The Effect of Post-chill Antimicrobials on *Salmonella*, *Campylobacter*, Shelf-life and Quality Attributes of Ground Chicken

by

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Abstract

Ground chicken is more likely to have higher microbiological loads than whole carcasses and parts. Therefore, it is necessary to identify antimicrobials that reduce pathogens and overall microbial loads without negatively impacting meat quality. The objectives of this research were to evaluate the effect of various post-chill antimicrobials on reducing *Salmonella* and *Campylobacter* and to determine the impact of these treatments on shelf-life and quality attributes of ground chicken. Five treatments (0.003% Chlorine, 0.07%, 0.1% Peracetic acid; PAA, 0.35%, 0.6% Cetylpyridinuium Chloride; CPC) were evaluated. Samples (4 lbs; n=120) of skin-on chicken breast and thigh meat were inoculated with *Salmonella* Typhimurium (10^8 CFU/mL) and *Campylobacter jejuni* (10^8 CFU/mL). Following a 30 minutes attachment time, parts were rinsed with either chlorine, PAA or CPC in a decontamination tank for 23 s. Parts were then ground, and then samples (25 g) were plated and reduction of *S.* Typhimurium and *C. jejuni* were determined. Non-inoculated ground breast and thigh meat were used for sensory and shelf-life determination. Samples (n=200) for shelf-life determination were collected on day 1, 4, 7 and 10 to estimate spoilage microflora of ground chicken stored at 4 ℃. Additionally, color measurement and sensory evaluation were conducted at day 1, 4 and 7. Ground chicken treated with 0.07% and 0.1% PAA had the greatest reductions in *Salmonella* and *Campylobacter*, followed by 0.35% and 0.6% CPC ($P \leq 0.05$). Chlorine (0.003%) was the least effective treatment ($P \leq 0.05$).

Treatments with 0.07% and 0.1% PAA also extended the shelf-life of ground chicken for 3 days. None of the treatments had negative impact on color or sensory attributes of ground chicken patties during the storage ($P \leq 0.05$).

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CHAPTER I

INTRODUCTION

According to estimations from the Center for Disease Control and Prevention (CDC), there are 48 million people in the US that suffer from foodborne illnesses, resulting in 128,000 hospitalizations and 3,000 deaths annually (CDC, 2011a). In addition, relevant economic losses are also great. It was estimated that there were approximately $ 77.7 billion every year spent on investigating foodborne illnesses caused by pathogens (Scharff, 2012).

Salmonella and *Campylobacter* are two major foodborne pathogens in the US. In total, they are responsible for approximate 20% of domestic foodborne illnesses, 50% of foodborne illnesses resulting in hospitalization and 34% of relevant fatal cases, respectively (CDC, 2011a). Contaminated raw or undercooked poultry and poultry product are considered to be the dominant vehicles of transmission for *Salmonella* and *Campylobacter* to humans (Atterbury *et al*., 2003; Shah *et al*., 2011). In fact, every year over 20% of salmonellosis and 70% of campylobacteriosis in the US were caused by poultry products (Batz *et al.*, 2011).

Compared with many other poultry products, ground poultry is more likely to be contaminated with these pathogens. The pathogens are mixed into the batch of meat through processes such as cutting and grinding. One of the most recent recalls related with ground poultry occurred in August, 2011, in which 36 million pounds of ground turkey was recalled due to the contamination by a multi-drug resistant strain of *Salmonella* Heidelberg (CDC, 2011b). In addition, 182 people were infected during this outbreak and 1 case resulted in death. Because of the potential health risk of consuming ground poultry product,

the United States Department of Agriculture (USDA) released new prevention-based regulations to improve the safety of ground poultry (9 CFR Parts 417; FSIS Notice 33-13). Therefore, it is necessary for the poultry industry to re-evaluate the current antimicrobial intervention strategies and to apply new antimicrobial agents and/or technologies in order to meet the stricter regulations.

Post-chill applications are one of the new antimicrobial strategies employed in the poultry industry, which is applied immediately after the primary chiller (McKee, 2012). The current available equipment that can be used in post-chill processing includes finishing chillers, post-chill dip tanks and drench cabinets. Compared with pre-chill and immersion chill tanks, the application of antimicrobials in a post-chill system has several benefits. Initially, water has less organic build up than the chill tanks and antimicrobials can be added higher levels because of the shorter contact times. Therefore, pathogens attached on chicken carcasses and parts, such as *Salmonella* and *Campylobacter*, can be effectively reduced at these steps. Moreover, as the contact time is typically shorter than immersion chill step (less than 30 s versus 1.5-3 h), higher concentration of antimicrobials can be used without bringing negative impacts on product quality. Last but not least, volumes of water needed for post-chill application is far less than that for immersion chill applications, which makes post-chill a cost effective step to treat product with antimicrobials.

The antimicrobials that are approved for use in the poultry industry during post-chill processing include acidified sodium chlorite, calcium hypochlorite, cetylpyridinium chloride (CPC), chlorine dioxide, chlorine gas, organic acids, ozone, peracetic acid (PAA), sodium hypochlorite, sodium metasilicate, trisodium phosphate among others (FSIS Directive

7120.1). Based on a survey conducted by McKee (2011), the most prevalent chemical used in post-chill applications in industry was PAA. While CPC was the predominant post-chill antimicrobial when drench cabinets were used. Chlorine did not become the major chemicals used during post-chill steps. Firstly, it was reported that the short contact time may limit the antimicrobial effects of chlorine (McKee, 2012). Secondly, Russia, the single largest export market for the domestic poultry meat (mainly dark meat), banned imports of chlorine-treated poultry meat from the US (Weyerbrock and Xia, 2000). Finally, Tamblyn and Conner (1997) found that chlorine levels must be at least 0.04% to kill the attached *Salmonella* on broilers, which is not allowed according to current regulations.

Currently, there are several published data indicating the antimicrobial effects of PAA and CPC on chicken carcasses (Yang *et al*., 1998; Bauermeister *et al*., 2008; Waldroup et al., 2010). However, few articles demonstrate the efficiency of post-chill antimicrobials on reducing pathogens from ground chicken and their effects on product quality and shelf-life. Therefore, the objectives of this research were to evaluate the effect of various post-chill antimicrobials used in a decontamination tank on reducing *Salmonella* and *Campylobacter* and to determine the impact on shelf-life and quality attributes of ground chicken.

CHAPTER II

LITERATURE REVIEW

Food Safety Concern

The safety of our food supply is a global concern. The Center for Disease Control and Prevention (CDC) estimates that 48 million people in the US get foodborne illnesses annually, of them 128,000 are hospitalized and 3,000 cases result in death (CDC, 2011a). Even so, these numbers may be underestimated, as a large number of cases may not be reported.

Foodborne pathogens are responsible for a large number of foodborne illnesses, other sources include foods contaminated with chemicals and toxins. Many outbreaks can be serious. For example, *Salmonella* non-typhoidal, *Toxoplasma qondii* and *Listeria monocytogenes* are the top 3 pathogens contributing to domestic foodborne illnesses fatalities, resulting in 378, 327 and 255 deaths annually, respectively (CDC, 2011a). In addition, foodborne outbreaks can lead to great economic losses. Scharff (2012) estimated that $ 77.7 billion was spent on investigating foodborne pathogens related illnesses every year, with $ 3 billion lost to salmonellosis alone (Bhunia, 2008).

In the poultry industry, *Salmonella* and *Campylobacter* have caused a large number of outbreaks and recalls. Batz *et al*. (2011) estimated that each year more than 220,000 people get salmonellosis through consuming poultry products and about 4,000 of them need hospitalization. The approximate numbers of campylobacteriosis and hospitalization cases associated with poultry are 600,000 and 6,000 per year, respectively. Moreover, the economic loss is also important. In the 1990s, the annual cost of poultry-associated salmonellosis and campylobacteriosis was estimated to range from $ 64 million to $ 114.5

million and $ 362 million to $ 699 million, respectively (Bryan and Doyle, 1995). In recent studies, those costs have been estimated to increase to $ 712 million and $ 1.3 billion (Batz *et al*., 2011).

Ground poultry, compared with other poultry products, is much easier to be contaminated and has a shorter shelf-life than other poultry products. This is mainly because of the cross-contamination that occurs during processing. Foodborne pathogens that initially attach on the meat parts and surface of equipment can be spread evenly and widely during the procedures of mixing and mincing, which increases the challenges to reduce and/or eliminate them from products. In fact, 19.4% of ground turkey or chicken samples (190 of 980) collected between 2005 and 2011 by the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) were *Salmonella* positive (USDA-FSIS, 2013). The most significant outbreak of multistate salmonellosis related to ground poultry occurred in 2011 due to the consumption of contaminated ground turkey produced by Cargill Meat Solutions Corporation (CDC, 2011b). A total of 182 people from 34 states were infected with a multi-drug resistant of *Salmonella* Heidelberg. Among the cases of foodborne illness reported in this recall, 50 individuals were hospitalized and 1 case was fatal. Cargill also recalled approximately 36 million pounds of ground turkey in August, 2011, which was the third largest meat recall in the US history, and an additional 185,000 pounds of ground turkey in September, 2011.

This and other similar outbreaks and recalls prompted the USDA to release new prevention-based steps in order to improve the safety of ground poultry products (USDA-FSIS, 2012). Specifically, FSIS asked companies producing not-ready-to-eat

(NRTE) ground or otherwise comminuted chicken and turkey products to conduct a Hazard Analysis and Critical Control Point (HACCP) reassessment (FSIS Notice 33-13). In addition, the *Salmonella* verification sampling program for raw meat and poultry product has been expanded to all forms of non-breaded, non-battered comminuted NRTE poultry products. The third change in this announcement is the increase in sampling size for analyzing *Salmonella* and *Campylobacter* in ground chicken and turkey. Previous methods used a 25 gram sample to analyze for pathogens and now a 325 gram sample of product is collected to analyze for pathogens. It is believed that the new sampling standard will dramatically increase the possibility of isolating target bacteria from samples and therefore increase the positive rate of *Salmonella* or *Campylobacter* of tested products. Furthermore, FSIS intends to conduct a new performance standard for ground poultry. The number of ground samples in a set would decrease from 53 to 26, and the performance standard for ground chicken and ground turkey will be 13 and 15, respectively (9 CFR Parts 417). It is considered that the new performance standard can provide a better reflection of current producing practices and increase the efficiency of FSIS resource utilization without compromising the detecting ability. Due to these new stricter regulations, the poultry industry must evaluate and select appropriate antimicrobials to improve the safety of ground poultry products, and employ various technologies to extend the shelf-life of products.

