

**Effectiveness of Several Antimicrobials Used in Parts Decontamination
Tank to Kill *Salmonella* and *Campylobacter* on Chicken Parts**
by

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Abstract

Research was conducted to determine the optimal contact time and effectiveness of antimicrobials (0.003% chlorine; 0.07% or 0.1% peracetic acid (PAA); or 0.35% or 0.60% Cetylpyridinium Chlorine (CPC)) added to a parts decontamination tank to reduce *Salmonella* and *Campylobacter* on chicken parts as well as the sensory attributes. Drumsticks were used for the contact time study and chicken parts (including breast, thigh, wings and drumsticks) were used in the parts decontamination tank study. Samples were inoculated with *Salmonella* Typhimurium (10^8 cfu/mL) and *Campylobacter jejuni* (10^8 cfu/mL) to test the effectiveness, and sensory evaluation was conducted by untrained panelists using both skin-on parts (drumette) and skin-off parts (breast meat).

Different contact times did only ($p < 0.05$) affect the effectiveness of 0.06% CPC to reduce the level of *Salmonella*, other antimicrobials were not affected ($p > 0.05$) by contact time in reducing either *Salmonella* or *Campylobacter*. The effectiveness of antimicrobials used in the study was significantly different ($p < 0.05$). Treatment with 0.35% or 0.60% CPC was found to be most effective in decreasing *Salmonella* and *Campylobacter*, and the higher concentration was more effective. While 0.07% and 0.1% PAA were the second effective ones, however, there were no difference between concentrations. Chlorine at 0.003% was least effective.

The sensory attributes that were affected ($p < 0.05$) were texture, juiciness, and overall acceptability, which were perceived as lower scores for 0.60% CPC and 0.1% PAA. For appearance and flavor which are the most important attributes for poultry meat were not affected, untrained panelists rated as “like moderately” to “like slightly”, they did not notice differences ($p > 0.05$) among different treatments.

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CHAPTER I

GENERAL INTRODUCTION

Poultry is the most popular consumed meat in the US, and the consumption has been steadily increasing through the years. Additionally, consumer demand has changed from whole birds to chicken parts over the years, because consumers want products that are ready to cook and easy to prepare. In 1962, 83% of broilers were marketed as whole birds, while 15% were marketed as parts, while the increasing trends have led to 12% of broilers consumed as whole birds and 42% as parts in 2009 (National Chicken Council, 2011).

Nowadays, consumers are more concerned about food safety than ever before. According to The Centers of Disease Control and Prevention (2011), 48 million people get ill in the US because of foodborne diseases, which includes 128,000 hospitalizations and 3,000 deaths each year. By using the enhanced cost-of – illness model, Scharff (2012) calculated the annual cost of illness is \$77.7 billion.

Salmonella and *Campylobacter* are the two pathogens commonly associated with poultry meat. These two pathogens are also in the top 3 food-borne pathogens responsible for the annual disease burden costs. *Salmonella* causes about \$3.3 billion in costs and *Campylobacter* causes about \$1.7 billion in costs

every year due to illness (Batz et al., 2011). Poultry meat can be contaminated by a variety of foodborne pathogens that may cause human illness by ingestion because of improper handling of raw meat, and undercooking or mishandling of the cooked meat. Hazard Analysis and Critical Control Points (HACCP) was introduced into the poultry plant and Quantitative Risk Assessment (QRA) is being developed to help control food safety. Increasing consumption of poultry products makes it a higher risk of relevant food-borne illness, and it is necessary to identify potential source of contamination and apply intervention strategies that will prevent or minimize the risk of contamination during processing. Over the past several years, an increasing number of poultry processing plants are using a post-chill intervention step to help in reducing microbial levels (McKee, 2011). Acidified sodium chlorite, trisodium phosphate, chlorine dioxide, hypochlorous acid, some organic acids, peracetic acid and cetylpyridinium chloride are now commonly used chemicals.

The Raw Chicken Parts Survey (2012) conducted by USDA-FSIS estimated the percent positive levels of *Salmonella* and *Campylobacter* and indicator bacteria on raw chicken parts. The results showed that there was no significant difference with skin-on versus skin-off parts of chicken when testing *Salmonella*. However, for *Campylobacter* the skin-on parts had a significantly higher number of positive results than skin-off parts. During this survey, FSIS tested both

Salmonella and *Campylobacter* simultaneously to determine the occurrence of pathogens on processed poultry parts. The results indicated that chicken neck parts(55%) had the highest percent positive results for both *Salmonella* and *Campylobacter*. The lowest percent positive results for *Campylobacter* is breast (16.1%), which is 13.5% for skinless breast and 25.6% for skin-on breast.

The Food Safety and Inspection Service (FSIS) have new performance standards for *Salmonella* and *Campylobacter* which are 4.3% and 10.4% (FSIS, 2012), respectively. For young chicken, the positive percent of *Salmonella* and *Campylobacter* are 5.9% and 10.6%, respectively. However, the percent positive samples in chicken parts is much higher, 26.3% for *Salmonella* and 21.4% for *Campylobacter* (RCPBS, 2012). It is possible that chicken parts are becoming cross-contaminated through cut-up and debone processes. Further intervention strategies are needed in a multi-hurdle approach to address the cross-contamination of poultry parts and to control *Salmonella* and *Campylobacter* in poultry parts. One new technology available is a parts decontamination tank that could help to reduce the bacteria levels on chicken parts after the cut up process. Using the parts decontamination tank in the poultry processing plant is one of the common choices to reduce the bacteria load on chicken parts. Similar to the finishing chiller, it has a short dwell time and uses a small volume of water with high concentration of antimicrobials. Thus, it is efficient and economic to apply antimicrobials using the parts decontamination tank in a poultry processing plant. This study was designed to determine the optimal contact time needed for each of the antimicrobials to be tested, and then six treatments (water, 0.003%

chlorine, 0.07% peracetic acid (PAA), 0.1% PAA, 0.35% cetylpyridinium chloride (CPC) and 0.6% CPC) would be tested in the parts decontamination tank to determine the effectiveness of antimicrobials on reducing *Salmonella* and *Campylobacter* on chicken parts. Additionally, the effect of the antimicrobials on the organoleptic properties was assessed based on the consumer's acceptability of treated chicken by sensory evaluation.

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CHAPTER II

LITERATURE REVIEW

Poultry Consumption

The US poultry industry is the world's largest producer of poultry, while in 2010 the United States produced 8.6 billion broilers weighing 49.2 billion pounds. Among this production, the top producers were Georgia, Arkansas, and Alabama, with liveweights of 6.9, 5.9 and 5.8 billion pounds, respectively (National Chicken Council, 2010). About 20% of chicken production is for export (Global Poultry Trends, 2010). Currently, Brazil is the number one exporter of chicken, from 2000 to 2007, their exports of chicken meat increased by 3 million tons, which was higher compared with the exports of any other countries (Global Poultry Trends, 2010). According to Food and Agricultural Policy Research Institute, in the next 10 years, Brazil will likely stabilize the market with approximately 3.6 million tons of poultry exported each year, while exports from the US are expected to rebound to approximately 3.5 million tons of poultry exports (Global Poultry Trends, 2010). The majority of the leg portions are exported to Russia and the paws to China. Russia imports approximately 700,000 tons of leg quarters each year and China imports nearly 500,000 tons of paws and approximately 250,000 tons of leg quarters yearly. This accounts for

around 40% of US chicken meat export (Global Poultry Trends, 2010). However, the US still imports chicken from Mexico, Canada, and Cuba, even though it is not a significant chicken–importing country. Mexico is the main exporter for the US. In 2000, the US imported 200,000 tons of chicken meat and the amount increased to more than 500,000 tons in 2010 (Global Poultry Trends, 2010).

According to USDA Economic Research Service, US consumption of poultry meat (broilers, other chicken, and turkey) is considerably higher than beef or pork, but less than total red meat consumption. Statistics from USDA data shows that, in 1965, the total poultry consumption was only 41.2 pounds, which included 33.7 pounds of chicken and 7.5 pounds of turkey. While in 2011, the amount was doubled with a total poultry consumption of 100.4 pounds, including 84.2 pounds of chicken and 16.1 pounds of turkey (National Chicken Council, 2013). This number increased until 2006, with a fluctuating period from 1971 to 1975. The consumption per capita of chicken dropped in 2007, 2008, and 2009 because of the economic recession, which has not happened since the USDA started keeping records for broiler consumption.

The broiler market is continually changing: the trend has shifted from a whole bird market to a market for cut-up parts and further processed products. Before 1950, more than 50% of the broilers were consumed as whole birds, while less than 40% were sold as cut-up parts for consumption, with only a small portion of

poultry were used for further processing. In 1995, only 10% of poultry was sold as whole birds, while 53% was sold as cut-up parts, 36% was for further processing. In 2009, cut-up parts and further processing accounted for approximately 90% of the total poultry consumption (National Chicken Council, 2011).

Introduction: *Salmonella* and *Campylobacter* in poultry

Poultry is the second most popular meat in the world, which accounts for about 30% of meat production with pork at 38% of the total meat production. The meat and poultry industry encompass a large part of US agriculture, while poultry is the most consumed meat in the US and consumption is continuously increasing. According to the data from USDA, the annual poultry meat consumption per capita in the US has increased from 27 pounds in 1970 to 60 pounds in 2005. From the years 1959 to 2005, the only time period that chicken consumption dropped a little was between 1973 to 1975 (Daily Livestock Report, 2011). The consumption of chicken has changed through these years, driven by, consumer trends for more convenient options.

The task of the USDA, Agricultural Research Service, and Poultry Microbiological Safety Research Unit, is to prevent commensal intestinal colonization of chickens by developing technology. *Salmonella* and *Campylobacter* are the two pathogens commonly associated with poultry meat and poultry products are frequent vehicles of these bacteria. They are also in the top 3 food-borne pathogens responsible for the annual disease burden costs. *Salmonella* causes about \$3.3 billion in costs and *Campylobacter* causes about 1.7 billion in costs every year

due to illness in the US (Batz et al., 2011). The route of contamination is related to the survival or growth during production, transportation, processing and preparation (Bryan and Doyle, 1995). Broilers can also be contaminated on farms and the bacteria are spread during transportation and processing. Before slaughtering, the live birds can be highly contaminated with *Salmonella* (Kotula and Pandya, 1995) and *Campylobacter* (Berrang and Dickens, 2000) on the skin, feathers, feed, and viscera, and it could become more prevalent when transporting the birds to plants. Contamination can occur during scalding, picking, eviscerating and chilling, with picking usually causing the most cross - contamination. After processing, transportation and storage of the chicken is needed. During these stages, the environmental condition is important, elevated temperature or moisture can lead to a change in meat quality which is related to growth of spoilage organisms or pathogens.

