POST-CHILL ANTIMICROBIAL TREATMENTS TO CONTROL *SALMONELLA, LISTERIA*, AND *CAMPYLOBACTER* CONTAMINATION ON CHICKEN SKIN USED IN GROUND CHICKEN

by

SUEJEE PARK

(Under the Direction of Mark A. Harrison)

ABSTRACT

Ground poultry products are frequently contaminated with foodborne pathogens. With increased regulatory guidelines from USDA-FSIS, it is important to employ sufficient intervention strategies to control pathogen levels effectively. In this research, antimicrobials including 50 ppm chlorine, 1,200 ppm peracetic acid (PAA) and a combination of these chemicals with 0.5% chlorine-stabilizer (T-128) were used in a post-chill system to reduce the number of inoculated *S.* Typhimurium, *L. monocytogenes*, and *C. coli*. Results showed that the chlorine+T-128 provided no significant effect in reducing the number of pathogens on ground chicken when compared to water treatment, but it did help decrease pathogen levels in chill water. PAA was found to be the most effective ($p \leq 0.05$) antimicrobial, not only in reducing the number of pathogens on ground chicken but also in post-chill water. Using PAA with/without a stabilizer can be an effective intervention strategy to lessen contamination on chicken skin prior to grinding.

INDEX WORDS: Ground chicken, *Salmonella, Listeria, Campylobacter*, Chlorine, Peracetic acid, Chlorine-stabilizer, Post-chill

POST-CHILL ANTIMICROBIAL TREATMENTS TO CONTROL *SALMONELLA, LISTERIA,* AND *CAMPYLOBACTER* CONTAMINATION ON CHICKEN SKIN USED IN GROUND CHICKEN

by

SUEJEE PARK

B.S., Seoul Women's University, South Korea, 2011

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2014

POST-CHILL ANTIMICROBIAL TREATMENTS TO CONTROL *SALMONELLA, LISTERIA*, AND *CAMPYLOBACTER* CONTAMINATION ON CHICKEN SKIN USED IN GROUND CHICKEN

by

SUEJEE PARK

Major Professor:	Mark A. Harrison
Committee:	Mark E. Berrang Joseph F. Frank

Electronic Version Approved:

Julie Coffield
Interim Dean of the Graduate School
The University of Georgia
August 2014

DEDICATION

This thesis is dedicated to my mom, Milan Ko, and my dad, Jongshik Park. They have provided unconditional love and support throughout my life. They always believed in me without question throughout the entirety of my education. My accomplishments are theirs. Thank you to my little sister, Eunjee Park, for looking out for me.

I would like to thank Professor Noh, Professor Min and Professor Hong from Seoul Women's University, and Professor Jun from University of Hawaii. They were always there for me with unconditional support and encouragement during my graduate school career. I would not have been successful without their help.

To all my precious friends in Athens, I won't mention each of your names since I do not want to leave anyone out, but I would like to express my feelings on how I love ya'll. Every moment I spent with you guys was so valuable. You guys were a great influence on helping me assimilate to American life…lol. Thanks for making good memories with me. I love ya'll.

To my friends in Korea, I miss all of you so much. All the letters, calls, and Korean foods that you have sent me kept me feeling loved and less homesick. I am so lucky to have all of you guys. I can't wait to see you. Love you all.

ACKNOWLEDGEMENTS

I would like to give my sincere gratitude to my major professor, Dr. Mark Harrison for the great opportunities and support he has given. Apart from academics, I always admire your humor and kindness. I am very fortunate to have you as my major professor. I will forever be grateful to you for your guidance. I would also like to express my gratitude to my committee members, Dr. Mark Berrang, and Dr. Joseph Frank, for your contributions. Every word from you was a great encouragement.

Thank you Eric Adams and Steve Knapp for your time and effort while training me at the USDA lab. You both were a big help whenever I had technical problems and I also thank you for consistently checking up on me. Thank you Gwen, Lauren, Charlotte and Cathy for helping me with my project. I seriously wouldn't have finished successfully without all the long hours of your unselfishly provided help. Outside of the lab, I enjoyed every moment we had. Thank ya'll for sharing good memories with me.

Last but not least I would like to thank the Department of Food Science and Technology for the financial support and encouragement provided to me throughout my graduate studies. I would also like to thank EnviroTech and SmartWash solutions for the materials and funding, so that I could successfully finish my project.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

According to the Centers for Disease Control and Prevention (CDC) Foodborne Disease Outbreak Surveillance Systems between 1998 and 2008, poultry products were the leading food groups linked to foodborne illness (17%) *(13)*. Seventy-two percent of campylobacteriosis, 35.1% of salmonellosis, and 2.7% of listeriosis were associated with poultry. Contaminated poultry ranked first in illness associated cost among foods (> $2.4 billion, annually) *(3)*. According to the pathogen-food combinations annual disease burden report, *Campylobacter*-poultry combination ranked first, *Listeria*-deli meat ranked third, and *Salmonella*-poultry ranked forth.

On July 1, 2011, USDA announced new performance standards for *Salmonella* and *Campylobacter* on post-chill poultry samples *(24)*. Percent positive *Salmonella* has to be less than 7.5% (5 positive samples out of 51), and percent positive *Campylobacter* must be below 10.4% (8 positive samples out of 51) on post-chill broiler carcasses. In addition, beginning June 1, 2013, sampling for *Salmonella* and *Campylobacter* was extended to not ready-to-eat (NRTE) comminuted poultry product which includes ground chicken, mechanically separated chicken and other comminuted chicken *(26)*. Based on the data from NRTE comminuted poultry product, pathogen reduction performance standards for *Salmonella* and *Campylobacter* will be established.

With intensified regulatory oversight, U.S. poultry processing plants should implement effective intervention strategies to control the prevalence of *Salmonella* and *Campylobacter* on poultry products. The prevalence of *Salmonella* in ground chicken is at least twice, and increases up to five times compared to unground chicken products *(27)*. Therefore, more attention needs to

be paid to ground poultry than poultry carcasses. When making ground chicken, skin is normally added to meat in order to bring up the target fat content. However, most bacteria associated with poultry carcasses are on the skin surface. The grinding process increases the chance of cross-contamination by increasing the exposed surface area and distributing pathogens throughout the product *(25)*.

Currently, peracetic acid (PAA) is the most frequently used antimicrobial in U.S. poultry processing industries, followed by chlorine *(46)*. PAA is an effective antimicrobial because its combined organic acid (acetic acid) and oxidant (hydrogen peroxide) produce a synergistic effect *(8)*. Historically, chorine has been used most commonly until PAA replaced it. The advantages of using chlorine are its low cost and availability, and it is also effective in preventing cross-contamination in chill water *(68, 75)*. However, chlorine is less effective in the elimination of bacteria from poultry skin than in chill water *(40, 42, 43, 66)*. Free available chlorine, which is the active form that kills microbes, is rapidly consumed by organic matter from carcasses *(68)*. Therefore, it would be useful to keep free available chlorine levels from dropping drastically.

In one study, the number of *Campylobacter* coming out of the primary chill tank was 1.5 log CFU/mL when an level of 30-50 ppm chlorine was used as an antimicrobial treatment at every processing step *(6, 42)*. This finding showed that carcass washing and immersion chilling reduces the number of pathogens but does not eliminate the bacterial contamination of poultry skin. Therefore, targeting two log reductions should eliminate the bacteria that would remain on carcasses after primary chill *(46)*, this further reduction can be achieved by approaching another "hurdle"*(58)*.

A post-chill dip system is another step or "hurdle" after the primary chilling *(46-48, 58)*. This system is considered as the most efficient step in reducing pathogens because the carcasses

are relatively clean at this point. This means there is a greater chance for antimicrobials to contact chicken skin that is contaminated with pathogens and interference with organic matter is the lowest at this point. Another factor making a post-cut up treatment before grinding effective is that the concentration of chemicals used as antimicrobials are allowed to be higher than the primary chill step *(28)*. The immersion time is relatively short (8 s-30 min), thus does not have a negative effect on quality *(17, 52)*.

The current research was conducted to evaluate the bactericidal activity of various antimicrobial formulations against *S.* Typhimurium, *L. monocytogenes*, and *C. coli* on ground chicken. Treatments of 50 ppm chlorine and 1,200 ppm PAA both with and without a 0.5% commercial chlorine-stabilizer (T-128) were tested to determine the optimal intervention method to decontaminate chicken skin and meat prior to grinding. This research also determined the efficacy of antimicrobial potential in preventing cross-contamination by examining the post-chill water *(30)*. The results of this research suggest an effective pathogen intervention in poultry processing facilities.

CHAPTER 2

LITERATURE REVIEW

Study Relevance

In 2011, the Centers for Disease Control and Prevention (CDC), estimated that 48 million people in the United States (or roughly 1 in 6 Americans) suffer annually from foodborne illness. Out of this 48 million, 128,000 are hospitalized and 3,000 die each year *(12)*. As a result, the health-related cost of foodborne illness is estimated to be 51 billion dollars *(62)*. Poultry products were the food commodity responsible for 17% of foodborne illness that were reported between 1998 to 2008 (Table 1) *(13)*.

The United States Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) has gathered data on *Salmonella* and *Campylobacter* testing of raw poultry since 1998 (Tables 2 and 3) *(27)*. The percent of positive *Salmonella* samples in ground poultry at least doubled in 2005, and increased up to six times higher than in unground samples in 2012. This lowers the pass rate and does not meet standards set forth by USDA for a processing facility, resulting in fewer passing samples compared to those in the unground form.

In 2011, there was a *S.* Hadar outbreak linked to ground turkey burgers, which sickened at least 12 patients, 3 of which were hospitalized *(14)*. A few months later in 2011, ground turkey was linked to *S.* Heidelberg, which sickened at least 136 people in 34 states *(12)*. After these two *Salmonella* outbreaks associated with ground poultry product, stricter pathogen controls for raw ground poultry product were implemented. USDA-FSIS announced a new policy to improve the safety of raw poultry.

In December 2012, FSIS required the reassessment of HACCP plans for Not-Ready-to-Eat (NRTE) comminuted poultry products *(26)*. In addition, testing for *Salmonella* was extended to non-breaded, non-battered comminuted poultry products. The sample size tested for *Salmonella* was increased from 25 g to 325 g. Lastly, as of June 1, 2013, inspection program personnel were notified to sample comminuted poultry products not only for *Salmonella* but also for *Campylobacter* in NRTE foods. The percent positive *Salmonella* and *Campylobacter* in the NRTE comminuted poultry report from 2013 is shown Table 4 *(27)*. *Salmonella* is present more frequently (42.26%) in NRTE Comminuted Poultry than *Campylobacter* (2.61%). This result suggests that better processing steps may be needed to lower the percent of pathogens on raw ground poultry.

***Salmonella* spp.**

The genus *Salmonella* belongs to the family Enterobacteriaceae. It consists of two species, *S. enterica* and *S. bongori*. *S. enterica*, which is of the greatest food safety concern, is divided into six subspecies based on biochemical traits and genomic relatedness *(9)*. *Salmonella* spp. are gram-negative, predominantly motile, non-spore forming, facultatively anaerobic, and rod-shaped bacteria *(20)*. They are resilient microorganisms that readily adapt to extreme environmental conditions; some can grow at 54℃, and some have ability to grow around at 2-4℃. Optimal pH for *Salmonella* growth is 4.5-9.5, with an optimum pH of 6.5 to 7.5. The ability to grow and survive over these wide growing conditions raise concerns about food safety *(20, 69)*.