***Salmonella* spp.**

Historical Background and General Characteristics

Salmonella was named after D. E. Salmon, an American bacteriologist, who isolated

and named a hog cholera bacillus in 1886 from a diseased pig as *Bacterium suipestifer* (later renamed *S.* Choleraesuis) (D'Aoust, 1989). By the early 1900s, many important species had been reported, such as *S.* Enteritidis, *S.* Typhi, *S.* Typhimurium, *S.* Paratyphi A and B. Now, at least 2,610 serotypes of *Salmonella* have been identified, and have been a major concern to almost all sectors of the food industry (Bell and Kyriakides, 2002).

Based on the host specificity and clinical pattern, *Salmonella* can be categorized into three types. The first one is highly adapted to animals, such as *S.* Dublin, *S,* Typhisuis and *S.* Pullorum. The second type, including *S.* Typhi and *S.* Paratyphi A, B and C, can easily host to human and is responsible for typhoid fever. The third type is zoonotic *Salmonella*, which can be detected in many species of animals that are used for consumption, and is responsible for many prevalent foodborne illnesses (Gantois *et al.*, 2009)

Salmonella are gram-negative, non-sporeforming, aerobic or facultative anaerobic short bacilli (0.7-1.5 × 2.5 μm). They can ferment sugars, produce H_2S, and most of them have motility with peritricheal flagella (Forshell and Wierup, 2006). The optimal pH and temperature for multiplication are around 7.0 and 35-37 ℃; although, *Salmonella* can tolerate pHs that range from 4.0-9.0, and temperatures that range from 5-47 ℃ and salt concentrations up to 9.0% (w/v) NaCl (Franco and Landgraf, 1996).

<u>*Salmonella* spp. in Poultry</u>

Rapid changes in consumer preferences and with the significant developments in the poultry industry, poultry products have become the primary sources of protein for people in the US. Therefore, the incidence of *Salmonella* in poultry flocks and products can significantly affect public health. Although great strides have been made to control

Salmonella, the prevalence of *Salmonella* in poultry product is still too high. According to the FSIS reports, 6.5% and 30.9% of chicken broilers and ground chicken, respectively, sold in domestic groceries were found to be *Salmonella* positive (USDA-FSIS, 2011). In terms of other countries, it was reported that the overall *Salmonella* prevalence in retailed raw poultry meat was 52.2% in China and 31.5% in Russia (Yang *et al.*, 2011; Alali *et al.*, 2012). In fact, among all the salmonellosis cases, over 20% of them were gotten through consuming poultry products (Batz *et al*., 2011). For the prevalence of different serotypes in ground poultry, *S.* Heidelberg, *S.* Hadar and *S.* Typhimurium were the most common serotypes isolated from ground chicken, and *S.* Hadar, *S.* Agona, *S.* Muenster and *S.* Senftenberg were the most prevalent ones identified from ground turkey (Schlosser *et al*., 2000). In addition, the top 5 serotypes isolated from the non-human animal sources (bovine, chicken, porcine and turkey) causing human illness in 2009 were *S.* Enteritidis (17.5%), *S.* Typhimurium (15.0%), *S.* Newport (9.3%), *S.* Javiana (4.9%) and *S.* Heidelberg (3.5%) (CDC, 2009).

A summary of the outbreaks related to consumption of *Salmonella* contaminated poultry meat in different states along with the illnesses and fatalities is presented in Table 1. There were 34 outbreaks that occurred from 2009 to 2011 resulting in a total of 1,239 illnesses, 150 hospitalizations and 1 fatality. Among all the outbreaks, 5 occurred in 2009, 14 in 2010 and 15 in 2011. Poultry meat and further processed products involved in these outbreaks included chicken broilers, BBQ wings, ground turkey and various Ready-to-Eat (RTE) poultry products. The dominated serotypes isolated from these outbreaks were *S.* Enteritidis, *S.* Heidelberg and *S.* Typhimurium, followed by *S.* Hadar, *S.* Anatum, *S.* Saintpaul, *S.* Ohio and *S.* Berta.

Table 1. *Salmonella* spp. outbreaks related to consumption of poultry meat

Year	State	Serotype/ Geno Group	Total Ill	Total Hos-pita-liza-tions	Total Dea-ths	Food Vehicle
2009	Oregon	Heidelberg	26	8	0	chicken
2009	Ohio	Typhimurium	2	1	0	chicken
2009	California	IIIa	9	5	0	turkey, other
2009	New Jersey	Enteritidis	39	5	0	chicken, marsala; pork, other
2009	Multistate	Heidelberg	35			chicken
2010	Florida	Anatum	37	1	0	chicken, BBQ; pork
2010	New York	Saintpaul	9	1	0	chicken, curry; kabobs, chicken
2010	Michigan	Enteritidis	4	2	0	chicken salad
2010	Pennsylvania	Enteritidis	21	1	0	chicken and rice
2010	Washington	Subspecies IIIa	5	4	0	turkey
2010	Rhode Island	Javiana	16	2	0	chicken salad
2010	New York	Ohio	13	1	0	chicken, roasted; rice
2010	Florida		17	2	0	chicken dishes
2010	Ohio	Heidelberg	6	5	0	turkey
2010	California	Heidelberg	21	1	0	chicken
2010	Multistate	Chester	44	7	0	Cheesy Chicken and Rice Frozen Meal
2010	Multistate	Berta	35	2	0	ground turkey, unspecified
2010	Multistate	Hadar	55			ground turkey, burger
2010	California	Typhimurium var Cope	7	0	0	chicken, baked
2011	Minnesota	Enteritidis	3	0	0	chicken
2011	Pennsylvania	Enteritidis	10	1	0	chicken picata
2011	Georgia	Typhimurium var Cope	17	3	0	chicken
2011	Pennsylvania	Montevideo	312	1	0	chicken, other
2011	New York	Typhimurium	21	2	0	turkey, roasted
2011	Massachusetts	Heidelberg	5	1	0	ground turkey, unspecified
2011	Illinois	Enteritidis	8	0	0	chicken
2011	New York	Enteritidis	15	5	0	turkey
2011	Idaho	Enteritidis	5	1	0	chicken
2011	Michigan	Enteritidis	6	3	0	chicken, shawarma
2011	Georgia	Infantis	54	3	0	turkey, BBQ; turkey, other; turkey, smoked

2011	California	Hadar	2	2	0	sandwich, chicken salad
2011	California	Heidelberg	8	0	0	sandwich, turkey
2011	Multistate	Heidelberg	190	30		liver, chicken
2011	Multistate	Heidelberg	182	50	1	ground turkey, unspecified

Source: CDC (2011c)

***Campylobacter* spp.**

Historical Background and General Characteristics

Campylobacter was first reported by McFadyean and Stockman in the early 1900s as "*Vibrio fetus*" causing an infectious abortion in sheep (Butzler, 1984). Veron and Chatelain (1973) found the differences of biochemical characteristics between "*V. fetus*" and Vibrionaceae and reclassified the former one as a new genus, *Campylobacter*. In the 1970s, studies of *Campylobacter* were developed rapidly due to the development of new culturing methods. The use of selective media combined with a microaerophilic environment made both diagnosis and study of *Campylobacter* possible (Skirrow, 1977; Allos and Taylor, 1998).

The word *Campylobacter* is derived from two Greek words "*campylos*" and "*baktron*", which mean "curved" and "rod", respectively. Therefore, the genus name *Campylobacter* is used to describe the appearance of this microorganism under microscopes (Blaser, 1986). Currently within the genus *Campylobacter*, there are 14 species in total. *C. jejuni* is one of the major species which cause foodborne human infections; other species include *C. coli, C. concisus, C. curvus, C. fetus, C. gracilis, C. helveticus, C. hyointestinalis, C. lari, C. mucosalis, C. rectus, C. showae, C. sputorum and C. upsaliensis* (Vandamme, 2000).

Campylobacter is gram-negative, non-sporeforming, curved rods that are approximately 0.5-5 μm long and 0.2-0.5 μm wide (Mahon and Manuselis, 2000). With a single polar flagellum, most *Campylobacter* species have the corkscrew darting type motility (Blaser, 1986). However, some species such as *C. showae* may have multiple flagella, and *C.gracillis* is not motile (Vandamme, 2000). *C. jejuni* is a strictly microaerophilic bacteria and can only grow in low levels of oxygen. The best atmosphere for growth of *Campylobacter* spp. contains about 5-15% CO_2 and 3-8% O_2 (Doyle, 1990). The optimal

temperature for *C. jejuni* growth is 42 ℃ (Doyle, 1990; Blaser, 2000).

Over 90 % of human campylobacteriosis are caused by *Campylobacter jejuni*, thus most studies and reviews have focused on this species (Janssen *et al*., 2008). Although it is usually assumed as spiral in shape, *C. jejuni* may show pleomorphism in different phases of a growing culture (Griffiths, 1993). In the exponential phase, morphology of *C. jejuni* cells is the typical short spiral; then the length of cells would be doubled in the stationary phase. If the culture enters the decline phase, most of the cells may become coccoid in shape, the remaining spiral cells may be 4 times the length of those in the exponential phase.