According to Raw Chicken Parts Survey (2012) conducted by The Microbiological Analysis and Data Branch, Science Division, Office of Public Health Science to estimate the percent positive, levels of *Salmonella* and *Campylobacter*, and indicator bacteria on raw chicken parts, 26.32% tested *Salmonella* positive, while 21.39% were *Campylobacter* positive. Chicken part samples (4 lbs) were randomly selected from production lines before packing. Samples were placed into a sterile bag with 400 ml Buffered Peptone Water (BPW) and shaken vigorously according to the Nationwide Raw Chicken Parts Microbiological Baseline Data Collection Program (USDA-FSIS, 2010). The BPW rinsate was poured into the screw-top container which was provided by

FSIS, and kept at 4 °C. The results showed that there was no significant difference with skin-on versus skin-off parts of chicken when testing *Salmonella*. However, for *Campylobacter* the skin-on parts had a significantly higher number of positive than skin-off parts. During this survey, USDA Food Safety Inspection Service (FSIS) tested both *Salmonella* and *Campylobacter* simultaneously to determine the prevalence of these pathogens on chicken parts. The outcome demonstrated that chicken necks (55%) had the highest percent positive for both *Salmonella* and *Campylobacter*. The lowest percent positive for *Campylobacter* is breast (16.1%), of which 13.5% for skinless breast and 25.6% for skin-on breast.

***Salmonella* spp**

Salmonella history and characteristics

Salmonella, which is a type of bacterium discovered by Theobald Smith in 1885, was named after an American pathologist whose name is Daniel Elmer Salmon. It is a genus of rod-shaped, gram-negative bacteria, and is also non-spore-forming (Jay et al., 2005). Because of the thin cell walls, gram-negative bacteria do not retain the crystal violet dye used in the gram staining. At the beginning, clinical symptoms were considered to name different *Salmonella* species, e.g., *Salmonella* Typhimurium (mouse typhoid fever), *S. Choleraesuis* (hog cholera) (F. Kauffmann, 1941). Later on, they started to name new strains according to the location where it was found. After that, due to the molecular research which supported the hypothesis that *Salmonella* had only one species (Minor and Popoff, 1987), *S. enterica*, and the serovar were classified into six groups

(Reeves et al., 1989). Currently, there are two recognized species: *S. bongori* and *S. enterica*, with six main subspecies: *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI) (Public Health Agency of Canada, 2011).

S. bongori has more than 2500 strains (serovars). Two of these strains are *Salmonella enterica serovar Paratyphi A* (causes paratyphoid fever) & *Salmonella enterica serovar Typhimurium* (causes salmonellosis). *S. bongori* has fewer strains and causes enteritis. *S. bongori* is the only subspecies that can cause illness in warm-blooded animals (Porwollik et al., 2004).

Salmonella is found worldwide in cold- and warm-blooded animals (including humans), and in the environment, including soil and water, which has been contaminated with animal excrement. It is often harmful to a host, which is the case for many of the *Salmonella* bacteria. When the conditions such as humidity, pH and temperature are appropriate, *Salmonella* can live for several weeks in water and several years in soil (Todar, 2009). *Salmonella* bacteria are zoonotic, meaning they can be transmitted from humans to animals or from animals to humans. Thus, it is very important to avoid contamination of the environment (Kaplan, 2002).

Growth characteristics

Salmonella is a kind of facultative anaerobic bacteria. It can grow in any food like beef, eggs, milk and poultry. The temperature for *Salmonella* growth is 7 to 48 °C

(Lawley, 2013). The optimal temperature for *salmonella* growth is between 35 and 37 °C. pH range is 4.2 to 9.5, while the optimum is between 7.0 and 7.5 (FDA, 2013). The lowest reported *Aw* for *Salmonella* growth is 0.93 (Shaw, 2013).

Salmonella serotypes

According to The Nationwide Microbiological Baseline Data Collection Program for raw chicken parts (2012), *S. Kentucky*, *S. Enteritidis*, *S. Typhimurium*, *S. Heidelberg*, and *S. Thompson* are the top 5 *Salmonella* serotypes in poultry parts, while *S. Enteritidis*, *S. Typhimurium*, and *S. Newport* are the top 3 *Salmonella* serotypes which have the highest number and incidence of *Salmonella* infections (CDC, 2011).

Foodborne *Salmonella* infections

Salmonellosis, which is an infection caused by the bacteria *Salmonella*, is the most common foodborne disease in the US. According to the Centers for Disease Control and Prevention (CDC), salmonellosis causes an estimated 1.4 million cases of foodborne illness and more than 400 deaths annually in the United States. In a research conducted by Blaser and Newman (1982), they showed that less than 1000 organisms could cause a *Salmonella* infection.

Poultry products are the most important sources of human infection with *Salmonella* serotypes in the United States, *Salmonella* is among the top 5, which

take roughly 90% of the cost of illness, each year illness caused by *Salmonella* costs \$3.3 billion (Sandra et al., 2012).

Salmonella causes two diseases in human: Enteric, or typhoid fever, and gastroenteritis.

Gastroenteritis, more commonly known as food poisoning, is the most common and is caused by *Salmonella* Enteritidis. This form of salmonellosis usually appears 12 to 72 h after ingesting the contaminated food and lasts from 4 to 7 d according to the CDC (2012). Symptoms include fever, abdominal cramps, diarrhea, which usually subside on their own without medical treatment. The bacteria can cross from the intestines into the bloodstream causing serious illness or even death if not treated with antibiotics.

Salmonella infections usually begin through a fecal-oral route. Once ingestion of the contaminated food or water, *Salmonella* coming from a natural environment need to pass through hostile environment of the stomach which has acidic conditions (Foster and Spector, 1995). The low acid will cause acid stress responses which are regulated by certain regulators of the bacteria. *Salmonella* can colonize the small intestine, cecum, and colon after it moves beyond the stomach. Adhesion is the first step in colonization. The adhesions are fimbriae which are usually arranged evenly but can be expressed in a polar orientation (Edwards and Puente, 1998). When necessary cells will produce adhesions while the expression will shut off when adhesions are not needed. Finally, following attachment, mucosal invasion will occur.

Outbreaks

In recent years, there have been a number of confirmed food-borne outbreaks of salmonellosis caused by *Salmonella*.

A recent outbreak resulting in 9 infected persons was reported from Tennessee on January 24, 2014. Due to possible *Salmonella* Heidelberg contamination, Tyson Foods recalled approximately 33,840 pounds of mechanically separated chicken products (CDC, 2014; USDA-FSIS, 2014). Another outbreak was posted on January 16, 2014. It was caused by multidrug-resistant *Salmonella* Heidelberg from Foster Farms Brand Chicken. 430 persons from 23 states and Puerto Rico were infected, 38% of them were hospitalized and no death was found (CDC, 2014). In August of 2013, 316 persons from 37 states were infected by *S. Typhimurium* in live poultry, which caused 51 hospitalization (CDC, 2013). Another outbreak linked to live poultry happened in July causing 23 hospitalizations (29% of the total infected persons) in 26 states, because of *Salmonella* Infantis, *Salmonella* Lille, *Salmonella* Newport, or *Salmonella* Mbandaka (CDC, 2013). Earlier in July, an outbreak linked to chicken caused 134 persons infected, and 33 of them were hospitalized (CDC, 2013).

There were several outbreaks involving live poultry in 2012 as well. One was caused by *S. Hadar* which lead to a total of 46 persons infected all over 11 states. 13 ill persons were hospitalized and no deaths were reported. Ill persons ranged in age from less than 1 year to 78 years, with a median age of 33 years, and 30% of ill persons were children 10 years old or younger (CDC, 2012). An

earlier one was caused by *Salmonella* Montevideo. In this case, 93 persons were infected from 23 states and Puerto Rico. 21 ill persons were hospitalized. One death was reported in Missouri, but *Salmonella* infection was not considered a contributing factor in this person's death. Ill persons ranged in age from less than 1 year to 83 years, with a median age of 20 years. Thirty-eight percent of ill persons were children 10 years of age or younger. Forty-eight percent of ill persons were female (CDC, 2012). *S. Infantis*, *S. Newport*, and *S. Lille* caused another case involving live poultry. A total of 195 persons from 27 states were infected. 34% of ill persons were hospitalized. Two deaths were reported. Ill persons ranged in age from less than 1 year to 100 years, with a median age of 32 years. Fifty-four percent of ill persons were female (CDC, 2012). In 2011, there were several outbreaks related to poultry products. A human *Salmonella* Heidelberg infection caused by Kosher broiled chicken livers from Schreiber Processing Corporation led to a total of 190 illnesses within 6 states (CDC, 2011). Another outbreak was from ground turkey caused by human *Salmonella* Heidelberg, 136 persons from 34 states were infected, including 37 hospitalized and one death (CDC, 2011).

Almost any kind of food has the potential to carry *Salmonella* which can infect humans, proper intervention from flocks to processing plant and final process is becoming more and more urgent.

Campylobacter spp

Campylobacter characteristics

It has been more than 100 years since *Campylobacter* infections have become one of the public health concern. In 1886, *Campylobacter* was first found by Escherich in stool samples of diarrhea from children (Samie et al., 2007). In 1913, *Campylobacter* (called related *Vibrio*) was identified by McFaydean and Stockman in aborted sheep fetal tissues. In 1957, King described the first found by Escherich, and in 1972, *Campylobacter* was first isolated from stool samples of patients with diarrhea clinically. Testing stool specimens for *Campylobacter* was allowed by development of selective growth media in the 1970s. Shortly after, *Campylobacter* species were established as common human pathogens (Altekruse et al., 1999).

Campylobacter is a genus of bacteria that are gram-negative, spiral, and microaerophilic. It grows best at 42°C (107°F) and low oxygen concentrations. Because of these characteristics, the intestines of warm-blooded birds and mammals have become the normal habitat for *Campylobacter*. There is wide diversity in the genus while *Campylobacter jejuni* and *Campylobacter coli* are the organisms responsible for approximately 95 percent of *Campylobacter*-attributed disease in humans (Lastovica and Skirrow, 2000).

Foodborne *Campylobacter* infections

Since 1992, food remains the most common vehicle for the spread of *Campylobacter*, and chicken is the most common food implicated. When a

person is infected and develops symptoms, the illness is called Campylobacteriosis. Fecal-oral, ingestion of contaminated food or water, and the consumption of raw meat are the most common routes of transmission (Stern, 1988). According to Kothary and Babu (2001), *Campylobacter jejuni* could cause an infection with as low as 500 organisms ingested.