Raw poultry meat and eggs are the principal vehicles of human foodborne salmonellosis *(3)*. *Salmonella* Typhimurium and *S.* Enteritidis cause most of these cases within this food group *(3, 13)*. Salmonellosis can cause nausea, vomiting, abdominal cramps, diarrhea, fever, and

headaches *(20, 69)*. However, its mortality rate is less than 1%, and is generally self-limiting among healthy adult with intact immune systems. According to the CDC's Foodborne Disease Outbreak Surveillance System between 1998 and 2008 *(13)*, *Salmonella* and poultry (145 outbreaks with 2,580 illnesses) were the third most common pathogen-commodity combination responsible foodborne illness most outbreaks.

In the broiler processing plant, fecal matter is reported to be the main source of *Salmonella* contamination of broiler chickens *(41)*. *Salmonella* can grow naturally in the intestinal tracts of chickens and can spread throughout the poultry processing stages, which can result in *Salmonella* ending up in retail products. Conner *(18)* reported *Salmonella* was recovered from 17 to 77% of freshly processed broilers. Schlosser *(63)* analyzed *Salmonella* serotypes from selected chicken carcasses and raw ground chicken. The most prevalent serotypes identified from chicken carcasses were Heidelberg, Kentucky, Hadar, and Typhimurium. The top most prevalent serotypes identified from ground chicken were Heidelberg, Kentucky, Schwarzengrund and Infantis.

***Listeria* spp.**

The genus *Listeria* contains ten species. Among the species, only *monocytogenes* is a human pathogen. There are 13 serotypes of *L. monocytogenes* which can cause disease, with 1/2a, 1/2b, and 4b accounting for more than 90% of foodborne infections *(64, 69)*. *Listeria* spp. are gram-positive, non-spore forming, motile, rod-shaped, facultatively anaerobic bacteria. They are different from most other pathogens in that they can grow slowly at temperatures as low as -1.5℃ and up to 45℃ *(64)*. *Listeria* spp. are also ubiquitous in the environment; they are resistant to diverse environmental conditions such as low pH (can grow in the range of 4.3 to 9.4), high NaCl concentrations (10-12%), and anaerobic or microaerobic conditions.

In the U.S., *L. monocytogenes* causes approximately 2,500 cases of infection (listeriosis) per year *(15)*. Listeriosis is a major public health concern because of the severity of the disease. The disease affects primarily pregnant women, neonates, immunocompromised adults, and the elderly. In non-pregnant adult groups, diseases such as meningitis, septicemia, and meningoencephalitis can occur, with a mortality rate of 20-30% *(64, 69)*. According to the CDC's Foodborne Disease Outbreak Surveillance System between 1998 and 2008 *(13), Listeria* was responsible for 5 outbreaks along with 127 illnesses within the poultry commodity group. In addition, outbreaks from *Listeria* and poultry combination were responsible for the most deaths (16 deaths).

L. monocytogenes can be introduced into food processing through a variety of sources including worker's shoes and clothing, transport equipment, and raw food of animal origin *(45)*. *L. monocytogenes* strongly attaches to the surface of raw meats, and this makes it difficult to remove. Cook et al. *(19)* examined the prevalence, counts, and subtypes of *L. monocytogenes* on raw, retail chicken breast samples with the skin on versus the skin off in Canada. Thirty-four percent of *L. monocytogenes* isolates were recovered from skin-on chicken breast, and 15% of isolates were recovered from skin-off chicken breast. *L. monocytogenes* serotype 1/2a was the most prevalent serotype on both skin-on and skin-off chicken breast, followed by 1/2b and 1/2c.

***Campylobacter* spp.**

Campylobacter spp. are microaerophilic, gram-negative bacteria belonging to the family Campylobacteraceae *(51)*. At present, the *Campylobacter* genus contains 18 species, with *C. jejuni* and *C. coli* being the most common species associated with human disease. *Campylobacter* spp. are curved or spiral rods that have a single polar flagellum, which makes them highly motile. They are fairly fragile in the ambient environment. They grow in the range

of 30 to 45℃, with an optimum growth temperature of 42℃. A unique characteristic of most *Campylobacter* spp., is they require a microaerobic (oxygen concentrations from 3% to 5%) environment for optimal growth *(51, 69)*. *Campylobacter* are sensitive to drying, heating, freezing, disinfectants, and acidic conditions. They are killed easily at pH 2.3 *(7)*. These characteristics limit their survival environments, which makes difficult for to survive outside of the host for a long period of time.

Campylobacter is the third leading cause of bacterial foodborne illness in the U.S. *(69)*. The CDC estimated 76 deaths per year in the U.S. are due to campylobacteriosis. Among *Campylobacter* spp., *C. jejuni* accounts for more than 80% of campylobacteriosis *(51, 69)*. The infectious dose is small (less than 500 cells), and symptoms appear between 1 to 11 days (typically 2-5 days) after infection. The most common symptoms seen in humans infected with *Campylobacter* are fever, diarrhea, abdominal cramps, and vomiting. Most cases of campylobacteriosis are self-limiting. According to the CDC's Foodborne Disease Outbreak Surveillance System between 1998 and 2008 *(13)*, *Campylobacter* spp. including *Campylobacter jejuni* was responsible for 22 outbreaks along with 163 illnesses within the poultry commodity.

Poultry (chickens, turkeys, ducks, and geese) meat and meat products are common reservoirs for *C. jejuni* and *C. coli (50)*. Conner *(18)* reported that 47 to 80% of *Campylobacter* were recovered from broilers from retail sources. Berrang and Dickens *(6)* examined *Campylobacter* populations of whole carcasses rinses at six different commercial processing sites, staring from the 'prior to scald' step and ending with 'post-chill'. The average number of *Campylobacter* cells on chilled carcasses (post-chill) was 1.5 log CFU/mL. *Campylobacter* is often isolated from commercial poultry products, since they can easily spread within poultry flocks through horizontal transmission through contaminated carcasses and water with fecal

material *(5, 6)*. *Campylobacter* primarily resides in the intestinal tracts of poultry, and carcass contamination can occur of the intestinal tract ruptures during processing.

Poultry Processing and Intervention Strategy

Poultry processing is a highly automated industry with many different opportunities for spread of pathogens. Therefore, multiple steps of intervention strategies such as heat treatments, water with chemical additives (antimicrobials), and mechanical methods are required to prevent spreading pathogens throughout processing *(54)*. Poultry processors apply a "multi-hurdle" approach to reduce pathogen levels throughout the processing plant. The hurdles approach means the more interventions that are applied, the less likely pathogens will be able to survive at the final step of the processing *(58)*.

Figure 1 shows a flow diagram of a typical commercial poultry processing plant *(11, 41)*. Poultry processing starts with live birds arriving in large coops. Birds are unloaded and hung on shackles. They travel through an electric stunning process, which includes their heads running through a water bath that conducts an electric current, resulting in unconsciousness, thus rendering them immobilized prior to slaughter. The next step is exsanguination by a mechanical rotary knife that cuts the jugular vein and the carotid arteries at the neck. Next, the birds go through a scald tank with water at 50 – 60°C. The bird's feathers are loosened due to scalding, and the feathers are plucked by rubber plucking fingers *(2)*. This step can significantly increase *Salmonella* and *Campylobacter* prevalence *(6)*. Rubber plucking fingers squeeze the carcasses, resulting in feces expelling from the birds. This can cause cross-contamination from carcass to carcass since the rubber fingers rub feces around on the skin and spread the pathogens *(11)*. In the next step, carcasses are dropped off the shackle when the head, neck, oil glands and feet are removed with a rotary knife and the birds are rehung for evisceration. During evisceration,

viscera are removed, and inspected by inspectors from the U.S. Department of Agriculture who look for signs of fecal contamination, disease or other problems.

Following inspection, carcasses are washed with antimicrobials using an inside-outside bird washer (IOBW) or an on-line reprocessing (OLR) system *(46)*. This step is responsible for removing visible contaminants such as ingesta and feces. However, IOBW and OLR system provide less than a one log reduction since the application is limited by an inadequate contact time and coverage *(47)*. Russell *(58)* reported that the poultry industry usually employs chlorine in an IOBW system, but research study conducted by Northcutt et al. *(54)* showed that the chlorine level up to 50 ppm did not result in significant reduction of total aerobic bacteria, *E. coli*, *Salmonella* and *Campylobacter* from the whole carcass wash. According to a more recent industry survey conducted by McKee *(46)*, which included 167 U.S. poultry processing plants, peracetic acid (PAA) was the most popular antimicrobial intervention used by the majority of processors for OLR and IOBW, followed by chlorine, acids with a pH of 2.0, acidified sodium chlorite, and cetylpyridium chloride.

Carcass chilling is one of the most critical steps for controlling pathogens. In the U.S., carcasses typically go through an immersion chiller where a counter-current flow of cold water is set up so that they move into increasingly cleaner water, and are chilled to a temperature below 4℃ *(2)*. Generally, the carcass dwell time in the chiller is 1-2 h. Chlorine has been commonly used in poultry chillers. However, the current trend has changed to using PAA, which has been reported to be more effective in reducing *Salmonella* and *Campylobacter* on poultry *(46-48)*. Thirty-five percent of the poultry industry uses PAA, while 27% of the industry uses chlorine during chiller intervention *(46)*. Bauermeister et al. *(4)* found that 85 ppm PAA reduced

Campylobacter prevalence by 43.4%, and *Salmonella* by 91.8%, whereas 30 ppm chlorine reduced *Campylobacter* prevalence by 12.8%, and *Salmonella* by 56.8% in poultry chillers.

A post-chill intervention may be applied directly after the primary chill step. Post-chill or finishing chiller antimicrobial application is a fairly new strategy in antimicrobial control. It is a last line of defense or "hurdle" against pathogens before carcasses exit the chilling system. Over the past few years, there has been a considerable increase in applying post-chill antimicrobial applications in U.S. poultry processing facilities *(48, 59)*. A majority of poultry processors apply post-chill antimicrobial applications *(48)*. There are two commonly used post-chill systems, spraying and tank dipping. Tank dipping resembles a traditional chiller, but the volume of the tank varies from 50 to 10,000 gal *(59)*.

In post-chill antimicrobial application, carcasses come into contact with clean water containing high concentrations of antimicrobials with a relatively short contact time (8 s to 30 s) *(46, 58)*. In addition, the likelihood of antimicrobials coming into contact with skin is the highest at this step since the interference from organic material is lowest at this point. Although high concentrations of antimicrobials are used, negative impacts on product quality have not been observed since the contact time is short *(52)*. PAA (23%) was the most predominant antimicrobial used in the post-chill applications, followed by chlorine (12%), cetylpyridium chloride (10%), acids with pH 2 (10%), and acidified sodium chlorite (7%) *(48)*.

Following post-chill treatment, further processing is implemented based on the final products. For ground chicken, carcasses are portioned, and deboned *(2)*. When poultry is ground, skin and its adhering fat are included in the formulation to bring the fat content to the desired target level. Grinding product increases the chance of introducing bacteria, since skin bacteria

can be transferred to the meat through the grinding process and distributed throughout the final product *(25)*.

