill dip

	Salmonella		*Campylobacter*	
Treatment	loosely attached (log CFU/ml)	strongly attached (log CFU/ g)	loosely attached (log CFU/ml)	strongly attached (log CFU/g)
Water	3.6[a]	2.6[a]	3.2[a]	2.1[a]
0.07% PAA	2.4[a]	1.8[a]	2.2[b,c]	1.6[a]
0.5%CPC	3.4[a]	2.5[a]	2.1[c]	1.7[a]
0.3% Propionic acid	2.9[a]	2.0[a]	3.0[a,b]	2.0[a]
1.5% Acidified lactic acid	3.5[a]	2.5[a]	2.9[a,b,c]	2.1[a]
0.5% Octanoic acid	3.4[a]	2.6[a]	2.6[a,b,c]	2.0[a]

[a-c]Means in a row within a column lacking a common superscript differ ($P < 0.05$).

Table 4.2 Antimicrobial efficacy on Reduction of S. Heidelberg and *C. jejuni* with 30s post-chill dip

	Salmonella		*Campylobacter*	
Treatment	loosely attached (log CFU/ml)	strongly attached (log CFU/g)	loosely attached (log CFU/ml)	strongly attached (log CFU/g)
Water	3.3[a]	2.5 [a]	3.1 [a]	2.4 [a]
0.04% PAA	2.8[b,c]	2.1 [a]	1.3 [e]	1.2 [c,d]
0.07% PAA	2.9[a,b,c]	1.4 [b]	1.5 [d,e]	0.9 [d]
0.07% PAA (pH 6.5)	2.5[c]	1.9 [a,b]	1.3 [e]	0.7 [d]
0.5%CPC	3.1[a,b]	2.3 [a]	2.8 [a,b]	1.7 [b,c]
0.3% Propionic acid	3.3[a]	2.4 [a]	2.4 [a,b,c]	1.9 [a,b]
1.5% Acidified lactic acid	3.1[a,b]	2.2 [a]	2.2 [b,c,d]	1.6 [b,c]
0.5% Octanoic acid	3.2[a,b]	2.5 [a]	1.9 [c,d,e]	1.7 [b,c]

[a-e]Means in a row within a column lacking a common superscript differ ($P < 0.05$).

CHAPTER V

CONCLUSION

PAA, CPC, and LAE dip treatments were proven to be effective in reducing *Salmonella* Heidelberg and *Campylobacter jejuni* in ground chicken frames. However, theses antimicrobials had no effect on total aerobic counts. PAA and LAE treatments achieved the highest reductions of *Salmonella* in ground chicken frames. PAA, LAE, and acidified lactic acid were effective in reducing *C. jejuni* in ground chicken frames without negatively impacting the pH or color of the comminuted product. These findings suggest that PAA, LAE, CPC, and acidified lactic acid could work as effective post-chill antimicrobials to control *Salmonella* and *Campylobacter* in raw comminuted poultry.

Similarly, the application of these antimicrobials as a 30 s post-chill immersion treatment can reduce loosely and strongly attached *Salmonella* and *Campylobacter* on chicken breast fillets. PAA (0.04%, 0.07%, and 0.07% pH 6.5) was more effective in reducing loosely attached *Salmonella.* Acidified lactic acid, octanoic acid, and PAA, and CPC were effective against loosely and strongly attached *C. jejuni*. However, further research is warranted to evaluate the impact of the concentration and contact time of the antimicrobials used in this study on the organoleptic properties of chicken parts and comminuted chicken product similar to MSC and functional properties of muscle proteins in the final product

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