The symptoms of *Campylobacter* infections were described in 1886 in infants by Theodor Escherich (Samie et al., 2007). It produces diarrhea, cramping, fever and abdominal pain. The diarrhea can be either inflammatory or blood. Although most cases of Campylobacteriosis are self-limiting, some infected people even do not have any symptoms, for some people the illness lasts more than a week. However, such infections can also be life-threatening, especially when other diseases like cancer, liver and, and immuno-deficiency diseases are present. The use of antibiotics, on the other hand, is controversial. Symptoms typically last for five to seven days (CDC, 2013).

Outbreaks

In 2006, FoodNet reported 5,712 confirmed cases of *Campylobacter* infections to the CDC. 321 more cases were reported in 2009, which indicates that in every 100,000 persons 13.03 would get infected in the United States (About *Campylobacter*, 2010).

Campylobacter is among most common causes of diarrheal illness in the United States. According to the CDC (2011) estimates of foodborne illness in the United States, 845,024 illness was estimated for *Campylobacter*, which takes 9% of

domestically acquired foodborne illnesses. Hospitalization and death were estimated 15% and 6%, respectively (CDC, 2013).

Regulations and prevention programs

Experts in the area of food safety estimated that in the United States, there are 76 million illnesses, 325,000 hospitalizations and 5000 deaths every year, which indicates a significant economic burden caused by foodborne illnesses (Dewaal, 2003). According to Batz and others (2012), *Salmonella* and *Campylobacter* are among 14 major foodborne pathogens in the US, which cause more than 95% of illnesses, hospitalizations, and deaths.

To improve the safety of poultry meat supply, there are several approaches that can be taken.

Firstly, strengthening the poultry inspection system. Inspection programs are designed to ensure the product safety for the consumers. Different forms of inspections are performed during poultry production depending on their way to be sold and consumed.

Secondly, educating consumers, retailers, and foodservice workers, and promoting safe food handling. As there are many ways to reduce contamination of the products, consumers, retailers, and foodservice workers can make an effort to reduce the illnesses associated with contaminated poultry products. Remember to chill or keep the product cool to help control microbial growth during processing, transportation, sale and storage. Cooking the food to a proper

temperature will kill the pathogen residue on the product. For example, *Salmonella* can be eliminated when the internal temperature of meat is 165 °F for 15 seconds.

Then, irradiating poultry products. Irradiation is the process which exposes products to radiation. It kills the cells bacterial pathogens that could be present in the products. However, both FDA and FSIS approval are required for irradiating foods. Compared with antimicrobials, it has no significant impact on the appearance of food.

Last but not least, using market-oriented approaches to food safety: labeling, branding, legal incentives, and providing food safety information about products and production methods.

Poultry meat can be contaminated by a variety of foodborne pathogens that may cause human illness by ingestion because of improper handling of raw meat, undercooking or mishandling of the cooked meat. As the emergent need for a systematic and universally applicable method to control food safety, HACCP was introduced into poultry plant and Quantitative Risk Assessments (QRA) are being developed. The HACCP system in poultry processing plants is designed to take care of those zoonotic agents that are not detectable by conventional meat inspection procedures. The system brings evident benefits in optimizing plant sanitation. Currently, it is being applied in two different situations worldwide: in the US, carcass decontamination is gradually achieved while carcasses pass through the processing line and are finally chilled in peracetic acid treated water.

Chemical-rinse treatments can be used for further reduction of microbial contamination. The second option, processors in the European Union (EU) are not allowed to use super-chlorinated water in processing or chilling, which provides an important washing effect (Mbata, 2005).

A program designed and performed by the FSIS was conducted to estimate the percent positive and level of microbiological pathogens and indicator bacteria on raw chicken carcasses sampled between July 2007 and June 2008. Broiler chicken carcasses (6,550) from 182 companies that slaughtered young chicken and produced whole carcasses under federal inspection were sampled. Samples were taken at both re-hang and post-chill locations within the plant and were collected from two different shifts. Samples were evaluated to assess the percent positive rate and levels of multiple pathogenic organisms including *Salmonella* and *Campylobacter*. The presence and number of these bacteria were compared to determine if significant differences existed between samples taken at re-hang and post-chill and during the separate shifts. Reduction in the percent positive rate was observed for both *Salmonella* (Post-Chill, 5.19%) and *Campylobacter* (Post-Chill, 10.66%). These results demonstrate that the poultry industry is doing an excellent job using intervention strategies to reduce pathogens during processing (The Poultry Site, 2009).

Antimicrobial Intervention Strategies in Poultry Processing

Increasing consumption of poultry products increases the risk of food-borne illnesses due to poultry, and it is necessary to identify potential sources of

contamination and apply intervention strategies that will prevent or minimize the risk of contamination during processing. Antimicrobials are one good example of a processing aid utilized in chicken processing. An antimicrobial is an agent that kills microorganisms or inhibits their growth. The US Poultry and Egg Association conducted a survey of the poultry industry in February of 2006 and found that following chemicals are using in the poultry industries: (1) acidified sodium chlorite (Sanova® - 33%), (2) chlorine dioxide (numerous companies – 15%), (3) hypochlorous acid (Zentox and TOMCO – 9%), (4) organic acids (6%), (5) peracetic acid (FMC 323 or Parasafe and Inspexx 100 - 5%), (6) cetylpyridinium chloride (Safefoods Cecure® - 3%) (Scott Russell, 2011).

(1) Acidified sodium chlorite (ASC)

The safety of acidified sodium chlorite (ASC) being used as a surface treatment antimicrobial agent was evaluated at the 68th meeting of Joint FAO/WHO Expert Committee on Food Additives. ASC is intended to be used as a spray or dipping solution for poultry carcasses and parts. ASC (500 to 1200 ppm) is used with acids that are general regard as safe (GRAS) to adjust the solution pH between 2.3 and 2.9. The final sodium chlorite concentration should not exceed 1200 mg/kg and the chlorine dioxide concentration should not exceed 30 mg/kg. It can be used in poultry chiller water as well, the sodium chloride concentration should be adjusted to 50 to 150 ppm in the chiller. The contact is usually several minutes at temperatures between 0 and 15 °C (Appendix 7120, 2013). It is classified as a “no-rinse” food grade sanitizer which has no corrosive actions at recommended concentrations. This antimicrobial can be used as a spray or dip

treatment on whole carcasses or parts, sausages or deli meats before or after chilling. ASC can also be used to treat poultry carcasses in a pre-chiller and chiller water at relatively low levels, where poultry carcasses are submerged (Rao, 2007).

The antimicrobial action effect of ASC is derived from chlorous acid, which is a very strong oxidizing agent and the level of chlorous acid is determined by the pH of the solution. Chlorous acid is uncharged, which allows it to be able to disrupt the permeability of the outer membrane of bacterial cell walls and penetrate them to disrupt protein synthesis (USDA, 2002b). Chlorous acid is thought to help proton leakage into cells and thereby increase the energy output of the cells to maintain their normal internal pH. The effect also adversely affects amino acid transport (Taormina, 2012).

(2) Chlorine dioxide

Chlorine dioxide is typically used in poultry processing in the United States either as sprays or washes for on-line reprocessing, or added to the chiller water to limit the potential for microbial cross-contamination. It is a synthetic yellowish green gas with chlorine-like pungent odor, which is the reason for the unsuccessful attempts to introduce the chemical in the industry in the early years. It is hard to control the level of ClO_2 during use, and gas off occurred frequently. Chlorine dioxide is an oxidizing biocide which kills microorganisms by directly acting on the cellular membrane and through disruption of fundamental cellular processes (USDA, 2002a). According to US regulations, chlorine dioxide may be used as an

antimicrobial agent in water used in poultry processing in an amount not to exceed 3 ppm residual chlorine dioxide (21 CFR 173.300).

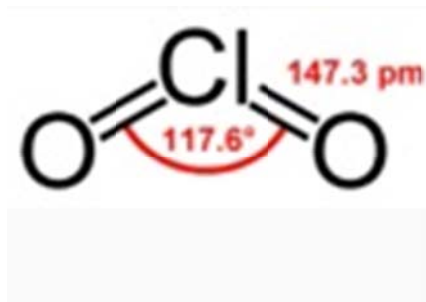


Figure-1 Chlorine dioxide

At high concentrations, cell wall is broken by chlorine dioxide to deactivate bacteria. Once chlorine dioxide has contact with the bacterial cell, the reaction will occur at once. Certain membrane proteins can react with chlorine dioxide and change the permeability of the cell membrane, or even damage the cell wall. While at lower concentrations, the outer membrane permeability is disrupted. With the ability to penetrate bacterial cell walls, protein synthesis is disrupted by its reaction with amino acids and nucleotides. This reaction stops the cell from producing proteins, which will kill the cell (ESFA, 2008).

(3) Hypochlorous acid

Hypochlorous acid (HClO) is highly unstable when isolated in a pure form, thus, it can only exist in solution as a weak acid. However, its strong oxidizing properties allow it to be used as a bleach and disinfectant. It is obtained by dissolving chlorine in water, or by reacting chlorine with mercury (II) oxide to create a pure form. It can be used on poultry carcasses as a spray or in chiller water which can

not exceed concentration of 50 ppm free available chlorine. Additionally, 20 ppm free available chlorine can be used for reprocessing contaminated poultry carcasses (Appendix 7120, 2013). A solution with a pH value between 5 and 6 is usually optimal when disinfecting, because when the solution pH is higher than optimal, hypochlorous acid breaks down forming hypochlorite ions, which will not function as an antimicrobial. This compound needs to be handled carefully to prevent it from gassing off which can occur when it is mixed with the wrong chemicals.

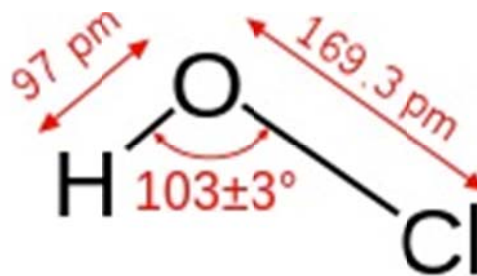


Figure – 2 Hypochlorous acid

(4) Organic or inorganic acids

It is unquestionable that acids can kill bacteria, however, they must be closely monitored to ensure that the contact time with the skin of the carcasses is appropriate so they do not create any product defects. In poultry plants, organic acids such as lactic, acetic, and citric acid can be used as a mist, fog, or small droplet rinse as part of a carcass wash applied pre-chill at concentration of 2.5% (Appendix 7120, 2013). Frequently, after being treated on the carcass with an acid, bacteria become acid stressed. These organisms become hard to recover when doing efficacy studies. This does not mean that the bacteria were killed and

will not be discovered by the USDA. Thus, when using acids, make sure that adequate neutralization and recovery steps are used during microbiological analysis or inaccurate results will be obtained (Scott, 2011).