Attachment of Bacteria on Chicken Skin

Most pathogens on poultry carcasses are found on the skin after evisceration *(36)*. When carcasses are immersed in chiller water, poultry skin swells, and water uptake increases as channels and crevices of the skin are exposed to water *(67)*. During water immersion, water uptake and surface film are important factors in the adhesion of bacteria to the skin. Even unattached floating pathogenic cells can migrate from the surface film to the skin during water immersion, and can be entrapped in crevices and feather follicles *(33, 38, 67)*. Once they are lodged in skin crevices and feather follicles, bacteria are protected and not easy to access with antimicrobial chemicals *(42)*.

Berrang and Dickens *(5)* examined the number of *Campylobacter* recovered from carcass skin and the meat beneath the skin of New York-dressed carcasses before going into a chill tank at a commercial processing plant. Skin samples showed an average of 2.5 CFU/g, whereas meat beneath the skin had no detectable counts. However, when they examined the number of *Campylobacter* colonies on cut up chicken parts from a retail market, the skin contained from 2.1 to 2.6 log CFU/part and each part of the meat beneath the skin contained a range of 2.1 to 2.6 log CFU/part. This suggests that during the cut-up operation, skin cross-contaminates the exposed meat edges by allowing transfer from water and other fluids from skin. Therefore, it is important to reduce/eliminate the number of pathogens on skin during processing before it can cross-contaminate meat under the skin.

Chlorine Intervention

Chlorine is one of the most popular antimicrobials used within poultry processing in OLR, IOBW, primary chill and post-chill systems *(46)*. Currently, the free available chlorine level is regulated and limited to 50 ppm within immersion chill tanks *(28)*. Due to its electronic configuration of chlorine, it acts as a strong oxidizing agent by possessing a strong tendency to acquire extra electrons. Therefore, chlorine in water reacts with organic materials until the chlorine atom loses its oxidizing properties by reduction to chloride; therefore, it loses its disinfectant properties *(72)*.

When chlorine is added to water, it creates hypochlorous acid (HOCl), a very potent bactericide *(23)*. The hypochlorous acid (HOCl) further dissociates into a hypochlorite ion (OCl^-). Both HOCl and OCl^- are forms of free available chlorine, meaning that they have not reacted or combined with organic material yet and is therefore "free" to react with ammonia, nitrogen-containing contaminants, or other organics *(23, 73)*.

HOCl is a far stronger disinfectant than OCl^-, and it is the most effective disinfectant form among chlorine species *(73)*. HOCl can easily penetrate the cell walls of bacterial pathogens over other chlorine species, since it is uncharged and has a relatively low molecular weight *(56)*. HOCl also releases oxygen, which combines with components of cell protoplasm, to destroy the organism *(73)*. On the other hand, the form OCl^- does not readily diffuse into cell walls of microorganisms since it is negatively charged; it is electrostatically repelled from cell walls, which are also negatively charged. The hypochlorite ion is also slower to diffuse through cells since it is strongly hydrated and becomes larger in size than an unhydrated molecule such as HOCl.

The percentage of chlorine in the form of HOCl and OCl^- is dependent on the pH and temperature *(23, 73)*. Therefore, the efficacy of chlorine can be controlled by adjusting these two factors in the solution. The dominance of HOCl goes up as the pH goes down and as the temperature decreases to 0℃ *(22)*.

The efficacy of chlorine decreases with organic load. Organic material reduces the availability of free available chlorine, and reduces its capacity for bactericidal activity *(22, 23)*. Chlorine combines with ammonia and other nitrogenous compounds to become combined available chlorine, which has no antimicrobial activity *(23, 73)*. The loss of chlorine due to the presence of organic matter needs to be overcome when chlorine is used as an antimicrobial treatment.

Studies *(40, 41, 43, 66)* have shown that chlorine treatment often reduces the number of pathogens, but rarely eliminates the pathogens on carcasses. Once pathogens attach to or embed into crevices in the skin, they become protected, which makes them inaccessible to certain external physical influences and antimicrobials. Lillard *(41)* also reported that it is more likely that chlorine mostly works on preventing cross-contamination in treated chill water, rather than working on elimination of attached bacteria.

Tamblyn et al. *(66)* showed a 2.3 and 2.5 log reduction of *Salmonella* attached to broiler skin when chlorine levels of 400 and 800 ppm were applied, respectively. However, high levels (400, 800 ppm) of chlorine can result in off-flavor and discoloration of the final product. In addition, chlorine treatment introduces strong and harmful odors due to the production of chlorine gas and trichloramines. It can irritate the plant worker's skin and also can be corrosive to the plant equipment *(49)*.

Chlorine-Stabilizer

A new formula, of antimicrobial solution, T-128 (SmartWash Solutions, Salinas, CA) was introduced in 2008 as an antimicrobial chemical blend. It has generally recognized as safe (GRAS) status. T-128 mainly composed of ortho-phosphoric acid, and propylene glycol. This chemical formulation, T-128, has been developed to improve the stability of chlorine and has been successfully used in the presence of high organic conditions encountered in fresh produce and chicken processing *(21, 61, 74).*

Xiangwu et al. *(74)* evaluated the ability of T-128 to stabilize free chlorine in wash solutions in the presence of high organic loads generated by the addition of lettuce extract and soil. Although the application of T-128 did not enhance the efficacy of chlorinated wash solutions for microbial (*E. coli* O157:H7, *S.* Typhimurium) reduction on contaminated iceberg lettuce, it helped delay degradation of the free chlorine caused by the presence of organic matter. However, a chlorine+T-128 treatment significantly lowered the number of bacterial pathogens in wash solutions regardless of the amount of organic matter (lettuce extract up to 2%) compared to chlorine treatment alone. In addition, when inoculated baby spinach leaves and uninoculated cut iceberg lettuce pieces were washed together to evaluate the effectiveness for prevention of cross-contamination, uninoculated spinach leaves from chlorine+T-128 treatment had significantly lower levels of pathogens than leaves from other treatments. The authors suggest the T-128 had weak bactericidal (against *E. coli* O157:H7) activity in suspension, unless used at high concentrations (> 2.5%) with an extended reaction time.

When T-128 was used as a chill additive to reduce the presence of *Salmonella* and *Campylobacter* on chicken wings during processing *(61)*, there were no significant differences between the control and the tested wash solutions (50 ppm chlorine, 0.5% T-128, 50 ppm

chlorine+0.5% T-128) in reducing *Salmonella* and *Campylobacter*. However, the chlorine+T-128 treatment solution provided significant reduction in pathogen levels present in the chill water. *Campylobacter* was found to be more susceptible to T-128 than *Salmonella* in chill water. T-128 treatment alone provided significant reduction (2.89 CFU/mL) in *Campylobacter* while it did not show any significant reduction in *Salmonella* levels in treated chill water. Cross-contamination was identified regardless of the treatment solution when inoculated and uninoculated chicken wings were washed together. However, all uninoculated wings contained significantly lower pathogen levels than the corresponding wings except for the control samples. The chlorine+T-128 treatment did not show significant differences from the other treatments on *Salmonella* contamination of uninoculated wings, but it yielded the lowest levels of contamination, other than control samples, by 1.15 logs. The chlorine+T-128 treatment provided significant reductions in *Campylobacter* populations by 2.05 logs when compared to the control.

Davidson et al. *(21)* assessed the efficacy of five commercial produce sanitizer treatments (30 ppm of peroxyacetic acid, 30 ppm of mixed peracetic, 30 ppm of free chlorine at pH 7.85, 30 ppm of available chlorine adjusted to pH 6.50 with citric acid, and 30 ppm of available chlorine adjusted to pH 6.50 with T-128) against *E. coli* O157:H7 on iceberg lettuce. None of the treatments showed significant differences in the recovered number of *E. coli* O157:H7 on lettuce. However, in flume water which was collected every 10 s to 90 s during lettuce processing, chlorine+citric acid and chlorine+T-128 treatments had significantly lower *E. coli* O157:H7 populations throughout the sampling times. *E. coli* O157:H7 levels were below the limit of detection of 0.02 log CFU/mL at 90 s when the water treatment contained 4.61 log CFU/mL.

Peracetic acid (PAA) Intervention

Peracetic acid, also known as peroxyacetic acid, is a potent disinfectant with a broad spectrum of antimicrobial activity even at low temperatures *(8)*. It has germicidal and sterilizing capabilities, high water solubility (>10% at 19℃), more lipid solubility than hydrogen peroxide, and yields no harmful residuals which include acetic acid, hydrogen peroxide, and diluted sulfuric acid. These characteristics have led to a wide range of applications in the food industry including use in beverage processing, meat and poultry processing, canneries, dairies and soft-drink plants. In poultry processing today, PAA is allowed to be used up to 2,000 ppm in a post-chill dip *(28)*.

PAA is produced by the reaction of acetic acid with hydrogen peroxide (HP) in the presence of sulfuric acid, which acts as a catalyst *(8, 35)*. Unlike HP, PAA is free from deactivation by catalase and peroxid and still remains effective in the presence of organic matter. This makes it a more potent and effective antimicrobial agent than HP and PAA. In addition, PAA is more effective than HP at lower concentrations against a broad spectrum of microorganisms. Metabolically active vegetative cells can donate electrons from transition metals located in the cell's surface to the radicals, which will become oxidized, and destroy the cell *(8)*.

Sagripanti et al. *(60)* compared PAA to other disinfectants. PAA showed generally better bactericidal properties that made it effective at a much lower concentration than other disinfectants. A ten percent peroxide treatment showed a similar effect as 0.03% PAA against 13 disease causing bacteria, including *L. monocytogenes* and *S.* Typhimurium. When compared, 90 ppm PAA was as lethal to *L. monocytogenes* and *Campylobacter* as 860 ppm chlorine *(55)*.

Nagel et al. *(52)* evaluated the efficacy of 40 ppm chlorine, 400 ppm PAA, and 1,000 ppm PAA in reducing *Salmonella* and *Campylobacter* on poultry carcasses in a post-chill immersion tank. The log reductions in *Salmonella* were 2.02 and 2.14 log for 400 ppm, and 1,000 ppm of PAA treatment, respectively, compared to the positive control. The 40 ppm chlorine treatment showed a less than one log reduction compared to the positive control and was not significantly different from the water treatment. The effect on *Campylobacter* was similar, with 400 ppm and 1,000 ppm of PAA reducing *Campylobacter* populations by 1.93 and 2.03 logs, respectively, while 40 ppm chlorine and the water only treatments showed less than one log reduction.

Xi *(17)* evaluated water, 30 ppm chlorine, 700 ppm and 1,000 ppm PAA treatments as post-chill antimicrobials in reducing *Salmonella* and *Campylobacter* on ground chicken. PAA treatments (700 ppm, 1,000 ppm) had the highest reduction in both *Salmonella* and *Campylobacter* populations; whereas chlorine showed the least effect, which was not significantly different from the water treatment. PAA treatment extended the shelf life of the product, and sensory evaluation did not show any deleterious effect on cooked ground chicken patties between treatments.