Campylobacter spp. in Poultry

Although most species of animals used for food have been found to be *C. jejuni* positive, chicken meat is considered to be one of the major sources for human *Campylobacter* infection (Doyle, 1990). In fact, the CDC reported 16 occurrences of *Campylobacter* outbreaks in the US from 2005 to 2011 which were suspected to be associated with consuming poultry meat products (Table 2). At least 140 people were infected and 17 of them required hospitalization. In 2009, contaminated poultry product was responsible for approximate 70% of campylobacteriosis in the US (Batz *et al*., 2011).

Moreover, several studies further show the role poultry plays. The first study was conducted in Iceland from 1999 to 2000 (Stern *et al*., 2003). During 1999, 116 cases of *Campylobacter* infections per 100,000 people were reported in Iceland, with 62% of broiler carcasses in the market tested *Campylobacter* spp. positive at the same time. In 2000, new regulations required that carcasses from poultry flocks which tested positive for *Campylobacter* must be frozen till distribution, as freezing was reported to be an effective

method to control *Campylobacter* in food (Humphrey and Cruickshank, 1985; Georgsson *et al*., 2006). As a result, only 15 % of broilers were contaminated with *Campylobacter* spp.. At the same time, incidence of campylobacteriosis dropped to 33 foodborne illnesses per 100,000 people.

Furthermore, a 12-month study conducted in the US from 1998 to 1999 found 1,316 patients from different states who were confirmed to have *Campylobacter* infections (Friedman *et al.*, 2004). Through using a standardized questionnaire, the infection route of every patient was identified. The results showed that contaminated chicken was responsible for approximately 24% of all campylobacteriosis, which was the largest population fraction.

Table 2. *Campylobacter*, spp. outbreaks related to consumption of poultry meat

Year	State	Serotype/ Geno Group	Total Ill	Total Hos-pita-liza-tions	Total Dea-ths	Food Vehicle
2005	Wisconsin		13	2	0	liver, chicken
2005	Washington		9	0	0	liver, chicken
2005	Oklahoma		28	1	0	fajita, beef; fajita, chicken; onions; peppers, unspecified
2006	Ohio		13	2	0	chicken, grilled; sausage, bratwurst
2006	Wisconsin	Enteritidis	2	2	0	turkey, baked
2006	California		10	2	0	chicken, soy sauce; pork, soy sauce
2007	Washington		3	1	0	chicken
2007	Michigan		11	0	0	chicken, unspecified
2007	Kansas		13	0	0	turkey, smoked
2008	Florida		2			sandwich, chicken
2008	North Carolina		8	2	0	chicken, BBQ
2008	Rhode Island		4	3	0	chicken
2008	Washington		4	1	0	turkey
2010	Pennsylvania		10	0	0	chicken
2010	Tennessee		6	1	0	BBQ, unspecified; chicken, BBQ; sausage
2011	Washington		4	0	0	chicken

Source: CDC (2011c)

Intervention Strategies for Pathogen Control in Processing Plants

To minimize the incidence of contamination of poultry carcasses, various antimicrobial strategies have been employed during different processing steps. Many factors including physical and chemical environment, properties of bacteria and adjustment of equipment can determine the effectiveness of those treatments in reducing pathogens from poultry products.

Multi-hurdle Approaches to Reduce Pathogens During Processing

Feather removal has been considered as an important contributor to contamination and cross-contamination of bacteria for many years (Lillard, 1986; Beresford *et al*., 2001; Arnold, 2007). The niches formed on picker fingers which contain water and processing debris provide a proper environment for attachment of bacteria and the development of biofilms. In addition, the integrity of the skin may be compromised during defeathering, allowing bacteria to lodge underneath the skin's superficial layers (Thomas and McMeekin, 1980).

To reduce the cross-contamination and overall microbial loads on carcasses and in the picker, several studies have focused on the effects of replacing fingers frequently and applying disinfectants. Arnold (2007) found that no bacteria growth occurred on new picker fingers collected from different processing plants. They also indicated that with employing disinfectants early in the defeathering process, sanitation practices can be enhanced.

Steps of spray-washing are widely used in various areas of poultry processing plants. Carcass rinsing with water or disinfectants not only removes most of the visible feces, but reduces the overall microbial levels on broilers as well. Generally, on-line steps where spray washers can be included are pre-scald washer, post-feather pick washer, inside/outside

washer, post-evisceration washer and final pre-chill washer. Although no single step significantly reduced bacterial contamination by itself, combined washing steps had a significant effect on pathogen control (Berrang and Bailey, 2009).

Studies have shown that the effectiveness of spraying washers in reducing bacteria on carcasses is determined by many factors, such as contact time, water temperature, and pressure of solution. Longer contact time (90 s) and higher water temperatures (55 and 60 ℃) were shown to enhance the efficacy of removing bacteria from carcasses without causing any color change (Li *et al*. 1997; Li *et al*., 2002). Treatments combined with proper pressure (30 psi) achieved good reduction, but high pressure (42 psi) actually increased the occurrences of cross-contamination by forcing bacteria into the tissue of broiler carcasses (Brashears *et al.*, 2001).

During processing, chilling is one of the most important steps in reducing bacteria and controlling cross-contamination. The most prevalent chilling method used in the US is counter-current immersion chilling. In this system, the movement of water in tanks is in the opposite direction to that of the carcasses, which allows carcasses to contact the cleanest, coldest water at the end of the chiller. The rate of overall water flow is determined carefully in order to minimize the microbiological loads. In addition, antimicrobials are added in the chiller to control the cross-contamination. The purpose of entire process is to inhibit the growth of microorganisms by reducing the carcass temperature. The USDA requires that a temperature of 4 ℃ or less must be achieved within 4 hours for broilers and 8 hours for turkeys (USDA, 1973).

Compared to air chilling, immersion chilling exposes all carcasses to pathogens present in the chill water, and is considered to have a greater chance of potential cross-contamination. A study on comparing different chilling systems showed that the incidence of bacteria in immersion-chilled broilers was significantly higher than that in air-chilled broilers, due to the increased prevalence of cross-contamination that can occur during water chilling (Sanchez *et al.*, 2002). Contrary to these findings, Huezo *et al.* (2007) reported no difference in concentration and prevalence of bacteria recovered from broiler carcasses between two chilling methods. Differences between these studies could be caused by increased handling of air-chilled carcasses, relative cleanliness of immersion chillers and species of bacteria tested.

Studies show varying results when evaluating the role of immersion chilling system plays in the carcass cross-contamination. Cason *et al.* (1997) reported no change in the prevalence of *Salmonella* and a decrease in *Campylobacter* prevalence following immersion chilling, which may be caused by the different positive rate of *Salmonella* and *Campylobacter* on carcasses (approximate 20% and 90%, respectively). If the initial prevalence of pathogen is high, the process of chilling can actually help to reduce the positive rate of carcasses. However, in situations where the proportion of contaminated carcasses is low, it is very likely that pathogens can spread to previously negative carcasses from contaminated ones. This conclusion was further supported by Izat *et al.* (1988) who reported a reduction of *Campylobacter* after immersion chilling when the initial microbial levels on carcasses were high; while there was no significant change found when *Campylobacter* levels were low prior to chilling.

Antimicrobial Agents Applied in Poultry Processing

In many cases, it is not effective to prevent cross-contamination and to reduce pathogen levels by only using physical treatments. A majority of poultry processing plants in the US apply different antimicrobial agents in processing to improve the safety of poultry products. The antimicrobials recognized as chemical interventions by FSIS for reducing pathogens in poultry products during post-chill processing are presented in Table 3 (FSIS Directive 7120.1).

As one of chemical agents applied in poultry industry, chlorine has been widely used in spray washers and immersion chillers in processing facilities. Through removing visible soil, protein residues and carbohydrate materials from contaminated areas, chlorine may clean the surface of processing equipment and reduce the bacterial level physically. The efficacy of chlorine as an antimicrobial is largely dependent on the pH and the presence of organic matter in solution (Lillard, 1979). In solutions containing high organic loads and alkaline detergents, chlorine's antimicrobial efficacy is drastically reduced, resulting in little or no antimicrobial activity.

In practice, chlorine is used in the form of hypochlorite in poultry chillers. The concentration of free available chlorine applied in chilling water can be up to 50 ppm (FSIS Directive 7120.1). Because of a relatively high organic level in chiller system, the efficacy of chlorine is often compromised. Besides this, the pH value of treatments is usually adjusted to be slightly acidic (pH 4.0-6.0) in order to convert the majority of chlorine to the most active antimicrobial form, hypochlorous acid (Byrd and McKee, 2005).

Though chlorine has been applied by poultry processing plant to extend shelf-life of product since 1950s, little direct effect on reduction of carcass bacteria has been found. Lillard (1993) reported that chlorine cannot rapidly access attached *Salmonella*, which affected the antimicrobial efficacy. Although, chlorine maintained at 45-50 ppm may reduce viable bacteria in chill-water, and may help in controlling cross-contamination of carcasses (Mean and Thomas, 1973).

Another chlorinated compound used in poultry chilling system is chlorine dioxide. Chlorine dioxide does not react with nitrogenous or ammonia compounds, thus has greater oxidizing capacity than chlorine. Also, the lethal effect on bacteria would not decrease at alkaline circumstance (Byrd and McKee, 2005). It is reported that chlorine dioxide was seven times more effective than chlorine on bactericidal effect due to its greater solubility, better oxidizing capacity and lower reactivity with organic materials (Lillard, 1979). In addition, Thiessen *et al.* (1984) showed that chlorine dioxide had a dose-dependent effect on reducing *Salmonella* existing in chilled water or on broiler carcasses.

Trisodium phosphate (TSP) is one of the alkaline detergents approved for spray applications and carcass dips in some poultry processing plants. As an antimicrobial, TSP can remove bacteria from carcass surfaces by high alkalinity and means of its surfactant properties. Also, it was reported that TSP killed bacteria via disrupting the cell membrane and causing leakage of cellular material (Giese, 1993). Slavik *et al*. (1994) reported a significant reduction of *Campylobacter* levels on poultry carcasses when a 10% TSP solution was used in carcass dip. In addition, TSP at 5% and 10% was found to be effective for removing *S*. Typhimurium on chicken skin (Xiong *et al.*, 1998).

However, the drawbacks of TSP cannot be ignored. Specifically, if TSP is used prior to carcasses entering the chiller, the resulting high pH may increase the alkalinity of chill water to approximately pH 11-12, which compromises the antimicrobial activity of added chlorine (Mu *et al*., 2007). Any attempt to rinse off the residual TSP from carcasses would cause extra cost and time. Additionally, large amount of phosphate in wastewater can cause environmental issue. Furthermore, the metal equipments may be corroded by those phosphate compounds after using for a long time (Byrd and McKee, 2005).

Peracetic acid (PAA), a strong oxidant, currently is the most widely used antimicrobial in poultry chillers. The mode of action of PAA as an antimicrobial is based on the release of active oxygen (Liberti and Notarnicola, 1999). Through oxidizing sensitive sulfur and double bonds in proteins, lipids and other metabolites, PAA can deactivate enzymes, rupture cell walls and eventually impair bacteria and viruses. Though its antimicrobial properties were first reported in 1902 (Freer and Novy, 1902), PAA did not incur enough attention until years later, when the technologies were developed for the commercial processing and production of PAA were developed (Block, 1991). Bauermeister *et al*. (2008) found that 0.0025% and 0.02% PAA effectively reduced *Salmonella* and *Campylobacter* attached on broilers. Also, the shelf-life of poultry carcasses treated by 0.015% or 0.02% PAA and stored at 4 ℃ was significantly extended from 15 days to 21 days.

Cetylpyridinium chloride (CPC), which is commonly found as a cationic quaternary ammonium compound in some mouthwashes, toothpaste and nasal sprays, has been approved for use on pre-chill and post-chill poultry in 2004 and 2006, respectively (21 CFR Parts

173.375; FAP No. 6A4767). Many studies have cited its antimicrobial properties for controlling a number of foodborne pathogens on poultry, including *Salmonella*, *Campylobacter*, *E. coli* O157: H7 and *Listeria monocytogenes*. Bai *et al*. (2007) found that the shelf-life of boneless, skinless broiler thigh meat was extended for 2 days after samples were treated by 1% or 1.5% CPC solution. In addition, there were significant reductions in Aerobic Plate Count (APC) of CPC treatments. In another study, a 4.87-log reduction of *S*. Typhimurium on chicken drumsticks was achieved by the treatment with a CPC concentration of 4 mg/ml and contacting time of 3 min (Breen *et al*., 1997).

Moreover, many antimicrobial agents can affect the shelf-life of food through accelerating or retarding the lipid oxidation. Jimenez-Villarreal *et al*. (2003) compared lipid oxidation status of ground beef that was treated by several antimicrobials. The results showed no difference between control group and CPC, TSP and chlorine dioxide treatments on day 0 and 3 of display, whereas chlorine dioxide had higher lipid oxidation than the rest of the treatments on day 7 of display. In another study, all the antimicrobial treatments used in ground beef (including potassium lactate, sodium metasilicate, peroxyacetic acid and acidified sodium chlorite) had lower lipid oxidation condition compared with control group on day 0, 3 and 7 of display (Quilo *et al*., 2009). In addition, peroxyacetic acid had the lowest TBARS values than the rest of the treatments on day 7 of display.

Table 3. Approved antimicrobials used for poultry products during post-chill processing.

Substance	Amount
Acidified sodium chlorine	500 to 1200 ppm
Calcium hypochlorite	Not to exceed 50 ppm as free available chlorine
Cetylpyridinium chloride	Not to exceed 0.8 percent
Chlorine gas	Not to exceed 50 ppm as free available chlorine
Chlorine dioxide	Not to exceed 3 ppm residual chlorine. Dioxide
DBDMH (1,3 dibromo-5,5-dimethylhydantion	Not to exceed 100 ppm active bromine
Electrolytically generated hypochlorous acid	Not to exceed 50 ppm as free available chlorine
An aqueous solution of citric and hydrochloric acids adjusted to a pH of 1.0 to 2.0	Applied as dip with a minimum contact time of 2 to 5 seconds
A blend of citric, phosphoric, and hydrochloric acids	Sufficient for purpose
Lactic acid bacteria mixture consisting of *Lactobacillus acidophilus*, *Lactobacillus lactic*, and *Pediococcus acidilactici*	10^5 to 10^6 CFU of lactobacilli per gram
Lauramide arginine ethyl ester (LAE)	Not to exceed 200 ppm
Ozone	In accordance with current industry standards
Solution of peroxyacetic acid, hydrogen peroxide, acetic acid, and hydroxethylidene-1,1-diphosphonic acid (HEDP)	In either application, peroxyacetic acid will not exceed 2000 ppm, hydrogen peroxide will not exceed 165 ppm, and HEDP will not exceed 14 ppm
Sodium hypochlorite	Not to exceed 50 ppm as free available chlorine
Sodium metasilicate	Not to exceed 2 percent by weight of the marinade
Trisodium phosphate	8-12 percent solution for up to 30 seconds, 8-12 percent solution within 65 ℉ to 85 ℉ for up to 15 seconds

Sources: FSIS Directive 7120.1

Micro Shelf-life of Poultry Products

Poultry meat is generally considered as a highly perishable food. The processing of broilers leaves the surface of products susceptible to pathogenic organisms, meat-spoilage microorganisms and cross-contamination that can accelerate the spoilage of product. Therefore, many antimicrobial strategies have been employed to protect the meat from external microbial contamination as well as extending their shelf-life.

Microbial Spoilage of Poultry

The microbiological quality of poultry meat is greatly determined by the physiological status of animal before slaughter, the contamination during processing, and the conditions of storage and distribution. Specifically, once animals are slaughtered, the inherent antimicrobial defense mechanisms would be destroyed rapidly, which causes the meat to be sensitive to microbial spoilage. The microorganisms either derived from the intestine of animals or from the environment with which animals or carcasses had contacted before or during processing can cause contamination of meat. Bolder (1998) reported that Enterobacteriaceae on meats are more likely to be acquired from the work surface than the feces. In addition, the original sources of psychrophile bacteria include carcasses and cut portions at all steps of processing.

The range of microbial genera isolated from poultry meat is listed in Table 4. A consortium of bacteria, dominated by *Pseudomonas* spp., is responsible for most of the spoilage cases of poultry stored aerobically at refrigerator temperature. When the amount of pseudomonad reaches approximately 10^7-10^8 per ml, off-odor and slime are apparent. This is the evidence when the lactate and glucose in meat have been exhausted and

nitrogenous compounds start to be metabolized by pseudomonad (Nychas and Tassou, 1997).

The genera type of microorganisms and their growth rate are largely dependent on temperature. Generally, a temperature reduction below the optimum can lead to an extension of generation time. Gill (1986) estimated that the growth rate of bacteria would approximately double with every 10 ℃ increase. While this effect is more pronounced under 10 ℃ that the growth rate will double with only 2-3 ℃ rise. Therefore, the generation time for pseudomonad is approximately 1 h at 20 ℃, 2.5 h at 10 ℃, 5 h at 5 ℃, 8 h at 2 ℃ and 11 h at 0 ℃ (James, 2005). The shelf-life of meat and poultry stored at refrigerator temperature would achieve the maximum at -1.5 ±0.5 ℃ without any surface freezing.

Cold-tolerant Enterobacteriaceae have also been isolated from meat stored aerobically at chilling temperature, while in terms of numbers they do not play an important role in the predominant microbial associations (Zeitoun *et al*., 1994). The total Enterobacteriaceae is usually used as an indicator of food safety.

Table 4. The bacteria and yeasts frequently found on poultry

	Genera		
Bacteria	*Acinetobacter*	*Aeromonas*	*Alcaligenes*
	Alteromonas	*Arthrobacter*	*Bacillus*
	Brochothrix	*Campylobacter*	*Carnobacterium*
	Chromobacterium	*Citrobacter*	*Clostridium*
	Corynebacterium	*Enterobacter*	*Enterococcus*
	Escherichia	*Flavobacterium*	*Hafnia*
	Kluyvera	*Kurthis*	*Lactobacillus*
	Leuconostoc	*Listeria*	*Micrococcus*
	Moraxella	*Neisseria*	*Pantoea*
	Pediococcus	*Planococcus*	*Proteus*
	Pseudomonas	*Serratia*	*Streptococcus*
	Streptomyces	*Staphylococcus*	
Yeasts	*Candida*	*Debaryomyces*	*Trichosporon*

Source: Stanbridge and Davies (1998)

Strategies to Extend Shelf-life in Industry

Modified atmosphere packaging (MAP) is one of the most effective techniques used for extending shelf-life and preventing meat from microbial contamination in industry. The mechanism of MAP is to generate an alternative gaseous environment for maintaining quality and safety of foods by altering the composition of air (78% N_2, 21% O_2, 0.03% CO_2 and trace gases) (Phillips, 1996). There are two different methods, namely active and passive MAP. Active modification is achieved by employing a desired mixture of gases to replace the air in the package. While passive modification packages the product in a gas-barrier film, in which the desired atmosphere can be developed naturally by microbial metabolism or residual respiration in the product.

The principal gases applied in MAP for preservation of poultry are CO_2, O_2 and N_2. In practice, a minimum concentration of 20-30% CO_2 is required to achieve an inhibitory effect (Saucier, 2000). Extended shelf-life of meat stored at anaerobic conditions is caused by the growth of lactic acid bacteria in meat. This kind of spoilage is generally described as souring, rather than putrefaction which is developed aerobically. The deliberate increase of lactic acid bacteria eventually suppresses the development of aerobic Gram-negative bacteria (pseudomonad and Enterobacteriaceae), and this approach is known as biopreservation (Holzapfel *et al*., 1995).

The effects of MAP on poultry meat have been evaluated by numerous studies. Baker *et al.* (1985) reported a significant extension of shelf-life in ground chicken was achieved by storing ground chicken in a MAP system, and treatments with higher CO_2 concentration had a longer shelf-life extension than treatments with lower CO_2 concentrations. It was further supported by Saucier *et al*. (2000) that growth of total

aerobic mesophilic, coliforms and *Escherichia, coli* on ground chicken packaged in MAP had been inhibited.

Another effective intervention strategy for maintaining the microbiological safety of meat product is irradiation. Because of its potential public health benefits, studies of irradiation treatment on poultry meat have been conducted since the early 1960s, (Mossel, 1966). The application of irradiation in food processing is based on the fact that cellular DNA exposed to ionizing radiation can be easily damaged, which leads to the inactivation of living cells of microorganisms, plant meristems and insect gametes. During inactivation, nucleic acids are damaged by oxidative free radicals which generate from the radiolytic breakdown of water molecules.

Irradiation has many advantages when employed during food processing. Specifically, the chemical changes in foods induced by radiation are minimal. Furthermore, irradiation, which causes virtually no temperature increase in foods, can be used on frozen product and product packaged with heat-sensitive materials. Therefore, irradiation is an ideal terminal antimicrobial treatment in processing, even after packaging, without increasing the possibility of recontamination. Through combining irradiation with the HACCP approach to risk management, it provides a physical Critical Control Point (CCP) for maintaining the safety of fresh or mildly-processed products. However, the application of irradiation in food industry is not very successful. It is mainly caused by its name, which has connotations of radioactivity, resulting in a low level of acceptance to consumers. Moreover, FDA establishes the regulations that irradiated foods must be labeled “Treated

with Radiation" or "Treated by Irradiation" and displays the irradiation logo (21 CFR Parts 179). This regulation further limits the development of food irradiation.

A number of studies have been conducted to determine the antimicrobial effect of irradiation combining with other interval strategies. Javanmard *et al.* (2006) evaluated the shelf-life of broilers which were treated by the combination of gamma irradiation and frozen storage. The results showed a significant reduction on overall microbial loads and dramatic extension on shelf-life of chicken meat. In another study, Kudra *et al*. (2012) found that *C. jejuni* was effectively eliminated from poultry meat packaged in vacuum or MAP after irradiation.

Meat Quality Determination

The definition of meat quality usually encompasses two parts: physical intrinsic qualities (color, shape, appearance, tenderness, juiciness and flavor) and extrinsic qualities (brand, origin, quality mark and production environment) (Steenkamp, 1997). In this study, the evaluation of meat quality was focused on the intrinsic characteristics.

Sensory evaluation is defined as a scientific method applied to evoke, measure, analyze and interpret the responses to samples as perceived by the human senses of sight, smell, tough, taste and hearing (Stone and Sidel, 2004). Panelists are used to assess the sensory attributes of products while providing responses, at the same time, instruments can be used to measure the physical or chemical characteristics which can influence the sensory stimulus perceived by panelists. Both human and instrumental methods are important for assessing sensory quality. Panelists differ in the experiences with foods and their innate

ability to sense stimuli allowing a base for the neurological categorizing of a stimulus and the subsequent varieties of responses. Instruments can be calibrated and programmed to respond consistently, which can be used to predict or relate to the anticipated sensory experience.

Sensory Methods to Evaluate Poultry Quality

Generally there are two types of sensory methods to evaluate poultry quality, namely analytical and affective methods (Lawless and Heymann, 2010). Analytical methods are to focus on determining whether differences exist in samples and how these differences can be described. Therefore a few panelists (6 to 12) are screened and trained for their sensory acuity and ability to describe samples. On the other hand, procedures to investigate how consumers react when evaluating given samples are called affective methods. The reactions that panelists are asked to convey are whether or how they like/dislike, accept/reject samples. Affective methods require a large number of people without training or screening, so the results of testing can represent the behavior of general consumers.

Besides conditions of panelists, sample preparation and environment control are very important for conducting a reliable sensory evaluation. Specifically, samples used for testing must come from a uniform source (De Wijk *et al*., 2003). One factor that needs to be decided when sampling poultry meat is how many samples are needed each time. This is largely depended on the type of testing and test questions. Another is how samples will be cooked, prepared and presented in order to provide nearly identical samples for each panelist under identical conditions. Both size and serving temperature of sample should be uniform. Water and unsalted crackers should be provided between samples to prevent taste carryover

(Lucak and Delwiche, 2009). Sometimes, questions, such as how to cook samples, how to check the internal temperature, what oven temperature to set, and what internal temperature to use should be considered as well.

It is necessary to do the environment controls for the room where panelists perform the tests. As human are designed to perceive stimuli constantly and unconsciously, panelists must be given an area that minimizes the environmental influence so that they can concentrate on the specific stimuli of test samples. In addition, individual booths are needed, which can isolate the panelist from others to avoid potential distraction and collaboration.

Instrumental Methods of Analysis

Instrumental methods to estimate the tenderness of poultry meat have been studied and widely applied by researchers and industries for decades. These procedures offer numerical values, which relate to tenderness. Most of the instrumental data used to describe tenderness in cooked poultry samples are based on the Warner-Bratzler (W-B) or the Allo-Kramer Shear Press (KSP) (Lyon and Lyon, 2001). The basic principle of them is that the tenderness/toughness of a sample should be related to the total force to cut through it. The force is historically recorded in weight measurements at first, then data can be converted to the force unit, if needed. Another technique, the Texture Profile Analysis (TPA), has been used to describe the texture information for poultry meat. Compared with shear test, the main drawback of the TPA test is the cost of purchasing the instrument and maintenance for the instruments are much higher (Lyon and Lyon, 2001).

Color is a major component of appearance in poultry products. Instrumental methods to measure color of objects involve a light source and a detector. When

instruments detect the light wavelengths reflected by objects, results must be converted to numerical values in order to understand the meaning. For example, in "L*a*b*" color coordinate system, "L* value" is associated with "lightness", "a* value" is associated with "redness" and "b* value" is associated with "yellowness". It was reported that the color of poultry meat can be affected by many factors, such as age, sex, strain, processing conditions, oxidation status, cooking temperature and freezing (Fletcher, 1995). Specifically, meat color can be greatly affected by oxidation during storage. It was found that the oxidation of heme pigments (hemoglobin and myoglobin) and lipid lead to the reduction of lightness and redness of various meat samples (Faustman *et al*., 1992; Mancini and Hunt, 2005; Xiao *et al*., 2011). Because color is one of the most direct ways to judge the quality and safety of poultry meat, any specific color defects or color variation may lead to the rejection from consumers.

Flavor of poultry product can be analyzed through extracting and identifying compounds, which are assumed to be related with aroma. Unlike the taste that is usually associated with five basic flavors of salt, sweet, sour, bitter and umami, aroma is generated by the stimulation of receptors in the nasal cavity by volatiles. The common instruments used to isolate components and determine their concentrations include high pressure liquid chromatography (HPLC), gas chromatography (GC) and sensing devices known as "electronic noses" (Lyon and Lyon, 2001).

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CHAPTER III

MATERIALS AND METHODS

Pilot Plant Study

A total of 320 lbs of boneless chicken breast and thigh with skin were used for the experiment (20 lbs per treatment × 2 replications × 8 treatments). During each replication, sufficient bone-in chicken breast and thigh meat with skin were obtained the day prior to the research and stored at 4 ℃ at the Auburn University Poultry Science Research Unit. Chicken parts were deboned and put in sterile rinse bags with 2 lbs breast and 2 lbs thigh per bag. Bags with parts were continually stored at 4 ℃ for 16 h.

Thirty-five bags of parts per replication (five bags per treatment) were inoculated with 1 mL of *Salmonella* Typhimurium (10^8 CFU/mL) and *Campylobacter jejuni* (10^8 CFU/mL) suspensions and massaged for 1 minute. Inoculated parts were set aside for a 30 min attachment period before being rinsed in the decontamination tank (COPE unit, Morris & Associates Inc., Garner, NC). The chill water treatments included 0.003% chlorine, 0.07% PAA, 0.1% PAA (Spectrum; FMC, Philadelphia, PA), 0.35% CPC, 0.6% CPC (SFC, North Little Rock, AR) and a water treatment. Chilled water (4 ℃) was used to bring treatments to the proper concentration. Positive and negative controls were included as well. Positive, or non-dipped inoculated parts, were used to determine the recovery of *Salmonella* and *Campylobacter* on ground chicken. Negative, or noninoculated parts were used to determine the prevalence of any background *Salmonella* or *Campylobacter*. Concentration of chlorine was measured using Aquachek Water Quality Test Strips (Hach Company, Loveland, CO). The concentrations of PAA and CPC were determined using titration drop test kits (FMC, Philadelphia, PA; Safe Foods Corporation, North Little Rock, AR). The pH

of treatments was recorded by a pH meter (HACH Company, Loveland, CO). The average pH of 0.07% and 0.1% PAA treatments were 3.40 and 3.32; the average pH of 0.35% and 0.6% CPC treatments were 7.10 and 6.96, respectively. The average pH of the chlorine treatments were adjusted to 5.62 by 1N HCl allowing for the development of hypochlorous acid. The temperature range of all the treatments was from 10 to 15 ℃.

Chicken parts were rinsed using a decontamination tank (13 gal) for approximate 23 s. The CPC treatments were sprayed with sterile water after dipping in the tank, which is requirement of the regulations for using CPC in poultry by the Food and Drug Administration (FDA) (21 CFR, Parts 173.375). In addition, cleaning and sanitation procedures were conducted between each treatment. Specifically, the remaining chicken tissue and antimicrobial agents were first rinsed off the decontamination tank using potable water. Then, chlorinated foam cleaner (Soft Jam Co., Atla Loma, CA) was applied on the decontamination tank, followed by scrubbing and rinsing. The next step was to apply BioQuatTM 20 disinfectant sanitizer (HACCO, Inc., Randolph, WI) to the tank. After setting for 20 min, the tank was again rinsed by water.

When finishing rinsing procedures, each batch of 4 lbs of parts was ground using mini-grinders (Megaforce 3000, STX, Lincoln, NE) and was aseptically collected in a sterile rinse bag. Samples were kept on ice and stored in coolers for 1 h before transporting to the laboratory at Auburn University, Department of Poultry Science for analysis.

Campylobacter and *Salmonella* Inoculum Preparation

One mL of the *Campylobacter jejuni* culture was added to 10 mL of Brucella-FBP

broth (Acumedia Manufactures Inc.). The culture was incubated at 42 ℃ for 48 h in a Anaero-Jar (Oxoid, Ogdensburg, NY) containing a CampyGen sachet (Oxoid) which generates a microaerophilic mixture of 5% O_2, 10% CO_2 and 85% N_2. The culture was streaked onto Campy-Cefex agar (Acumedia) and incubated at 42 ℃ for 48 h in microaerophilic conditions as previously described. Isolated *C. jejuni* colonies were picked from the Campy-Cefex plates and added to 10 mL of Brucella-FBP broth tubes before incubating at 42 ℃ for 48 h in microaerophilic conditions. One mL of solution was further incubated with 99 ml of fresh Brucella-FBP broth for 48 h in the same conditions. Cultures were centrifuged (Sorvall Legent RT+ Centrifuge, Thermo Scientific, Thermo Electron Corp., Germany) at 8000 × g for 10 min at 4 ℃. The pellets were suspended in equivalent amounts of buffered peptone water (BPW; Acumedia) and centrifuged again. After resuspended with BPW, a stock culture of 10^8 cells/mL *C. jejuni* was prepared.

Ten mL of tryptic soy broth (TSB) was inoculated with 10 μL of frozen nalidixic acid-resistant (35 μL/mL) strain of *Salmonella* Typhimurium and the culture was incubated at 37 ℃ for 24 h. The *Salmonella* culture was streaked onto xylose lysine tergitol 4 agar (XLT4, Acumedia Manufactures Inc., Baltimore, MD) containing 35 μL/mL of nalidixic acid. After incubating at 37 ℃ for 24 h, isolated black colonies were picked from the XLT4 plates and were added to 10 mL of TSB tubes with 1 colony per tube. The tubes were incubated at 37 ℃ for 24 h. One mL of *Salmonella* culture was added to 99 mL of TSB in a conical flask and incubated at 37 ℃ for 12 h. Cultures were centrifuged and suspended twice following the procedures previously described. A stock culture of 10^8 cells/mL of *Salmonella* Typhimurium was prepared and mixed with 10^8 cells/mL *C. jejuni* before inoculating

samples.

Ground Chicken Sampling and Enumeration of *Salmonella* and *Campylobacter*

A raw meat and poultry product sampling method described in the *Microbiological Laboratory Guidebook* was used for sampling and enumeration with several modifications (FSIS, 2004). Once the samples arrived at the laboratory, the fresh ground meat was thoroughly mixed by hand. Four 25 g samples were weighed from each 4 lb batchs and placed into their own sterile filtered Whirl Pak bags (69 oz/2041 mL; Nasco, Fort Atkinson, WI) containing 50 mL of BPW. Individual bags were stomached for 1 minute using a lab blender (400 Circulator, Seward, England) in order to homogenize samples. Based on the results of a pre-test (unshown data), reducing the BPW volume from 225 mL to 50 mL did not negatively affect the recovery of *Salmonella* or *Campylobacter*. In fact, 50 mL of the BPW had the better recovery than 100 mL or 225 mL.

Direct-plating methods were used for enumerating inoculated samples. The ground meat was diluted by the BPW (1:10) in order to make serial dilutions. Then, samples were added to selective media in duplicate. For *Salmonella,* 0.1 mL of the sample from the proper dilution was added onto the XLT4 media containing nalidixic acid (35 μL/mL) and spread evenly using a sterile plastic disposable spreader before it was dried. After that, the plates were inverted and incubated at 37 ℃ for 24 h. Bacterial populations were converted and reported as log CFU/g of ground meat.

Plating methods for *Campylobacter* were similar to those for *Salmonella*, while Campy-Cefex agar was used for enumerating *Campylobacter* (Stern *et al.*, 1992). Then

plates were inverted and incubated at 42 ℃ in AnaeroPack rectangular jars (Mitsubishi Gas Chemical America, Tokyo, Japan) in a microaerophilic environment as previously described for 48 h. Results were also converted to log values with 75 g of homogenate representing the sample and reported as log CFU/g of ground meat.

Shelf-life and Quality Determination

A total of 200 lbs of ground chicken meat (5 lbs per treatment × 2 replications × 5 treatments × 4 days) were prepared at the Auburn University Poultry Science Research Unit. Specifically, boneless chicken breast and thigh meat were transported from a local supplier (1 day after processing) and stored at 4 ℃ at the Auburn University Poultry Science Research Unit before use. On day 0, chicken parts were randomly divided among the five post-chill antimicrobial treatments. The treatments used in this section included 0.003% chlorine, 0.07% PAA, 0.1% PAA, 0.35% CPC and 0.6% CPC. Parts were dipped for approximately 23 s and ground twice through a grinder (Mini-32, Biro Manufacturing Marblehead, OH). Following grinding, the ground meat was stuffed into E-Z Pak Poly bags (Manchester Packaging, Saint James, MO) using a piston stuffer (SC-50 Hydraulic, Koch Equipment, Kansas City, MO) with 1 lb of meat per bag. Then, bags were clipped using a poly-clipper (Poly-clip system LLC, Mundelein, IL) and distributed in a walk-in refrigerator (Thermo-Kool, Mid-South Industries Inc., MS) at Auburn University Department of Poultry Science and maintained at 4 ℃.

Aerobic plate count (APC) and psychrotroph (PSY) were analyzed to determine the level spoilage in the ground meat. Specifically, tests were conducted in duplicate at days 1,

4, 7 and 10. At each storage period, 10 bags from each treatment (5 bags in the morning and 5 bags in the afternoon) were randomly selected for analysis. Samples (4-25 g samples of ground meat/bag) were aseptically collected in a sterile whirl-pak bag (69 oz/2041 mL; Nasco, Fort Atkinson, WI). Samples were homogenized; 25 g of ground meat with 50 mL of BPW and serial dilutions were made following the procedures previously described. One hundred microliter of the appropriate dilution was added to Standard Methods Agar (Acumedia) using spread-plating method in order to enumerate APC and PSY. The APC plates were incubated at 37 ℃ for 24 h and PSY were incubated at 4 ℃ for 10 days.

Quality determination, which included color measurement and sensory evaluation, was conducted in duplicate at days 1, 4 and 7. Two 40 g samples of ground meat were first collected and made as a patty size. The color value of each patty was recorded using a Minolta Colorimeter (DP-301, Minolta Corp., Ramsey, NJ) prior to baking. Results were converted to numerical values using the Hunter L* a* b* color coordinate system in which L* value, a* value and b* value are associated with lightness, redness and yellowness, respectively.

All the sensory tests were approved by Auburn University Institutional Review Board (IRB). Sensory evaluations were conducted in duplicate (1 panel in the morning and 1 panel in the afternoon) with untrained panelists (30 panelists in the morning and 30 panelists in the afternoon) in the Auburn University, Department of Poultry Science. After color measurement, all the chicken patties were baked to an internal temperature of 74 ℃ in muffin top trays in a convection oven (Viking Professional Series, VESC Series, Greenwood, MS) set at 177 ℃. Cooked patties were cut into bite-sized pieces and placed into coded sampling

cups with lids (Solo Cup Company, Highland Park, IL). Samples were stored in a warmer at 93 ℃ for less than 1 h until they were served to panelists. Samples were served one at a time and panelists were asked to evaluate chicken patties based on a modified 8-point hedonic scale. Attributes in the hedonic scale included appearance (like extremely to dislike extremely), odor (like extremely to dislike extremely), flavor (like extremely to dislike extremely), texture (extremely tender to extremely tough), juiciness (extremely moist to extremely dry) and overall acceptability (like extremely to dislike extremely).

Statistical Analysis

All the experiments were conducted in duplicate. Data was analyzed with ANOVA in the GLM of SAS 9.1 (SAS Institute, Cary, NC). Differences were considered significant at $P \leq 0.05$.

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CHAPTER IV

RESULTS AND DISCUSSION

Antimicrobial Effects of Various Treatments on *Salmonella* and *Campylobacter*

The reduction values of *S.* Typhimurium and *C. jejuni* on ground chicken that were treated with various antimicrobials (0.003% chlorine, 0.07% PAA, 0.1% PAA, 0.35% CPC and 0.6 % CPC) were evaluated (Figure 1 and 2). The PAA and CPC treatments significantly decreased *Salmonella* and *Campylobacter* on ground chicken compared with the positive control, water control and chlorine treatments. Specifically, 0.07% PAA and 0.1% PAA had the greatest reductions on *Salmonella* and *Campylobacter*, which were approximate 1.5-log and 1.3-log reductions, respectively. 0.35% CPC and 0.6% CPC also achieved an approximate 0.8-log reduction on both *Salmonella* and *Campylobacter*. Treatment with chlorine (0.003%) was no different than the treatment with water and was the least effective treatment. Specifically, the chlorine treatment resulted in levels of *Salmonella* and *Campylobacter* that were only slightly lower than levels of positive control. There was no difference in the reduction of *Salmonella* and *Campylobacter* ($P > 0.05$) between the two different concentrations of PAA and CPC. Other researchers also reported no significant difference ($P > 0.05$) of the recovery of *Salmonella* and *Campylobacter* on chicken carcasses rinsed in a post-chill immersion tank between different levels of PAA (0.04% and 0.1%) (Nagel *et al*., 2013). *Salmonella* spp. was not recovered from the negative control, indicating that there was no background *Salmonella* in this study. However, there was *Campylobacter* spp. on negative control, indicating chicken parts were contaminated with *Campylobacter* before initiation of the study. The *Campylobacter* positive chicken is not uncommon to see in the US, as researchers reported that *Campylobacter* were recovered from

over 70% of retailed chicken meat in several states (Zhao *et al*., 2001; Cui *et al*., 2005).

Although chlorine has been widely applied in the poultry industry, it was less effective in reducing *Salmonella* and *Campylobacter* from ground chicken in the current study. It may be caused by the high organic load in the chill water, which can drastically reduce the antimicrobial effectiveness of chlorine (Bauermeister *et al.*, 2008). On the other hand, many studies demonstrated the efficacy of PAA and CPC on removing pathogens from poultry and meat product. As the most prevalent chemical used in post-chill applications in poultry industry (McKee, 2011), PAA, like other oxidizing agents, the mechanism of action is denaturing proteins, disrupting cell wall permeability and oxidizing sulfur bonds in proteins and other metabolites (Block, 2001; Middleton *et al*., 1997). Rio *et al*. (2007) reported that, through dipping inoculated chicken legs into a treatment of 0.022% PAA for 15 min, a significant reduction of various microflora including *Enterobacteriaceae*, *Micrococcaceae*, enterococci, *Brochothrix thermosphacta*, pseudomonads, lactic acid bacteria, molds and yeasts, was achieved. In another study, Ellebracht *et al*. (2005) reported a reduction of *Salmonella* Typhimurium by 1.0 $\log_{10}$ CFU/cm^2 when beef trimmings (100 cm^2 × 3 cm) were dipped into 0.02% PAA treatments for 15 s. Meanwhile, the antimicrobial mechanism of CPC was discussed by many researchers. It was shown that CPC can damage the cell membrane and cause the leakage of cellular materials while strongly interacting with negatively charged surfaces of microorganisms (Maeda *et al*., 1996; Schep *et al*., 1995). Yang *et al*. (1998) reported a reduction of *Salmonella* by 2 $\log_{10}$ CFU/carcass when chicken carcasses were sprayed by CPC (0.5%) at 35 ℃ at a pressure of 413 kPa for 17 s.

Microbiological Quality of Ground Chicken Treated with Various Treatments

Non-inoculated ground chicken samples treated with 0.003% chlorine, 0.07% PAA, 0.1% PAA, 0.35% CPC and 0.6% CPC were analyzed at day 1, 4, 7 and 10 for growth of total APC and PSY (Table 1).

The initial recovery of APC and PSY on day 1 was significantly lower ($P \leq 0.05$) in PAA treatments when compared to other treatments. Moreover, the growth of APC and PSY in PAA treatments maintained lower levels than the other treatments through day 7 of storage. The APC and PSY counts increased at a lower rate between sampling days in PAA treatments compared to the CPC and chlorine treatments during the same storage period. By day 10, APCs were still the lowest for 0.07% PAA and 0.1% PAA; whereas, all the treatments were similar for PSY. The fact that PAA treatments extending shelf-life was further supported by organoleptic observation using sensory analysis. By day 7, the only two treatments not exhibiting off-odors were 0.07% PAA and 0.1% PAA.

On the other hand, 0.35% CPC and 0.6% CPC had slightly lower APC levels than 0.003% chlorine at day 1, while no differences ($P > 0.05$) between them were detected in the following sampling days. In terms of PSY levels, CPC treatments were also significantly lower ($P \leq 0.05$) than chlorine on days 1 and 10. Both 0.003% chlorine and CPC with 0.35% and 0.6% had reached spoilage levels by day 7, so they were no longer served for sensory analysis. Greater shelf-life extensions of samples treated with CPC were achieved in other studies. Bai *et al*. (2007) found a 2 day extension in shelf-life of boneless, skinless chicken thigh meat compared with non-sprayed control, when samples were sprayed by 1.0% CPC and stored at 2.5 ℃.

Color Measurement of Ground Chicken

Uncooked chicken patties were subjected to objective analysis on day 1, 4 and 7 to determine the differences in lightness (L*), redness (a*) and yellowness (b*) due to rinsing by various antimicrobial treatments before grinding (Table 2).

On day 1 through day 4, PAA treatments were lighter in color than CPC treatments or chlorine control, but by day 7 the 0.35% CPC had the lightest color, followed by PAA, 0.6% CPC and chlorine control. In terms of the redness values, both CPC and chlorine were significantly higher than PAA on day 1 and 7, while only 0.35% CPC had higher a* ($P > 0.5$) than others on day 4. Yellowness, or b* value, did not show any particular trends throughout the storage study. Only chlorine on day 4 and 0.1% PAA on day 7 were significantly lower than other treatments. The lighter color occurred in meat treated with PAA was also reported by other researchers. Bauermeister *et al*. (2008) reported that chicken carcasses chilled by various concentrations of PAA (0.01%, 0.015% and 0.02%) at 4 ℃ for 2 h had higher L* and lower a* than those treated by 0.003% chlorine. The lighter color of meat was probably caused by the bleaching effect of PAA, which is an oxidizing agent. In addition, the pH change of meat can also lead to the lighter color (Allen *et al.*, 1997). The PAA treatments can decrease the pH of meat closed to the isoelectric point of the myofibrillar proteins, which cause the loss of water from meat. As less moisture in the meat results in more light to be reflected, the meat of lower pH will be lighter in color. In terms of the studies on CPC, Pohlman *et al*. (2002) found that 0.5% CPC increased the redness (a*) of ground beef stored at 4 ℃, which may due to its effect on improving oxymyoglobin stability.

Organoleptic Evaluation of Ground Chicken

Non-inoculated chicken patties treated with 0.003% chlorine, 0.07% PAA, 0.1% PAA, 0.35% CPC and 0.6% CPC stored at 4 ℃ were evaluated up to day 7 for their organoleptic acceptance. The products were evaluated by untrained panelists for the sensory attributes of odor, appearance, flavor, texture, juiciness and overall acceptability. On day 1 (Table 3), panelists failed to determine any differences in sensory attributes between treatments, except for the juiciness between 0.07% PAA and 0.35% CPC ($P \leq 0.05$). The ranking in odor, appearance, flavor and overall acceptance of PAA treatments (0.07% and 0.1%) were slightly lower than ranking of CPC and chlorine, while those differences cannot be considered as significant ($P > 0.05$). By day 4, panelists can only determine the difference ($P \leq 0.05$) in appearance between chlorine, 0.1% PAA and 0.35% CPC. While 0.07% and 0.1% PAA still had the lowest ranking in odor, texture and juiciness among all the treatments. On day 7, only two PAA treatments were still served to panelists and there was no difference between them. All the sensory scores of 0.07% PAA and 0.1% PAA decreased by day 7, which may be because those samples were nearing spoilage. Similar results were also reported in other studies. Bai *et al.* (2007) reported that sprayed CPC treatments with 0.5%, 1.0% and 1.5% did not result in any observable differences in odor or color on boneless, skinless broiler thigh meat during a 10-day shelf-life study. In another study, no difference was found in sensory quality of chicken broilers when chilled in various levels of PAA (0.1%, 0.015% and 0.02%), chlorine (0.003%) and control for 2 h and stored at 4 ℃ in two weeks (Bauermeister *et al.*, 2008).

Based on the results from this study, it can be concluded that PAA and CPC can be applied in parts decontamination tank to control the recovery of *Salmonella* Typhimurium

and *Campylobacter jejuni* on chicken ground meat. Furthermore, PAA can be effectively used to extend the shelf-life of ground chicken through inhibiting the growth of APC and PSY at 4 ℃. Although raw ground meat treated with PAA was slightly lighter in color, a negative impact on sensory attributes of cooked chicken patties was not detected ($P \leq 0.05$). Therefore, treatment of chicken parts before grinding with PAA or CPC may not only improve ground chicken safety, but maintain or enhance patty shelf-life and quality.

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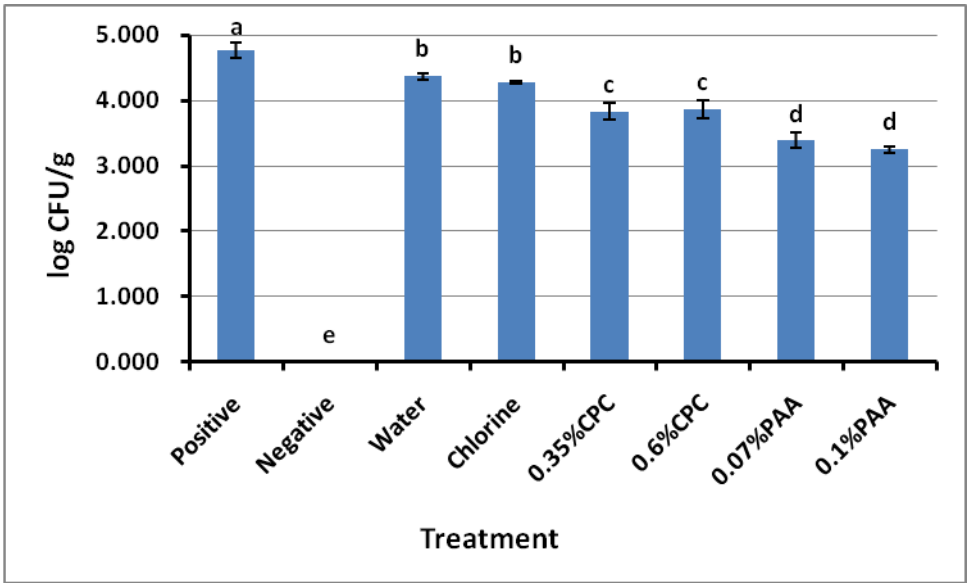

Figure 1. Effects of various antimicrobial treatments against *Salmonella* Typhimurium. [a-e] Means with no common letter differ significantly ($P \leq 0.05$).