(5) Peracetic acid

Peracetic acid (PAA) is an equilibrium mixture of an organic acid (acetic acid), an oxidant (hydrogen peroxide) and peracetic acid in a water solution. It is a bright colorless liquid with a pungent odor and a low pH value. PAA is an ideal antimicrobial agent because of its high oxidizing potential. It can be used as a spray, rinse, dip, in chiller water or scald water for poultry carcasses and parts, which requires the concentration of peracetic acid not to exceed 2000 ppm (Appendix 7120, 2013).

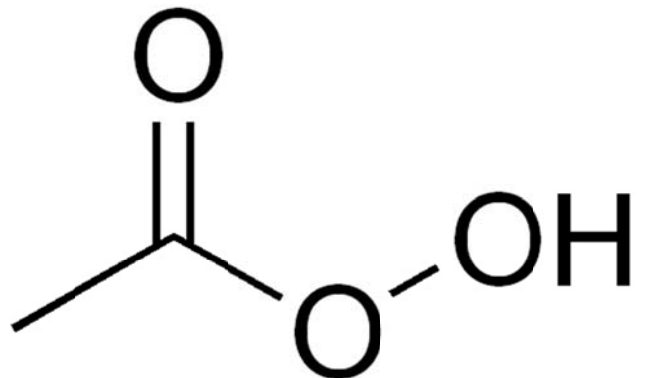


Figure – 3 Peracetic Acid

It kills microorganisms by oxidation and subsequent disruption of their cell membrane. It can damage virtually all types of large molecule weight substance associated with a microorganism: carbohydrates, nucleic acids, lipids and amino acids, which will eventually lead to cell lysis and microbial death. It also breaks

down in food and the residues (acetic acid and hydrogen peroxide) are safe and environmentally friendly, thus, it does not need a rinse procedure following application (EnviroTech, 2013). It can be used over a wide range of temperatures (0 to 40 °C), wide pH ranges (3.0 to 7.5), in clean-in-place (CIP) processes, in hard water conditions, and is not affected by protein residues. Temperature is also important, PAA is more effective when the pH value is 7 than at a pH range between 8 and 9. At pH 7 and a temperature of 15 °C five times more peracetic acid is required to affectively deactivate pathogens than at a temperature of 35 °C (LennTech, 2013).

(6) Cetylpyridinium chloride

Cetylpyridinium chloride (CPC 1-hexadecylpyridinium chloride) has been shown to have antimicrobial effects in decontamination of raw beef, produce and poultry. It is a chemical that destroys or cleanses harmful bacteria from organic surfaces and is often found in various oral sprays, mouthwashes, and lozenges, as well as some other types of surface disinfectants. In its purest form, CPC is a fine white powder without taste or odor. CPC is currently used as an active antimicrobial ingredient in mouthwash and throat lozenges (Coleman, 2000).

When used in a poultry plant, a fine mist spray is always used to treat the surface of poultry carcasses or parts (skin-on or skinless) prior to immersion in a chiller; the concentration is required not to exceed 0.3 g CPC per pound raw poultry carcass or parts. If used as a liquid aqueous solution before or after chilling, the concentration needs to be lower than 0.8% and the amount of aqueous solution

should not exceed 5 gallons per carcass. When applied in a dip tank, the maximum dwell time is 10 seconds. Additive is also needed in the system to recapture the solution (Appendix 7120, 2013).

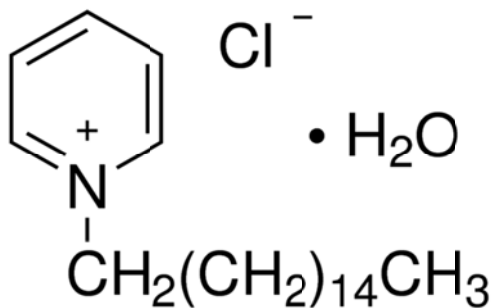


Figure-4 Cetylpyridinium chloride

Cetylpyridinium chloride is a cationic surface-active agent belonging to the group of quaternary ammonium compounds (QACs), which are the most useful antiseptics and disinfectants (McDonnell and Russell, 1999). Thus, it has both a positively charged hydrophilic region and a hydrophobic region. QACs are membrane active agents and are known to have lower cellular surface tension, disrupt the bacterial cell membrane and cause loss of selective permeability of the bacterial cell membrane (Talaro and Talaro, 1993). The CPC mechanism of action is dependent upon the ability of the positively charged molecule to interact with negatively charged anionic sites on the cell walls of bacteria. Bacterial cells carry a net negative charge under physiological conditions, which is because of the presence of negatively charged molecules on the surface. When bacteria are exposed to CPC, the positively charged hydrophilic group associated with the negatively charged groups on the bacterial surface allowing the hydrophobic

portion of CPC to interact with the cell membrane resulting in leakage of cellular components, disruption of bacterial metabolism, inhibition of cell growth, and cell death (Scheie, 1989; Smith et al., 1991; Merianos, 1991).

As of April 2, 2004, the Food and Drug Administration (FDA) has amended the food additive regulations in FR Doc 04-7399 to provide for the safe use of cetylpyridinium chloride as an antimicrobial agent in poultry processing (Federal Register, 2004). The FDA has regulated that CPC be used to treat the surface of raw poultry carcasses.

During first processing, which is slaughter though chilling, there are processing steps that are designed to reduce the microbial loads during processing.

First, for scalding, it is a necessary step to remove those well-attached feathers and reduce the bacteria level. Scald condition can effect bacteria reduction, higher flow rate can further dilute particulate matter and help reduce bacteria contamination from bird's feathers and skin (Owens et al., 2010). Counter-flow, which means the scald water and birds move in opposite directions, gives reductions of fecal material and microbial loads both on carcasses and in scald water (Hafez, 1999). In addition, proper scalding temperature will maintain the property of poultry meat, low temperature could cause growth of bacteria, while high temperature could lead to loss of yield (Owens et al., 2010). However, chemicals can be added to scald water to further reduce cross-contamination. Increased death rates of *Salmonella* and *Campylobacter jejuni* were achieved in the water by adding 0.1 – 0.2% acetic acid to scald water (Okrend et al., 1986).

Adding 5% acetic acid to scald water resulted in 2 log₁₀ reduction of *S. Typhimurium* on chicken skin (Tamblyn et al., 1997). In McKee and others' (2008) study, they found that when hard scald temperature is used, RP scald may help reducing *S. Typhimurium* on carcasses.

Picking can be an area where a large amount of cross contamination occurs, a post-picking carcass rinse can be used to help to reduce bacteria on the carcasses (Owens et al., 2010). The picking area has a warm and humid atmosphere, making conditions ideal for the survival and growth of bacteria in these areas. Additionally, rubber picker fingers can be easily contaminated and contamination will be passed along to every carcass (Mead et al., 1980). Rubber picker fingers are prone to forming small cracks which can be a niche for bacteria to grow and an area that is difficult to clean and sanitize (McKee, 2009). Furthermore, bacteria can be pushed into the skin tissue and feather follicles by contact with picking fingers (Bryan et al., 1968). Thus, it is important to reduce bacterial levels on carcasses before picking and frequent rinses of picking finger is necessary.

Evisceration is a step to remove edible and inedible viscera from the carcasses. A vent-opening machine (vent cutter) is used to pull the terminal part of lower intestine out of the carcass. This step can easily cause fecal and bacterial contamination by cutting the intestine if the machine is improperly adjusted. In addition, every bird contacts this machine by the blade and a probe reaching into the bird to open the vent. Thus, it is necessary to spray the machine with chlorinated water between carcasses. The cropper, which is used to remove the

chicken crop, is another machine that can cause cross-contamination during evisceration and intervention strategies such as a chlorinated spray are needed to ensure food safety (Owens et al., 2010).

An inside/outside bird wash (IOBW) is important for reducing bacteria on carcasses. The inside of the bird is washed by high-pressure water sprayed from a spinning nozzle through both the abdominal opening and the neck opening, while the outside of bird is washed by fixed mounted nozzles spraying mid-pressure water (Barbut, 2001). Chemicals can be added in the water to increase the effectiveness of reducing microbial levels. Peracetic acid, followed by chlorine (in levels below 0.002%), is the most commonly used, along with other organic acids can be used as well (McKee, 2011).

Chilling is done to meet the regulatory requirements to reduce carcass temperature to 4 °C or less within 4 h of slaughter to reduce microbial growth on carcasses. Countercurrent flow is applied in the chiller to maximize heat exchange from carcasses to water and minimize the bacteria level on carcasses. The water in the chiller usually contains antimicrobials to prevent cross-contamination, historically, chlorine was most commonly used antimicrobial in chillers (Owens, et al., 2010). However, PAA is now commonly used due to some export issues (Food Safety News, 2010). Finishing chillers can be used immediately after the chiller, they are smaller than the chiller which uses smaller volumes of water and shorter dwell times compared to the chiller used to reduce carcass temperature (Owens et al., 2010). Due to the shorter dwell times, higher concentrations of antimicrobials can be used (McKee, 2011).

FSIS's new performance standards for *Salmonella* and *Campylobacter* are 7.5% and 10.4%, respectively. For young chicken, the positive percent of *Salmonella* and *Campylobacter* are 4.3% and 10.4% (FSIS, 2012), respectively. However, the chicken parts baseline study found that the occurrence of pathogens are much higher in parts, 26.3% for *Salmonella* and 21.4% for *Campylobacter* (RCPBS, 2012). Parts may become cross-contaminated through the additional handling steps and further intervention strategies are needed in a multi-hurdle approach to food safety. One new technology available is a parts decontamination tank that could help to reduce the bacteria level on chicken parts after being cut up.

Preventative Measures

Food-borne illness is a major public health concern. The largest number of food-borne illness cases attributed to poultry and poultry products are caused by paratyphoid serotypes of *Salmonella* and by *Campylobacter jejuni*. In order to make significant improvements in food safety, measures must be taken at all points from farm to table including production, transportation, slaughter, processing, storage, retail, and food preparation. Since pathogens can get into and grow anywhere along the food chain, intervention measures should be considered and compared along the whole chain (Hogue et al., 1998).