Color Determination

Color is one of the most important contributing factors influencing consumer buying decisions. It also represents the freshness of the product. Lynch et al. *(44)* reported that 74% of consumers reported color was important in ground meat purchasing decisions. In 1976, the Commission Internationale de l'Eclairage (CIE) developed the CIE L*a*b*. CIE L* represents brightness (L*=100: White, L*=0: Black). CIE a* represent redness. A positive a* indicates red, and a negative a* indicates green (scale from +60 for red to −60 for green). A positive b*

indicates yellow, and a negative b* indicates blue (scale from +60 for yellow to −60 for blue). Chroma is a measure of color saturation, and hue is the color angle *(1)*.

There are many factors that affect meat color. Extrinsic factors include animal genetics, gender, age, diet energy density, time-on-feed, seasonality etc. Intrinsic factors include pH, muscle type, areas within a muscle, muscle fiber composition, and myoglobin concentration.

TABLE 1. Number of outbreaks and outbreak-associated illnesses attributed to poultry in 1998-2008, by etiology — Foodborne Disease Outbreak Surveillance System, United States, 1998–2008 *(13)*.

	No. outbreaks	No. illnesses
Bacillus cereus	15	132
Campylobacter jejuni	16	110
Campylobacter spp.	6	53
Clostridium perfringens	71	3,452
Escherichia coli, Shiga toxin–producing	2	38
Listeria	5	127
Salmonella	145	2,580
Shigella sonnei	4	54
Staphylococcus enterotoxin	39	655
Vibrio parahaemolyticus	1	47
Other bacterial	5	82
Total bacterial	309	7,330

Table 2. Percent positive *Salmonella* sample sets meeting the *Salmonella* USDA-FSIS Performance in the Pathogen Reduction/HACCP Verification Testing Program, 1998 – 2013 *(27)*.

Year	1998-2013		2011		2012		2013	
Product	# Samp	% Pos	# Samp	% Pos	# Samp	% Pos	# Samp	% Pos
Broiler	129,951	9.5	4,744	6.5	10,933	4.3	11,124	3.9
Ground chicken	6,489	25.2	466	30.9	1,376	28.0	453	18.0
Turkeys	13,670	4.2	1,541	2.4	2,183	2.2	2,412	2.3
Ground turkey	13,264	20.3	511	12.3	1,155	11.0	217	15.0

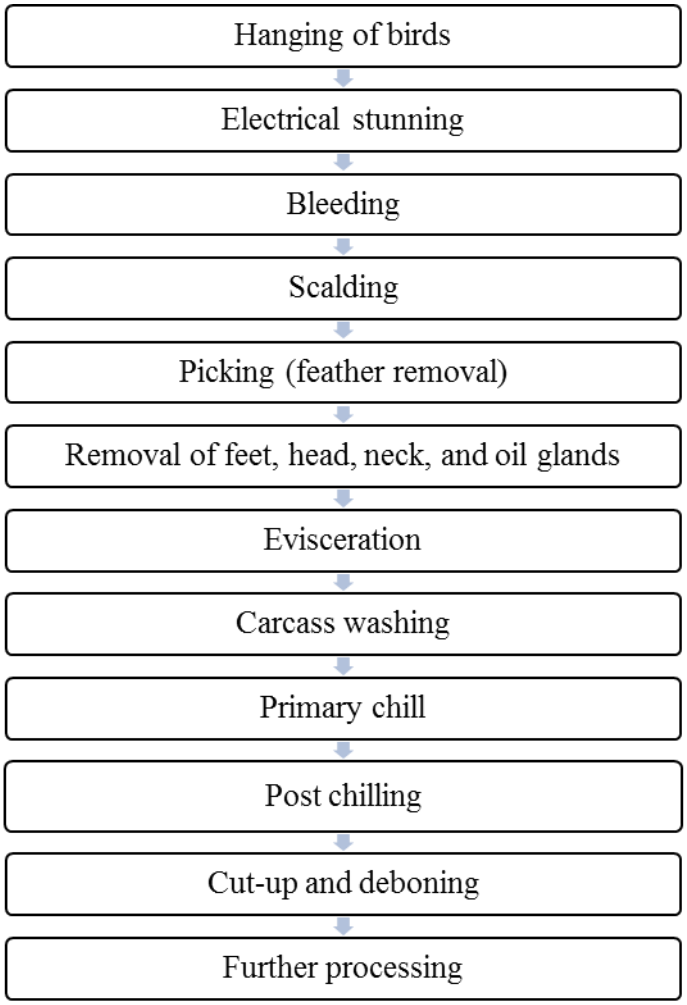
Table 3. Percent positive *Campylobacter* tests in the Pathogen Reduction/HACCP Verification Testing Program, 2011 – 2013 *(27)*.

	2011-2013		2011		2012		2013	
Product	# Samp	% Pos	# Samp	% Pos	# Samp	% Pos	# Samp	% Pos
Young chicken	24,327	7.33	2,433	9.30	10,770	7.00	11,124	7.20
Turkeys	5,614	2.69	1,089	4.20	2,114	2.30	2,411	2.40

Table 4. Percentage of *Salmonella and Campylobacter* positive samples in the not ready to eat comminuted poultry sampling project in 2013 *(27).*

		Salmonella		*Campylobacter*	
		# Samp	% Pos	# Samp	% Pos
Chicken	Ground Chicken	691	42.26	689	2.61
	Mechanically Separated Chicken	697	82.93	696	20.69
	Other Comminuted Chicken	189	41.80	190	1.05
	Chicken Subtotal	1,577	60.18	1,575	10.41
Turkey	Ground Turkey	660	21.36	659	0.91
	Mechanically Separated Turkey	66	45.45	66	4.55
	Other Comminuted Turkey	139	18.71	139	0.00
	Turkey Subtotal	865	22.77	864	1.04

Figure 1. Flow diagram of a typical commercial poultry processing plant *(11, 40)*.



CHAPTER 3

MATERIALS AND METHODS

Inocula Preparation

All cultures used were from the USDA-Agricultural Research Service Berrang culture collection. The *Salmonella enterica* serovar Typhimurium was previously adapted to be resistant to nalidixic acid at a concentration of 200 ppm. This strain were maintained as frozen stock and has been used in previous poultry-related projects reported by Schambach *(61)*. For the inoculum preparation, one frozen bead of 200 ppm nalidixic acid-resistant *S.* Typhimurium was added to 9 mL of tryptic soy broth (TSB, Becton Dickinson, Sparks, MD) and incubated at 35℃ for 24 h. Cultures were activated by at least two successive TSB transfers before using. Inoculum was used at the initial concentration of approximately 1.0×10^9 CFU/mL without dilution. Inoculum concentrations were estimated by dilution spread plating onto brilliant green agar with sulfapyridine (BGS, Acumedia, Lansing, MI) containing 200 ppm of nalidixic acid (Sigma, St. Louis, MO.). Plates were incubated at 35℃ for 24 h, and the colony forming units (CFUs) that showed *Salmonella* characteristics on BGS plates were enumerated.

L. monocytogenes strain used was previously adapted to streptomycin at a concentration of 200 ppm. For the inoculum preparation, one frozen bead of 200 ppm streptomycin-resistant *L. monocytogenes* was added to 9 mL of TSB and incubated at 35℃ for 24 h. The culture was activated by at least two successive TSB transfers before use. The inoculum was used at the initial concentration of approximately 1.0×10^9 CFU/mL without dilution. Inoculum concentrations were estimated by dilution spread plating onto Oxford *Listeria* agar (Acumedia,

Lansing, MI) plates containing 20 ppm of moxalactam sodium salt (Sigma, St. Louis, MO), and 200 ppm of streptomycin sulfate salt (Sigma, St. Louis, MO) (MOX). The plates were incubated at 35℃ for 24 h, and the CFUs that showed *Listeria* characteristics on MOX plates were enumerated.

The *C. coli* strain used was originally adapted to be gentamicin-resistant at a concentration of 200 ppm. This inoculum has been used in previous poultry-related projects reported by Schambach *(61)*. For the inoculum preparation, one frozen bead of 200 ppm gentamicin-resistant *C. coli* inoculum was added to 9 mL of Bolton broth (BB, Acumedia, Lansing, MI), and incubated at 42℃ for 48 h in sealed bags containing a gas mixture of 5% O_2, 10% CO_2, and 85% N_2 (Airgas, Athens, GA). The culture was activated by at least two successive BB transfers before using. Inoculum was used at the initial concentration of approximately 1.0 x 10^9 CFU/mL without dilution. Inoculum concentrations were estimated by dilution spread plating onto Campy-Cefex agar (Acumedia, Lansing, MI) plates with 5% (v/v) lysed horse blood (Lampire, Pipersville, PA), 33 ppm cefoperazone (Sigma, St. Louis, MO), 200 ppm cyclohexamide (Sigma, St. Louis, MO), and 200 ppm gentamicin (Sigma, St. Louis, MO). Sterile lysed horse blood was added to the medium under aseptic conditions, while the rest of the supplements were filter sterilized through a 0.22 µm filter (Thermo Fisher Scientific, Waltham, MA) before adding to the medium. The plates were incubated at 42℃ for 48 h in sealed bags containing a gas mixture of 5% O_2, 10% CO_2, and 85% N_2, and the *Campylobacter* CFUs were confirmed by characteristic growth on the Campy-Cefex plates.

Antimicrobial Solution Preparation

Treatments included tap water, 0.5% T-128 (v/v) (SmartWash Solutions, Salinas, CA), 50 ppm chlorine (Clorox germicidal bleach, 8.25% sodium hypochlorite), a combination of 50 ppm

chlorine and 0.5% T-128, 0.12% peracetic acid (PAA; Perasan MP-2; EnviroTech, Modesto, CA), and a combination of 0.11% PAA and 0.5% T-128. For the negative control, neither pathogens nor treatment were applied to the ground product. For the positive control, pathogens were inoculated, but no treatment solutions were applied. Tap water was used for making solutions since it has been reported that 72% of commercial broiler processing facilities process birds with city water *(53)*. All treatment solution containers were sterilized, and pre-chilled before use. Four hundred grams of chicken breast meat and chicken skin were immersed in 2,170 mL of treatment solution. pH (HI 99163; Haana Instruments, Carrollton, TX), temperature (Multimeter 410; Extech Instrument, Waltham, MA), and PAA concentration, total and free chlorine concentration (V-2000 Multi-Analyte Photometer; CHEMetrics, Midland, VA) of treatment solutions were measured before and after immersion of samples.

