# The Effects of Different De-feathering Methods on *Salmonella* Attachment to Chicken Skin and Antimicrobial Efficacy

by

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ABSTRACT

Chickens usually arrive at processing facilities with high populations of microorganisms. *Salmonella* is a major concern in poultry and poultry products in regards to food safety. During the initial steps of slaughter (i.e., scalding and de-feathering) the topography of chicken skin changes, which in turn might impact *Salmonella* attachment to skin. Experiments were conducted to determine the impact of de-feathering methods (dry – hand-picked, tap water scalded and mechanically de-feathered, soft scalded and mechanically de-feathered, and hard scalded and mechanically de-feathered), attachment time (5, 10, 15, or 20 min), and presence of *E. coli* on *Salmonella* attachment to chicken skin. The results showed the de-feaering methods had a minor effect on chicken *Salmonella* attachment rate to the skin. Longer attachment time did not result in more attached cells, and presence of *E. coli* did not affect *Salmonella* attachment on chicken skin. However, within the same attachment time, the presence of *E. coli* increased the number of “firmly attached” *Salmonella*.

Antimicrobials applied during processing can be an efficient intervention strategy of achieving poultry product safety, and chlorine and peracetic acid (PAA) are the two common chemicals used in poultry processing plants. In the second experiment, 0.5% sodium dodecyl sulfate (SDS) was combined with 0.005% chlorine or 0.2% PAA solutions to improve the efficacy of both antimicrobials in reducing *Salmonella* level on chicken skin. The results showed 0.2% PAA was more effective against *Salmonella*

than 0.005% chlorine, especially on dry hand de-feathered chicken skin. SDS (0.5%) enhanced the efficacy of 0.005% Cl, but not PAA used in this study.

A direct intervention to minimize the chance for cross-contamination is to prevent *Salmonella* attachment to chicken skin, and coating of the skin could be a possible intervention method. Beeswax and carnauba wax micro-emulsions were prepared and used to coat chicken skins to validate their effectiveness on reducing *Salmonella* attachment on skins de-feathered by different methods. Carnauba wax coating helped to prevent *Salmonella* attachment to chicken skin significantly, while beeswax was not able to reduce *Salmonella* attachment.

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# CHAPTER I

# INTRODUCTION

The United States (US) is the World's largest producer of poultry products. In 2015, the US produced 8.8 billion broilers weighing 53.0 billion pounds. (National Chicken Council, 2015a). The top production states were Georgia, Arkansas, and Alabama, with average live weights of 6.9, 5.9, and 5.8 billion pounds in 2010, respectively (National Chicken Council, 2010). The consumption of poultry meat has increased over time. The per capita consumption of poultry and poultry products were 105.6 lb in 2015, up from 44.4 lb in 1965 (National Chicken Council, 2015b).

*Salmonella* is a foodborne pathogen that ranks second among the top five pathogens contributing to domestically acquired foodborne illness and the first resulting in hospitalization and death. It is estimated to cause 1,027,561 illnesses, 19,336 hospitalizations, and 378 deaths annually in the U.S. (CDC, 2014a). Salmonellosis is one of the most common and widely distributed foodborne diseases (WHO, 2013) in the World, with animals and their environment as the main sources. *Salmonella* is usually found in the intestines of both cold and warm- blooded animals. Salmonellosis is a common cause of foodborne disease in the U.S. (MedlinePlus, 2015). It was estimated that non-typhoidal *Salmonella* causes approximately 1.2 million illnesses and 450 deaths every year in the U.S. (Scallan et al., 2011); the *Salmonella* annual infection

incidence reported by FoodNet was 15.2 illnesses for every 100,000 persons (CDC, 2014b). In the US, the total basic annual loss for illnesses caused by *Salmonella* was estimated at $4.43 billion, which includes treatments, lost wages, etc (Scharff, 2011). *Salmonella* is considered an important pathogenic microorganism contributing to public health risks (Nauta et al., 2005), and poultry products were associated with approximately 29% of the *Salmonella* outbreaks from 2004 to 2008 (GAO, 2014).

In order to reduce microorganisms on the final products, many interventions are applied during poultry processing, including pre-scald bird brushes, multi-stage scalding, and antimicrobial applications. Pre-scald bird brushes physically remove the adhering feces from skin and feathers and can help to lower *Salmonella* numbers. Scalding is one of the major points of cross-contamination. Scalded temperatures can affect the skin topography to enhance bacterial attachment. Most processing plants use multi-stage scalders which includes 2 or 3 scalding tanks with increasing water temperatures from the first to the last stage (Russell, 2001). Antimicrobials are applied at multiple steps, including online reprocessing, during inside/outside bird wash, and during carcass chilling. Chlorine and peracetic acid (PAA) are the two common antimicrobials that are used in the poultry processing plants in the US.

Sodium dodecyl sulfate (SDS) has the ability to solubilize fats and oils, lower the surface tension of aqueous solutions, or form micro-emulsions, thus, it is widely used as an ingredient in consumer products, as an aid in manufacturing process, and as a biological research tool (Singer and Tjeerdema, 1993). It is possible that combining SDS with antimicrobials will help to reduce the time needed for microbial killing and enhance the antimicrobial activity at the same time.

Wax coating is a common practice applied on a variety of fruits and vegetables like apples, oranges, lemons, limes, cucumbers, eggplants, pumpkins, and tomatoes. Moisture content in fruits and vegetables are usually high (80 – 90%), wax is applied to help retain moisture during shipping and at the market (Shellhammer and Krochta, 1997). Coating improves the appearance of the products, but not the quality (Anonymous, 2004). Wax coated produce has a shine and fresh appearance for longer time, which prolongs the shelf-life of the product (Anonymous, 2004). Moisture content typically determines the quality grade of a produce. Application of a thin layer of wax helps to reduce the weight loss by 30 - 40 % (Anonymous, 2004). In general, wax coating on fruits and vegetables can extend shelf-life and make the product available all the way through the season by delaying the color changes and ripening processes, reducing moisture loss, maintaining the texture (Puttalingamma, 2014) as well as inhibit mold growth, and prevent microorganisms from entering the products (Brin, 2014). Waxes are also used in poultry processing (mostly water fowl) to remove the remaining vestiges of plumage.

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Chapter II

LITERITURE REVIEW

### ***Poultry Meat Consumption***

Poultry, like pigeons, geese, turkeys, and ducks have been grown in China for more than 3,000 years (World Poultry Industry, 2010). Chicken, originating from the Red Jungle Fowl, was first domesticated in China 10,000 years ago (Gray, 2014), and was introduced into America in the sixteenth century (Anonymous, 2010) providing eggs, fresh meats and feathers (Garrigus, 2015). Chicken is considered a lean, low-fat, and high-protein source for a healthy and balanced diet, and also provides good quantity and quality of phosphorous and other minerals, as well as B-complex vitamins (Anonymous, 2010).

The US is the largest producer of poultry worldwide. About 35 companies carry business with chicken production, processing, and marketing; approximately 25,000 farmers have contracts with poultry companies (National Chicken Council, 2016). In 2015, the United States produced almost 9 billion broilers weighing 53 billion pounds (National Chicken Council, 2015a). The top production states were Georgia, Arkansas, and Alabama, with total live slaughter weights of 6.9, 5.9 and 5.8 billion pounds, respectively (National Chicken Council, 2010). Due to the high production efficiencies, chicken has a lower price compared to beef and pork (National Chicken Council, 2012); easy preparation is another advantage of chicken consumption over another animal

proteins (Anonymous, 2010). Thus, the consumption of poultry products is increasing over time. Per capital consumption of poultry and livestock has steadily increased since 1965 from 44.4 lb to 105.6 lb in 2015 (National Chicken Council, 2015b).

US is also the second largest broiler meat exporter in the world after Brazil, exporting about 19.2% of production in 2014 (National Chicken Council, 2015b). The poultry products are mainly exported to Mexico, China, and Canada (U.S. Poultry and Egg Association, 2014).

### ***Salmonella***

*Salmonella*, ranking second among the top five pathogens contributing to domestically acquired foodborne illnesses and the first of resulting in hospitalization and death, is estimated to cause 1,027,561 illnesses, 19,336 hospitalizations and 378 deaths annually in the U.S. (CDC, 2014a). Salmonellosis caused by the infection of *Salmonella*, is one of the most common and widely distributed foodborne diseases (WHO, 2013). Foods, animals, and environments are the main sources of *Salmonella*. *Salmonella* is usually found in the intestines of both cold and warm- blooded animals. Foods, such as chicken, turkeys, cows, and pigs, are the most common animal carriers of *Salmonella*; contaminated water is also a source of *Salmonella* infection.

#### ***1. Characteristics***

*Salmonella* is a genus of bacterium that includes over 2,000 different serotypes (Arnold, 2009). Currently, there are two recognized species which are *S. bongori* and *S. enterica* (Chandler, 2011). *S. enterica* has six subspecies of *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), *salamae* (II), *indica* (VI), and *enterica* (I) (Desai et al., 2013).

*Salmonella* is a gram-negative, rod-shaped, non-spore-forming and facultative anaerobic bacterium belonging to Enterobacteriaceae. The size of the bacterium is 0.7 to 1.5 µm in diameter and 2 to 5 µm in length; flagella help the bacteria moving around (Arnold, 2009). *Salmonella* survives at temperatures ranged from 8 to 45 °C, and pH between 4 and 8 (Cornell Chronicle, 1997). The optimal growth conditions for *Salmonella* are 35 to 43 °C, pH 7 to 7.5, and water activity ($a_w$) around 0.99 (Food Safety, 2011). It was reported that *Salmonella* is not able to grow under dry conditions and the minimum $a_w$ for growth is 0.94 (Food Safety Watch, 2013).

### 2. *Infections*

Salmonellosis, is a common cause of foodborne disease in the U.S. (MedlinePlus, 2015). It was estimated that non-typhoidal *Salmonella* causes approximately 1.2 million illnesses and 450 deaths every year in the U.S. (Scallan et al., 2011); the annual *Salmonella* infection incidences reported by FoodNet was 15.2 illnesses in every 100,000 persons (CDC, 2014c).

Salmonellosis affects the intestinal tract and causes enteric, or typhoid fever, and gastroenteritis (MNT, 2014). The infection usually begins through a fecal-oral route; contaminated water and foods are common causes of salmonellosis. Beef, poultry, milk, and eggs are the top commodities infected with *Salmonella* (WebMD, 2014). There is a seasonal trend in infection, and it is more common in the summer than winter (CDC, 2009a). The symptoms of *Salmonella* infection include nausea, vomiting, abdominal cramps, diarrhea, fever, chills, headache, and blood in the stool (Mayo Clinic, 2014a); symptoms usually last for 4 to 7 days. Most people recover without treatment, but

elderly, infants, and persons with impaired immune systems are more likely to become serious illnesses (WebMD, 2014).

### ***3. Outbreaks***

In 2012, CDC reported that 831 foodborne outbreaks caused by a variety of pathogens, with 106 related to *Salmonella* (CDC, 2015a).

A recent *Salmonella* outbreak related to poultry was in July 2015. Three people were infected by *Salmonella* Enteritidis after consuming raw, frozen, stuffed chicken entrees produced by Aspen Foods, and two of them had been hospitalized. Aspen Foods recalled approximately 1.9 million lb of the products potentially contaminated with *Salmonella* (CDC, 2015e). In 2014, Tyson recalled approximately 33,840 lb of mechanically separated chicken due to possible *Salmonella* Heidelberg contamination at the Tennessee correctional facility. Nine people were infected and two of them were hospitalized. Additional 23 persons from 15 other states were infected by the same strain but not related to the outbreak in Tennessee (CDC, 2014b). Two outbreaks by consumption of chicken products happened in 2013 were both related to *Salmonella* Heidelberg. One outbreak had 634 cases reported from 29 states and Puerto Rico. Among the 634 ill persons - ranged from under 1 to 93 years old, 50% were male, and 38% were hospitalized, without deaths (CDC, 2014e). Another outbreak infected 134 persons from 13 states; 33 of the infected persons were hospitalized. Among those 134 persons, ranged from under 1 to 94 years old, 55% were female, and no death was reported (CDC, 2013). There was a previous outbreak with ground turkey, in which 136 persons from 34 states were infected in 2011. The company recalled approximately 36

million lbs of ground turkey products that possibly contaminated with *Salmonella* Heidelberg (CDC, 2011).

There were other *Salmonella* outbreaks happened in the past years in pork (CDC, 2015f), live poultry (CDC, 2015d), frozen raw tuna (CDC, 2015e), cucumbers (CDC, 2015b), and raw cashew cheese (CDC, 2014d) which had been contaminated by different strains of *Salmonella*. *S*. Enteritidis, *S*. Typhimurium, *S*. Newport, *S*. Javiana, and *S*. Heidelberg are the most popular strains that caused foodborne illnesses (Robinson, 2013).

### ***4. Detection***

As *Salmonella* has been a major cause of foodborne illness, the inspection and detection of *Salmonella* contamination in food and food ingredients has become urgent and important. A desired method for *Salmonella* detection should be sensitive enough to detect one cell in a defined food sample (Lee et al., 2015) since the infective dose of *Salmonella* is as low as only one cell (FDA, 2012). Conventional culture methods and a number of rapid methods have been developed.

Culture methods are the most commonly used techniques for *Salmonella* detection because of their sensitivity and selectivity (Odumeru and León-Velarde, 2012). Typically, culture methods have a pre-enrichment procedure to recover the injured cells in non-selective media (e.g. buffered peptone water), and followed by a secondary selective enrichment step using medium broths, such as Rappaport Vasiliadis Soy broth (RVS), Tetrathionate broth (TT) and Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn) (Odumeru and León-Velarde, 2012; ISO, 2002). After incubation in selective broth, bacteria are sub-cultured onto selective agars (e.g.

Xylose Lysine Desoxycholate (XLD) agar, Brilliant Green agar (BGA), or Hektoen Enteric agar (HEA)) (Hendriksen, 2003). Suspect colonies are then confirmed by biochemical and serological tests. The biochemical tests are based on some of the characteristics of *Salmonella,* such as fermentation of glucose, fermentation of dulcitol, $H_2S$ production, and negative urease reaction (Odumeru and León-Velarde, 2012); while polyvalent antisera for flagellar (H) and somatic (O) antigens are the basis for serological tests (Gebreyes and Thakur, 2010). The conventional detection method is easy, sensitive, specific, and economic; however, it is time consuming, and usually takes 5 to 7 days to obtain the results. Because of this, there is an immediate need - to develop the technology of rapid detection methods (Lee et al., 2015).

### 5. Regulations and preventions

The United States Department of Agriculture's (USDA) Food Safety Inspection Service (FSIS) have required testing for *Salmonella* since the mid-1990s. Initially, the national baselines for broiler, ground chicken and ground turkey were established at 12 or fewer positive out of 51 samples, 26 or fewer positive out of 53 samples, and 29 or fewer positive out of 53 samples, respectively (FSIS, 1998; GAO Highlights, 2014). In order to reduce the contamination of *Salmonella* in poultry products, new standards for *Salmonella* levels in post-chiller carcasses were recently introduced by FSIS. Currently, the *Salmonella* performance standard for young chicken is 7.5%, or 5 positive test results out of 51 samples (USDA-FSIS, 2015); the performance standard for young turkey is 1.7%, or 4 positive test results out of 56 samples (Acheson, 2011). Hazard Analysis and Critical Control Point (HACCP) system is a tool that the poultry industry uses to prevent and control *Salmonella* contaminations in poultry products.

As *Salmonella* is not resistant to high temperatures (Food Safety, 2011), proper cooking and pasteurization can be applied to eliminate the pathogen and prevent contamination (Foodsafety.gov, 2015). Some safety tips for consumers can be followed to prevent the infections, such as (1) keeping everything clean: wash hands thoroughly with warm water before and after handling raw products (Davis, 2014); washing utensils, cutting boards, dishes, and countertops carefully with warm soapy water after preparing each item; (2) separating raw and ready-to-eat food, never placing cooked food on a plate contained raw foods (Mayo Clinic, 2014b); (3) cooking the food thoroughly (e.g., cooking poultry products till the internal temperature reach 165 °F) and holding cooked products at safe temperatures; (4) Chilling the leftovers promptly and properly.

### *Poultry Processing*

Poultry processing is the preparation of meat from live birds for consumption. In general, poultry processing includes receiving and weighing, shackling, stunning, bleeding, scalding, de-feathering, evisceration, chilling, cutting and packaging, distribution and further processing.

Of special interest to this research are the processes involved in the removal of feathers (i.e., scalding and de-feathering), as poultry are often marketed with skin-on whole carcass or portioned parts. Scalding is the process of submerging the birds in warm water to loosen the feathers. Hot water is used in this procedure to transfer heat to the feather follicles and loosen the feathers (Mead, 2004). This step is one of the most important points during poultry processing to remove adhering fecal material, thus,

there is a chance for *Salmonella* cross-contamination among carcasses (Russell, 2001). Counter-current flow (water moving against the carcasses) is critical to wash off and remove fecal material and bacteria on carcasses while they are conveyed in the scalder. The water at the exit of scalder should be the cleanest to give birds a final rinse and the surfaces of carcasses should be feces free before entering the de-feathering machine (Russell, 2001).

Most processing plants are using multi-stage scalders which include 2 or 3 scalding tanks with increasing water temperatures from the first to the last stage. To control bacterial growth by temperature alone, the temperature should be maintained at least 5.6 °C higher than the temperature the target bacteria can grow (Russell, 2007). *Salmonella* grows at temperature up to 45°C, so scald water is usually kept above 50 °C (Russell, 2007). The pH is another way to control pathogen growth during scalding as well as to combat yield loss. Adding acidic disinfectant chemicals (Tasker Blue, peracetic acid) to scalder helps to lower the pH, inhibit *Salmonella* growth, and make it easier for de-feathering birds (Russell, 2007).

The scalding method affects the appearance of the final product significantly and also influences yield and shelf life of products. Soft-scalding and hard-scalding are two commonly applied scalding schemes.

Soft-scalding is performed at relatively lower temperatures of 50 – 53 °C with 60 to 180 s immersion time (Barbut, 2002). It is commonly used for young birds because the temperature is mild and does not cause significant damage on the skin surfaces, leaving the cuticle layer of the skin intact, to allow air chilling to be applied afterwards (Mead, 2004). Hard-scalding is usually at temperatures between 58 and 60 °C for 90 s

to 120 s (Mead, 2004). Higher scalding water temperatures have a better effect on loosening feathers from the follicles, but also causes the epidermis to become softer. Usually, hard scalded carcasses are either sold as frozen products or used for the production of breaded and battered products.

In order to minimize *Salmonella* cross-contamination during scalding step, a new technology has been developed – AeroScalder. Moisturized hot air is used instead of hot water to scald birds. The AeroScalder blows moisturized hot air forcefully on the carcasses, and heat is transferred to the feather follicles effectively.

After scalding, feathers are removed immediately while carcasses are still warm. Typically, several de-feathering machines equipped with rubber fingers are used to remove the feathers. During this process, carcasses move suspended upside down on an overhead conveyor line, while the rubber fingers rotate and rub the carcasses to remove feathers (Barbut, 2002). When comparing soft-and hard- scalded bird feather removal, soft scalded birds need more de-feathering equipment (Mead, 2004).

The rubber fingers are designed with grooved surfaces for better feather removal; however, this can serve as one of the avenues of causing cross-contamination during de-feathering. During de-feathering a contaminated carcass may expel, microorganisms that may contaminate the grooves allowing the microorganisms to pass to other carcasses. Another risk of causing cross-contamination is the compression of the carcass results in expulsion of internal fecal material to the carcass surface. Chlorine dioxide is approved as an antimicrobial during poultry processing, Berrang et al. (2010) tested by spraying 50 ppm $ClO_2$ water on carcasses during de-feathering. The results showed that $ClO_2$ water reduced the numbers of *Campylobacter*, *E. coli*,

and *Salmonella* significantly compared with water spray. Spraying $ClO_2$ water during feather removal may be a potential method to lower cross-contamination risk.

Other processing steps include evisceration, giblet harvest, carcass washing and chilling, all of which can contribute to bacterial contamination and cross-contamination of the carcasses.

To reduce the fecal contamination and microbial risk, good manufacturing practices are employed by the industry. Weight uniformity of birds being processed is important for proper equipment function. Feed withdrawal times have an impact on the strength and integrity of intestines, with a longer feed withdrawal time decreasing the strength and integrity of intestines rapidly (Owens et al., 2010). In order to reduce cross-contamination during this step, chlorinated water sprays are applied at the blade and the spoon.

There are several different points during evisceration that carcasses can be washed with various antimicrobials. Inside/outside bird washer (IOBW) is a common equipment that follows evisceration prior to chilling. This equipment is designed to remove any visible contamination (e.g. debris, blood clot, feces) in order to meet the zero-tolerance policy stated in pathogen reduction and Hazard Analysis and Critical Control Point Systems (PR/HACCP) (Barbut, 2002; USDA, 1996; USDA, 1998). It also reduces the organic load, which may affect the antimicrobial effectiveness in chilling (Owens et al., 2010). The device used in IOBW has multiple nozzles that cover the outside of the carcass. The equipment is driven by an overhead conveyor and carcasses pass through the equipment, with legs hanged vertically and the back towards the center of the equipment. Inside washing is accomplished by a spinning

nozzle or a rotating brush which is able to enter the abdominal cavity; outside washing is performed by nozzles spraying breast and back sides. High-pressure, low-volume nozzles are recommended to remove debris effectively, while proper position for the nozzle and carcasses is important to improve the cleaning efficacy as well (Barbut, 2002).

Research conducted by Northcutt et al. (2003) stated that IOBW does not significantly reduce coliform or *E. coli* counts on carcasses, but it is able to decrease total aerobic bacteria counts. Thus, antimicrobials should be applied in this step to improve the efficacy of microbial reduction. Chlorine has been used for years in the U.S. at concentrations between 20 ppm and 50 ppm in poultry processing (Mead, 2000). Mead and Scott (1994) demonstrated that application of 20 ppm chlorine after evisceration had little effect on reducing *E. coli*, however, it helped to control the bacteria buildup on equipment and destroyed the spoilage bacteria present in the water supply. As an alternative antimicrobial, lactic acid rinses have been reported effectively at reducing *Salmonella* on turkey carcasses (Bautista et al., 1997).

Carcasses chilling is required to suppress the growth of pathogenic and spoilage microorganisms unless the products are ready to be frozen or cooked immediately (9 CFR 381.66). Air chilling and immersion chilling are the two most common methods used for poultry carcasses chilling.

Air chilling has been a common method in Western Europe for more than 45 years (Jung, 2008), and it is accomplished by blasting cold air on carcasses. The carcasses are hanged individually and pass through cold air for several hours, which allows the carcasses to cool down slowly and independently (Urban, 2014). However,

air chilling was reported to have an average of 2.5% weight loss (Huezo et al., 2007a). In order to resolve the weight loss, the process can include a water spray or mist for more than one hour (Barbut, 2002). Air chilling controls microbial growth by surface desiccation achieved by high air velocity; however, high air velocity may cause quality issues for poultry carcasses (Loretz et al., 2010) It was demonstrated that air chilling (150 min, -1.1°C, air speed: 3.5 m/s) reduced *Campylobacter jejuni* by 1.4 log CFU/mL (Huezo et al., 2007b). A similar result was reported by James et al. (2007) that air chilling reduced *C. jejuni* by 1.8 log CFU/$m^2$.

Immersion chilling is the major chilling method used in the US. It is achieved by submerging all carcasses together in tanks with cold water or water-ice mixture (Urban, 2014). Immersion chilled chicken showed 9.3% more moisture retention than those air chilled (Huezo et al., 2007a). Water has 25 times higher heat transfer rate than air. One disadvantage of immersion chilling is that it takes a large amount of fresh water (Urban, 2014). Each carcass needs at least 1.5 L of water at the first stage and 1.0 L at the last chiller (Cavani, 2010). As all carcasses are putting together in one tank, the water makes it easy for cross-contamination from carcass to carcass. To reduce microbial load on carcasses, antimicrobials are applied in the chilling tank and antimicrobial efficacies have been evaluated intensively. Chlorine, peracetic acid (PAA), and cetylpyridinium are the common antimicrobials used in chilling. Nagel et al. (2013) tested on 0.004% chlorine in post-chill tank for reducing *Salmonella* Typhimurium and *Campylobacter jejuni*, and the results showed less than 1 log bacteria reduction on broiler carcasses. Smith et al. (2015) reported that broilers immersed in 200 ppm PAA

for 60 s reduced *Campylobacter* by 1.42 log CFU/mL, which is significantly higher than spray application of 200 ppm PAA.

Both air chilling and immersion chilling are effective in eliminating bacteria on carcasses, however, it is shown that air chilling results in more tender breast meat and lower cook loss than immersion chilling (The Poultry Site, 2008a). The lower cooked yield was due to the birds with high moisture content absorbed during immersion chilling (The Poultry Site, 2008b).

### *Avian Skin Topography*

Skin or integument, the largest organ of the body, is the most important physical barrier of the animal to the environment. The functions of skin are protection (to keep out pathogens and other potentially harmful substances), regulation (retain vital fluids and gases), and sensation (serve as a sensory organ). The skin of birds also produces and supports feathers. With feathers, the skin also plays an important role in thermoregulation (Lucas and Stettenheim, 1972; Spearman, 1980).

Avian skin consists of two layers, the epidermis and dermis. The outer layer, the epidermis, is generally very thin and flexible. The epidermis is the thinnest in areas covered by feathers (pterylae) and the thickest in featherless areas (apteria). The dermis lies between the epidermis and the subcutaneous tissues. The dermis contains blood vessels, fat deposits, nerves and free nerve endings, several types of neuroreceptors, and smooth muscles that move the feathers. The dermis is very pliable. The main component of the dermis is collagen with a small amount of elastin. Each tendon runs into the end of a smooth muscle belly. These muscles run throughout the

dermis interconnecting between the feather muscles which move the feathers and the apterial muscles that interconnect the feather tracts (Bereiter-Hahn et al., 1986).

Scalding and de-feathering procedures impact the structure of skin because these two procedures result in the removal of the upper layer (cuticle) of epidermis (Clark, 1968; Thomson et al., 1974; Thomson and McKeekin, 1980). A study conducted by Dougherty and Seibold (1965) stated that the histology of skin changed in a definite progression with the increase of scald water temperature. They slaughtered the broilers following kosher method, and the broilers were scalded under different temperatures (110, 120, 130, 140, 150, and 160 °F) and different times (30 s and 60 s), all birds were hand de-feathered. Four birds were dry-picked as a kosher control. Skin samples were collected from the left breast and lumbar region of the back. After histological study on these skin samples, contraction was found on both epidermis and dermis, and the contracted epidermis developed ridges or corrugations on skin surfaces. When scalded at or below 130 °F, cells were visible on the epidermis, and connective tissue had a slight contraction on the dermis; while the changes above 130 °F were more marked: cells were indistinguishable on the epidermis, and well-marked to extreme contraction of connective tissues were observed on the dermis. Ridges were starting forming at 150 °F, and very marked ridges were found at 160 °F. However, the scalding time did not show an impact on the histological changes.

Some studies showed that only flagellated bacteria attached the skin significantly, and the rate and extent of attachment were effected by bacteria strain, time of exposure, temperature, and the number of organisms present in the water-film on the skin surface (Notermans and Kampelmacher, 1974, 1975a). Microorganisms that are

suspended in the surface water-film can be easily washed off (loosely attached), while those that are entrapped (firmly attached) cannot be removed by rinsing alone and is more resistant to removal by chemical or physical methods (Notermans and Kampelmacher, 1975b). McMeekin and Thomas (1978) reported that mobility of bacteria had a negligible effect on attachment.

McMeekin and Thomas (1978) also demonstrated that “hard” scalding and de-feathering of chicken exposed a new surface for microbial contamination, which contained many capillary-size channels and crevices. However, research showed that bacteria were firmly attached to the skin even before being processed. The attachment of bacteria on skin began within 15 s and increased with time in a linear manner. The rate of attachment was not significantly different between “soft” and “hard” scalded carcasses (Mead, 2006).

### *Microbial Intervention Strategies during Slaughter*

Poultry processing is a complex and high-speed operation. Thus, there is a high possibility of cross contamination if the operation of processing equipment is not well controlled (Barbut, 2002). In order to minimize cross-contamination during processing, a number of potential intervention strategies can be applied. First, a proper feed withdrawal time, typically 8 to 12 hours must be applied at the farm, helps to reduce food and fecal contamination on carcasses. Longer feed withdrawal time (>12 h) can cause fragile internal organs which may be broken during processing; shorter feed withdrawal time (<8 h) leads to intestines full of ingesta which is not desirable to reduce cross-contamination (Curtis, 2007). Adding an organic acid in drinking water during feed

withdrawal time is another method to minimize the cross-contamination caused by leakage of crop, which is a main source of *Salmonella* contamination during processing. Byrd et al. (2001) tested acetic, lactic, and formic acid in drinking water during feed withdrawal, and the results showed that lactic acid was the most effective one in reducing *Salmonella* Typhimurium. They also demonstrated that 0.44% lactic acid reduced 80% of *Salmonella* contaminated crops.

Before entering the processing plant, broilers are transported, unloaded and held. Cleaning and sanitation are important to prevent cross contamination. Washing the cages with water and air-drying for 48 h is the most effective way to clean the cage, and cleaning and sanitation of unloading and holding areas are important as well (Curtis, 2007).

During processing, a number of interventions can be applied to prevent microbial contamination. In the scalding step, counter-current water flow can rinse off bacteria and fecal material to reduce cross contamination. Using ample fresh water into the scalding tank also reduces bacterial populations. Chemicals can be added to scald water for further reducing cross-contamination. Increased death rates of *Salmonella* and *Campylobacter jejuni* were achieved in the water by adding 0.1 – 0.2% acetic acid to scalding water (Okrend et al., 1986). Adding 5% acetic acid to scald water resulted in 2 log reduction of *S*. Typhimurium on chicken skin (Tamblyn et al., 1997). De-feathering step provides a warm and humid atmosphere which is ideal for bacterial survival and growth, but cross-contamination can be controlled by rinsing the equipment and carcasses using chemical solutions. Chlorine, chlorine dioxide, acetic acid, and hydrogen peroxide are recommended chemicals to be applied in this step (Curtis,

2007). Evisceration is a step to remove edible and inedible viscera from the carcasses. This step can easily cause fecal and other bacterial contamination by cutting the intestine if the machine is improperly adjusted. In addition, every bird contacts this machine with the blade and probe reaching into the bird to open the vent. Thus, it is necessary to spray the machine with chlorinated water between carcasses to prevent cross-contamination. An inside/outside bird wash (IOBW) is important for reducing bacteria on carcasses. Chemicals can be added in the water to increase the effectiveness of reducing microbial levels. Peracetic acid, followed by chlorine or other oganic acids (< 0.002%), are the most commonly used (McKee, 2011). During chilling, counter-current flow can be applied as well; adjusting the chemical level and pH is important to reduce bacteria loads; keep the water temperature below 4.4 °C to reduce bacteria growth.

All these intervention strategies applied during processing make a significant reduction of bacterial levels. In 2012, the levels of *Salmonella* had an average of 4.3% positive on chicken carcasses at processing plants nationwide, which is well below the USDA performance standard of 7.5% in raw chicken products (National chicken Council, 2013).

### *Chemical Applications*

Applying chemicals as disinfectants or antimicrobials in poultry processing is one of the most important intervention strategies to maintain the safety of products. 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 industry: (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%) (Russell, 2011).

### *1. Chlorine*

Chlorine was first introduced into water treatment and used by municipal water treatment facilities in Chicago and Jersey City in 1908. It has been used as a sanitizer for many years, and it is applied as wash, spray, and flume water in the food industry. In poultry processing, chlorine is applied as the forms of sodium hypochlorite (bleach), calcium hypochlorite tablets or chlorine gas for carcasses and equipment rinses (Russell, 2009). The USDA Food Safety and Inspection Service (FSIS) regulates the addition of chlorine to processing water at levels not exceed 50 ppm in carcass wash and chiller make-up water (FSIS Directive 6410.3, 2012). When it was used for reprocessing, the FSIS requires chlorinated water containing at least 20 ppm available chlorine on inner surfaces of carcasses (FSIS Directive 7120.1, 2015). When chlorine used as surface disinfectant on food contact surfaces, the free chlorine levels can be between 50 ppm and 200 ppm. Higher concentration of chlorine increases the effectiveness of killing microorganisms, but it is not recommended because chlorine can cause corrosion and explosions, and may have adverse effects on health. The pH of water is an important factor that affects chlorine efficiency. The active form of chlorine as an antimicrobial is hypochlorous acid (HClO), quantity of which depends on the pH of the water. The optimal pH to obtain the highest content of HClO is at 6. Thus, adjusting the pH level around 6 provides the best effectiveness.

The mechanism of chlorine killing bacteria is not completely understood, and it may involve many factors: oxidation of amino acids, ring chlorination of amino acids, loss of intracellular contents, inhibition of protein synthesis, decrease of oxygen uptake, decrease of nutrient uptake, and decrease of adenosine triphosphate production (CDC, 2009b).

A number of studies have been conducted to investigate the effectiveness of chlorine as an antimicrobial. Northcutt et al. (2005) demonstrated that spraying 55 ppm chlorine on chicken carcasses caused 0.9 – 1.1 $log_{10}$ CFU/mL reduction on *Salmonella* and 2.5 – 2.6 $log_{10}$ CFU/mL reduction on *Campylobacter*. One study conducted by Killinger et al. (2010) to test the effectiveness of chlorine levels between 50 ppm and 100 ppm applied on chicken carcasses for 3 min spray, showed that it reduced aerobic plate count and coliform by 0.4 log CFU/carcass and 0.21 log CFU/carcass, respectively.

***2. Chlorine dioxide ($ClO_2$)***

Chlorine dioxide is typically used in poultry processing in the U. S. 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).

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.

***3. Acidified sodium chloride (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 chlorite concentration should be adjusted to 50 to 150 ppm in the chiller. The contact time is usually several minutes at temperatures between 0 and 15 ˚C (FSIS Directive 7120.1, 2015). 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. This action also adversely affects amino acid transport (Taormina, 2012).

### *4. Hypochlorous acid (HClO)*

Hypochlorous acid (HClO) is highly unstable when isolated in a pure form, thus, it can only exit 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 (FSIS Directive 7120.1, 2015). 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 from gassing off possibly when it is mixed with other chemicals.

### *5. 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 without creating any product defects. In poultry processing 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% (FSIS Directive 7120.1, 2015). Frequently, after being treated on the carcass with an acid, bacteria become acid stressed, and hard to recover which will affect antimicrobial 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 (Russell, 2011).

### *6. Peracetic acid (PAA)*

Peracetic acid (PAA) is an equilibrium mixture of an acetic acid (organic acid), and hydrogen peroxide (an oxidant) in a water solution. It is a colorless liquid with a pungent odor and low pH value. At the level of 600 ppm the pH is around 2.5 (EFSA, 2014). 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 2,000 ppm (FSIS Directive 7120.1, 2015). 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 and in hard water conditions, and the antimicrobial efficacy is not affected by protein residues (Lenntech, 2013).

The antimicrobial mechanism of PAA is through oxidation of organic materials (lipids, ionic protein bond, etc.) and disruption of bacterial cell membranes (Viticulture & Enology, 2014). Oxidation is accomplished by transferring electrons of hydroxyl radicals (OH·), to compounds including carbohydrates, nucleic acids, lipids, and amino acids, damaging or killing the microorganisms (Kaya, 2010).

A number of studies on killing efficacy of PAA in different applications on poultry products have been conducted. Bauermeister et al. (2008) tested 85 ppm of PAA in chiller and obtained 92% reduction on *Salmonella* and 43% reduction on *Campylobacter*. In Nagel et al. (2013) research, they suggested that PAA utilized in post-chill immersion tanks as an antimicrobial is effective for reducing *Salmonella* and *Campylobacter* on carcasses without impacting product quality. In their research, 0.04% and 0.1% PAA were applied in post-chill immersion tank to treat *Salmonella* and *Campylobacter* inoculated broiler carcasses. The results showed that 2.02 log CFU/mL reduction on *Salmonella* and 1.93 log CFU/mL reduction on *Campylobacter* were obtained by treating with 0.04% PAA; while 0.1% PAA treatment reduced *Salmonella* and *Campylobacter* by 2.14 and 2.03 log CFU/mL, respectively.

***7. 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 (FSIS Directive 7120.1, 2015).

CPC 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 hydropholic 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 (FR Doc 04-7399) to provide for the safe use of CPC as an antimicrobial agent in poultry processing (Federal Register, 2004).

### ***Surfactants***

Surfactants or surface-active agents, contain both hydrophobic and hydrophilic moieties. These compounds can reduce surface and interfacial tensions between different molecules when used at very low concentrations (Ranasalva et al., 2014). Surfactants are widely applied in detergents, personal care, cosmetics, and in foods. They are frequently used to modify the interface properties. Several kinds of surfactants are used in the food industry, such as ionic (anionic, cationic, amphoteric) and non-ionic surfactants which are the most common types (Solanki, 2001).

#### ***1. Sodium dodecyl sulfate***

Sodium dodecyl sulfate (SDS) or sodium lauryl sulfate (SLS), an anionic surfactant, is an organic compound which has a formula of $CH_3(CH_2)_{11}SO_4 \cdot Na$ and the structure is shown in Figure 1. The negatively charged sulfonate group is the hydrophilic end, and the saturated 12-carbon chain is the hydrophobic end, which is nonpolar and soluble in oils/fats (Anonymous, 2006). SDS is a white solid with a slight odor. The pH is between 8.5 and 10 in 1% aqueous solution (Fisher Scientific, 2011). It is commonly used as household and industrial cleaners, personal care products, and cosmetics; it is also used in industrial manufacturing processes, such as delivery aid in

pharmaceuticals and biochemical research involving electrophoresis (Singer and Tjeerdema, 1993).

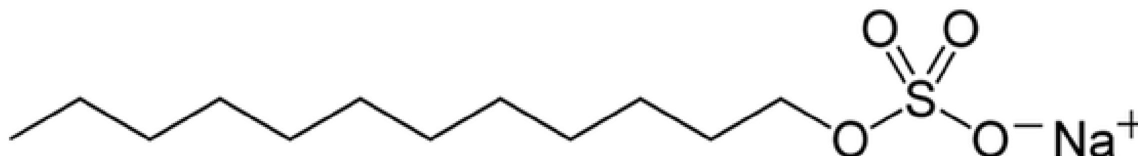


Figure 1. The chemical structure of sodium dodecyl sulfate

Combination of SDS with antimicrobials has been studied on poultry products to reduce microorganisms. Zhao et al. (2009) reported that a combination of levulinic acid and SDS as a wash solution may be practical to inactivate foodborne enteric pathogens on poultry products. Four organic acids including lactic acid, acetic acid, caprylic acid, and levulinic acid at 0.5% individually or in combination with 0.05 to 1% SDS were tested in reducing *Salmonella* and *E. coli*. The results showed that individual compounds reduced pathogens less than 2 log CFU/mL within 20 min at 21 °C, while any combinations with 0.5% SDS reduced the pathogens by more than 7 log CFU/mL within 10 s at the same temperature. However, Lu and Wu (2012) tested 0.2 mg/mL thymol and 2 mg/mL acetic acid with or without 5% (w/v) SDS on chicken breast to reduce *Salmonella enterica*, and found that SDS did not improve the antimicrobial activity in either solutions.

### *Waxing Technology*

Wax coating, applying a thin layer of edible wax onto the surface of product, is commonly used in fruits and vegetables such as apples, oranges, lemons, limes,

cucumbers, eggplants, pumpkins, and tomatoes. Moisture content in fruits and vegetables is usually high (80 – 90%), and wax is applied to help retain moisture during shipping and in the market place. Wax coated produce has a shine and fresh appearance for a longer time, and prolongs the shelf-life of the product (Anonymous, 2004). For vegetables, moisture loss is one of the main concerns for both sellers and consumers (Shellhammer and Krochta, 1997). A lower moisture content will typically give a lower grade for the produce; application of a thin layer of wax helps to reduce the weight loss by 30 - 40 % (Anonymous, 2004). In general, wax coating on fruits and vegetables can extend shelf-life and make the product available all the way through the season by delaying the color changes and ripening processes, reducing moisture loss, and maintaining the texture (Puttalingamma, 2014), as well as inhibiting mold growth and preventing microorganisms from entering the products (Brin, 2014). Waxes are also used in poultry (mostly water fowl) to remove the remaining vestiges of plumage.

Carnauba wax, and beeswax are the common types that are used as food-grade waxes (Shellhammer and Krochta, 1997).

***1. Carnauba wax***

Carnauba wax (also called Brazil wax and palm wax) is a wax obtained from the leaves of palm tree, with a high melting point between 82 and 86 °C (Marie, 2014), and is used as ingredient in many foods, cosmetics and polishes, such as candies, chocolates, confectionery, and fruit coating; skin care, hair care, and shave cream; polish waxes for car, floor, leather, and furniture (Anonymous, 2011). The production of carnauba wax in Brazil was 22,409 tons in 2006, among them 3,130 tons were solid, and 19,279 tons were powder. Brazil exports approximately 15,000 tons of carnauba

wax mainly to USA, Germany, Japan, Holland, and Italy (Anonymous, 2011). The application of carnauba wax in foods is regulated and cannot exceed 200 mg per kg food in Europe, except in confectionery, where it may be used up to 500 mg/kg and 1,200 mg/kg in chewing gum (ESFA, 2012; Scott-Thomas, 2012).

#### *2. Beeswax*

Beeswax is a natural wax from honey bees, and it has been known since ancient time (Root and Root, 1923). Beeswax has been considered as the best material for making candles for centuries, and it also played an important role in ancient seals (Crane, 1999). The melting point of beeswax is between 61 and 65 °C (Bogdanov, 2009). The wax is very stable and can only be deteriorated by several solvents (MAAREC, 2005). Beeswax is widely used in cosmetics, food, and medicine, for examples lipsticks, mascara, ointment and cream, food packaging, confectionary, cigarette filters, etc. (Krell, 1996). It is hard to obtain accurate data on beeswax production as the majority is used in beekeeping for producing comb foundations (Bogdanov, 2009). It is estimated that the beeswax production takes 1.5-2.5% of honey production (Crane, 1990). For using in food, it was regulated that beeswax should not exceed 0.065% in chewing gum, and 0.005% in confections and frostings. In soft and hard candies the levels should be less than 0.1% and 0.04%, respectively; for all other food categories, the maximum level is 0.02% (CFR 21.3, 2015).

### *Salmonella Attachment Model (SAM)*

*Salmonella* Attachment Model (SAM) was first developed by Conner and Bilgili (1994) to test in-vitro the efficacy of broiler carcass disinfectants against *Salmonella* on

chicken skin. This method allows rapid screening of potential carcass disinfectants and allows fine-tuning optimum conditions of application.

Skin samples are collected from breast skin of freshly processed and chilled broilers, cut into 10 cm diameter circle that includes both pectoral pterylae and apteria. Each sample is packaged in sterile plastic bags individually and gamma irradiated at 10 to 12 kGy at ≤ -20 °C in order to inactivate background microflora. The irradiated skin samples are stored at -20 °C before use. In the original study, Conner and Bilgili (19940 two serotypes of *Salmonella*: *S.* Typhimurium, and *S.* Montevideo. Each skin sample was inoculated with each serotype by a coarse spray at three different levels: $10^2$, $10^3$, and $10^4$ cells/skin; then allow 10, 20 and 30 min contact time before rinsing the skin with 20 ml fresh phosphate buffer (PB) to remove loosely attached cells, then the skin was transferred to fresh PB (20 ml) and stomached for 2 min to remove firmly attached cells. Blended samples were cultured on tryptic soy agar (TSA) at 37 °C for 24 h to enumerate cells remaining on the skin.

The subsequent studies with SAM showed that inoculum level or contact time had no effect on attachment of *S.* Typhimurium where 31 to 37% of the inoculated cells attached to chicken skin. However, an interaction effect between inoculum level and contact time was found. Increasing contact time increased the attachment rate when inoculum level was $10^2$ or $10^3$ cells/skin; while at $10^4$ cells/skin, attachment rate decreased over time (Tambyln et al., 1997).

SAM was developed for *Salmonella* initially, however, it can be used to test other pathogenic and spoilage bacteria as well. There are several advantages of SAM method to evaluate potential carcass disinfectants, the irradiation of the skin before

inoculation eliminates any original microorganisms existing; therefore, TSA, a non-selective recovery media, can be used, which allows the recovery of both viable and injured cells.

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# CHAPTER III

## Study 1: The Effect of Different De-feathering Methods on *Salmonella* Attachment to Chicken Skin

### INTRODUCTION

*Salmonella*, ranking second among the top five pathogens contributing to domestically acquired foodborne illnesses and the first of resulting in hospitalization and death, is estimated to cause 1,027,561 illnesses, 19,336 hospitalizations and 378 deaths annually in the US. (CDC, 2014).

Scalding and de-feathering are the two procedures used during the initial stages of poultry slaughter that can cause a change of skin structure, which in turn affect the rate of bacterial attachment to chicken skin. Scalding is the process of submerging the birds in warm water to wet and loosen the feathers. Hot water is used in this procedure to transfer heat to the feather follicles (Mead, 2004). This step is also one of the most important points to remove adhering fecal material, thus, there is a chance for Salmonella cross-contamination among carcasses (Russell, 2001). High scalding water temperatures have a better effect on loosening feathers from the follicles, but also cause the epidermis to become softer. During de-feathering, the rubber fingers can serve as one of the avenues of cross-contamination. The objective of this study was to assess *Salmonella* attachment rates to chicken skin scalded and de-feathered by various methods to alter the skin topography.

## MATERIALS AND METHODS

### *Preparation of Bacterial Culture*

***Salmonella* culture.** Frozen nalidixic acid resistant strains of *Salmonella* Typhimurium, *S.* Enteritidis, and *S.* Heidelberg were cultured in Trypticase Soy Broth (TSB; Becton, Dickinson and Company, Sparks, MD) containing 0.01% Nalidixic acid at 37 $^{o}$C for 24 h, respectively. Then, the cultures were streaked onto Trypticase Soy Agar (TSA; Becton, Dickinson and Company, Sparks, MD) with 0.01% Nalidixic acid and incubated at 37 $^{o}$C for 24 h separately. Single colonies from TSA with Nalidixic acid plates were transferred into 15 mL sterile TSB separately and incubated at 37 $^{o}$C for 12 h. *Salmonella* inoculum cocktail was prepared by transferring 1 mL of each strain into a 15 mL sterile centrifuge tube and centrifuged (Thermo Electron Corporation, ZI Aze Bellltoume, France) at 2,600 × g for 3 min at 4 $^{o}$C. The bacterial pellet was washed twice with buffered peptone water (BPW; HiMedia, HiMedia Laboratories Pvt. Ltd, India) through centrifugation. Finally, the bacteria were re-suspended in BPW. The bacterial population was estimated by the optical density (OD) at 640 nm measurement.

***Salmonella* plus *E. coli* culture.** A loopful of frozen Nalidixic acid resistant strains of *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Salmonella* Heidelberg were cultured in sterile TSB containing 0.01% Nalidixic acid at 37 $^{o}$C for 24 h separately, and *E. coli* was cultured in TSB at 37 $^{o}$C for 24 h. The *Salmonella* cultures were then streaked onto TSA with 0.01% Nalidixic acid and incubated at 37 $^{o}$C for 24 h separately, while *E. coli* was streaked onto regular TSA and incubated at 37 $^{o}$C for 24 h. Single colony picked from each *Salmonella* and *E. coli* was transferred into 15 mL sterile TSB

separately and incubated at 37 ºC for 12 h. One milliliter of each culture was mixed and transferred into a sterile centrifuge tube and centrifuged at 2,600 × g for 3 min at 4 ºC. The pellet was washed with BPW through centrifugation. The final bacteria were suspended in 3 mL of BPW in order to keep the *Salmonella* concentrations at the same level as *Salmonella* original culture.

***Collection of Chicken Skin***

This study utilized the SAM developed by Conner and Bilgili (1994) Chicken skins were collected at the Auburn University Poultry Science Research Unit the day before experiment. Broilers were stunned and bleed for 90 s, then skins from the breast area were collected from (1) euthanized, bled and dry - hand-picked carcasses, (2) carcasses bled, scalded (Cantrell, Galnesville, GA) in tap water at 20 ± 2 ˚C for 65 s and mechanically de-feathered (Meyn, Armsterdam, Netherlands), (3) carcasses bled, scalded at 51 ± 2˚C for 65 s (soft-scalding) and mechanically de-feathered, and (4) from carcasses bled, scalded at 60 ± 2˚C for 65 s (hard-scalding) and mechanically de-feathered. Skin samples were cut into 5 cm diameter pieces. Each skin sample included both the pterylae and apteria. Four samples from each bird were obtained, and stored at 4 ºC for use.

***Procedures***

A total of 384 (n = 4 contact times × 4 skin types × 2 inoculum × 4 sample per treatment × 3 replications) chicken skin samples were prepared. They were placed on sterilized aluminum foil, and inoculated with 50 µl of 2 × $10^8$ CFU/mL bacterial

suspension. After contact times of 5, 10, 15, and 20 min, each sample was rinsed with 10 mL BPW to remove “loosely attached” cells. Then the skin was transferred to 10 mL fresh BPW and stomacher blended for 1 min to remove “firmly attached” cells. All rinsed and blended samples were diluted and plated on TSA with Nalixidic acid (Alfa Aesar, Ward Hill, MA) plates. Plates were incubated at 37 °C for 24 h for enumeration.

***Statistical Analysis.***

All microbial data were converted to $log_{10}$ CFU/sample (each chicken skin was counted as a sample) before analysis in the statistical model. Statistical analysis was conducted using SAS 9.3 software (SAS Institute, Cary, N.C.). General Linear Model of SAS was used to analyze the data and comparisons were made using LSMEANS, when significant effects at $p \leq 0.05$ were identified.

## RESULTES AND DISCUSSION

Results from this study are presented in Tables 1 to 3 and Figures 2 to 5. A cocktail of *Salmonella* Typhimurium, *S*. Enteritidis, and *S*. Heidelberg was used in this study. *E. coli* was also used in this experiment to investigate the effect of co-existing bacteria on *Salmonella* attachment (*Salmonella* + *E. coli*).

There were significant skin type and inoculum interaction for “loosely” and “firmly” attached cells, whereas, contact time was not significant (Tables 2 and 3). The “loosely attached” *Salmonella* recovery by chicken skin types is shown in Table 2 and Figures 2 and 4. For “loosely attached” *Salmonella*, there were no significant differences ($p >$ 0.05) with increasing contact time up to 20 min (Figure 2). *Salmonella* attachment was

not affected ($p > 0.05$) by the presence of *E. coli* over the time. The recovery of “loosely attached” *Salmonella* cells was approximately 6.00 log CFU/sample for each group. The “firmly attached” *Salmonella* recovery by chicken skin types is shown in Table 3 and Figures 3 and 5. For “firmly attached” *Salmonella*, there were no significant differences ($p > 0.05$) with increasing contact time up to 20 min (Figure 3). *Salmonella* attachment was not affected ($p > 0.05$) by the presence of *E. coli* over the time except for tap water scalded which *E. coli* increased the recovery of “firmly attached” *Salmonella* by approximately 1 log: tap water scalded chicken skin inoculated with *Salmonella* had a recovery of around 5.20 log CFU/sample, while *Salmonella* + *E. coli* inoculated samples recovered approximately 6.20 log CFU/sample.

In conclusion, neither longer contact time nor the presence of *E. coli* affect ($p > 0.05$) *Salmonella* attachment on various chicken skins types except for tap water scalded which *E. coli* was able to promote “firmly attached” *Salmonella* attachment. There was no trend of scalding temperature effects on *Salmonella* attachment. However, when comparing contact times, the presence of *E. coli* could increase the “firmly attached” *Salmonella* attachment.

Kim and others (1993) studied the effect of scalding temperature on *Salmonella* Typhimurium attachment. Breast skins obtained from birds scalded at 52, 56, and 60 °C were tested, and the attachment was quantified by both scanning electron microscopy (SEM) and microbiological plating method. The results obtained from SEM showed breast skin scalded at 60 °C had 1.1 -1.3 log more *Salmonella* Typhimurium attached compared with lower temperature scalded chicken breast skin. However, no differences were found by plating methods. They also suggested that skins began to

lose the majority of epidermal layers when scalded at 60 °C, and suggested avoiding the removal of whole epidermis during processing to reduce *Salmonella* attachment to skin. Notermans and Kampelmacher (1974) stated that attachment rate of flagellated bacteria was greatly dependent on temperature and pH, and found the optimal temperature for flagellated bacteria attachment was approximately 20 °C.

When leaving poultry skin in a bacterial suspension, some bacteria become physically entrapped, some will directly attach to the skin tissue, and the others will float in the water film surrounding the skin (Thomas and McMeekin, 1980). Lillard (1985) stated that large numbers of cells adhere rapidly and attachment increases with time, which is not consistent with the results from this study. Another study conducted by Thomas and McMeekin (1984) demonstrated that physical entrapment of bacteria on chicken skin is related to water uptake. The results of confocal micrographs showed *Salmonella* Typhimurium deep within skin crevices, where they are assumed to be protected from removal by rinsing or chemical inactivation; while *Salmonella* Typhimurium hydrated skin can be seen floating freely deep within feather follicles with other cells apparently adhering to the interior follicle surface. Thomas et al. (1987) found that soft scalding did not cause significant removal of the epidermis, but did cause partial separation of the stratum corneum (cuticle) and damage to the underlying tissue. Lillard (1985) observed that soft-scalded (52 °C) and hard-scalded (56 °C) skin did not show significant difference in bacterial attachment, which is consistent to the result of this study.

A study conducted by Dougherty and Seibold (1965) stated that the histologic of skin changed in a definite progression with the increase of scald water temperature.

They slaughtered the broilers following kosher method, and the broilers were scalded under different temperatures (110, 120, 130, 140, 150, and 160 °F), all birds were hand de-feathered. Four birds were dry-picked as a kosher control. Skin samples (2×2 cm) were collected from the left breast and lumbar region of the back. Histological observations showed contraction on both epidermis and dermis, and the contracted epidermis developed ridges or corrugations on skin surfaces. When scalded at or below 130 °F, connective tissues had slight contraction on the dermis; while the changes above 130 °F were extreme. Ridges were starting forming at 150 °F, and very marked ridges were found at 160 °F. Thus, the higher the temperature of scalder water, the more ridges were present on the skin. While in this study, different scalding temperatures did have an impact on *Salmonella* attachment, but no trend was shown that higher temperature scalded chicken skin had more cells attached. McMeekin and Thomas (1978) demonstrated that "hard" scalding and de-feathering of chicken exposed a new surface for microbial contamination, which contained many capillary-size channels and crevices. However, research showed that bacteria were firmly attached to the skin even before being processed. The attachment of bacteria on skin began within 15 s and increased with time in a linear manner. The rate of attachment was not significantly different between "soft" and "hard" scalded carcasses (Mead, 2006).

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# CHAPTER IV

## Study 2: The Use of Sodium Dodecyl Sulfate to Improve the Efficacy of Antimicrobials against *Salmonella* on Chicken Skin from Different De-feathering Methods

### INTRODUCTION

*Salmonella* is an important pathogen domestically contributing to foodborne illness annually in the US. (CDC, 2014). *Salmonella* is frequently associated with poultry and poultry products (Bryan and Doyle, 1995). In order to reduce the contamination of *Salmonella* in poultry products, new standards for *Salmonella* were introduced by FSIS. Currently, the post-chill *Salmonella* performance standard for young chicken is 7.5%, or 5 positive test results out of 51 samples (USDA-FSIS, 2015).

During processing, a number of interventions can be applied to prevent or reduce microbial contamination. A number of antimicrobial compounds (acidified sodium chlorite, chlorine, chlorine dioxide, organic acid, peracetic acid, and cetylpyridinium chloride) are available to the processors to reduce contamination and cross-contamination throughout slaughter and processing.

Sodium dodecyl sulfate (SDS) or sodium lauryl sulfate (SLS), an anionic surfactant, is commonly used for cleaning agent. Surfactants break the surface tension and separates molecules to allow better interaction between the chemical and target object. Combination of surfactants with antimicrobials has been studied on poultry products to reduce microorganisms. Zhao et al. (2009) reported that a combination of

levulinic acid and SDS as a wash solution may be practical to inactivate foodborne enteric pathogens on poultry products. Four organic acids including lactic acid, acetic acid, caprylic acid, and levulinic acid at 0.5% individually or in combination with 0.05 to 1% SDS were tested in reducing *Salmonella* and *E. coli*. The results showed that individual compounds reduced pathogens less than 2 log CFU/mL within 20 min at 21 °C, while any combinations with 0.5% SDS reduced the pathogens by more than 7 log CFU/mL within 10 s at the same temperature (Zhao et al., 2009). However, Lu and Wu (2012) tested 0.2 mg/mL thymol and 2 mg/mL acetic acid with or without 5% (w/v) SDS on chicken breast to reduce *Salmonella enterica*, and found that SDS did not improve the antimicrobial activity in either solution. The objective of study was to determine the effect of SDS added to chlorine and PAA on their antimicrobial efficacy.

## MATERIALS AND METHODS

### ***Salmonella* culture preparation**

Frozen nalidixic acid resistant strains of *Salmonella* Typhimurium, *S.* Enteritidis, and *S.* Heidelberg were cultured in Trypticase Soy Broth (TSB; Becton, Dickinson and Company, Sparks, MD) containing 0.01% Nalidixic acid at 37 ºC for 24 h, respectively. Then, the cultures were streaked onto Trypticase Soy Agar (TSA; Becton, Dickinson and Company, Sparks, MD) with 0.01% Nalidixic acid and incubated at 37 ºC for 24 h separately. Single colony from TSA with Nalidixic acid plate was transferred into 15 mL sterile TSB separately and incubated at 37 ºC for 12 h. *Salmonella* inoculum cocktail was prepared by transferring 1 mL of each strain into a 15 mL sterile centrifuge tube and centrifuged (Thermo Electron Corporation, ZI Aze Bellltoume, France) at 2,600 × g

for 3 min at 4 ºC. The bacterial pellet was washed twice with buffered peptone water (BPW; HiMedia, HiMedia Laboratories Pvt. Ltd, India) through centrifugation. Finally, the bacteria were re-suspended in BPW. The bacterial population was estimated by the optical density (OD) at 640 nm measurement.

### *Collection of Chicken Skin*

Chicken skins were collected at the Auburn University Poultry Science Research Unit the day before experiment. Broilers were stunned and bled for 90 s, then 4 types of skins from the breast area were collected from (1) euthanized-dry hand-picked carcasses, (2) carcasses scalded (Cantrell, Galnesville, GA) in tap water at 20 ± 2 ˚C for 65 s and mechanically de-feathered (Meyn, Armsterdam, Netherlands), (3) carcasses scalded at 51 ± 2˚C for 65 s (soft scalding) and mechanically de-feathered, and (4) from carcasses scalded at 60 ± 2˚C for 65 s (hard scalding) and mechanically de-feathered. Skin samples were cut into 5 cm diameter pieces. Each skin sample included both the pterylae and apteria. Four samples from each bird were obtained, and stored at 4 ºC for use.

### *Procedures*

A total of 336 chicken skin samples (n = 7 treatments × 4 skin types × 4 samples per treatment × 3 replications) were used. The 7 treatments were positive control, negative control, 0.005% chlorine (Great value, Bentonville, AR), 0.2% peracetic acid (PAA; Spectrum; FMC, Philadelphia, PA), 0.5% sodium lauryl sulfate (SDS; AMRESCO, Solon, Ohio), 0.005% chlorine with 0.5% SDS, and 0.2% PAA with 0.5% SDS. Positive

control (inoculated without any treatment) samples were used to determine the physical recovery rate of bacteria on the skin samples, while negative control (chicken skin only) samples were included to determine any background of *Salmonella*. Concentration of chlorine was measured using Aquachek Water Quality Test Strips for High-Range Chlorine (HACH company, Loveland, CO), and pH was adjusted to 5.5 to 6.0 with 1 N HCl; PAA concentration was measured using titration drop test kit (FMC; Safe Foods Corporation, North Little Rock, AR). *Salmonella* Attachment Model (SAM) was applied to recover “loose” and “firm” cells (Conner and Bilgili, 1994). Chicken skin samples were placed on sterilized aluminum foil, and inoculated with 50 μl of the inoculum ($2 \times 10^8$ CFU/mL of *S.* Typhimurium, *S.* Enteritidis, and *S.* Heidelberg cocktail). After 10 min attachment, samples were treated with 3 sprays (3 mL) of the chemical solutions. After approximately 5 s, the treated samples were aseptically placed in sterile rinse bags containing 10 mL BPW per bag, and rinsed for 1 min to remove “loosely attached” cells. After rinsing, skins were transferred to fresh BPW (10 mL/bag) and stomacher blended for 1 min to remove “firmly attached” cells. All rinsed and blended samples were diluted and plated on TSA with Nalixidic acid (Alfa Aesar, Ward Hill, MA) plates. Plates were incubated at 37 °C for 24 h for enumeration.

***Statistical analysis.***

Statistical analysis was conducted using SAS 9.3 software (SAS Institute, Cary, N.C.). General Linear Model of SAS was used to analyze the data and comparisons were made using LSMEANS, when the significant effects at $p \leq 0.05$ were identified.

## RESULTS AND DISCUSSION

The results of this study are expressed as a reduction (%) relative to positive control. *Salmonella* was not detected in the negative control.

Significant antimicrobial treatment by skin type interaction was detected for both "loosely" and "firmly" attached cells (Table 4). The antimicrobial efficacy of antimicrobials against "loosely attached" *Salmonella* is illustrated in Figure 6. Chlorine was the least effect treatment among all five treatments, 0.2% PAA and 0.2% PAA with 0.5% SDS were the most effective treatments. SDS alone was effective in reducing the "loosely attached" *Salmonella* from 70-80 % depending upon the skin type. Only the antimicrobial activity of chlorine was significantly enhanced with SDS. PAA resulted in the highest reduction of *Salmonella*, regardless of skin type, SDS was less effective against the "firmly attached" cells *Salmonella*, with reduction rate of about 60%. Again, SDS only enhanced the antimicrobial activity of chlorine. SDS did not show a synergistic effect against the "firmly attached" cells, regardless of skin type.

Overall, the results showed that 0.2% PAA was more effective against *Salmonella* than 0.005% Cl. SDS (0.5%) enhanced the antimicrobial efficacy of 0.005% Cl, but not PAA.

SDS is generally recognized as safe by FDA for additives (21 CFR 172.822). It can denature protein surfaces and damage cell membranes, its antimicrobial effects can be enhanced at pH value between 1.5 and 3.0. Adding SDS to organic acids for promoting the antimicrobial efficacy against *Salmonella enterica* on chicken skin was studied by Zaki et al. (2015). In their study, *S. enterica* Kentucky inoculated chicken skins were dipped into organic acids (Lactic, Levulinic, and Acetic acids; 1-2 %), SDS

(0.5-1 %) or their combinations for 1 to 3 min. The results showed that more than 5 log reduction of *S. enterica* Kentucky were obtained by adding SDS to lactic acid or acetic acid. SDS alone at 0.5% resulted in 0.21 and 0.36 log reduction of *Salmonella* for 1 and 3 min dipping time, respectively; while at 1% SDS, the reduction rates were increased to 0.24 log (1 min) and 0.66 log (3 min).

Another study conducted by Zhao et al. (2009) demonstrated that adding SDS to levulinic acid as a wash solution could be used for killing foodborne enteric pathogens on fresh produce and uncooked poultry. The results showed that adding 0.05% SDS to 0.5% levulinic acid, increased the reduction of bacteria in pure culture (*S*. Typhimurium, *S*. Enteritidis, or *E.coli* O157:H7) from ≤ 2 log CFU/mL within 20 min at 21 °C to > 7 log CFU/mL within 10 min at 21 °C. The combination of 0.5% levulinic acid and 0.05% SDS (pH 3.0) reduced *S.* Enteritidis on chicken skin by 0.4, 2.9, 3.7 and 5.3 log CFU/cm$^2$ for 0, 1, 2, and 5 min contact time, respectively. *S*. Enteritidis was reduced by 0.5, 2.6, 1.6, and 3.6 log CFU/g on chicken wings when applied 2% levulinic aicd and 1% SDS (pH 3.0) mixture for 0, 1, 2, and 5 min, respectively. Levulinic acid at 0.3% plus 1% SDS had better bacterial reduction on chicken wings. The treatment of 3% levulinic acid plus 2% SDS (pH 4.0) on water heavily contaminated with chicken feces could cause more than 7 log CFU/mL reduction within 20 min.

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# CHAPTER V

## Study 3: The Application of Wax Coating to Prevent *Salmonella* Attachment on Chicken Skin with Different De-feathering Methods

### INTRODUCTION

*Salmonella*, ranking second among the top five pathogens contributing to domestically acquired foodborne Salmonellosis in the US. (CDC, 2014). In order to reduce the contamination of *Salmonella* in poultry products, new standards for *Salmonella* levels in post-chiller carcasses were introduced. Currently, the *Salmonella* performance standard for young chicken is 7.5%, or 5 positive test results out of 51 samples (USDA-FSIS, 2015).

In order to reduce microorganisms on the products, many intervention strategies are applied during poultry processing, including pre-scald bird brushes, multi-stage scalding, and chemical interventions. Pre-scald bird brushes physically remove the adhering feces from skin and feathers to lower organic matter in the scalder, and reduces the spread of feces during de-feathering. Scalding is one of the major points of cross-contamination. Most processing plants are using multi-stage scalders with counter-current flow which is critical to reducing cross-contamination (Russell, 2001). Chemical interventions are applied at multiple steps, including online reprocessing,

inside/outside bird wash, during and after chilling. Chlorine, and peracetic acid (PAA) are few of the most common antimicrobials that are used in the poultry processing plant.

Wax coating is a common practice applied on a variety of fruits and vegetables like apples, oranges, lemons, limes, cucumbers, eggplants, pumpkins, and tomatoes. Moisture content in fruits and vegetables are usually high (80 – 90%), wax is applied to help retain moisture during shipping and at the market (Shellhammer and Krochta, 1997). Coating improves the appearance of the products, but not the quality (Anonymous, 2004). Wax coated produce has a shine and fresh appearance for longer time, which prolongs the shelf-life of the product (Anonymous, 2004). A lower moisture content will typically give a lower grade for produce. Application of a thin layer of wax helps to reduce the weight loss by 30 - 40 % (Anonymous, 2004). In general, wax coating on fruits and vegetables can extend shelf-life and make the product available all the way through the season by delaying the color changes and ripening processes, reducing moisture loss, maintaining the texture (Puttalingamma, 2014) as well as inhibit mold growth, and prevent microorganisms from entering the products (Brin, 2014). Waxes are also used in poultry (mostly water fowl) to remove the remaining vestiges of plumage. Wax coating of chicken carcasses to reduce contamination and cross-contamination during slaughter and processing have not been investigated. The purpose of this study was to evaluatew the attachment rates of *Salmonella* on wax-coated chicken skins de-feathererd by different methods.

## MATERIALS AND METHODS

### *Salmonella culture preparation*

Frozen nalidixic acid resistant strains of *Salmonella* Typhimurium, *S.* Enteritidis, and *S.* Heidelberg were cultured in Trypticase Soy Broth (TSB; Becton, Dickinson and Company, Sparks, MD) containing 0.01% Nalidixic acid at 37 ºC for 24 h, respectively. Then, the cultures were streaked onto Trypticase Soy Agar (TSA; Becton, Dickinson and Company, Sparks, MD) with 0.01% Nalidixic acid and incubated at 37 ºC for 24 h separately. Single colonies from TSA with Nalidixic acid plates were transferred into 15 mL sterile TSB separately and incubated at 37 ºC for 12 h. *Salmonella* inoculum cocktail was prepared by transferring 1 mL of each strain into a 15 mL sterile centrifuge tube and centrifuged (Thermo Electron Corporation, ZI Aze Bellltoume, France) at 2,600 × g for 3 min at 4 ºC. The bacterial pellet was washed twice with buffered peptone water (BPW; HiMedia, HiMedia Laboratories Pvt. Ltd, India) through centrifugation. Finally, the bacteria were re-suspended in BPW. The bacterial population was estimated by the optical density (OD) at 640 nm measurement.

***Collection of Chicken Skin***

Chicken skins were collected at the Auburn University Poultry Science Research Unit the day before experiment. Broilers were stunned and bleed for 90 s, then skins from the breast area were collected from (1) euthanized-dry hand-picked carcasses, (2) carcasses scalded (Cantrell, Galnesville, GA) in tap water at 20 ± 2 ˚C for 65 s and mechanically de-feathered (Meyn, Armsterdam, Netherlands), (3) carcasses scalded at 51 ± 2˚C for 65 s (soft scalding) and mechanically de-feathered, and (4) from carcasses scalded at 60 ± 2˚C for 65 s (hard scalding) and mechanically de-feathered. Skin samples were cut into 5 cm diameter pieces. Each skin sample included both the

pterylae and apteria. Four samples from each bird were obtained, and stored at 4 ºC for use.

### *Preparation of Wax Microemulsions*

**Carnauba wax.** Carnauba wax microemulsion was prepared following Hagenmaier (2004) protocol with modification. In an aluminium container (6.5 cm diameter × 15.2 cm high), 40 g carnauba wax (Alfa Aesar, Ward Hill, MA), 15 g water, 4 g mysristic acid (TCI, Tokyo Chemical Industry Co., LTD, Tokyo, Japan), 4 g oleic acid (Fisher Chemical, Fair Lawn, New Jersey) and 0.15 g of 5% polydimethylsiloxane (Dow Corning Corporation, Midland, MI) were added, and the container was submerged into a boiling water bath at depth of 7 cm. The mixture was mixed with an overhead stirrer at 700 rpm for 10 min. Then, 8 g of 28% ammonium hydroxide (BDH, Radnor, PA) were added slowly and stirred at 700 rpm for 3 min. Hot water (160 mL) at 85 ˚C was added into the mixture slowly and stirred at 700 rpm for another 3 min. The wax microemulsion was kept in water bath at 80 ˚C for use.

**Beeswax.** Beeswax microemulsion was prepared following Hagenmaier and Baker's method (1997). In an aluminum container (6.5 cm diameter × 15.2 cm high), 20 g beeswax (Fisher Chemical, Fair Lawn, New Jersey), 2.4 g palmitic acid (Alfa Aescar, Heysham, LA32XY, England), 2.4 g mysristic acid, 12 mg of 5% polydimethylsiloxane, 2.7 g 28% ammonium hydroxide, and 35 g water were added and heated at 93 ˚C for 10 min while stirring at 700 rpm, and another 5 min with the speed of 800 rpm. Hot water (95 ˚C) was added in three increments: 20, 20, and 80 mL at intervals of 1 min, then the

mixture was stirred at 700 rpm for another 5 min. The wax microemulsion was kept in water bath at 50 ˚C before use.

### *Procedures*

A total of 240 (n = 5 treatments × 4 skin types × 4 sample per treatment × 3 replications) chicken skins were prepared the day before experiment. The 5 treatments included positive control, negative control, water rinse control, carnauba wax coated and beeswax coated groups. Positive control was the *Salmonella* inoculated samples without wax coating to determine the real inoculum level; negative control was non-coated and non-inoculated samples to check the background microorganisms; water rinse control was designed to recover the unattached cells. Chicken skins were placed on sterile weighing boat, and coated with wax microemulsion. Beeswax was applied as spray (0.4 g/sample) while carnauba wax was applied using a brushing method (0.4 g /sample) due to its high viscosity and high melting point. After 5 min drying time, each sample was inoculated with 50 μL of *Salmonella* suspension ($2 \times 10^9$ CFU/mL *S.* cocktail). After 10 min attachment period, sample was dipped into 30 mL of sterile water in a weighing boat and removed immediately. Then, each treated sample was aseptically placed in a sterile rinse bag containing 10 mL BPW, and rinsed for 1 min to remove "loosely attached" cells. Then chicken skin was transferred to fresh BPW and blended with stomacher for 1 min to remove "firmly attached" cells. Both BPW rinsate were diluted and plated on TSA with Nalixidic acid plates for enumeration. Plates were incubated at 37 °C for 24 h and colonies were recorded for analysis. Negative control

samples were plated on regular Xylose Lysine Deoxycholate (XLD) agar as well for *Salmonella* background examination.

***Statistical analysis.***

All microbial data were converted to $\log_{10}$ CFU/sample (each chicken skin was counted as a sample) before analysis in the statistical model. Statistical analysis was conducted using SAS 9.3 software (SAS Institute, Cary, N.C.). General Linear Model of SAS was used to analyze the data and comparisons were made using LSMEANS, when significant effects at $p \leq 0.05$ were identified.

## RESULTS AND DISCUSSION

Table 5 summarizes the main effects of wax treatment and skin type. Significant wax treatment effect was observed for “firmly attached” *Salmonella*, whereas the “loosely attached” S*almonella* recovery varied by skin type. The inocula was determined from *Salmonella* recovered from inoculated chicken skins without wax coating and averaged about 7.4 log CFU/sample. Water control group was the sample rinsed with water only and used to determine the unattached and attached *Salmonella*. The “loosely attached” *Salmonella* are presented in Figure 8. Carnauba wax coating significantly ($p \leq 0.05$) reduced *Salmonella* attachment on all four chicken skin types. The reduction was 1.57, 0.71, 0.74, and 0.84 log on dry hand de-feathered, tap water scalded, soft and hard scalded chicken skins, respectively. Beeswax coating was not also able to reduce *Salmonella* attachment on chicken skin. Results from “firmly attached” *Salmonella* are

presented in Figure 9, and the trend was similar with “loosely attached” *Salmonella*. However, the “firmly attached” cells populations were not affected by various skin types.

In conclusion, *Salmonella* attachment on chicken skin can be reduced by a water resistant coating, such as illustrated with carnauba wax in this study.

Ugur et al. (2004) conducted a study to determine the effect of oil-wax coating on the quality of refrigerated chicken meat. The coating was prepared by homogeneously mixing 46.6% vegetable oil, 1.8% beeswax, and 51.6% water with heating until all wax melted. The results showed that oil-wax coated treatment significantly ($p \leq 0.01$) inhibited *Pseudomonas* growth and was also more effective than the control group in inhibiting *Salmonella* growth. This might be caused by creating an anaerobic environment from the coating resulting in the inhibition of microbial growth. However, the inhibition efficiency of mesophilic aerobic bacteria was not as good as the control group.

As scalding and de-feathering steps can change the topography of chicken skin, higher temperature scalded chicken skin has more ridges (Dougherty and Seibold, 1965). The change of the skin could provide a new surface for microbial contamination (McMeekin and Thomas, 1978). It is possible that the application of wax coating could fill the ridges and leave a smoother surface to prevent microbial attachment. Chicken skin is not smooth, as it contains numerous crevices and feather follicles. Wax coating may seal the crevices and feather follicles, which can be another possible niche for bacteria.

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# CHAPTER VI

# CONCLUSION

In summary, the bacterial contact time, or existing of *E. coli* did not have remarkable impacts on *Salmonella* attachment on chicken skins scalded and de-feathered by different methods, except for tap water scalded which *E. coli* was able to promote “firmly attached” *Salmonella* attachment. The scalding temperature did not show effects on *Salmonella* attachment. PAA (0.2%) had more antimicrobial effectiveness against *Salmonella* than 0.005% chlorine water, especially on dry hand de-feathered chicken skin. SDS (0.5%) was able to enhance the antimicrobial efficacy of chlorine, but not PAA. Carnauba wax coating was effective in preventing *Salmonella* attachment to chicken skin, while beeswax was not.

Table 1 Overall ANOVA with the probabilities indicating the main effects of skin type, inoculum, and contact time.

| Effect | Probability | |
|---|---|---|
| | Loosely attached cells | Firmly attached cells |
| Skin type | 0.0021 | <0.0001 |
| Inoculum | 0.4627 | <0.0001 |
| Contact time | 0.4923 | 0.4853 |
| Skin type* Inoculum | 0.0138 | <0.0001 |
| Skin type*Contact time | 0.0838 | 0.1587 |
| Inoculum*Contact time | 0.4611 | 0.4640 |
| Skin type*Inoculum*Contact time | 0.4831 | 0.8419 |

N = 384

Table 2 "Loosely attached" *Salmonella* recovery (log CFU/sample) at different contact times on various chicken skin types.

| Inoculum | Skin Type | Contact time (min) | | | |
|---|---|---|---|---|---|
| | | 5 | 10 | 15 | 20 |
| *Salmonella* | Dry | 6.24 $^{Ax}$ | 6.13 $^{Ax}$ | 6.21 $^{Ax}$ | 6.00 $^{Bx}$ |
| | Tap | 6.02 $^{Dx}$ | 5.93 $^{Bx}$ | 6.00 $^{BCx}$ | 6.10 $^{ABx}$ |
| | Soft | 6.10 $^{BCDx}$ | 6.04 $^{ABx}$ | 6.15 $^{ABCx}$ | 6.16 $^{ABx}$ |
| | Hard | 6.25 $^{Ax}$ | 6.17 $^{Ax}$ | 6.19 $^{ABx}$ | 6.22 $^{Ax}$ |
| *Salmonella* + *E. coli* | Dry | 6.19 $^{ABx}$ | 6.13 $^{Ax}$ | 6.24 $^{Ax}$ | 6.14 $^{ABx}$ |
| | Tap | 6.16 $^{ABCx}$ | 6.09 $^{ABx}$ | 5.98 $^{Cx}$ | 6.07 $^{ABx}$ |
| | Soft | 6.06 $^{CDx}$ | 6.04 $^{ABx}$ | 6.09 $^{ABCx}$ | 6.15 $^{ABx}$ |
| | Hard | 5.98 $^{Dx}$ | 6.12 $^{Ax}$ | 6.08 $^{ABCx}$ | 6.13 $^{ABx}$ |

Dry = dry hand de-feathered chicken skin;
Tap = tap water scalded and mechanically de-feathered chicken skins;
Soft = soft scalded (51 °C) and mechanically de-feathered chicken skin;
Hard = hard scalded (60 °C) and mechanically de-feathered chicken skin.
Pooled SEM = 0.1144.
$^{A-D}$ Means within a column without common letter different significantly ($p \leq 0.05$)

$^{x}$ Means within a row without common letter different significantly ($p \leq 0.05$)

Table 3 "Firmly attached" *Salmonella* recovery (log CFU/sample) at different contact times on various chicken skin types.

| Inoculum | Skin Type | Contact time (min) | | | |
|---|---|---|---|---|---|
| | | 5 | 10 | 15 | 20 |
| *Salmonella* | Dry | 5.93 $^{Bx}$ | 6.15 $^{Ax}$ | 6.04 $^{ABx}$ | 5.91 $^{Ax}$ |
| | Tap | 5.18 $^{Cx}$ | 5.23 $^{Bx}$ | 5.12 $^{Cx}$ | 5.21 $^{BCx}$ |
| | Soft | 5.04 $^{Cx}$ | 5.15 $^{Bx}$ | 5.06 $^{Cx}$ | 5.24 $^{BCx}$ |
| | Hard | 5.23 $^{Cx}$ | 5.07 $^{Bx}$ | 5.19 $^{Cx}$ | 5.02 $^{Cx}$ |
| *Salmonella* + *E. coli* | Dry | 6.06 $^{ABx}$ | 6.21 $^{Ax}$ | 5.75 $^{Bx}$ | 5.86 $^{Ax}$ |
| | Tap | 6.27 $^{Ax}$ | 6.34 $^{Ax}$ | 6.18 $^{Ax}$ | 6.12 $^{Ax}$ |
| | Soft | 5.32 $^{Cx}$ | 5.22 $^{Bx}$ | 5.25 $^{Cx}$ | 5.30 $^{Bx}$ |
| | Hard | 5.29 $^{Cx}$ | 5.18 $^{Bx}$ | 5.20 $^{Cx}$ | 5.13 $^{BCx}$ |

Dry = dry hand de-feathered chicken skin;
Tap = tap water scalded and mechanically de-feathered chicken skins;
Soft = soft scalded (51 °C) and mechanically de-feathered chicken skin;
Hard = hard scalded (60 °C) and mechanically de-feathered chicken skin.
Pooled SEM = 0.2071.
$^{A\text{-}C}$ Means within a column without common letter different significantly ($p \leq 0.05$)

$^{x}$ Means within a row without common letter different significantly ($p \leq 0.05$)

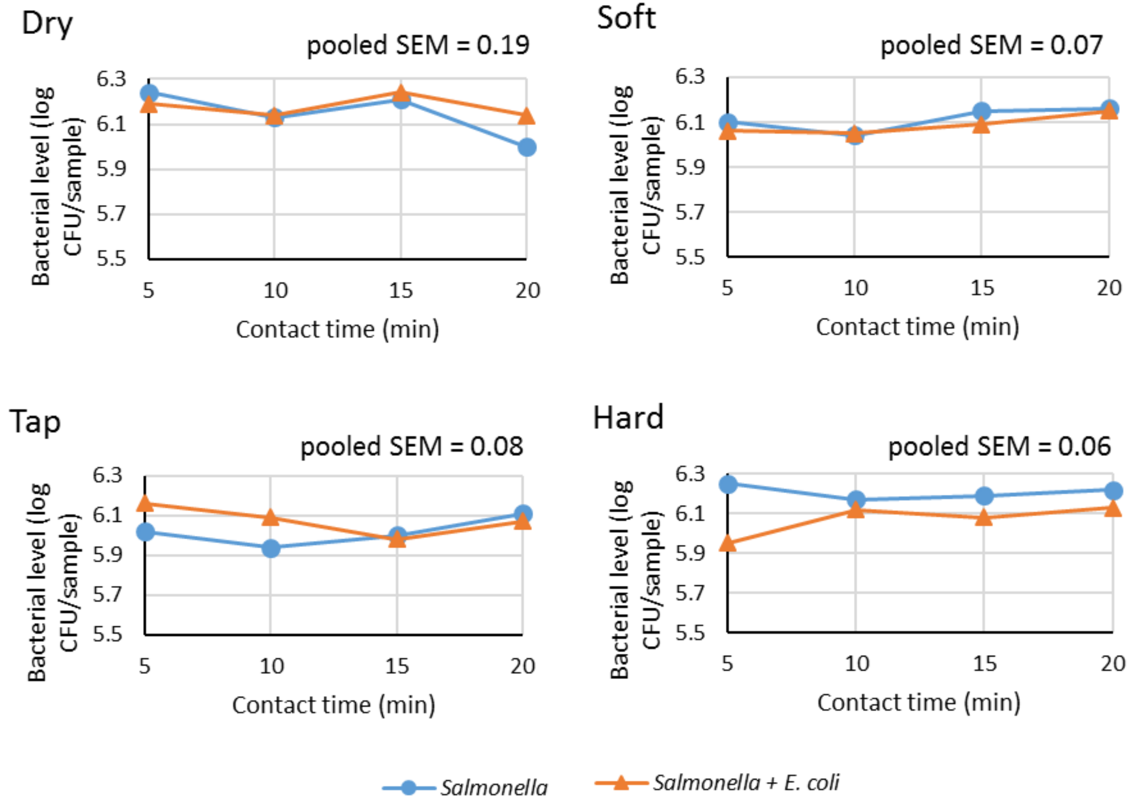


Figure 2. "Loosely attached" *Salmonella* recovered at different contact times by skin type. Dry = dry hand de-feathered chicken skin; Tap = tap water scalded and mechanically de-feathered chicken skins; Soft = soft scalded (51 °C) and mechanically de-feathered chicken skin; Hard = hard scalded (60 °C) and mechanically de-feathered chicken skin.

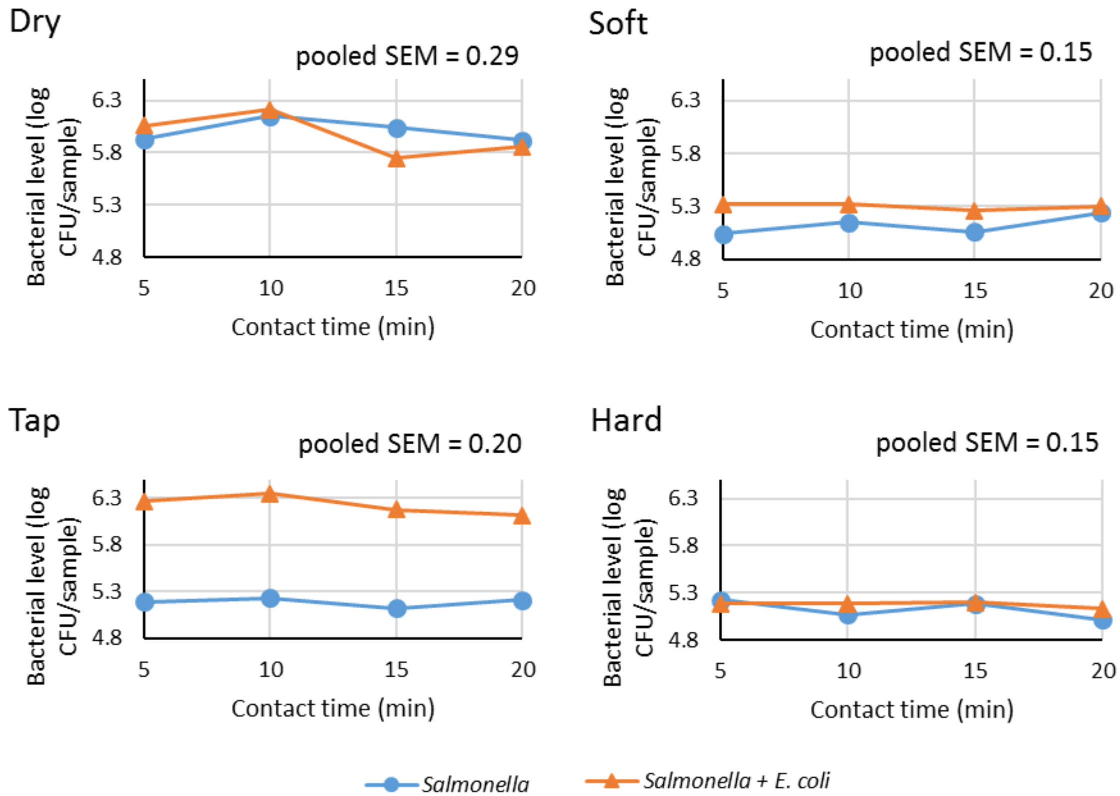


Figure 3. "Firmly attached" *Salmonella* recovered at different contact times by skin type. Dry = dry hand de-feathered chicken skin; Tap = tap water scalded and mechanically de-feathered chicken skins; Soft = soft scalded (51 °C) and mechanically de-feathered chicken skin; Hard = hard scalded (60 °C) and mechanically de-feathered chicken skin.

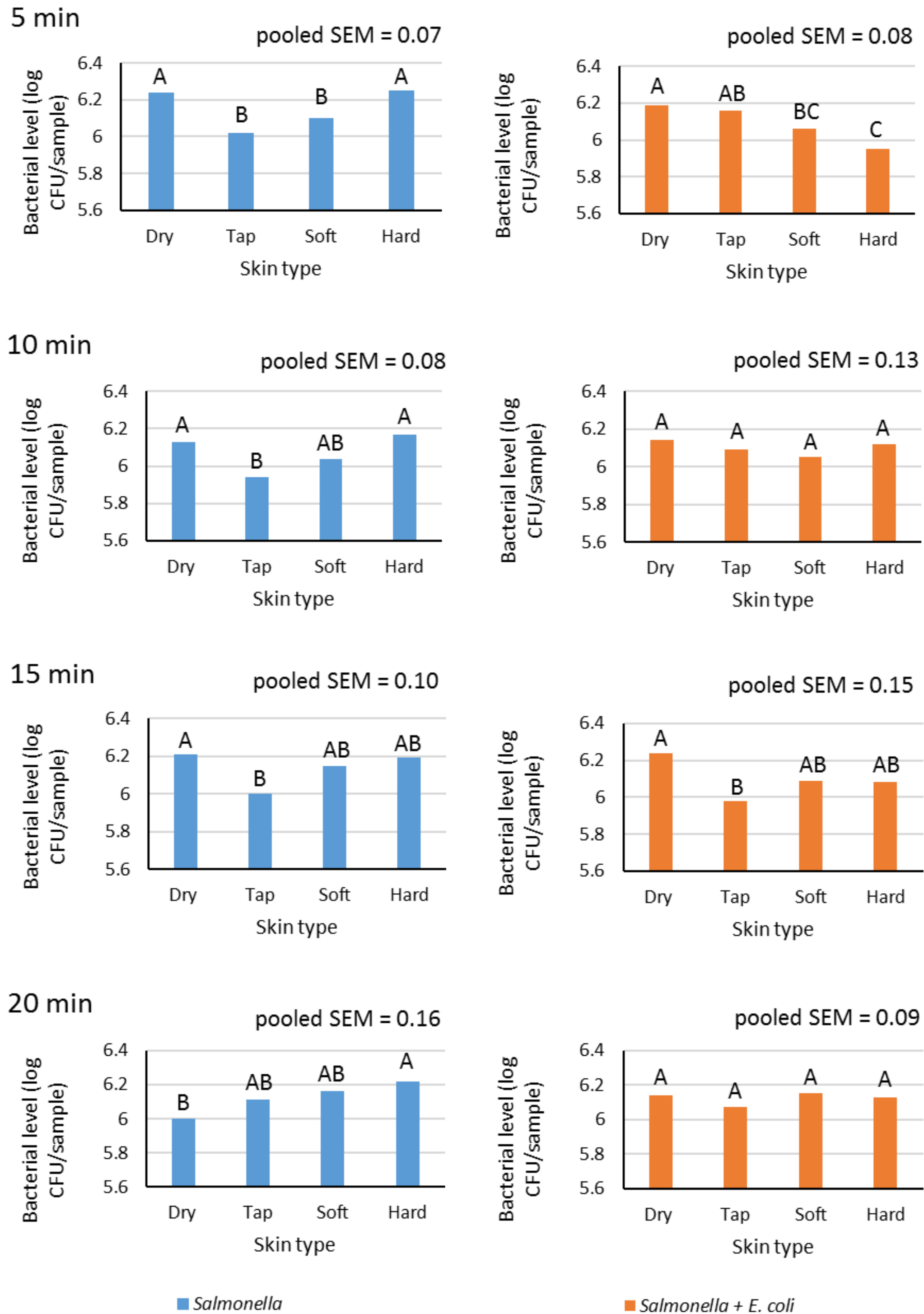


Figure 4. "Loosely attached" *Salmonella* recovery from various skin types by contact time. Dry = dry hand de-feathered chicken skin; Tap = tap water scalded and mechanically de-feathered chicken skin; Soft = soft scalded (51 °C) and mechanically de-feathered chicken skin; Hard = hard scalded (60 °C) and mechanically de-feathered chicken skin. Letters A through C mean without common letter different significantly ($p \leq 0.05$).

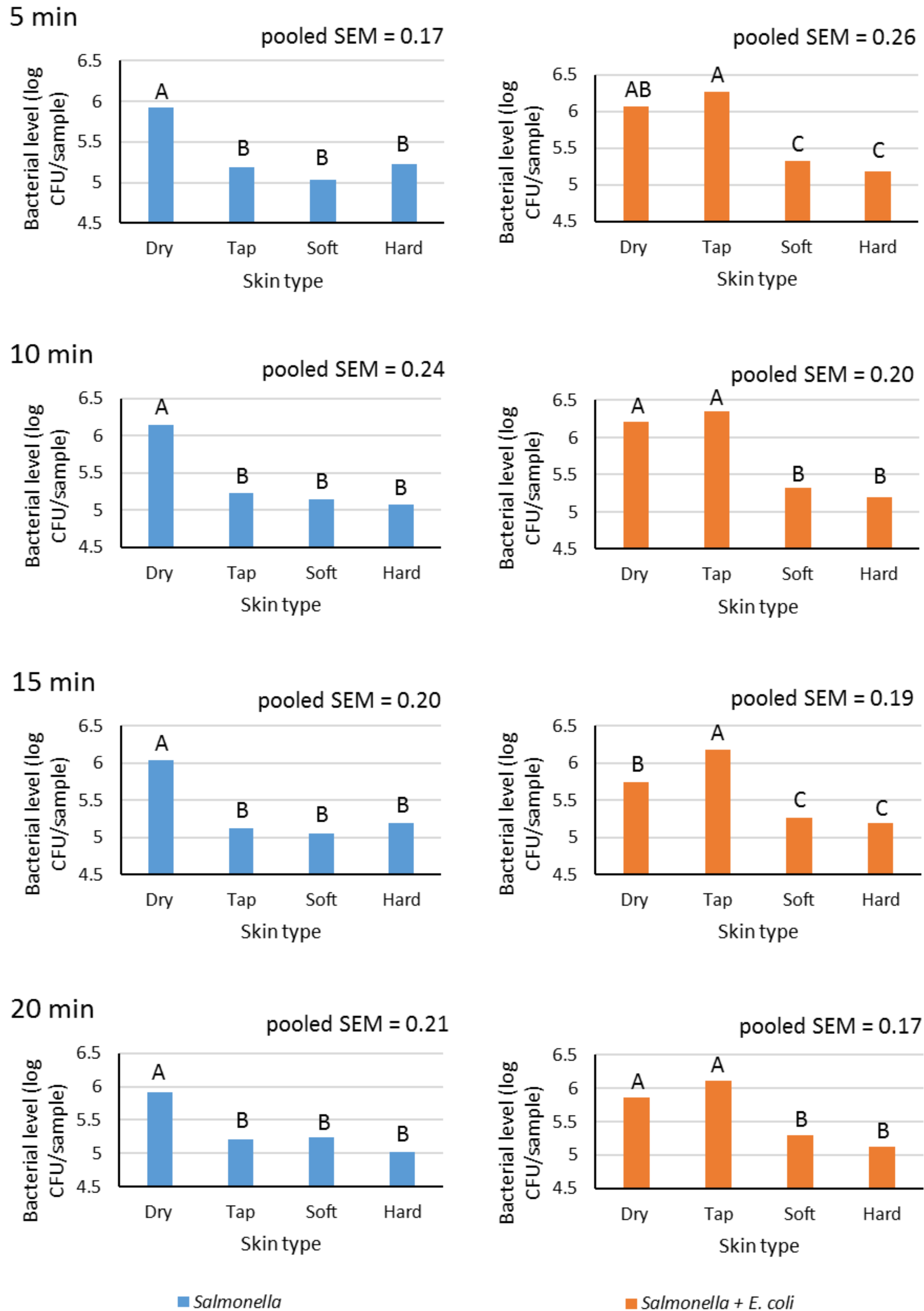


Figure 5. "Firmly attached" *Salmonella* recovery from various skin types by contact time. Dry = dry hand de-feathered chicken skin; Tap = tap water scalded and mechanically de-feathered chicken skin; Soft = soft scalded (51 °C) and mechanically de-feathered chicken skin; Hard = hard scalded (60 °C) and mechanically de-feathered chicken skin. Letters A through C mean without common letter different significantly ($p \leq 0.05$).

Table 4 Overall ANOVA with the probabilities indicating the main effects of antimicrobial treatment and skin type.

| Effect | Probability | |
|---|---|---|
| | Loosely attached cells | Firmly attached cells |
| Antimicrobial treatment | <0.0001 | <0.0001 |
| Skin type | 0.0020 | 0.0358 |
| Interaction | <0.0001 | 0.0147 |

N = 336

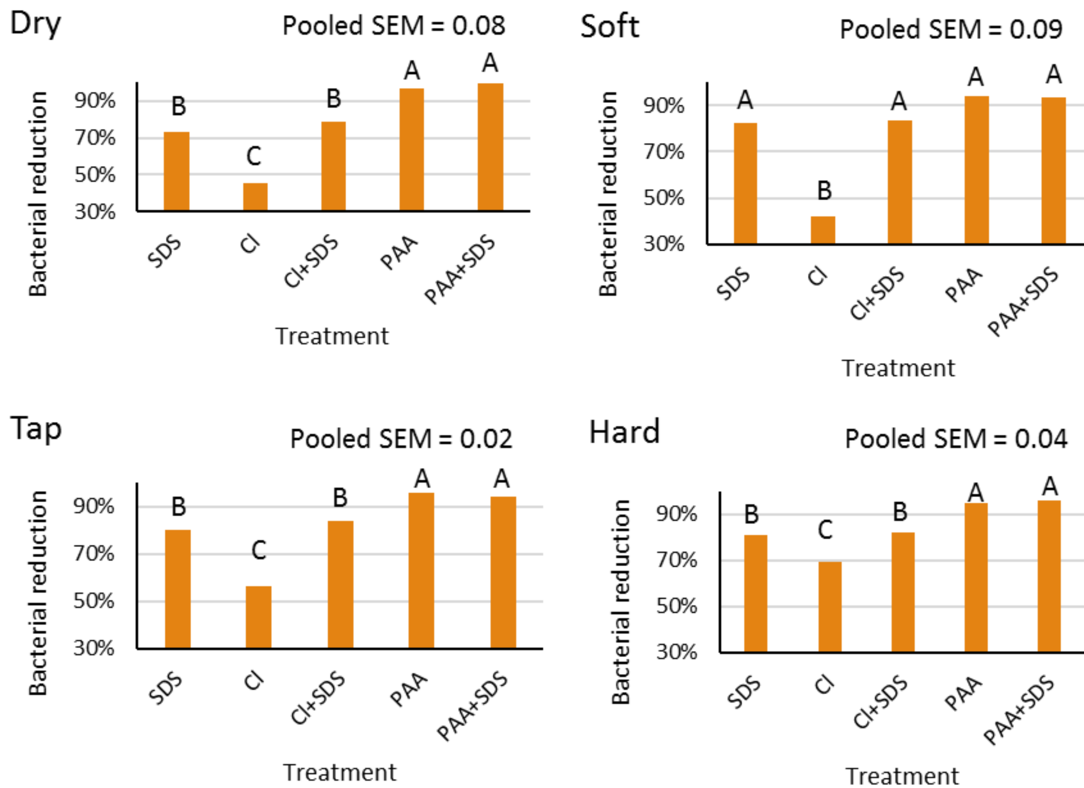


Figure 6. Reduction rate (%) of "loosely attached" *Salmonella* on various skin types treated with antimicrobials. Dry = dry hand de-feathered chicken skin; Tap = tap water scalded and mechanically de-feathered chicken skin; Soft = soft scalded (51 °C) and mechanically de-feathered chicken skin; Hard = hard scalded (60 °C) and mechanically de-feathered chicken skin. Letters A through C mean without common letter different significantly ($p \leq 0.05$).

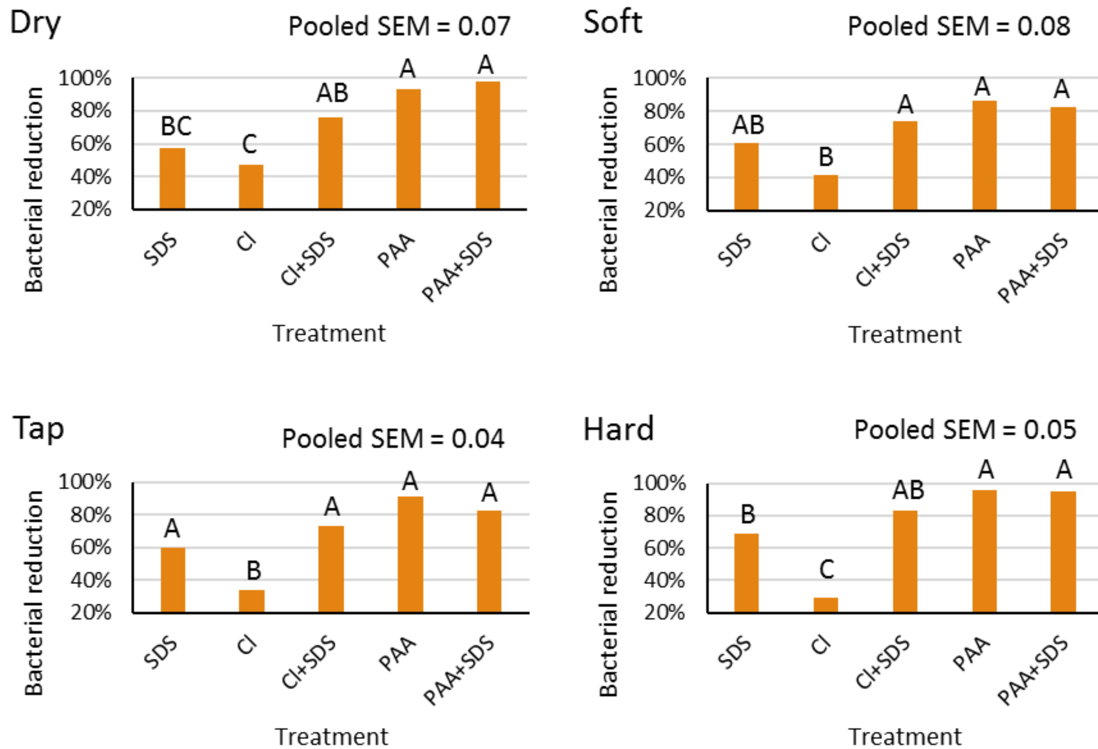


Figure 7. Reduction rate (%) of "firmly attached" *Salmonella* on various skin types treated with antimicrobials. Dry = dry hand de-feathered chicken skin; Tap = tap water scalded and mechanically de-feathered chicken skin; Soft = soft scalded (51 °C) and mechanically de-feathered chicken skin; Hard = hard scalded (60 °C) and mechanically de-feathered chicken skin. Letters A through C mean without common letter different significantly ($p \leq 0.05$). Pooled standard error (SEM) = 1.0991.

Table 5 Overall ANOVA with the probabilities indicating the main effects of waxing treatment and skin type.

| Effect | Probability | |
|---|---|---|
| | Loosely attached cells | Firmly attached cells |
| Waxing treatment | <0.0001 | <0.0001 |
| Skin type | <0.0001 | 0.5080 |
| Interaction | <0.0001 | 0.1000 |

N = 240

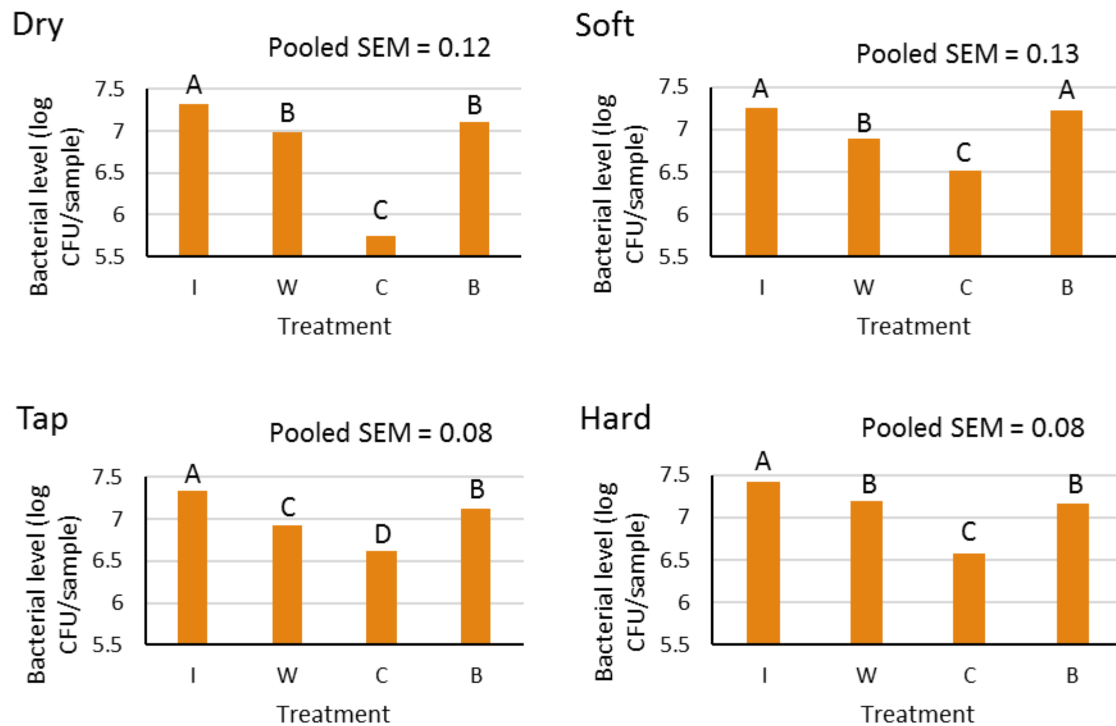


Figure 8. "Loosely attached" *Salmonella* recovery from wax coated skin types. Dry = dry hand de-feathered chicken skin; Tap = tap water scalded and mechanically de-feathered chicken skin; Soft = soft scalded (51 °C) and mechanically de-feathered chicken skin; Hard = hard scalded (60 °C) and mechanically de-feathered chicken skin; I = Inoculum; W= Water; C = Carnauba; B = Beeswax. Letters A through D mean without common letter different significantly ($p \leq 0.05$).

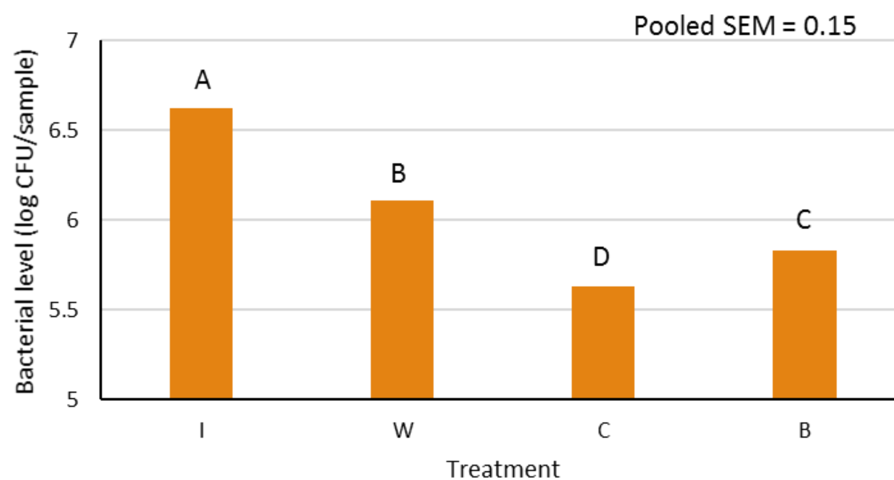


Figure 9. “Firmly attached” *Salmonella* recovery from wax coated skins. I = Inoculum; W= Water; C = Carnauba; B = Beeswax. Letters A through D mean without common letter different significantly ($p \leq 0.05$).