First, the design of the house is important. It should be easy to clean and disinfect. Cracks should be repaired to decrease the likelihood for survival of bacteria and parasites. Also, the house should be bird and rodent proof, because

these can be vectors of contamination by spreading bacteria to the feed with their feces, which makes it difficult to control. Good ventilation is required to prevent the build-up of ammonia for the chickens (Silverside and Jones, 1992). During processing, it is very important to keep the dirty and clean parts of the processing plant separate, for example, processing before and after evisceration should be conducted in separate spaces. This is to prevent fecal contamination from feathers. Also, all the equipment should be washed and disinfected before and after processing to minimize the possibility of cross-contamination. After processing, storage conditions of the processed meat become the primary thing that plant should consider. From the farm to the store, meat and poultry products must be chilled and kept chilled packaged and handled properly so it will be safe for consumers to buy.

HACCP has been introduced into meat and poultry processing plants to identify potential sources of pathogen contamination and prevent foodborne illnesses by applying science-based controls including practical applications of the new technologies and extensive knowledge of microbiology and risk assessment (Keener, 2007). The USDA-FSIS adopted HACCP Systems and established finished product standards for *Salmonella* in slaughter plants to improve food safety for meat and poultry (FSIS, 1998).

Importance of Quality Determination

Poultry have been on the earth for more than 150 million years since the original wild jungle fowl. Chicken meat and eggs are the best source to provide quality

protein, as well as essential vitamins and minerals, and are badly needed by many people who live in poverty. A major advantage of poultry meat as human food is that there are no cultural or religious obstacles for consumption. In addition, the price is relatively low compared to other meats. As for the nutritional aspects, poultry meat contains less fat which fits the current demand for consumers. Additionally, poultry meat has a high degree of unsaturated fatty acids, is naturally low in sodium and cholesterol which makes poultry meat a better choice to buy (Petracci and Cavani, 2012). It also provides bioactive substances with favorable effects on human health, such as, conjugated linoleic acid, vitamins and antioxidants, and a balanced n-6 to n-3 polyunsaturated fatty acids (PUFA) ratio (Barroeta, 2006; Given, 2009). Thus, worldwide poultry meat production and consumption have increased rapidly and, in many parts of the world, per capita consumption of poultry meat will continue to grow (Cavani et al., 2009).

Meat quality has several dimensions according which is individual to each consumer; generally, they are interested in appearance, flavor, texture, and juiciness.

Before purchasing a product, the consumers will visually inspect a product to see if it is appealing. They would avoid product with bruises or broken bones, which indicates that the appearance of the meat is critical for making a purchasing decision. After purchasing and cooking product, consumers will further evaluate product based on taste, flavor, texture, convenience, and health, which can

determine if a consumer would purchase a product again. Therefore, sensory evaluation is important before putting products into the market.

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CHAPTER III

Effectiveness of Several Antimicrobials Used in Parts Decontamination Tank to Reduce *Salmonella* and *Campylobacter* on Chicken Parts

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INTRODUCTION

Poultry meat is the most popular consumed meat in the US, and the consumption has been steadily increasing through the years. The consumption of chicken has shifted from whole birds to more convenient parts. In 1962, 83% of broilers were whole birds, while 15% were marketed as parts, and the shift in the market trend for more convenient items has lead to 12% consumed as whole birds, 42% as parts and 46% as further processed products in 2009 (National Chicken Council, 2011).

Salmonella and *Campylobacter* are the two pathogens commonly associated with poultry meat and poultry products are frequent vehicles of these bacteria. They are also among the top 3 food-borne pathogens responsible for the annual

disease burden costs. Combined they result in approximately \$5 billion in costs every year related to illness (Batz et al, 2011).

According to the Raw Chicken Parts Survey (2012) conducted by United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) to estimate the percent positive levels of *Salmonella*, *Campylobacter*, and indicator bacteria on raw chicken parts. Chicken part samples were randomly selected from the production line before packing, and 1.82 kg of selected parts were placed into a sterile bag with 400 ml Buffered Peptone Water (BPW), and shaken vigorously for 1 min. The shaken BPW rinsate was poured into the screw-top container, which was provided by FSIS, and kept at approximately 4 °C. The results showed that there was no significant difference with skin-on versus skin-off parts of chicken when testing *Salmonella*. However, for *Campylobacter*, the skin-on parts had a significantly higher number of positive samples than skin-off parts. During this survey, FSIS tested both *Salmonella* and *Campylobacter* simultaneously to determine the incidence of chicken parts that are positive for pathogens. The outcome demonstrated that chicken necks (55%) had the highest percent positive samples for both *Salmonella* and *Campylobacter*. The lowest percent positive samples for *Campylobacter* was breast meat (16.1%), which is 13.5% for skinless breast and 25.6% for skin-on breast. Because *Salmonella* and *Campylobacter* are associated with poultry and are found on

poultry parts, it is imperative that effective intervention strategies are found to reduce these pathogens.

The first objective of the current research was to determine the optimal contact time for water, 0.003% chlorine, 0.07% PAA, 0.1% PAA, 0.35% CPC and 0.6% CPC. These concentrations were chosen based on the typical use in industry that was also within the regulatory limits. Next, the effectiveness of these antimicrobials was determined when they were used in combination with a parts decontamination tank to reduce *Salmonella* and *Campylobacter* on chicken parts. In order to evaluate the effect of the treatments on the consumer's acceptability of chicken parts treated with antimicrobials, sensory evaluation was also performed.

Materials and Methods

Preparation of bacteria culture

Salmonella Typhimurium (Nalidixic Acid resistant strain) was cultured in 10 mL sterile Trypticase Soy Broth (TSB; Acumedia Manufactures Inc., Lansing, MI) with Nalidixic Acid (0.05%) to achieve approximately 10^8 CFU/ml after 12h of incubation at 37°C. Cultures were centrifuged (Sorvall Legend RT+ Centrifuge, Thermo Scientific, Thermo Electron Corp., Germany) at 8,000 rpm for 10 min at 4°C.

Campylobacter jejuni was cultured in Brucella Broth supplemented with FBP (FBP, Acumedia Manufactures Inc., Lansing, MI) (10 mL) to achieve approximately 10^8 log CFU/ml after 24h of incubation at 42°C. Cultures were centrifuged at 8,000 rpm for 10 min at 4°C. The *Salmonella* and *Campylobacter*

pellets were combined and resuspended in equivalent amounts of sterile buffered peptone water (BPW; Acumedia Manufactures Inc., Lansing, MI) to maintain the bacteria population.

Preparation and treatment of chicken parts

For the contact time study, drumsticks (n=160) were used. In each of 2 replications, 4 non-marinated drumsticks were prepared for each treatment (positive control, negative control, water, 0.003% chlorine, 0.07% PAA, 0.1% PAA, 0.35% CPC and 0.6% CPC). Each drumstick was inoculated by dripping 250 μ L of the inoculum containing both 10^8 CFU/ml *Salmonella* Typhimurium and 10^8 CFU/ml *Campylobacter jejuni*. After inoculation, a sterile spreader was used to spread the bacteria evenly on the surface of the drumstick and a 30 - min attachment period was allowed before treatments were applied. Inoculated drumsticks were dipped in the water control and antimicrobials for various contact time (10 s, 20 s, and 30 s).

For the parts decontamination tank study, cut-up poultry part samples (n=80) were used. In each of 2 replications, 5 samples (1.8 kg chicken parts per sample) were prepared for each treatment (positive control, negative control, water, 0.003% chlorine, 0.07% PAA, 0.1% PAA, 0.35% CPC and 0.6% CPC). Each 1.8 kg sample had at least one breast, one thigh, one wing, and one drum. They were put in separate carcass rinse bags, and inoculated with 1 spray (1 ml) of the inoculum containing both 10^8 CFU/ml *Salmonella* Typhimurium and 10^8 CFU/ml *Campylobacter jejuni*. A 1 min massage was conducted to spread

the bacteria evenly in the sample. An attachment period of 30 minutes was allowed before applying any treatments to the samples.

Sample Collection

Six chilled treatments (water, 0.003% Chlorine, 0.07% PAA (Spectrum; FMC, Philadelphia, PA), 0.1% PAA, 0.35% CPC (Cecure; Safe Food Corporation, North Little Rock, AR) and 0.6% CPC) were prepared for both studies. Water used to mix the treatments was 10 °C.

For contact time study, 900 ml of each antimicrobial solution was held in a 1,500 ml beaker. Inoculated drumsticks (one at a time) were added to the chilled water treatments and agitated with a stick to mimic the movement of the parts in the parts decontamination tank (Morris & Associates Inc., Garner, NC). Samples were treated for either 10 s, 20 s, or 30 s contact time with treatments listed above. After moving the drumstick from the beaker, each sample was placed into a sterile filter bag with 25 ml BPW. Briefly each sample was shaken for 1 min to rinse bacteria off of the drumstick. Serial dilutions were made with the rinsate using BPW and plated on the appropriate media plate for bacteria detection.

For parts decontamination tank study, the same six treatments (water, 0.003% Chlorine, 0.07% PAA, 0.1% PAA, 0.35% CPC and 0.6% CPC) were used on the parts in the parts decontamination tank. Prepared poultry parts (1.8 kg samples described previously) were randomly assigned to each treatment and inoculated with *Salmonella* and *Campylobacter*. The tank held approximately

45 L of water, and the temperature of the chilled water was approximately 10 °C. Antimicrobials were added into the parts decontamination tank, and concentrations of antimicrobials were verified before samples were tested in the decontamination tank. Chlorine was tested using Aquachek Water Quality Test Strips for High-Range Chlorine (HACH company, Loveland, CO), while PAA and CPC concentrations were tested using PAA test kit (FMC Corporation, Philadelphia, PA) and Cecure Titration kit (LaMotte Company, Chestertown, MD), respectively. The concentrations of chemicals were tested before use and the pH of the chlorine treatment was adjusted to a pH less than 6.0. The parts decontamination tank speed was set at 23 s per cycle, and samples were treated with one cycle. After samples were treated, chicken parts were aseptically collected, and placed in a sterile carcass rinse bag. BPW (200 ml) was added to the carcass rinse bag and sample was rinsed for 1 min to remove any remaining bacteria. Serial dilutions were made using the rinsate and plated on appropriate selective media for bacterial detection.

Salmonella detection procedure

S. Typhimurium in carcass rinse samples was enumerated by serially diluting samples and spread plating on selective media. Appropriate dilutions were plated on Xylose-Lysine-Tergitol 4 (XLT4) with nalidixic acid (30µg/ml) for the selective plating and was incubated for 24 h at 37°C. Black colonies were counted and results were reported as log₁₀ CFU/ml comparing untreated samples to treated samples.