Sampling and Enumeration of Ground Chicken

According to the USDA National Nutrient Database for Standard Reference release 26 *(70)*, the main composition of chicken meat is: 74.36 g of water, 23.20 g of protein, and 1.65 g of total lipid (fat) per 100 g of chicken meat. The main composition of chicken skin is: 54.22 g of water, 13.33 g of protein, and 32.35 g of total lipid (fat) per 100 g of chicken skin. Based on this, 75% chicken meat and 25% chicken skin were combined to yield 9.3% fat content ground chicken in the final product. Retail cut-up chicken breasts with skin-on (Tyson Foods, Springdale, AR) were obtained from a local Athens, GA distributer. Three hundred grams of chicken breast meat was separated from 100 g of chicken skin and stored at 4℃ before use. On the day of experiment, the pH (HI 99163; Haana Instruments, Carrollton, TX) and temperature (Multimeter 410; Extech Instrument, Waltham, MA) of the meat was measured and the skin was spread on a pre-chilled sterile stainless steel tray. One milliliter of each of overnight culture of *S.*

Typhimurium, *L. monocytogenes*, and *C. coli* (approximately 1.0 x 10^9 CFU/mL, each) was spot inoculated on the outer surface of pre-weighed pieces of chicken breast skins using a micropipetor and the inoculum droplets was evenly spread using a sterile plastic spreader. Skin was kept at room temperature (approximately 25℃) for 10 min. Following attachment, inoculated skin and non-inoculated chicken breast meat were immersed for 16 s in the designated treatment solution and hand agitated with sterilized forceps. Samples were removed from the solutions and drained on sterile stainless steel mesh strainer for 1 min. After the 1 min draining period, chicken skin and chicken breast meat were cut into smaller pieces with sterilized scissors to facilitate grinding (MI-1800A; Rancho Cucamonga, CA). Chicken breast meat and skin were mixed, and fed into the hopper. The mixture was ground through a coarse cutting plate and then through a fine cutting plate. The pH and temperature of ground chicken were measured and ground product was divided into four-50 g portions. Each portion was placed on a white foam tray (Webstaurant Store, Lititz, PA) covered with polyvinyl chloride food wrap film (Boardwalk, Radnor, PA) (O_2 transmission rate: 8-25 cc/m^2/24 h) and stored at 4℃. Samples were analyzed along with color (L*, a*, b*) measurements using a chroma meter (CR-410; Konica Minolta, Tokyo, Japan) on days 0, 3, 6, and 9. On designated sampling days, 25 g of ground product was placed in a sterile filter stomacher bag containing 100 mL of 0.1% peptone water, and stomached (Stomacher 400 Circulator; Seward, Davie, FL) for 2 min at 230 rpm. Appropriate dilutions (0.1% peptone) were spread plated onto BGS, MOX, and Campy-Cefex plates supplemented with 200 ppm nalidixic acid, 200 ppm streptomycin, 200 ppm gentamicin, respectively. Plates were incubated at designated temperature and time for enumeration of *Salmonella*, *Listeria* and *Campylobacter*, respectively and characteristic colonies were counted.

Enumeration of Post-Chill water

After sample immersion in antimicrobial treatment solutions, the number of *Salmonella*, *Listeria* and *Campylobacter* recovered from post-chill water was enumerated. Water samples were direct plated while the rest of the treatments were enumerated after membrane filtration. One percent (w/v) sodium thiosulfate *(32)* was added to 100 mL of post-chill treatment solution in order to inactivate residual oxidative activity of the sanitizers. Two samples of treatment solutions were filtered through 0.45 µm filters. One filter paper was put into 20 mL of universal pre-enrichment broth (UPB, Becton Dickinson, Sparks, MD) and directly plated on BGS and MOX plates. The remaining UPB was incubated at 35℃ for 24 h for the simultaneous recovery of *Salmonella* and *Listeria* as a primary enrichment. If there were no CFUs on the plates from direct plating, 0.1 mL of UPB culture was inoculated into Fraser broth (FB, Becton Dickinson, Sparks, MD) with a Fraser supplement (SR0156, Thermo Scientific, Lenexa, KS) as a secondary enrichment for *Listeria*, and incubated at 35℃ for 24 h for *Listeria* detection. For *Salmonella* detection, 0.1 mL of UPB culture was transferred to Rappaport-Vassiliadis (RV, Becton Dickinson, Sparks, MD) enrichment broth and incubated at 42℃ for 48 h. The incubated broths were subcultured by streaking onto MOX and BGS plates, respectively, that were incubated at 35℃ for 24 h. The second filter paper was placed into 20 mL BB with a Bolton broth selective supplement (SR0183, Thermo Scientific, Lenexa, KS) for the recovery of *Campylobacter* that was incubated at 42℃ for 48 h. Following incubation, portions were spread plated onto Campy-Cefex plates that were incubated at 42℃ for another 48 h.

Statistical Analysis

For each of three replications (n=3), one sample for each treatment was analyzed. CFU were log transferred and were analyzed by analysis of variance (ANOVA) using the general

linear model (GLM) procedure. Significant differences for each treatment were determined by Duncan's multiple range test. Significance was reported at a level of $P \leq 0.05$.

CHAPTER 4

RESULTS

The most effective treatments for the reduction ($P \leq 0.05$) of *S.* Typhimurium (Table 5) and *C. coli* (Table 6) in ground chicken were 1,200 ppm PAA and 1,100 ppm PAA+0.5% stabilizer, resulting in approximately 2- log reduction. Treatments with water, chlorine-stabilizer alone, chlorine, and chlorine+stabilizer resulted in a significant reduction ($P \leq 0.05$) of *Salmonella* levels, the decrease was less than a 1-log compared to the positive control. The non-inoculated (negative control) was below the detection limit of 50 CFU/g (< 1.7 log), signifying low levels or no background nalidixic acid-resistant *S.* Typhimurium or gentamicin-resistant *C. coli* initially present on the chicken. During the 9 days of storage at 4±1℃, the number of *S.* Typhimurium and *C. coli* remained similar to the day 0 counts.

Similar trend in results was seen with *L. monocytogenes.* Ground chicken treated with PAA and PAA+stabilizer had significantly ($P \leq 0.05$) lower numbers of *L. monocytogenes* compared to the ground chicken that was treated with water, chlorine-stabilizer alone, chlorine, and chlorine+stabilizer, resulting in approximately 2-log reduction compared to the positive control (Table 7). Treatments with chlorine-stabilizer alone, chlorine, and chlorine+stabilizer were not significantly different ($P \geq 0.05$) from the positive control in reduction of *Listeria* levels. The non-inoculated (negative control) was below the detection limit of 50 CFU/g (< 1.7 log) of sample, signifying low levels or no background streptomycin-resistant *L. monocytogenes* existed initially on the chicken. Unlike *S.* Typhimurium and *C. coli*, the number of *L. monocytogenes* on ground chicken increased during the 9 days of storage at 4±1℃. A significant ($P \leq 0.05$) increase

in the number of *L. monocytogenes* on ground chicken was detected on day 6 in all treatments except for PAA treated ground chicken. However, by day 9, the number of *L. monocytogenes* from PAA treated ground chicken also significantly ($P \leq 0.05$) increased compared to day 0.

The level of residual free available chlorine in chlorine+stabilizer solution remained numerically higher (4.4 ppm) than chlorine alone treatment (1.8 ppm) (Table 8) after sample immersion. However, chlorine+stabilizer treatment did not show any significantly better ($P \geq 0.05$) effect than chlorine treatment alone in reducing the 3 pathogens (Table 5-7).

Post-chill water and stabilizer alone solution rinses were direct plated while the rest of the treatments (chlorine, chlorine+stabilizer, PAA, PAA+stabilizer) were filtered and enriched. PAA treatment provided more than five log reduction of *S.* Typhimurium, *L. monocytogenes*, and *C. coli* in post-chill rinse (Table 9). After enriching, none of the pathogens treated with PAA were recoverable below the detection limit of 0.2 CFU/ml. PAA+stabilizer post-chill rinse also was equally effective for *L. monocytogenes* and *C. coli* after 16 s of sample immersion. While treatment with chlorine alone as a post-chill rinse resulted in at least a 4 log reduction for *S.* Typhimurium, *L. monocytogenes*, and *C. coli* compared to the water control after enrichment, they were recovered by enrichment. Treatment with stabilizer alone reduced *Salmonella* and *Listeria* population by 2.3 and 1.2 log CFU/ml, respectively. Although the data was collected on one replication in this case, *C. coli* was susceptible to stabilizer. Treatment with chlorine+stabilizer reduced the levels of the three pathogens, by more than 4 logs.

The average temperature of antimicrobial solutions was 8℃, before sample immersion (Table 10). When chlorine was combined with 0.5% stabilizer, the pH of the solution dropped drastically as low as stabilizer alone. The average pH of chicken breast meat before dipping into treatment solution was 5.92 (Table 11). After chicken breast meat and chicken skin were exposed

to treatment solutions and ground, there was no significant changes in pH between chicken breast meat and ground chicken. This is positive for maintenance of water holding capacity (WHC) *(57, 76)*. The higher the pH of the meat, the better its WHC will be. Quiao et al. *(57)*, reported ground breast meat with a pH of 6.23 showed the highest WHC, followed by ground breast meat at pHs 5.96 and 5.81.

There was a slight color change noted by the investigator between treatments. PAA and PAA+stabilizer treated ground chicken appeared whiter than rest of the treatments. However, the value from the colorimeter showed there were no significant differences observed in the L* values, a means to measure lightness or darkness of samples *(1)*, during three days of storage (Table 12). After day 3, L* values of negative control, positive control, water and stabilizer alone treated ground chicken started to drop through day 9. The a* measurement, which represents red and green, showed PAA treated ground chicken samples were less red than the rest of the treated samples throughout storage. Ground chicken treated with chlorine, water, PAA+stabilizer, chlorine+stabilizer, and PAA showed color shifts to less yellow than the other samples.

CHAPTER 5

DISCUSSION

The results obtained in this research agreed with the results of other studies *(17, 52)*. When *S.* Typhimurium and *C. jejuni* inoculated chicken meat breast were immersed in 400 ppm PAA and 1,000 ppm PAA post-chill immersion tank for 20 s, *S.* Typhimurium and *C. jejuni* decreased about 2 log CFU/mL *(52)*. When 700 ppm PAA and 1,000 ppm PAA were used as a post-chill antimicrobial treatment in inoculated ground chicken, *S.* Typhimurium and *C. jejuni* also resulted in 2 log CFU/mL reduction *(17)*. However, treatment with water, 30 ppm and 40 ppm chlorine were found to be less effective ($P \leq 0.05$) than PAA, which showed less than a 1-log reduction in reducing populations of *S.* Typhimurium and *C. jejuni*.

The limitation of using chlorine is that its bacterial reactivity decreases with the presence of organic compounds. The free available chlorine in the form of hypochlorous acid is quickly counteracted by organic materials (ammonia and nitrogenous compounds) present in water to form chloramines *(71, 73)*. The current study's results agreed with Schambach's study *(61)*, in which chlorine+stabilizer treatment was applied to *S.* Typhimurium and *C. jejuni* inoculated chicken wings in chill water for 45 min. The current study and Schambach's study *(61)* show that the time exposure of chlorine+stabilizer (16 s vs. 45 min) is not a significant factor in reducing attached pathogens.

It has been reported that pathogens (*Salmonella* spp. *C. jejuni, L. monocytogenes, E. coli* O157:H7) are firmly attached to or entrapped in poultry skin, even when broilers first arrive at the processing plant *(19)*. Chlorination of chill water reduces but does not eliminate pathogens

attached on the chicken skin on poultry carcasses *(18, 37, 40-43, 75)*. Numerous studies indicate that embedded/firmly attached bacteria in follicles or crevices are resistant to carcass treatments because they are protected in these areas *(37, 40-42, 65, 66)*.

Lillard's study *(43)* supports this idea, when chlorine with or without sonication were applied to *Salmonella* attached to or entrapped in chicken skin. Treatment with chlorine and sonication for 15 min reduced *Salmonella* counts by 2.09 log CFU/12 cm^2 the skin whereas the *Salmonella* levels on skin immersed in chlorine solution for 30 min without sonication was reduced only by 0.33 log CFU/12 cm^2. Furthermore, when sonication time was extended to 60 min, the number of *Salmonella* decreased by 2.90 log CFU/12 cm^2. Sonication helped detach cells that were attached/entrapped in skin so that they have a better chance to react with chlorine.