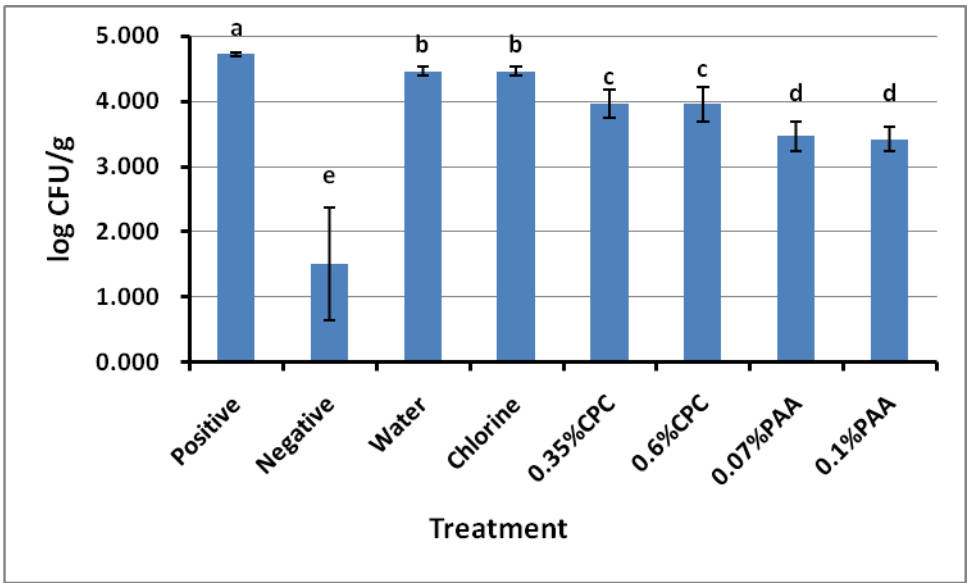

Figure 2. Effects of various antimicrobial treatments against *Campylobacter jejuni*. [a-e] Means with no common letter differ significantly ($P \leq 0.05$).

Figure 3. The picture of decontamination tank (COPE unit, Morris & Associates Inc., Garner, NC) used in this study.

Table 1. Microbial analysis of ground chicken treated with various antimicrobials during storage[1]

Storage Period	Treatment	Aerobic Plate Count (log CFU/g)	Psychrotroph (log CFU/g)
Day 1	0.003% Chlorine	3.80 ±0.17[a]	5.64 ±0.08[a]
	0.07% PAA	2.94 ±0.24[c]	4.70 ±0.17[d]
	0.1% PAA	2.67 ±0.28[d]	4.82 ±0.12[e]
	0.35% CPC	3.52 ±0.21[b]	5.50 ±0.09[b]
	0.6% CPC	3.35 ±0.32[b]	5.34 ±0.09[c]
Day 4	0.003% Chlorine	5.22 ±0.14[a]	6.63 ±0.13[a]
	0.07% PAA	3.49 ±0.75[b]	5.49 ±0.17[c]
	0.1% PAA	3.25 ±0.51[b]	5.71 ±0.09[d]
	0.35% CPC	5.32 ±0.25[a]	6.51 ±0.12[ab]
	0.6% CPC	5.02 ±0.46[a]	6.42 ±0.15[b]
Day 7	0.003% Chlorine	6.68 ±0.28[a]	8.03 ±0.12[b]
	0.07% PAA	4.81 ±0.35[b]	6.72 ±0.10[c]
	0.1% PAA	5.12 ±0.46[b]	6.77 ±0.10[c]
	0.35% CPC	6.79 ±0.13[a]	8.14 ±0.10[a]
	0.6% CPC	6.88 ±0.08[a]	8.03 ±0.06[b]
Day 10	0.003% Chlorine	6.49 ±0.22[a]	8.60 ±0.25[a]
	0.07% PAA	4.54 ±0.34[c]	8.46 ±0.21[ab]
	0.1% PAA	5.52 ±0.92[b]	8.36 ±0.30[b]
	0.35% CPC	6.82 ±0.38[a]	8.37 ±0.16[b]
	0.6% CPC	6.71 ±0.21[a]	8.28 ±0.18[b]

[a-e] Values with the same letter within each storage period and columns are the same ($P \leq 0.05$)
[1] Mean ± SD; n = 200.

Table 2. Color of chicken patties during storage treated with various antimicrobials[1]

Storage Period	Treatment	L* Value[2]	a* Value[3]	b* Value[4]
Day 1	0.003% Chlorine	66.87 ±2.01[c]	5.78 ±1.43[ab]	10.73 ±2.67[a]
	0.07% PAA	69.44 ±2.18[a]	5.72 ±2.36[ab]	10.76 ±1.89[a]
	0.1% PAA	69.42 ±1.13[a]	5.58 ±1.31[b]	11.57 ±1.31[a]
	0.35% CPC	69.25 ±1.13[ab]	6.38 ±1.32[a]	11.60 ±1.55[a]
	0.6% CPC	67.38 ±2.91[bc]	6.41 ±1.29[a]	10.80 ±1.19[a]
Day 4	0.003% Chlorine	63.18 ±0.93[b]	7.58 ±0.77[b]	10.78 ±1.57[b]
	0.07% PAA	65.12 ±0.92[a]	7.57 ±0.88[b]	11.81 ±0.91[a]
	0.1% PAA	65.39 ±0.82[a]	7.21 ±1.03[b]	12.15 ±1.10[a]
	0.35% CPC	64.02 ±0.73[ab]	8.37 ±0.81[a]	11.93 ±0.75[a]
	0.6% CPC	64.24 ±0.67[ab]	7.34 ±0.78[b]	11.40 ±0.89[ab]
Day 7	0.003% Chlorine	69.37 ±0.99[c]	7.09 ±1.05[a]	11.13 ±1.42[ab]
	0.07% PAA	70.36 ±0.94[bc]	6.11 ±1.32[b]	10.83 ±0.69[ab]
	0.1% PAA	71.39 ±0.82[b]	5.86 ±0.59[b]	10.71 ±1.29[b]
	0.35% CPC	72.17 ±0.98[a]	6.49 ±0.93[ab]	11.74 ±0.99[a]
	0.6% CPC	69.73 ±1.13[c]	7.13 ±0.59[a]	11.29 ±1.36[ab]

[a-c] Values with the same letter within each storage period and columns are the same ($P \leq 0.05$)

[1] Mean ± SD; n = 150.

[2] Where L* = 0 is black, L* = 100 is white.

[3] Where +a* is red, -a* is green.

[4] Where +b* is yellow, -b* is blue.

Table 3. Sensory analysis of ground chicken during storage treated with various antimicrobials[1]

Storage Period	Treatment	Odor[2]	Appearance[2]	Flavor[2]	Texture[3]	Juiciness[4]	Overall[2]
Day 1	0.003% Chlorine	5.59 ±1.19[a]	5.44 ±1.39[a]	5.61 ±1.25[a]	6.37 ±1.14[a]	5.85 ±1.46[ab]	5.73 ±1.20[a]
	0.07% PAA	5.39 ±1.39[a]	5.20 ±1.51[a]	5.53 ±1.27[a]	6.36 ±1.17[a]	6.02 ±1.37[a]	5.53 ±1.45[a]
	0.1% PAA	5.20 ±1.24[a]	5.25 ±1.37[a]	5.36 ±1.18[a]	6.14 ±1.24[a]	5.66 ±1.36[ab]	5.51 ±1.10[a]
	0.35% CPC	5.61 ±1.23[a]	5.49 ±1.37[a]	5.70 ±1.30[a]	6.22 ±1.10[a]	5.42 ±1.33[b]	5.73 ±1.14[a]
	0.6% CPC	5.53 ±1.29[a]	5.39 ±1.34[a]	5.59 ±1.24[a]	6.19 ±1.15[a]	5.76 ±1.34[ab]	5.63 ±1.23[a]
Day 4	0.003% Chlorine	5.55 ±1.40[a]	5.29 ±1.50[b]	5.68 ±1.02[a]	5.62 ±1.27[a]	5.41 ±1.42[a]	5.43 ±1.22[a]
	0.07% PAA	5.52 ±1.20[a]	5.54 ±1.39[ab]	5.57 ±1.37[a]	5.57 ±1.46[a]	5.29 ±1.67[a]	5.63 ±1.38[a]
	0.1% PAA	5.43 ±1.22[a]	5.25 ±1.47[b]	5.07 ±1.35[a]	5.46 ±1.23[a]	5.21 ±1.35[a]	5.07 ±1.20[a]
	0.35% CPC	5.78 ±1.19[a]	5.86 ±1.10[a]	5.77 ±1.20[a]	5.74 ±1.21[a]	5.35 ±1.43[a]	5.59 ±1.27[a]
	0.6% CPC	5.86 ±1.03[a]	5.52 ±1.35[ab]	5.47 ±1.38[a]	6.02 ±1.36[a]	5.57 ±1.51[a]	5.67 ±1.22[a]
Day 7	0.003% Chlorine	—	—	—	—	—	—
	0.07% PAA	5.19 ±1.20[a]	5.11 ±1.22[a]	5.09 ±1.29[a]	5.44 ±1.30[a]	4.98 ±1.28[a]	5.04 ±1.32[a]
	0.1% PAA	5.18 ±1.36[a]	5.23 ±1.27[a]	4.97 ±1.59[a]	5.40 ±1.41[a]	5.12 ±1.43[a]	4.97 ±1.50[a]
	0.35% CPC	—	—	—	—	—	—
	0.6% CPC	—	—	—	—	—	—

[a-b] Values with the same letter within each storage period and columns are the same ($P \leq 0.05$).

[1] Mean ± SD; n = 174 (n = 59 for day 1, n = 58 for day 4 and n = 57 for day 7).

[2] Where 1 = Dislike extremely; 8 = Like extremely.

[3] Where 1 = Extremely tough; 8= Extremely tender.

[4] Where 1 = Extremely dry; 8 = Extremely moist.

— Data not collected.

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