Campylobacter detection procedure

Campylobacter spp. in carcass rinse samples was analyzed for the enumeration of *Campylobacter* following the steps described here. Carcass rinse containers were serially diluted and a spread plated on Campy CEFEX for selective plating. Plates were incubated for 48 h at 42°C in AnaeroPack rectangular jars (Mitsubishi Gas Chemical America, Tokyo, Japan) with a microaerophilic environment of 5% O₂, 10% CO₂, and 75% N₂, generated by CampyGen sachets (Oxoid). Colonies were counted and results were reported as log₁₀ CFU/ml comparing untreated samples to treated samples.

Sensory Evaluation

Sensory analysis was performed to evaluate the effect of the treatments on the consumer's acceptability of chicken parts treated with antimicrobials. An untrained test panel (n = 60; 30 in the A.M. (rep 1) and 30 in the P.M. (rep 2)) was recruited using e-mail postings and flyers from employees and students at Auburn University to evaluate samples (drumette and breast meat) for appearance, flavor, texture, juiciness, and overall acceptability using an 8-point hedonic scale as suggested by the Institute of Food Technologists (IFT, 1981). The recommended number of panelist responses per sample product is listed between 50 and 100 (IFT, 1981). Selection criteria for panelists was based on age (>19 years) and a willingness to participate.

Drumettes and breast meat were baked in the oven until the internal temperature reached 74 °C, whole drumettes were served warm on plates labeled with random 3-digit codes. Breast meat samples were cut into 2 cm *2

cm *1 cm pieces and placed into capped (PL2 clear plastic souffle' lids, Solo® Cup Company, Highland Park, IL) plastic sample cups (59.2 mL B200 plastic souffle, Solo® Cup Company) labeled with a random 3-digit code number and kept warm (28 °C) in a warming oven (FlavorView C175-C(1)N Heated Cabinet, Intermetro Industries Corp., Wilkes-Barre, PA) until serving. Each panelist was given an Institutional Review Board (IRB) approval letter from Auburn University's Office of Human Subjects Research and an evaluation form for each sample and was asked to score each drummettes/ breast meat sample based on the degree of liking ("like extremely" to "dislike extremely"). Each of the 5 sensory attributes (appearance, flavor, texture, juiciness, and overall acceptability) were scored from the same 8-point hedonic scale that ranged from, (1) Like Extremely; (2) Like Very Much; (3) Like Moderately; (4) Like Slightly; (5) Dislike Slightly; (6) Dislike Moderately; (7) Dislike Very Much; (8) Dislike Extremely (Appendix A). Room-temperature water and unsalted crackers were provided to cleanse panelists' palates between samples.

Statistical analysis.

All microbial data were converted to \log_{10} CFU/ml before analysis in the statistical model. Zero cannot be statistically analyzed, so 0.1 was used instead of 0. Statistical Analysis of the data was conducted using SAS 9.1 software (SAS Institute, Cary, N.C.). For the sensory data, hedonic scores were assigned a numerical value and data was analyzed. General Linear Model of

SAS was used to analyze the data and comparisons were made using LSMEANS and significant differences ($P < 0.05$) were identified.

Results and Discussion

Contact Time Analysis

One of the objectives monitored during this study was to determine the optimal contact time with the antimicrobials to get the most effective reduction of *Salmonella* and *Campylobacter*.

Salmonella

As shown in Figure 5, for the positive control, inoculated with 10^8 CFU/ml *Salmonella*, the recovery of *Salmonella* was 2 \log_{10} less at 10^6 CFU/ml. For the negative control, which was not inoculated, the bacterial level was under the detection limit which is 0.69 CFU/ml, thus, it was concluded that there was no *Salmonella* background on the drumsticks. For those six treatments, there were significant differences ($p < 0.0001$) among each treatment. Water and 0.003% chlorine resulted in a 1 \log_{10} reduction compared to the positive control, while both concentrations of PAA had a 1 \log_{10} reduction. As for CPC, the higher concentration (0.6%) worked more efficiently than the 0.35% CPC. However, different contact times did not increase the effectiveness of these antimicrobials ($p > 0.05$) with the exception of the CPC treatment. Specifically, the 0.35% and 0.6% CPC reduced *Salmonella* more effectively with increased contact time. Li and others (1997) said that there was limited research on contact time and

concentration. In their research, the efficacy of 30 s and 90 s spraying time of different antimicrobials including 0.1% CPC were tested on chicken carcasses, and the results showed that longer time (90 s) worked better than shorter ones (30 s). They concluded that the longer the contact time in their study, the better the effectiveness of the antimicrobial which also supported the results found in the current study. A major difference in their study was that, the concentration of CPC (0.1%, applied as a spray) used in Li and others' (1997) study was much lower than the concentration of CPC (0.35% or 0.6%, applied as a dip) used in this study. Based on earlier studies, spraying methods are less effective than dipping methods in applying antimicrobials. However, the range of contact times used by Li and others (1997) was 30 s to 90 s which was a wider time range than the contact times used in this study (10, 20, and 30 s). In this study, reductions were found in *Salmonella* with 0.35% and 0.6% CPC, with 0.6% CPC which was due to the increased contact time but may have also been due the better coverage when applied as a dip.

Campylobacter

As shown in Figure 6, drumsticks inoculated with 10^8 CFU/ml *Campylobacter* resulted in a 5.5 log₁₀ CFU/ml recovery for the positive control. For negative control, the bacterial level was under the detection limit. Thus, it can be concluded that there was no *Campylobacter* background on the drumsticks. For those six treatments, there were significant differences ($p < 0.0001$) among each

treatment. Both concentrations of CPC (0.35% and 0.6%) were more effective than the other antimicrobials resulting in an approximate 4 log₁₀ reduction compared to the positive control. While both concentrations of PAA (0.07% and 0.1%) resulted in a 2 log₁₀ reduction when compared to the positive control. The mechanical action of water alone resulted in a 1 log₁₀ reduction of *Campylobacter* compared to the positive control and when 0.003% chlorine was added to the water the chlorine was no more effective than the mechanical action of water. However, contact times had no effect ($p>0.05$) on the effectiveness of any of these antimicrobials.

In a research conducted by Walairut and others (2004), the effectiveness of chlorine (40 or 100 ppm) and PAA (40 or 100 ppm) for 2 or 15 min was tested. The results showed that chlorine at 40 ppm had no significant differences when applied for 2 or 15 minutes. However, 100 ppm chlorine had 0.97 log₁₀ CFU/ml reduction of *Campylobacter jejuni* when applied for 15 minutes while only 0.65 log₁₀ CFU/ml reduction with a 2 min contact time. For PAA, the 15 min contact time was more effective than the 2 min for both concentrations. This is most likely due to the different mechanisms of action for PAA and chlorine. For PAA, it eliminates microorganisms by oxidation and subsequent disruption of their cell membrane (EnviroTech, 2013). However, chlorine eliminates microorganisms by directly acting on the cellular membrane and disruption of fundamental cellular processes (USDA, 2002a). Thus, chlorine needs longer contact time than PAA to be effective as an antimicrobial.

In conclusion, the higher concentration of CPC (0.6%) was the only treatment that was effected by contact time where the longer the contact time the more effective it was on *Salmonella*. However, there were no differences in effectiveness based on contact time for *Campylobacter*.

Parts Decontamination Tank Analysis

The objective of this trial was to determine the effectiveness of these six antimicrobials when applies with the use of new technology, a parts decontamination tank.

Salmonella

It is shown in figure 7 that the positive control resulted in 5.5- log₁₀ CFU/ml *Salmonella* recovery, while the negative control was undetectable. However, different antimicrobials did have an effect on the reduction of *Salmonella* recovered from treated samples ($p < 0.0001$).

For *Salmonella* reduction of each treatment, water was used as a control to determine the amount of bacteria that was washed off due to the mechanical rinsing from the decontamination tank and this resulted in a less than 1 log₁₀ reduction in *S. Typhimurium*. Chlorine (0.003%) was no more effective than the mechanical rinsing provided by water alone. Other researchers have found similar results with chlorine, Northcutt and co-workers (2005) found no reduction of *Salmonella* using Chlorine (0 or 50 ppm) with an exposure time of 5 seconds. With an increased concentration, spraying 55 ppm chlorine on chicken

carcasses, they had a 0.9-1.1 log₁₀ CFU/ml reduction of *Salmonella*. For PAA (0.07% and 0.1%) in this study, both concentrations worked equally as well, they both had about a 1.5- log₁₀ CFU/ml reduction on *Salmonella*. Research conducted using PAA in chiller water as an antimicrobial on chicken carcasses demonstrated that at a concentration of 0.0025% it was effective in reducing *Salmonella* Typhimurium: however, 0.02% was more efficient (Bauermeister et al., 2008). In research conducted by Nagel and others (2013), concentrations (0.04% and 0.1%) had no significant difference, and both had a 2 log₁₀ CFU/ml reduction.

While CPC was the most effective antimicrobial tested in this study resulting in a 2.5 log₁₀ CFU/ml reduction with a concentration of 0.35% and a 3.5 log₁₀ CFU/ml reduction with a concentration of 0.6%. Li and others (1997) conducted a pre-chill spray test on chicken carcasses reducing *S. Typhimurium*, 0.1% CPC was tested resulting in a 1.6-log₁₀ CFU/ml reduction with 90 sec spraying time when the pressure was 827 kPa. 0.5% CPC was tested to be the most effective antimicrobials among 0.5% CPC, 5% sodium bisulfate (SBS), 2% lactic acid (LAC), and 10% trisodium phosphate (TSP), which had 2.16 log₁₀ CFU/carcass when applied with spray method of 17 sec to reduce *Salmonella* (Yang et al., 1998). Applying 0.1 to 0.5% CPC had a 1.5-1.9 log₁₀ CFU/ml reduction *Salmonella* (Xiong et al., 1998). Similarly, Kim and Slavik (1996) found that chicken breasts treated with 0.1% CPC in an immersion application for 1 – 3 minutes had a 1.0 – 1.6 log₁₀ CFU/cm².

Campylobacter

Figure 8 shows that recovery of *Campylobacter* in the positive control was 10^6 CFU/ml, while the negative control had less than 1-log_{10} CFU/ml *Campylobacter* recovered. Background *Campylobacter* spp. was recovered because the inoculation strain was not resistant to antibiotics and additional antibiotics were not added in to the recovery medium. The same was not true for *Salmonella* as the strain used in this study was Nalidixic acid resistant and this was added to the XLT4 to eliminate any background *Salmonella* spp. on chicken parts tested. As with *Salmonella* and regardless of the background *Campylobacter* spp., the different antimicrobials did have an effect on the reduction of *Campylobacter* recovered from treated samples ($p < 0.0001$).