However, Chantarapanont et al. *(16)* suggested that crevices, folds, or follicles do not protect bacteria from accessibility to sanitizers. They showed that a greater number of viable *Campylobacter* cells were observed at the surface of the chicken skin than in the crevices or folds of chlorine treated skin. Yang et al. *(75)* suggested that oil from chicken skin protects bacteria from sanitizers. Tamblyn and Conner *(65)* also suggested high lipid content and topography of chicken skin are the primary protective factors for pathogens from sanitizers. The heterogeneity of chicken skin seems to complicate bacterial attachment, by allowing multiple attachment sites through multiple mechanisms, making it difficult to eliminate attached bacteria *(34)*.

Tamblyn and Conner *(65)* used a combination of transdermal compounds (emulsifiers) combined with organic acids in an effort to solubilize skin components and enhance delivery of organic acids to attached or embedded cells. Izat et al. *(31)* evaluated the microbial effects of lactic acid in combination with propylene glycol on *Salmonella* inoculated broiler carcasses. The

results showed that two treatments: 0.25% lactic acid+20% propylene glycol and 0.5% lactic acid+20% propylene glycol were effective in completely eliminating *Salmonella* from the carcass compared to lactic acid alone. However, both treatments resulted in discoloration of the skin and propylene glycol caused a "sickeningly sweet odor". The main component of T-128 is phosphoric acid and propylene glycol, a transdermal compound. However, T-128 showed less than a 1-log reduction on skin attached bacteria. Since the concentration of phosphoric acid and propylene glycol in T-128 is unknown due to proprietary reasons, it is not possible to draw comparative conclusions.

PAA is a mixture of acetic acid and hydrogen peroxide. It is considered to have better disinfectant potential than chlorine and chlorine dioxide *(35)*. By combining organic acids with an oxidant, it brings a greater antimicrobial efficacy. PAA also does not cause negative organoleptic effects such as flavor and color changes *(8, 10)*. The concentration of PAA (400, 700, 1,000, 1,200 ppm) with different contact time (16, 20, 23 s) in the presence of organic matter does not seem to greatly affect antimicrobial efficacy *(17, 52)*. This supports that of Brinez et al. *(10)*, who used different concentrations of PAA in combination with hydrogen peroxide (PAHP at 0.05, 0.4 %) with different contact times (10, 30 min) on *L. monocytogenes* inoculated eggs. There was no significant difference with concentration of PAHP and exposure time in antimicrobial efficacy.

Many studies have been conducted to determine the population of recovered pathogens from primary chill water, but not many on post-chill water, where higher levels of antimicrobials are used. Yang et al. *(75)* tested chlorinated chill water at the concentration of 30 and 50 ppm. Both concentrations resulted in more than 5 log CFU/ml reduction of *C. jejuni,* but they did not effectively reduce the bacteria attached on chicken skin. In the study by Schambach *(61)*, *C. coli*

was also more susceptible (3 log reduction) to chlorine+stabilizer chill water than *Salmonella* (1.7 log reduction).

Examining post-chill water is an important factor since bacterial adherence is rapid, within 15 s of exposure *(38)*. The bacteria transfers from surface film formed on poultry skin to the skin. The adhesion of bacteria to skin occurs when "capillary-sized channels and spaces" in the surface layers are opened during water immersion *(67)*. Even though post-chill water immersion is short (8 s to 30 s) compared to primary chill (1-2 h) *(46, 58)*, it is important to examine post-chill water, because the percentage of bacteria adhered to the skin increases from 6% to 40% when the percentage of bacteria formed on the surface film decreases from 94% to 60% in between 15 s and 30 min immersion*(39)*. This means that the bacteria in the surface film could transfer to the skin during immersion. Pathogens recovered from post-chill antimicrobial rinse can also be an indicator of cross-contamination. During immersion, the liquid film on carcass skin surfaces can be replaced by the antimicrobial solutions in which they were they are dipped *(67)*.

Sensory properties were not determined in the current research, however, Xi *(17)* reported that sensory attributes including odor, appearance, flavor, texture, juiciness, and overall acceptability of ground chicken, that was treated with 700 ppm PAA and 1,000 ppm PAA, were not significantly different from 30 ppm chlorine treated ground chicken.

Color is a significant factor that determines consumer purchasing. In the current research, although color measurements were not statistically analyzed, the results were similar to the trends identified by Xi *(17)*. Xi reported a* and b* values were not significantly different for chlorine and PAA treated ground chicken, but PAA (700, 1,000 ppm) treated ground chicken samples were significantly lighter than 30 ppm chlorine on storage day 0. Xi's L* values results

do not agree with this the current results, where the L* values were similar for chlorine and PAA treated samples. The discrepancy might be explained by the design of the experiment with different levels of the chlorine (30, 50 ppm) and fat contents.

Ability of antimicrobials is determined by target microorganisms and their growth conditions, interfering substances (primarily organic matter), pH, temperature, contact time, and concentration of the antimicrobial solutions *(29).* In the current research, various antimicrobials were used in reducing the population of *S.* Typhimurium, *L. monocytogenes,* and *C. coli* in ground chicken. Results from this research, along with other studies, suggest that utilizing 400 ppm PAA as a post-chill, pre-grind antimicrobial would be an effective intervention strategy in reducing all three pathogens firmly attached to the chicken skin used in the formulation of ground chicken without any negative organoleptic effect. PAA also will be an effective antimicrobial for preventing cross-contamination between the carcasses.

REFERENCES

1. American Meat Science Association. 2012. Meat color measurement guidelines. Available at: http://www.meatscience.org/uploadedFiles/Publications_Resources/AMSA%20Meat%20Color%20Guidelines%20Second%20Edition.pdf. Accessed 10 June 2014.

2. Barker, D., J. Lankhaar, and P. Stals. 2004. Primary processing of poultry. p. 90-102. *In* G. C. Mead (ed.), Poultry meat processing and quality, vol. 1. CRC Press, Washington D.C.

3. Batz, M. B., S. Hoffmann, and J. G. Morris. 2011. Ranking the risks: The 10 pathogen-food combinations with the greatest burden on public health. Emerging Pathogens Institute. University of Florida, Gainesville. p. 1-70.

4. Bauermeister, L. J., J. W. J. Bowers, J. C. Townsend, and S. R. McKee. 2008. Validating the efficacy of peracetic acid mixture as an antimicrobial in poultry chillers. *J. Food Prot.* 71:1119-1122.

5. Berrang, M. E., R. J. Buhr, and J. A. Cason. 2000. *Campylobacter* recovery from external and internal organs of commercial broiler carcass prior to scalding. *Poult. Sci.* 79:286-290.

6. Berrang, M. E., and J. A. Dickens. 2000. Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant. *J. Appl. Poultry Res.* 9:43-47.

7. Blaser, M. J., H. L. Hardesty, B. Powers, and W. L. Wang. 1980. Survival of *Campylobacter fetus* subsp. *jejuni* in biological milieus. *J. Clin. Microbiol.* 11:309-313.

8. Block, S. S. 2001. Peroxygen compounds. p. 191-199. *In* S.S. Block (ed.), Disinfection, sterilization, and preservation. Lippincott Williams & Wilkins, philadelphia, PA.

9. Brenner, F. W., R. G. Villar, F. J. Angulo, R. Tauxe, and B. Swaminathan. 2000. *Salmonella* nomenclature. *J. Clin. Microbiol.* 38: 2465-2467.

10. Brinez, W. J., A. X. Roig-Sagues, M. M. H. Herrero, T. Lopez-Pedemonte, and B. Guamis. 2006. Bactericidal efficacy of peracetic acid in combination with hydrogen peroxide against pathogenic and non pathogenic strains of *Staphylococcus* spp., *Listeria* spp., and *Escherichia coli. Food Control*. 17:516-521.

11. Bryan, F. L., and M. P. Doyle. 1995. Health risks and consequences of *Salmonella* and *Campylobacter jenuni* in raw poultry. *J. Food Prot*. 58:326-344.

12. Centers for Disease Conrol and Prevention. 2011. CDC estimates of foodborne illness in the United States. Available at: http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html. Accessed 14 June 2014.

13. Centers for Disease Conrol and Prevention. 2013. Surveillance for foodborne disease outbreaks — United States, 1998–2008. *Morb. Mortal. Wkly. Rep.* 62: 2-8.

14. Centers for Disease Control and Prevention. 2011. Multistate outbreak of *Salmonella* Hadar infections associated with turkey burgers. Available at: http://www.cdc.gov/salmonella/hadar0411/040411/index.html. Accessed 19 June 2014.

15. Centers for Disease Control and Prevention. 2013. *Listeria monocytogenes*. Available at: http://www.cdc.gov/pulsenet/pathogens/listeria.html. Accessed 29 May 2014.

16. Chantarapanont, W., M. E. Berrang, and J. F. Frank. 2004. Direct microscopic observation of viability of *Campylobacter jejuni* on chicken skin treated with selected chemical sanitizing agents. *J. Food Prot*. 67:1146-1152.

17. Chen, X. 2013. The effect of post-chill antimicrobials on *Salmonella, Campylobacter*, shelf life and quality attributes of ground chicken. T1-06. IAFP Annual Meeting Proceedings. International Association for Food Protection. Charlotte, North Carolina. 29 July.

18. Conner, D. E., and S. F. Bilgili. 1994. Skin attachment model for improved laboratory evaluation of potential carcass disinfectants for their efficacy against *Salmonella* attached to broiler skin. *J. Food Prot.* 57:684-688.

19. Cook, A., J. Odumeru, S. Lee, and F. Pollari. 2012. *Campylobacter, Salmonella, Listeria monocytogenes*, verotoxigenic *Escherichia coli*, and *Escherichia coli* prevalence, enumeration, and subtypes on retail chicken breasts with and without skin. *J. Food Prot.* 75:34-40.

20. D'Aoust J. Y., J. Maurer. 2007. *Salmonella* species. p. 187-202. *In* M. P. Doyle and L. R. Beuchat (ed.), Food microbiology: Fundamentals and frontiers, vol. 3. ASM press, Washington, D.C.

21. Davidson, G. R., A. L. Buchholz, and E. T. Ryser. 2013. Efficacy of commercial produce sanitizers against nontoxigenic *Escherichia coli* O157:H7 during processing of iceberg lettuce in a pilot-scale leafy green processing line. *J. Food Prot.* 76:1838-1845.

22. Deborde, M., and U. von Gunten. 2008. Reactions of chlorine with inorganic and organic compounds during water treatment—kinetics and mechanisms: A critical review. *Water Res.* 42:13-51.

23. Dychdala, G. R. 2001. Chlorine and chlorine compounds. p. 135-141. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation. Lippincott Williams & Wilkins, Philadelphia, PA.

24. Food Safety and Inspection Service, United State Department of Agriculture. 2013. *Salmonella* and *Campylobacter* verification program for raw meat and poultry products. Available at: http://www.fsis.usda.gov/wps/wcm/connect/ebf83112-4c3b-4650-8396-24cc8d38bf6c/10250.1.pdf?MOD=AJPERES. Accessed 28 June 2014.

25. Food Safety and Inspection Service, United State Department of Agriculture. 1996. Nationwide raw ground turkey microbiological survey. Available at: http://www.fsis.usda.gov/OPHS/baseline/rwgrturk.pdf. Accessed 1 June 2014.

26. Food Safety and Inspection Service, United State Department of Agriculture. 2013. Not ready-to-eat comminuted poultry exploratory sampling project. Available at: http://www.fsis.usda.gov/wps/wcm/connect/8489ea6c-c230-474f-a86e-2a32348d427b/06-14.pdf?MOD=AJPERES. Accessed 28 May 2014.

27. Food Safety and Inspection Service, United State Department of Agriculture. 2014. Progress report on *Salmonella* and *Campylobacter* testing of raw meat and poultry products, 1998-2013. Available at: http://www.fsis.usda.gov/wps/wcm/connect/885647f4-2568-48bf-ae5c-4a0d8279f435/Progress-Report-Salmonella-Campylobacter-CY2013.pdf?MOD=AJPERES. Accessed 13 June 2014.

28. Food Safety and Inspection Service, United State Department of Agriculture. Directive 7120.1. 2014. Safe and suitable ingredients used in the production of meat, poultry, and egg products. Available at: http://www.fsis.usda.gov/wps/wcm/connect/bab10e09-aefa-483b-8be8-809a1f051d4c/7120.1.pdf?MOD=AJPERES. Accessed 19 June 2014.

29. Holah, J. T. 1995. Progress report on CEN/TC 216 working group 3: Disinfectant test methods for food hygiene, institutional, industrial and domestic applications. *Int. Biodeter. Biodegr.* 36:355-365.

30. Holser, R. A. 2011. Water chemistry and antimicrobial treatment in poultry processing. *Internet J. Food Safety*. 13:339-341.

31. Izat, A. L., R. E. Hierholzer, J. M. Kopek, M. H. Adams, J. P. McGinnis, and M. A. Reiber. 1990. The use of propylene glycol and/or lactic acid in chill water for reducing *Salmonella* on broilers. *J. Food Process. Preserv.* 14:369-374.

32. Kemp, G. K., and K. R. Schneider. 2000. Validation of thiosulfate for neutralization of acidified sodium chlorite in microbiological testing. *Poult. Sci.* 79:1857-1860.

33. Kim, K. Y., J. F. Frank, and S. E. Craven. 1996. Three-dimensional visualization of *Salmonella* attachment to poultry skin using confocal scanning laser microscopy. *Lett. Appl. Microbiol.* 22:280-282.

34. Kim, K. Y., H. S. Lillard, J. F. Frank, and S. E. Craven. 1996. Attachment of *Salmonella* Typhimurium to poultry skin as related to cell viability. *J. Food Sci.* 61:439-441.

35. Kitis, M. 2004. Disinfection of wastewater with peracetic acid: A review. *Environ. Int.* 30:47-55.

36. Lecompte, J. Y., A. Kondjoyan, S. Sarter, S. Portanguen, and A. Collignan. 2008. Effects of steam and lactic acid treatments on inactivation of *Listeria innocua* surface-inoculated on chicken skins. *Int. J. Food Microbiol.* 127:155-161.

37. Lillard, H. S. 1980. Effect on broiler carcasses and water of treating chiller water with chlorine or chlorine dioxide. *Poult. Sci.* 59:1761-1766.

38. Lillard, H. S. 1985. Bacterial cell characteristics and conditions influencing their adhesion to poultry skin. *J. Food Prot.* 48:803-807.

39. Lillard, H. S. 1986. Distribution of attached *Salmonella* Typhimurium cells between poultry skin and a surface-film following water immersion. *J. Food Prot.* 49:449-454.

40. Lillard, H. S. 1988. Effect of surfactant or changes in ionic strength on the attachment of *Salmonella* Typhimurium to poultry skin and muscle. *J. Food Sci.* 53:727-730.

41. Lillard, H. S. 1989. Factors affecting the persistence of *Salmonella* during the processing of poultry. *J. Food Prot.* 52:829-832.

42. Lillard, H. S. 1989. Incidence and recovery of *Salmonella* and other bacteria from commercially processed poultry carcasses at selected pre-and post-evisceration steps. *J. Food Prot.* 52:88-91.

43. Lillard, H. S. 1993. Bactericidal effect of chlorine on attached *Salmonella* with and without sonification. *J. Food Prot.* 56:716-717.

44. Lynch, N. M., C. L. Kastner, and D. H. Kropf. 1986. Consumer acceptance of vacuum packaged ground-beef as influenced by product color and educational-materials. *J. Food Sci.* 51:253-255.

45. Martin, S. E. 2003. *Listeria monocytogenes.* p. 257-263. *In* M. E. Torrence, and R. E. Isaacson (ed.), Microbial food safety in animal agriculture: Current topics, vol. 1. Iowa State Press, Ames, IA.

46. McKee, S. R. 2011. *Salmonella* and *Campylobacter* control during poultry processing. International Poultry Scientific Forum. Atlanta, Georgia. Jan. 24-25.

47. McKee, S. R. 2012. *Campylobacter* update in poultry. 65th Annual Reciprocal Meat Conference. Fargo, ND. June 17-20.

48. McKee, S. R. 2012. *Salmonella* control in poultry processing. AMAS 65th Annual Reciprocal Meat Conference. Fargo, ND. Jun. 17-20.

49. Menconi, A., S. Shivaramaiah, G. R. Huff, O. Prado, J. E. Morales, N. R. Pumford, M. Morgan, A. Wolfenden, L. R. Bielke, B. M. Hargis, and G. Tellez. 2013. Effect of different concentrations of acetic, citric, and propionic acid dipping solutions on bacterial contamination of raw chicken skin. *Poult. Sci.* 92:2216-2220.

50. Miller, W. G., and R. E. Mandrell. 2005. Prevalence of *Campylobacter* in the food and water supply: Incidence, outbreaks, isolation and detection. p. 101-104. *In* J. M. Ketley, and M. E. Konkel (ed.), *Campylobacter*: Molecular and cellular biology, vol. 1. Horizon Scientific Press, U.K.

51. Nachamkin, I. 2007. *Campylobacter jejuni.* p. 237-244. *In* M. P. Doyle and L. R. Beuchat (ed.), Food microbiology: Fundamentals and frontiers, vol. 3. ASM Press, Washington, D.C.

52. Nagel, G. M., L. J. Bauermeister, C. L. Bratcher, M. Singh, and S. R. McKee. 2013. *Salmonella* and *Campylobacter* reduction and quality characteristics of poultry carcasses treated with various antimicrobials in a post-chill immersion tank. *Int. J. Food Microbiol.* 165:281-286.

53. Northcutt, J. K., and D. R. Jones. 2004. A survey of water use and common industry practices in commercial broiler processing facilities. *J. Appl. Poult. Res.* 13:48-54.

54. Northcutt, J. K., D. P. Smith, M. T. Musgrove, K. D. Ingram, and A. Hinton. 2005. Microbiological impact of spray washing broiler carcasses using different chlorine concentrations and water temperatures. *Poult. Sci.* 84:1648-1652.

55. Orth, R., and H. Mrozek. 1989. Is the control of *Listeria*, *Campylobacter* and *Yersinia* a disinfection problem? *Fleischwirtschaft.*

56. Pullar, J. M., M. Vissers, and C. C. Winterbourn. 2000. Living with a killer: The effects of hypochlorous acid on mammalian cells. *IUBMB life*. 50:259-266.

57. Qiao, M., D. L. Fletcher, D. P. Smith, and J. K. Northcutt. 2001. The effect of broiler breast meat color on pH, moisture, water-holding capacity, and emulsification capacity. *Poult. Sci.* 80:676-680.

58. Russell, S. M. 2010. *Salmonella* reduction calls for multi-hurdle approach. Available at: http://www.wattagnet.com/Salmonella_reduction_calls_for_multi-hurdle_approach.html. Accessed 22 May 2014.

59. Russell, S. M. 2006. Processing tip. Post-chill dip tanks are gaining popularity. Available at: http://www.poultry.uga.edu/extension/tips/documents/01200620p.pdf. Accessed 19 June 2014.

60. Sagripanti, J. L., C. A. Eklund, P. A. Trost, K. C. Jinneman, C. Abeyta, C. A. Kaysner, and W. E. Hill. 1997. Comparative sensitivity of 13 species of pathogenic bacteria to seven chemical germicides. *Am. J. Infect. Control*. 25:335-339.

61. Schambach, B. T. 2013. Use of a chill additive to reduce levels of *Salmonella* and *Campylobacter* during poultry processing. M.S. thesis. University of Georgia, Athens, GA.

62. Scharff, R. L. 2012. Economic burden from health losses due to foodborne illness in the United States. *J. Food Prot.* 75:123-131.

63. Schlosser, W., A. Hogue, E. Ebel, B. Rose, R. Umholtz, K. Ferris, and W. James. 2000. Analysis of *Salmonella* serotypes from selected carcasses and raw ground products sampled prior to implementation of the pathogen reduction; hazard analysis and critical control point final rule in the US. *lnt. J. Food Microbiol.* 58:107-111.

64. Swaminathan, B., D. Cababes, W. Zhang, and P. Cossart. 2007. *Listeria monocytogenes.* p. 457-460. *In* M. P. Doyle and L. R. Beuchat (ed.), Food microbiology: Fundamentals and frontiers, vol. 3. AMS Press, Washington, D.C.

65. Tamblyn, K. C., and D. E. Conner. 1997. Bactericidal activity of organic acids against *Salmonella* Typhimurium attached to broiler chicken skin? *J. Food Prot.* 60:629-633.

66. Tamblyn, K. C., D. E. Conner, and S. F. Bilgili. 1997. Utilization of the skin attachment model to determine the antibacterial efficacy of potential carcass treatments. *Poult. Sci.* 76:1318-1323.

67. Thomas, C. J., and T. A. McMeekin. 1982. Effect of water immersion on the microtopography of the skin of chicken carcasses. *J. Sci. Food Agr*. 33:549-554.

68. Tsai, L. S., J. E. Schade, and B. T. Molyneux. 1992. Chlorination of poultry chiller water: Chlorine demand and disinfection efficiency. *Poult. Sci.* 71:188-196.

69. Nayak, R. 2012. *Campylobacter jejuni*. p. 14-17. *In* K. A. Lampel, S. Al-Khaldi, and S.M. Cahill (ed.), Bad bug book, foodborne pathogenic microorganisms and natural toxins. College Park, MD.

70. United States Department of Agriculture,. 2013. National nutrient database for standard reference release 26. Basic report 05015, chicken, broilers or fryers, skin only, raw.

71. Wabeck, C. J., D. V. Schwall, G. M. Evancho, J. G. Heck, and A. B. Rogers. 1968. *Salmonella* and total count reduction in poultry treated with sodium hypochlorite solutions. *Poult. Sci.* 47:1090-1094.

72. Weidenkopf, S. J. 1953. Water chlorination. *U S Armed Forces Med J.* 4:253-261.

73. Black & Veatch Corporation. 2010. White's handbook of chlorination and alternative disinfectants, 5th ed. John Wiley & Sons, Inc., Hoboken, NJ.

74. Xiangwu, N., L. Yaguang, L. Hollar, Y. Yang, F. Hao, P. Millner, and D. Shelton. 2011. Chlorine stabilizer t-128 enhances efficacy of chlorine against cross-contamination by *E. coli* O157:H7 and *Salmonella* in fresh-cut lettuce processing. *J. Food Sci.* 76:218-224.

75. Yang, H., Y. B. Li, and M. G. Johnson. 2001. Survival and death of *Salmonella* Typhimurium and *Campylobacter jejuni* in processing water and on chicken skin during poultry scalding and chilling. *J. Food Prot.* 64:770-776.

76. Young, J. F., A. H. Karlsson, and P. Henckel. 2004. Water-holding capacity in chicken breast muscle is enhanced by pyruvate and reduced by creatine supplements. *Poult. Sci.* 83:400-405.

TABLE 5. Mean log CFU/g *Salmonella* recovered from ground chicken formulated with inoculated and antimicrobial treated broiler skin.

[1]Treatment	Storage period (Days) at 4℃			
	0	3	6	9
Positive Control	6.51±0.36^{A}	6.66±0.56^{A}	6.50±0.26^{A}	6.37±0.61^{A}
Water	5.79±0.21^{B}	5.74±0.32^{B}	5.69±0.09^{B}	5.58±0.47^{B}
T-128	5.83±0.27^{B}	5.82±0.40^{B}	5.65±0.26^{B}	5.34±0.49^{B}
Cl	5.88±0.29^{B}	5.82±0.53^{B}	5.51±0.37^{B}	5.66±1.17^{B}
Cl + T-128	5.80±0.17^{B}	5.83±0.16^{B}	5.52±0.95^{B}	5.43±0.61^{B}
PAA	4.43±0.67^{C}	4.50±0.46^{C}	4.43±0.20^{C}	4.29±0.09^{C}
PAA + T-128	4.46±0.87^{C}	4.49±0.98^{C}	4.40±0.33^{C}	4.27±0.42^{C}

[1]Positive control; Water; 0.5% T-128; Cl: 50 ppm chlorine; Cl+T-128: 50 ppm chlorine+0.5% T-128; PAA: 1,200 ppm peracetic acid; PAA+T-128: 1,100 ppm peracetic acid+0.5% T-128.
[A-C] Values within the columns with different superscripts represent significantly different among treatments by Duncan's test ($P \leq 0.05$).

TABLE 6. Mean log CFU/g *Campylobacter* recovered from ground chicken formulated with inoculated and antimicrobial treated broiler skin.

[1]Treatment	Storage period (Days) at 4℃			
	0	3	6	9
Positive Control	5.78±0.24^{A}	5.72±0.48^{A}	5.68±0.16^{A}	5.53±0.68^{A}
Water	5.11±0.36^{B}	5.04±0.34^{B}	5.01±0.51^{B}	4.74±1.15^{B}
T-128	5.16±0.62^{B}	4.97±0.51^{B}	4.99±0.49^{B}	4.75±0.09^{B}
Cl	5.04±0.30^{B}	4.85±0.47^{B}	4.74±0.83^{B}	4.55±1.15^{B}
Cl + T-128	4.84±1.15^{B}	4.72±1.16^{B}	4.69±1.17^{B}	4.58±1.26^{B}
PAA	3.47±0.97^{C}	3.31±0.22^{C}	3.21±0.57^{C}	2.94±0.79^{C}
PAA + T-128	3.55±0.80^{C}	3.51±0.71^{C}	3.25±1.01^{C}	3.09±1.03^{C}

[1]Positive control; Water; 0.5% T-128; Cl: 50 ppm chlorine; Cl+T-128: 50 ppm chlorine+0.5% T-128; PAA: 1,200 ppm peracetic acid; PAA+T-128: 1,100 ppm peracetic acid+0.5% T-128.
[A-C] Values within the columns with different superscripts represent significantly different among treatments by Duncan's test ($P \leq 0.05$).

TABLE 7. Mean log CFU/g *Listeria* recovered from ground chicken formulated with inoculated and antimicrobial treated broiler skin.

[1]Treatment	Storage period (Days) at 4 ℃			
	0	3	6	9
Positive Control	6.26 ± 0.79^{Ax}	6.51 ± 0.44^{Ax}	7.39 ± 0.89^{Ay}	8.05 ± 0.10^{Ay}
Water	5.50 ± 0.82^{Bx}	5.79 ± 0.60^{Bx}	6.88 ± 0.66^{ABy}	7.59 ± 0.38^{Az}
T-128	5.70 ± 0.38^{ABx}	5.78 ± 1.10^{Bx}	6.51 ± 0.15^{By}	7.72 ± 0.47^{Az}
Cl	5.61 ± 0.64^{ABx}	6.21 ± 1.17^{ABx}	7.37 ± 1.17^{Ay}	8.02 ± 0.87^{Ay}
Cl + T-128	5.57 ± 1.29^{ABx}	5.74 ± 1.33^{Bx}	6.55 ± 0.95^{By}	7.40 ± 1.49^{Az}
PAA	4.31 ± 1.46^{Cx}	4.23 ± 1.28^{Cx}	4.70 ± 1.75^{Cx}	5.70 ± 1.71^{By}
PAA + T-128	4.25 ± 0.35^{Cx}	4.23 ± 0.29^{Cx}	4.96 ± 0.53^{Cy}	5.42 ± 0.83^{By}

[1]Positive control; Water; 0.5% T-128; Cl: 50 ppm chlorine; Cl+T-128: 50 ppm chlorine+0.5% T-128; PAA: 1,200 ppm peracetic acid; PAA+T-128: 1,100 ppm peracetic acid+0.5% T-128.
[A-C] Values within the columns with different superscripts represent significantly different among treatments by Duncan's test ($P \leq 0.05$).
[x-y] Values within the rows with different superscripts represent significantly different over time by Duncan's test ($P \leq 0.05$).

TABLE 8. Chemical parameters of treatment solution before and after immersing inoculated skin and uninoculated breast meat for 16 s.

[1]Treatment	Total Cl (ppm)		Free Cl (ppm)		PAA (ppm)	
	Before	After	Before	After	Before	After
Water	0.6	0.4	0.7	0.4		
T-128	0.6	0.1	0.5	0.4		
Chlorine	51.5	22.1	52.9	1.8		
Cl + T-128	52.7	27.7	52.8	4.4		
PAA					1169.4	1180.1
PAA + T-128					1101.1	1105.2

[1]Positive control; Water; 0.5% T-128; Cl: 50 ppm chlorine; Cl+T-128: 50 ppm chlorine+0.5% T-128; PAA: 1,200 ppm peracetic acid; PAA+T-128: 1,100 ppm peracetic acid+0.5% T-128.

TABLE 9. Populations of pathogens (CFU/mL) recovered from post-chill antimicrobial rinses after 16 s treatment.

Treatment[a]	*S.* Typhimurium	*L. monocytogenes*	*C. coli*
Water	6.8×10^5	2.8×10^5	1.7×10^5
T-128	1.4×10^4	3.2×10^4	<0.2
Cl	<7.5 (3/3)[b]	<3.7 (3/3)	<1.0 (3/3)
Cl + T-128	<10 (3/3)	<1.4 (3/3)	<0.2 (0/3)
PAA	<0.2 (0/3)	<0.2 (0/3)	<0.2 (0/3)
PAA + T-128	<0.2 (1/3)	<0.2 (0/3)	<0.2 (0/3)

[1]Positive control; Water; 0.5% T-128; Cl: 50 ppm chlorine; Cl+T-128: 50 ppm chlorine+0.5% T-128; PAA: 1,200 ppm peracetic acid; PAA+T-128: 1,100 ppm peracetic acid+0.5% T-128.
[b]Obtained through enrichment (# Positive/# Total of replication) for all treatment except the water and T-128.

TABLE 10. pH and temperature of the treatment solution, before and after immersing inoculated skin and uninoculated breast meat for 16 s.

[1]Treatment	pH		Temperature (°C)	
	Before	After	Before	After
Water	7.50	7.57	7	8
T-128	2.31	2.54	8	9
Chlorine	6.00	5.16	8	9
Cl + T-128	2.33	2.47	8	8
PAA	2.94	3.16	9	10
PAA + T-128	2.41	2.34	8	7

[1]Positive control; Water; 0.5% T-128; Cl: 50 ppm chlorine; Cl+T-128: 50 ppm chlorine+0.5% T-128; PAA: 1,200 ppm peracetic acid; PAA+T-128: 1,100 ppm peracetic acid+0.5% T-128.

TABLE 11. pH and temperature of the chicken breast meat, before treatment and grinding and after antimicrobial treatment and grinding.

[1]Treatment	pH		Temperature (℃)	
	Before (Breast meat)	After (Ground meat)	Before (Breast meat)	After (Ground meat)
Negative Control	5.99	6.05	8	17
Positive Control	6.04	6.01	10	17
Water	6.09	6.15	6	17
T-128	5.84	6.24	7	17
Chlorine	5.75	6.08	8	16
Cl + T-128	5.53	5.96	8	19
PAA	6.11	6.12	8	17
PAA + T-128	6.03	5.99	5	16

[1]Positive control; Water; 0.5% T-128; Cl: 50 ppm chlorine; Cl+T-128: 50 ppm chlorine+0.5% T-128; PAA: 1,200 ppm peracetic acid; PAA+T-128: 1,100 ppm peracetic acid+0.5% T-128.

TABLE 12. Effect of antimicrobial treatments on CIE *L**, *a**, *b** of ground chicken during 9 days of storage at 4 ± 1℃.

	[1]Treatments	Storage period (Days)			
		0	3	6	9
[2]*L**	Negative Control	68.65	69.15	66.71	65.07
	Positive Control	69.38	69.32	68.49	66.70
	Water	67.39	67.70	66.80	65.74
	T-128	70.11	69.27	67.85	65.77
	Chlorine	69.60	68.69	69.63	69.32
	Cl + T-128	69.13	69.32	69.57	69.26
	PAA	69.71	69.66	70.19	69.39
	PAA + T-128	70.07	70.59	72.05	70.68
[3]*a**	Negative Control	7.32	7.41	7.98	7.77
	Positive Control	6.81	7.21	5.19	7.98
	Water	5.28	5.11	5.60	6.85
	T-128	7.04	6.75	7.94	8.31
	Chlorine	5.30	5.59	5.94	6.36
	Cl + T-128	6.38	6.52	4.29	7.35
	PAA	6.02	6.36	5.83	6.46
	PAA + T-128	4.21	4.09	3.45	4.03
[4]*b**	Negative Control	16.69	16.33	16.83	16.41
	Positive Control	16.17	15.98	15.92	16.58
	Water	13.95	13.98	14.31	15.67
	T-128	17.02	17.02	17.54	16.54
	Chlorine	13.79	13.76	14.59	14.65
	Cl + T-128	14.87	15.28	15.21	15.53
	PAA	15.21	15.66	15.40	15.75
	PAA + T-128	14.53	14.34	14.72	16.21

[1]Positive control; Water; 0.5% T-128; Cl: 50 ppm chlorine; Cl+T-128: 50 ppm chlorine+0.5% T-128; PAA: 1,200 ppm peracetic acid; PAA+T-128: 1,100 ppm peracetic acid+0.5% T-128.
[2] Where L*= 100 is white, L*= 0 is black
[3] Where a*=60 is red a*=-60 is green
[4] Where b*=60 is yellow b*=-60 is blue