The results for *Campylobacter* followed the same trend as *Salmonella*, CPC was the most effective with approximately a $4 \log_{10}$ CFU/ml reduction at concentration of 0.35%, and the higher concentration (0.6%) was even more effective resulting in approximately a $5 \log_{10}$ CFU/ml reduction compared to the positive control. PAA gave about a $1.5 \log_{10}$ CFU/ml reduction compared to the positive control with both concentrations tested in this study. Similar to the results for *Salmonella*, water was tested as a control to demonstrate the amount of reduction due to the mechanical rinsing of the chicken in the parts decontamination tank and had approximately a $1 \log_{10}$ CFU/ml reduction compared to the positive control. Chlorine applied in the parts in the parts decontamination was no more effective than water.

Northcutt et al (2005) had 2.5-2.6 log₁₀ CFU/ml reduction on *Campylobacter* by spraying 55 ppm chlorine on chicken carcasses. Different concentrations of chlorine as spray treatment had no difference in reducing *Campylobacter* on chicken carcasses (Northcutt et al., 2005). An earlier study conducted by Arritt et al (2002) demonstrated that higher concentrations of CPC were more effective in reducing *Campylobacter*. In their study 0.1% and 0.5% CPC was applied as a spray, and the results showed 2.89 log₁₀ CFU/skin was achieved by 0.5% CPC, while concentration at 0.1% resulted in a 1.42 log₁₀ CFU/skin. In another study, 0.02% PAA resulted in a 1.5 log₁₀ CFU/ml reduction on *Campylobacter*, while the lower concentrations (0.0025%, and 0.01%) achieved similar reductions as 0.003% chlorine (Bauermeister et al, 2008). Moreover, Riedel and others (2009) found that with an immersion application and exposure time of 1 min, 0.5% CPC resulted in more than 4.2 log₁₀ CFU/ml reduction in *Campylobacter* on poultry carcasses. Other research has demonstrated chlorine (0.003%) to be the least effective treatment for either *Salmonella* or *Campylobacter* with a short contact time that limits the effectiveness of chlorine (Nagel et al, 2013). Instead, 1 to 1.5 h is suggested to be the optimal contact time for chlorine (McKee, 2011).

To conclude, treatment with 0.35% or 0.6% CPC was found to be most effective in decreasing *Salmonella* and *Campylobacter* on poultry parts in this study, followed by 0.07%, 0.1% PAA. For CPC, the higher concentration was more effective; however, for PAA, there were no differences in effectiveness between concentrations for this application. Chlorine at 0.003% was no different than the water control in this study.

Sensory Analysis

The most important aspect of poultry meat is its eating quality. Organic acids can impart negative quality attributes to products that they are used to treat, sensory panel evaluations were conducted to determine whether antimicrobials used in the study had negative effects on the products evaluated. Both skin-on (drumette) and skin-off (breast meat) samples were evaluated. These sensory attributes are important to consumers because they will not consume the products, which are viewed as unpalatable.

Sensory panel evaluation results are listed in Table 1 and Table 2. Panelists were asked to evaluate the effect of the treatments on the consumer acceptability of non-inoculated chicken drumettes and breast meat treated with antimicrobials for appearance, flavor, texture, juiciness, and overall acceptability using an 8-point hedonic scale. The higher the number rating, the more favorable the sensory score.

Appearance

It is appearance of food products that plays a major role for a consumer to make a purchasing decision. In this study, according to the evaluation of untrained consumer panelists, all six treatments (0.003% Chlorine, 0.07% PAA, 0.1% PAA, 0.35% CPC and 0.6% CPC) had no significant differences ($p>0.05$) for both skin-on and skin-off chicken meat for the cooked appearance. Panelists rated the appearance as “liked moderately” for breast meat (Table 1) and “like slightly” for drumettes (Table 2). These observations indicate that application of different antimicrobials did not ($p>0.05$) alter the consumers’ response to the likeness of

appearance of both skin-on and skin-off chicken meat.

Flavor

Flavor is another quality attribute that consumers use to determine the acceptability of poultry meat. Meat flavor mainly develops during cooking, and fat is a major contributor to the flavor of meat. The chemical changes that develop the flavor are not unique to poultry, but the lipids and fats, which lead the changes are unique. According to the panelist's evaluations, there were no differences in the degree of likeness indicated ($p>0.05$) for flavor of the chicken meat (Table 1 and Table 2) treated with the various antimicrobials used in this study. These results were similar to those for appearance, in that the addition of different antimicrobials did not ($p>0.05$) affect the consumers' acceptability of the chicken for both skin-on and skin-off meat.

Texture

The composition of the food can affect the texture of food products, and texture is an important attribute of the product. Sensory evaluation is a method that can reflect the consumers' acceptability of texture in different products. It is demonstrated in the study that 0.1% PAA applied used in the parts decontamination tank had a slight positive impact ($p<0.05$) on the texture of skin-off poultry meat. The result of chicken breast meat shows that texture score of 0.1% PAA (5.38 ± 1.52) was lower than other treatments, which indicates that the product was more tender than other treatments.

Juiciness

Juiciness is a sensory measurement of water-holding capacity of food products. The results showed that juiciness was affected ($p < 0.05$) by applying some of the antimicrobials. The results for chicken breast meat showed that the 0.1% PAA treatment was perceived as more juicy compared to other treatments for chicken breast meat, but not for the skin on parts (drumettes) tested. No differences were observed in drumettes and this likely had to do with the skin-on product reducing the contact of the antimicrobial with the meat.

Overall acceptability

Overall acceptability is an indicator that can be used to estimate a consumers' willingness to purchase a particular product. For skin-on samples, the application of the antimicrobials had no effect ($p > 0.05$) on drumettes, however, for skin-off samples, 0.1% PAA had the lowest score ($p < 0.05$) for overall acceptability, however, these differences were small and may not be noticed by a consumer if it is was not sampled alongside other treatments.

In research conducted by Bauermeister et al (2008), they found that 0.01% PAA had negative effect on appearance, flavor, and texture when contact times of 30 min were used. However, Nagel et al (2013) found no negative impacts on sensory attributes including appearance, flavor, texture, juiciness, and overall acceptability when 0.004% chlorine, 0.04% or 0.1% PAA were used in a post chill application with a 30 sec contact time. Nagel et al (2013) also suggested that the shorter contact time may be the reason that no significant differences in the sensory attributes were shown.

Limited sensory studies have been conducted on cooked chicken testing the impacts on product after applying antimicrobial treatments. However, some researches have focused on the effect of antimicrobial treatment on meat quality. Hinton and Corry (1996) found that because of its bleaching action, PAA had an unexpected discoloration action on chicken carcasses. In Jimenez-Villarreal et al (2003) research, they reported that ground beef flavor was not impacted by treating with 0.5% CPC.

Conclusion

In conclusion, contact time did not influence the reduction of *Campylobacter*, however, the longer the contact time for the higher concentration of CPC the greater the reduction of *Salmonella* in this study. Treatment with 0.35% or 0.6% CPC was found to be most effective in decreasing *Salmonella* and *Campylobacter* followed by 0.07%, 0.1% PAA for parts treated in the decontamination tank. For CPC, the higher concentration was more effective in reducing *Salmonella* and *Campylobacter* on the poultry parts, with longer contact times improving reductions of *Salmonella* at 0.6% CPC. For PAA, there were no differences in reduction between concentrations, or contact time for *Salmonella* or *Campylobacter*. Chlorine was least effective treatment and similar to water for a short contact time. It was no more effective than the water control used to determine the reduction of *Salmonella* and *Campylobacter* due to the mechanical rinsing action of the decontamination tank. As for sensory attributes, texture, juiciness, and overall acceptability were slightly affected by 0.1% PAA on skin-off

samples. While appearance and flavor, which are the most important attributes for meat quality, were not affected. Thus, we can conclude that lower concentrations of CPC and PAA combined with a parts decontamination tank are effective for reducing *Salmonella* and *Campylobacter* on chicken parts while maintaining product quality.

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Tables and Figures

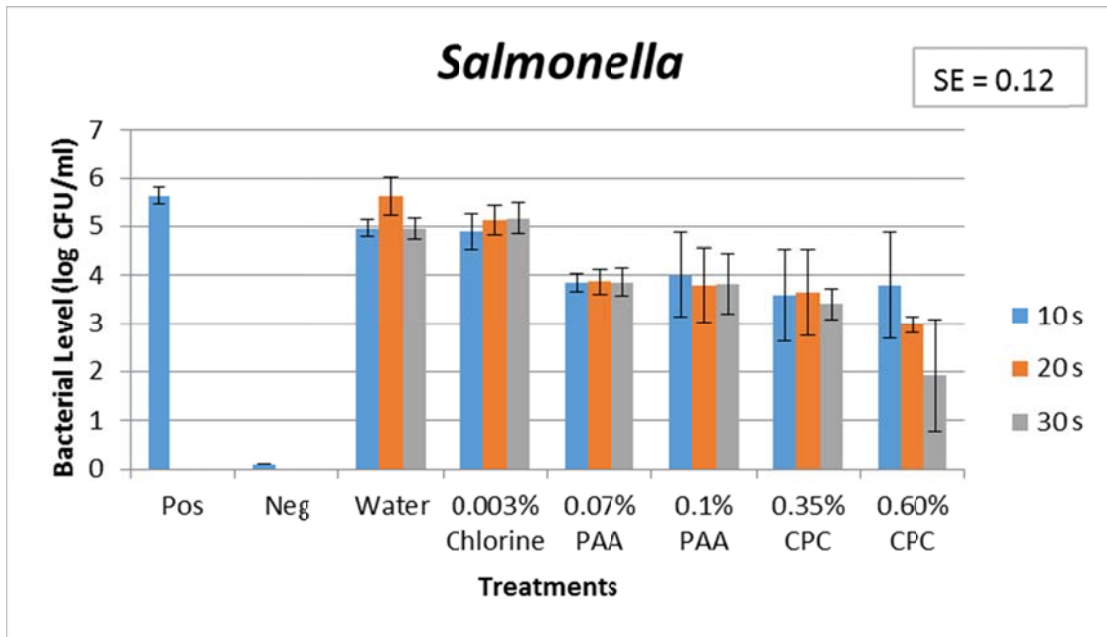


Figure – 5 Contact time study results for *Salmonella*

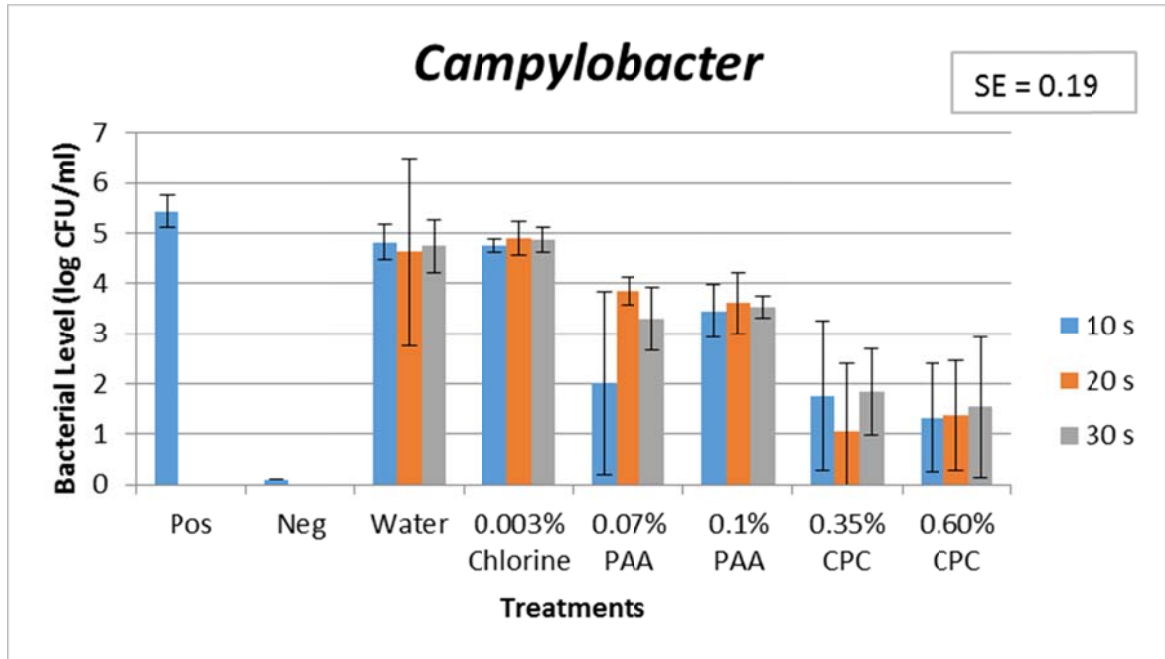


Figure – 6 Contact time study results for *Campylobacter*

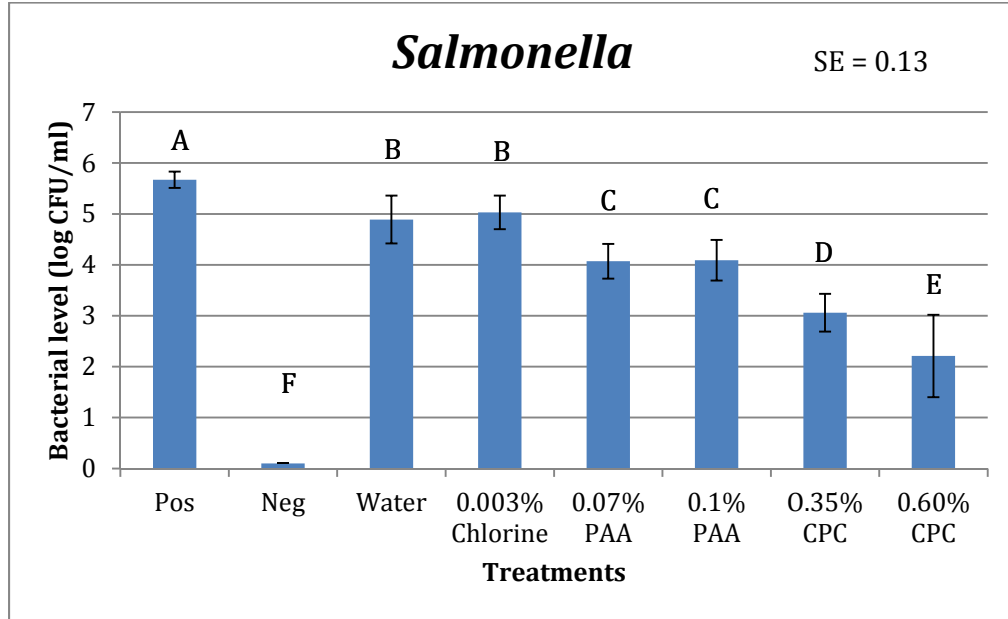


Figure – 7 Parts decontamination tank study results for *Salmonella*

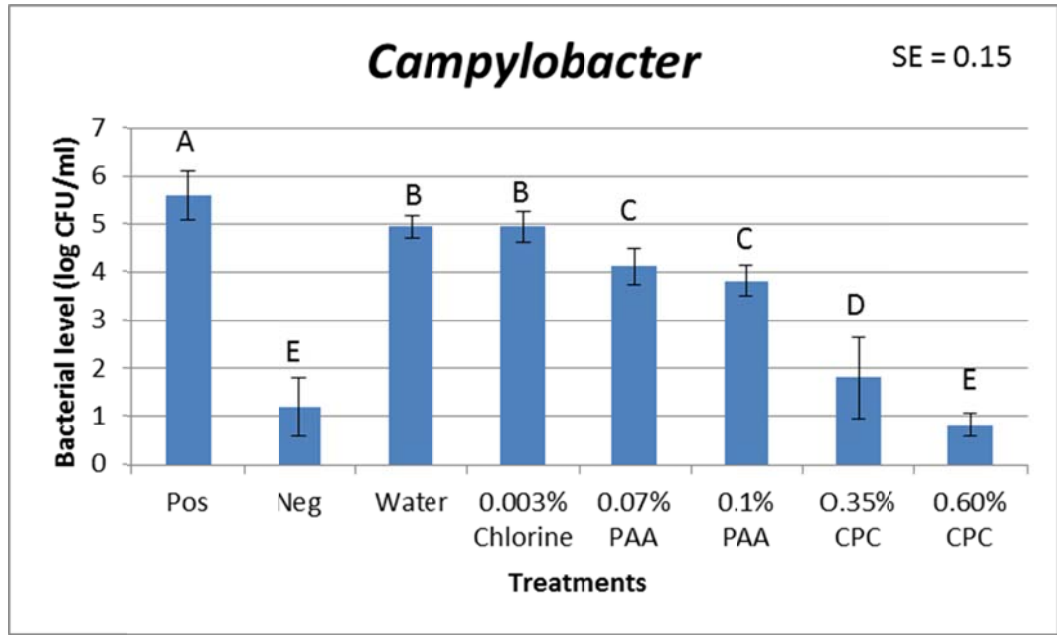


Figure – 8 Parts decontamination tank study results for *Campylobacter*

Tables

Table – 1 Sensory Results for Chicken Breast Meat

Treatment	Appearance ^{*1}	Flavor ^{*1}	Texture ^{*2}	Juiciness ^{*3}	Overall Accptability ^{*1}
0.003% Chlorine	6.03±1.18	5.92±1.07	5.93 ^a ±1.21	5.70 ^{ab} ±1.39	5.87 ^a ±1.07
0.07% PAA	6.34±1.06	5.84±1.10	5.90 ^a ±1.26	5.90 ^a ±1.26	5.87 ^a ±1.04
0.1% PAA	6.02±1.30	5.49±1.43	5.38 ^b ±1.52	4.75 ^c ±1.69	5.33 ^b ±1.39
0.35% CPC	6.30±0.95	5.72±1.18	6.02 ^a ±1.30	5.85 ^a ±1.31	5.87 ^a ±1.12
0.6% CPC	6.05±1.16	5.58±1.45	5.55 ^{ab} ±1.59	5.25 ^{bc} ±1.69	5.45 ^{ab} ±1.56
P-value	0.3184	0.3185	0.0467	0.0003	0.0279

*1 1 = Dislike Extremely 8 = Like Extremely

*2 1 = Extremely Tender 8 = Extremely Tough

*3 1 = Extremely Moist 8 = Extremely Dry

Table – 2 Sensory Results for Chicken Drumette

Treatment	Appearance ^{*1}	Flavor ^{*1}	Texture ^{*2}	Juiciness ^{*3}	Overall Accptability ^{*1}
0.003% Chlorine	5.48±1.62	5.43±1.57	5.72±1.35	5.72±1.41	5.48±1.51
0.07% PAA	5.41±1.60	5.36±1.63	5.76±1.22	5.77±1.19	5.41±1.60
0.1% PAA	5.58±1.53	5.54±1.42	5.59±1.53	5.64±1.35	5.64±1.31
0.35% CPC	5.42±1.64	5.37±1.40	5.53±1.47	5.46±1.49	5.25±1.40
0.6% CPC	5.59±1.44	5.36±1.58	5.51±1.64	5.46±1.50	5.36±1.48
P-value	0.9543	0.9610	0.8401	0.6364	0.6718

*1 1 = Dislike Extremely 8 = Like Extremely

*2 1 = Extremely Tender 8 = Extremely Tough

*3 1 = Extremely Moist 8 = Extremely Dry

Appendices

Appendix A

Sensory Evaluation of Chicken Meat

Sample Code (3-digit): _____ **Date:** _____

You will be given a total of 6 chicken meat samples. Please take a drink of water and a bite of cracker before each sample to cleanse your palate. Please record the 3-digit sample code and evaluate the sample for the characteristics listed below. Indicate your degree of liking according to the scales below. In addition, please include any comments you have about this product.

Appearance

- _____ Like Extremely
- _____ Like Very Much
- _____ Like Moderately
- _____ Like Slightly
- _____ Dislike Slightly
- _____ Dislike Moderately
- _____ Dislike Very Much
- _____ Dislike Extremely

Flavor

- _____ Like Extremely
- _____ Like Very Much
- _____ Like Moderately
- _____ Like Slightly
- _____ Dislike Slightly
- _____ Dislike Moderately
- _____ Dislike Very Much
- _____ Dislike Extremely

Texture

- _____ Extremely Tender
- _____ Very Much Tender
- _____ Moderately Tender
- _____ Slightly Tender
- _____ Slightly Tough
- _____ Moderately Tough
- _____ Very Much Tough
- _____ Extremely Tough

Juiciness

- _____ Extremely Moist
- _____ Very Moist
- _____ Moderately Moist
- _____ Slightly Moist
- _____ Slightly Dry
- _____ Moderately Dry
- _____ Very Dry
- _____ Extremely Dry

Overall Acceptability

- _____ Like Extremely
- _____ Like Very Much
- _____ Like Moderately
- _____ Like Slightly
- _____ Dislike Slightly
- _____ Dislike Moderately
- _____ Dislike Very Much
- _____ Dislike Extremely

Comments: