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Post-harvest reduction of Salmonella in pork trimming

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The objective of the current study was to determine the efficacy of 3% acetic acid in reducing *Salmonella* in pork trimming and the effects of such treatment on meat quality. For 15-s dipping and 5-log CFU/pork cube inoculation, only 0.2- to 0.3-log reduction was observed ($P \leq 0.026$). Acetic acid worked best at 75 s and 50°C, providing 1.4-log reduction ($P < 0.001$), damaging *Salmonella* cell membranes. When an inoculated pork cube was placed at the geometrical center of 2.3-kg pork trimming, dipping at 50°C for 75 s only reduced *Salmonella* by 0.2 log ($P = 0.040$). Although dipping slightly increased lightness ($P < 0.001$) and decreased redness ($P \leq 0.008$) on the meat surface, no inside color change was detected ($P = 0.120$). Neither lipid oxidation (TBARS, $P = 0.644$), protein solubility ($P = 0.187$), nor water-holding capacity ($P = 0.076$) were affected by treatments.

DEDICATION

This thesis is dedicated to my beloved family, teachers, and friends who supported me throughout my life. I would like to express my sincere gratitude and love to my father Mr. K. Sajeev and my mother Mrs. C. Deepa for spending a major part of their life nurturing me physically and mentally. I am grateful to my sister for always being there for me as a friend. A special dedication to my lovely fiancée Rajalakshmi for her sweet words and smile that always gave me a positive energy.

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CHAPTER I

INTRODUCTION

Salmonella is one of the most common foodborne bacteria that threaten the food safety system of the U.S., and salmonellosis is one of the leading causes of a large number of hospitalizations in the U.S. (CDC, 2019). In 2018, 40% of the total meat consumed in the world was pork, and the U.S. ranked 8th in the world per capita pork consumption, at 65.3 lbs./person (NPB, 2019), making pork the second most consumed meat worldwide. As a recognized source of human salmonellosis (Rostagno et al., 2012), *Salmonella* contamination in pork is a major food safety issue for the meat industry and has a negative impact on public health and consumer confidence. From 2016 to 2019, approximately 549,102 lbs. of pork and pork products were recalled due to *Salmonella* contamination (USDA/FSIS, 2019). Regardless of both pre- and post-harvest safety measures were employed in pork production sector, incidence due to *Salmonella* contamination has been increasing every year. *Salmonella* contamination unavoidably occurs at any stage of pork production because *Salmonella* colonizes in the intestinal tract of pigs. Pigs, as monogastric animals, are stressed easily either during transportation or in lairage and shed pathogenic bacteria that contaminate the animals' batch (Kumar et al., 2019). Current practices to control *Salmonella* infection in pre-harvest pork production are vaccination, antibiotic treatments, good management, and biosecurity procedures (Baer et al., 2013). In the post-harvest production, hot-water scalding, washing, and singeing are used (Loretz et al., 2011). Apart from these conventional methods, novel interventions such as ultraviolet lights, bacteriophages, and chemical agents. Ultraviolet light at a

wavelength of 250-260 nm is effective for decontaminating the surface of the meat (Guo, Huang, &Chen, 2017), but it can result in quality damage of meat due to lipid oxidation (Wambura & Verghese, 2011). Bacteriophages are approved by the FDA to use as a decontaminating agent in the food industry (Jahid & Ha, 2012); however, they do not have broad-spectrum effects (Wei et al., 2019). Chemical decontamination agents such as acetic acid, citric acid, and lactic acid (organic acids), hydrogen peroxide, acidified sodium chlorite, peroxyacetic acid, or trisodium phosphate are widely used for bacterial decontamination (Hamilton et al., 2010; Loretz et al., 2011). Antimicrobial interventions, especially acid spraying or washing, have been extensively studied for beef and poultry processing, whereas limited research has been conducted for pork processing (Britton, 2018). In the pork industry, the use of chemical antimicrobials is limited to organic acids (Loretz et al., 2011), which are economical, efficient, and generally recognized as safe by regulatory agencies (GRAS; FDA, 2017). Current pork safety practices place most emphases during slaughter (Hendricks et al., 2018). However, safety interventions for pork trimming are lacking. Therefore, following a literature review of various safety interventions to reduce *Salmonella* in pork, the current study was aimed to determine the efficacy of 3% acetic acid in reducing *Salmonella* in pork trimming and the effects of such treatment on meat quality.

CHAPTER II

LITERATURE REVIEW

Pork production and *Salmonella* contamination

Production and trade

Development in the pork market is very dynamic as a result of globalization of the economy and its associated outcomes (Szymanska, 2017). National Agricultural Statistical Service (NASS) reported that total commercial pork production in the U.S. was 27.7 billion lbs. in 2019, 1.3 billion lbs. greater than 2018, which was a new exporting record, at 26.9% of 2019 pork production with a total volume of 5.89 billion lbs. of pork and pork variety meats valued at $6.95 billion (NASS/USDA, 2019; NPB, 2020). Four countries accounted for 75% of U.S pork exports, including Mexico (31%), Japan (23%), China (12%), and Canada (10%). From 1990 to 2016, U.S. pork export jumped from 2 to 21% (USDA, 2019). Since 2000, the U.S. has been one of the top five pork-exporting countries, shipping more than 5 billion lbs. of fresh and frozen pork to foreign markets. However, there was a decline in pork import to the U.S., which was less than 10% of global pork imports (USDA, 2020) because of an increase in the U.S hog production. In 2019, U.S. hog inventory increased by 4% compared to that of 2018 (NASS/USDA, 2019). In 2019, Smithfield led the pork-packing industry with a total capacity of 130,300 heads/day, followed by JBS with 93,000 heads/day.

Pork production in the U.S. is concentrated heavily in the Midwest-Iowa and Southern Minnesota (ERS/USDA, 2019). Low production cost, safety and quality of production, efficient production, and authenticity of supply (Young, 2005) make the U.S. one of the largest pork exporters, with approximately 20% of commercial pork production every year. During 2014, the world's pork production was 110.5 million tons, and it was 1.5% higher than in 2013 (USDA, 2016). From 2005 to 2015, there was an increase of 14.6% in pork production on a global scale, with more than 110.3 million tons in HCW, which accounted for 42.8% of the total volume of meat production. Pork production in the U.S. is vertically integrated, a production system following a rapid shift in pork production since 1990, when the number of pig farms declined by 70%, and individual enterprises for pork production grew by more than 30% due to increased specialization in a single phase of production. Earlier hogs were produced on farrow-to-finish operations that managed the production from breeding to the sale for slaughter. Today, most hog operations specialize only in one of the three major life-cycle phases of production, either breeding to weaning stage, weaning to a feeder pig, or feeder pig to slaughter stage. In 1992, 65% of market hogs came from farrow-to-finish farms. By 2009, hogs from farrow-to-finish were reduced to 20%, and more than 70% of market hogs were produced by specialized feeder-to-finish farms (ERS/USDA, 2014). This shift in production was also associated with improved technologies and economic relationships among producers, packers, and consumers. The specialized single phase of production operates under contractual agreements, by which hog owners pay fees to producers to grow the animals through different stages (Maples et al., 2019). This contractual basis consisted of 5% of hog production in 1992 but shifted to more than 70% in 2009 (Key, 2014). The development of technologies in genetics, nutrition, handling practices, and safety contributed remarkably to the development of the pork production industry. Lusk

(2013) reported that 71% of the enhanced pork productivity is due to the contribution of technological advancement. Due to the increasing demand for pork in the international market, pork production is still a growing industry (Vermeulen et al., 2015). Therefore, controlling pathogenic bacteria in pork, especially *Salmonella* contamination, is an important component of the pork production chain.

Salmonella **and salmonellosis in meat**

The genus *salmonella* has two species, *Salmonella enterica,* and *Salmonella bongori*. *Salmonella enterica* consists of six sub-species. More than 2,500 serotypes of *Salmonella* exist, classified by types of antigen, either O or H antigen. The majority of *Salmonella* harmful to humans comes under the species *S. enterica.* Prevalent serotypes of *Salmonella* that cause foodborne outbreaks are *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Salmonella* Heidelberg, and *Salmonella* Newport (CDC, 2011). *Salmonella* is a facultative gram-negative rod-shaped anaerobic bacterium of the *Enterobacteriaceae* family. It colonizes in the intestinal tract of animals, mainly livestock, wildlife, and humans. *Salmonella* is a chemoorganotrophic organism, metabolizing nutrients through oxidative and chemical pathways (Eng et al., 2015). Consumption of *Salmonella*-contaminated food is the primary source of infection in humans (Hugas et al., 2014). Therefore, common sources of *Salmonella* contamination are from foods of animal origin, such as meat, milk, egg, or fish (USDA/FSIS, 2011). According to Gonzalez et al. (2015), 10 to 20% of foodborne illnesses are caused by *Salmonella* associated with pork and pork products. According to the Center for Disease Control and Prevention (CDC), after consuming contaminated foods, symptoms of salmonellosis start to appear from 12 to 72 h. They can last for 4 to 7 days, depending on the severity of the infection (CDC, 2017). Common symptoms are diarrhea, abdominal cramps, and fever. Momin et al. (2020) and Antunes et al. (2016) reported

that for a healthy person, the infectious level is typically ≥ 5 log *Salmonella* cells, and for susceptible individuals, even a few cells can cause infection. Therefore, it is important to understand how bacterial contamination spread through the pork production chain and the technologies available to reduce such a risk.

Salmonella contamination during pork production

Salmonella contamination in pork occurs at both pre-harvest (animal production) and post-harvest (animal processing) stages. The muscle tissues of animals are sterile. Dickson & Acuff (2017) reported that microbiological contamination of the sterile muscle tissue occurs when they are converted from live animal to meat. *Salmonella* spp. and *Campylobacter* spp. are the bacteria commonly found in pig intestinal tract. In the finishing barns, pigs can be infected with bacteria through infected feed, contact with an infected pig, contaminated environment, contaminated surface, equipment, or person (Fois et al., 2017), which are the main routes of *Salmonell*a transmission in pigs. Clinical symptoms of salmonellosis exhibit when pigs are exposed to a high dose of *Salmonella*. According to Fedorka-cray et al. (1994), pigs infected with 4 log of *Salmonella* are still a carrier, and they do not exhibit any clinical symptoms. However, Gray et al. (1995) reported that pigs infected with 8 log *Salmonella* showed clinical signs such as diarrhea, high temperature, and loss of appetite due to salmonellosis. A potential source of pre-harvest contamination is contaminated feed and water. Other sources are rodents, birds, and humans. *Salmonella* can survive for a long period of time under an unfavorable environment. Therefore, it is difficult to eliminate *Salmonella* by cleaning the finishing barns (Zamora-Sanabria & Alvarado, 2017).

Transportation of live pigs from finishing barns to the packing plants is a primary step involved in the process of slaughter. Loading and unloading of pigs during transportation are stressful to pigs (Goumon & Faucitano, 2017). After arriving at the packing plants, the pigs are moved into resting pens. The use of these pens in a slaughterhouse is to provide a continuous supply of pigs to the slaughter line and to serve as a resting place for the pigs after transportation (Brandt & Aaslyng, 2014). Transportation of the pigs is a primary source of bacterial contamination because it creates various stressors such as feed withdrawal, high stocking density, changes in the environment such as temperature and noises. Pigs, as monogastric animals, are stressed easily, leading to bacterial shedding, especially *Salmonella,* in carrier pigs (Andres & Davies, 2015). In the resting place of packing plants, most pigs will be potential carriers of *Salmonella* with the intestinal tract and lymph nodes being the most common locations where *Salmonella* colonizes and proliferates. Stress during transportation and in resting area of carrier pigs increases *Salmonella* shedding rate, which further cross-contaminates other animals, kill floor, and equipment during slaughter (Snary et al., 2016). The length of the time in lairage also contributes to the level of *Salmonella* infection in pigs (Bonardi, 2017). The more time pigs spend in lairage, the higher the risk of *Salmonella* infection because of higher fecal contamination from the previous or within the same group of animals (González Santamarina, 2019).

Carcass contamination is correlated with skin contamination of live pigs before stunning, and the skin contamination is affected by lairage duration and hygienic conditions (Bonardi et al., 2016). The primary source of bacterial contamination during slaughter is animals. Slaughter of infected pigs and slaughter of a large number of pigs in the same line with the same equipment and

surfaces contribute to direct or indirect bacterial contamination on the carcass surface (Li et al., 2016). Badvela et al. (2016) reported that 70% of all carcass contamination resulted from the animals themselves being carriers, with the rest being cross-contamination from other positive carcasses. In pork processing, from stunning to fabrication, the probability of *Salmonella* contamination is high because approximately 50% of the pig carcasses after exsanguination are positive for *Salmonella* (Casanova-Higes et al., 2017). These authors found that positive pigs shed *Salmonella* approx. 30 times higher than negative pigs during the slaughter. Therefore, processing steps along the slaughter line are critical to control bacterial contamination on the carcass (Sohaib et al., 2016).

On the day of slaughter, pigs are moved into the stunning chamber manually or by automatic push gates. Stunning happens before sticking to render the animal insensitive to pain until the animal is dead (Velarde & Dalmau, 2018). The most common stunning methods used in pigs are electrical stunning and gas stunning. In electrical stunning, it is required to restrain the animal first, and the electrical probes are placed on the head behind the ears with a voltage of 110 V and current of 1.25 amp. In gas stunning, the animals are not restrained, and the gas used for stunning is CO_2. The unconscious animal is further exsanguinated, and this process must be performed efficiently to prevent the pigs from regaining consciousness and experiencing pain (Brandt & Aaslyng, 2014). During stunning, potential contamination source is limited to bacteria that are on the animal. After exsanguination, scalding is performed in a water tank at 60 - 62°C for 3 to 5 min. The primary purpose of scalding is to reduce bacterial counts and assist in the dehairing. Scalding of pigs destroys most bacteria on the skin (Swart et al., 2016). Bolton et al. (2002) reported a reduction of 1.5 log CFU/cm^2 of bacterial count after the scalding. However, Hill et al.

(2016) reported that after the scalding process, the rectum of the pigs would be loosened and scratching with a dehairing device, which potentially introduces bacteria to the skin surface because of fecal material exiting the rectum of the pig. This probability of cross-contamination is high if a large number of pigs in the same batch are processed. Dehairing is performed after scalding to remove hairs from the animal body. Scalding reduces the overall bacterial population on the surface of the carcass due to high temperature, whereas dehairing is a major source of bacterial contamination. Many reports supported that the bacterial contamination of the dehairing machine jeopardized pork safety. Rivas et al. (2000) found a bacterial count of 4.4 to 6.2 log CFU/cm^2 in the dehairing machine three hours after slaughter. Regardless, scalding and dehairing reduce *Salmonella* prevalence from 91.2% to 19.1% (Schmidt et al., 2012).

After dehairing, the internal organs are removed from the animal through the evisceration, a process reportedly being a significant source of bacterial contamination on pork carcass (Belluco et al., 2015). Davies et al. (1999) found an increase of 4 to 32 % in *Salmonella* count on pork carcass after evisceration. During the head drop, the mandibular lymph node present in the head can be a potential source of contamination because *Salmonella* colonizes the lymph nodes of the animal and can spread to the knife, resulting in cross-contamination during slaughter (Bonardi et al., 2016). The evisceration of carcass is a manual operation that has the highest potential for *Salmonella* contamination if not practiced properly. Damage to internal organs while eviscerating a carcass can spread bacteria to the carcass surface and the equipment. Approximately 55 and 90% of carcass contamination occurs during evisceration because there are limited methods to control contamination at this stage, which mostly depends on the skill and efficiency of employees (De Busser et al., 2013). Hygiene and skill of the workers are also a

potential factor of bacterial contamination (Bakhtiary et al., 2016). Trimming and final washing of the carcass have a great impact on the bacterial population (Belluco et al.,2015). This step helps reduce the bacterial population to an almost undetectable level. It was reported that aseptic trimming of the carcass reduces total bacterial count by approximately 3 log on the beef carcass (Dickson and Acuff, 2017). Singeing of the carcass is performed after evisceration to remove the remaining bacteria and hair from the carcass surface. Corbellini et al. (2016) reported a reduction of 2.5 log in *Salmonella* count on pig carcass after singeing. The carcass undergoing singeing is then washed with warm or cold water to remove the bone dust and blood clots. Pig slaughter is finalized with chilling of the carcasses to remove heat from the hot carcass surface as quickly as possible (Huff-Lonergan & Page, 2001). Chilling also helps prevent the proliferation of bacteria on the surface of the hot carcass (Tomovic et al., 2011). During chilling, reducing the temperature of the carcass surface helps minimize bacterial growth and further contamination. However, if the chilling conditions permit the carcasses to remain moist and warm for an extended period, psychotropic bacteria will grow and contaminate the carcasses (Reid et al., 2017). During chilling, carcass temperature, water activity (a_w), carcass surface pH, are the main factors limiting microbial growth (Reid et al., 2017). In spray chilling, the chance of contamination is higher than that of blast chilling because of the moisture present on the carcass surface.

During fabrication, the potential sources of *Salmonella* cross-contamination are equipment, knives, cutting boards, or inedible tissues (Martínez-Chávez et al., 2015). Several studies discussed the sources associated with *Salmonella* in meat and meat products in the U.S. *Salmonella* contamination occurs not only through infected animals and humans, feed, and

processing at packing plants but also at retail establishments, and during distribution or preparation at home (Andres & Davies, 2015; Gonzalez et al., 2015). Although the majority of *Salmonella* contamination occurs from pre- and post-harvest practices as mentioned previously, contamination does happen at other points of the pork merchandising. Pork chops have 1 to 3.3% and ground pork, having approximately 16% *Salmonella* prevalence (Foley et al., 2008). Ground meat has the highest probability of *Salmonella* contamination (FSIS, 2017) because the trimming is sourced from various carcasses and suppliers. *Salmonella* surviving safety interventions can cross-contaminate ground meat during mixing and grinding (Møller et al., 2016). The meat industry has used decontamination technologies to reduce *Salmonella* in pork, during slaughter and fabrication to minimize *Salmonella* risk in minimally and further processed meats (Arguello et al., 2013).

Outbreaks and recalls

Salmonella is one of the most prevalent foodborne pathogens worldwide, causing infections in animals and humans that can be fatal (Zhu et al., 2019). Globally, *Salmonella* is responsible for approximately 153 million cases of gastroenteritis every year, that leads to hospitalization and death. In the U.S., 30% of food infections are salmonellosis, making *Salmonella* the second leading source of foodborne illness in the U.S. (Brunette, 2017). Approximately 75% of *Salmonella* infections in humans are caused by the consumption of contaminated meat and meat products (Momin et al., 2020), with pork being one of the main sources of human *Salmonella* infection (Sanchez-Maldonado et al., 2017). FSIS listed numerous recalls of pork and pork products due to *Salmonella* contamination in the last few years. In 2019, 6,444 lbs. of ready-to-eat (RTE) pork sausage patties were recalled (USDA/FSIS 2019). In 2017, 1,076 lbs. of salami were recalled. In 2016, approximately 550,287 lbs. of pork products were recalled due to

Salmonella contamination (USDA/FSIS 2016/ USDA/FSIS 2017). In 2015, 523,380 lbs. of whole roaster hogs and assorted pork were recalled due to *Salmonella* contamination (USDA/FSIS 2015). In Canada, contamination of pork due to *Salmonella* is a major public concern. Canadian Food Inspection Agency (CFIA) reported three pork recalls during 2019 and 18 pork recalls during 2016 due to *Salmonella* contamination. Raw Pork Products Exploratory Sampling Program Phase I from July 1, 2015, to June 30, 2017, reported a steady increase in *Salmonella* incidence rate in comminuted pork, from 21.24% in 2015 to more than 30.93% in 2017 (FSIS, 2017). From June 2017 through May 2018, Raw Pork Products Exploratory Sampling Program continued to Phase II. A total of 4145 raw pork samples (comminuted, non-intact, and intact cuts) were collected, and the prevalence of *Salmonella* was 26.74% for comminuted, 10.03% for intact, and 5.99% for non-intact pork (Scott et al., 2019).

Performance standards

Efforts to control *Salmonella* contamination in pork have focused on various product testing programs as well as regulatory policies on *Salmonella* as a contaminant in foods. In 1996, Food Safety and Inspection Service (FSIS) developed a *Salmonella* verification program as a part of the Pathogen Reduction under Hazard Analysis and Critical Control Points (PR/HACCP) systems final rule (FSIS, 1996). This PR/HACCP final rule established *Salmonella* performance standards used to verify and control *Salmonella* in meat and poultry processing facilities (FSIS/USDA, 2019). The performance standards come under the Code of Federal Regulations (CFR), within Title 9 - Chapter III - Subchapter A - Part 310.25 for meat and 381.94 for poultry (CFR/USDA, 2020). The FSIS selected *Salmonella* as the target organism because of the incidence of a large number of *Salmonella*-related foodborne infections every year and because *Salmonella* is present in all primary food animals. The purpose of the implementation of

PR/HACCP performance standards was to reduce *Salmonella* contamination of raw products and allow the FSIS to verify whether processing and slaughtering facilities have effective strategies to address *Salmonella* hazard. Based on the performance standards, no more than 8.7% of samples from swine carcasses at pork processing facilities are to be positive for *Salmonella*. By 2011, the data showed that swine slaughter establishments had achieved *Salmonella* positive incidence below the performance standard by 2.6% in 2008, 2.3% in 2009, 2.4% in 2010, and 3.3% in 2011 (Self et al., 2017). The FSIS suspended sampling to redirect resources and has not generally considered *Salmonella* to be a serious contaminant in raw pork because *Salmonella* incidence has been more prevalent in the poultry products with outbreaks than pork products. However, in response to the modernization of pathogen control and the lack of progress in controlling *Salmonella* in pork, in 2013, the FSIS developed a *Salmonella* action plan aimed to determine the level of *Salmonella* contamination in FSIS-regulated pork products and devise effective measures to reduce such contamination. From 2015 to 2017, the FSIS launched a Raw Pork Products Exploratory Sampling Project phase I and detected *Salmonella* in 16.7% of pork product samples, including ground pork. The agency continued the sampling program in 2017 into phase II and is planning to use the results to develop new *Salmonella* performance standards for pork. In 2016, the FSIS revised the performance standards to include *Campylobacter* along with *Salmonella* in all beef and pork slaughtering and processing facilities along with new performance standards for *Salmonella* and *Campylobacter* in not ready-to-eat (NRTE) comminuted chicken and turkey products. *Salmonella* and *Campylobacter* performance standards are implemented not for an individual product but for the establishment's overall process control. Products are not tested for their quality and safety, but the sampling is to evaluate the effectiveness of the slaughter and grinding processes to achieve minimal contamination. The

FSIS can request a re-assessment of the HACCP plan if an establishment does not meet the performance standards (8.7%). If still not meeting the standards, such an establishment will be suspended until they submit written assurances detailing the corrective actions in their HACCP system and other appropriate measures to reduce the prevalence of *Salmonella* (CFR/USDA, 2020).

Decontamination technologies to reduce *Salmonella* in pork

Pre-harvest interventions

Decontamination technologies are applied in both pre- and post-harvest stages of pork production to reduce *Salmonella* contamination. In pre-harvest pork production, common practices are management, biosecurity procedures, vaccination, and antibiotic treatments (Baer et al., 2013). Maes et al. (2001) studied the efficacy of vaccination in pigs and found a reduction of 6.6% of *Salmonella* that colonized in the lymph nodes of 3- to 16-week old pigs. Moreover, dietary feeding of probiotics or prebiotics, along with or without vaccination, showed better results in the reduction of *Salmonella* in pigs. However, probiotics or prebiotics with proper vaccination is effective than without vaccination (Kreuzer et al., 2012). Recently, bacteriophage therapy has been introduced in the swine industry to decrease *Salmonella.* This technology was previously established in the beef industry to reduce *E-coli* (Carter et al., 2012). The efficacy of feeding encapsulated phages to pigs was 2- to 3-log reduction in *Salmonella* Typhimurium. However, the effectiveness of phage therapy lasts only for a short period of approx. 6 to 12 h (Wall et al., 2010). In lairage, reducing the holding time and maintaining the hygiene of the holding environment has been considered an effective method to reduce *Salmonella* infection. Typical holding time for pigs is 2 to 3 h to decrease transportation stress (De Busser et al., 2013).

Preventing *Salmonella* contamination during slaughter

In post-harvest pork production, abattoir hygiene and decontamination of carcasses are the key actions to control *Salmonella* contamination (Van Hoek et al., 2012). Biasino et al. (2018) reported 4 log CFU/cm^2 of total aerobic bacteria and 2 log CFU/cm^2 of *Salmonella* on pig carcass after slaughter and a positive correlation between hygiene indicators (total aerobic bacteria and *Enterobacteriaceae*) and *Salmonella* count, which showed that proper decontamination of facilities must be implemented to control pathogenic bacteria. There are many critical control points along the slaughter line to control *Salmonella* contamination, such as during scalding, removal of internal organs, removal of pluck set, singeing, and during the meat inspection process (Arguello et al., 2013). Pigs entering the slaughter area have a high prevalence of food pathogens, especially *Salmonella,* in their skin and stomach. It was reported that the occurrence of *Salmonella* in the skin of pigs is as high as 28 to 40% (Blagojevic et al., 2011), and in some cases, 60% pig carcasses are *Salmonella* positive (Biasino et al., 2018). Compared to other ruminants, pigs are not skinned after slaughtering; therefore, the probability of contamination is higher for pigs. Scalding, dehairing, and singeing were usually performed to minimize this type of contamination, which makes skin as an edible part. It was reported that the scalding reduced approximately 2 log of bacterial count from pigskin. (Buncic & Sofos, 2012). However, irregular changing of scalding water and dirtiness of the pigs are the two risk factors (Blagojevic et al., 2011). It is better to use mechanized bung cutter along with plastic bags to close the rectum to eliminate leakage of feces into the carcass after scalding (Houf, 2012). The careful evisceration of slaughtered pigs can also minimize pathogenic contamination on carcasses. The risk of *Salmonella* contamination is still high because of potential spillage of the intestinal contents onto carcasses either from natural openings or when a puncture occurs (De Busser et al., 2013). Van

Ba et al. (2019) observed 1-log CFU/cm^2 reduction in *Salmonella* count after proper evisceration of a pig carcass. There is also a high probability of cross-contamination by knives and hands during the evisceration process. (Buncic et al.,2012) as well as during the splitting of carcass using electric saw. The prevalence of *Salmonella* cross-contamination during carcass breaking range from 0% to 31.1% (Wong & Hald, 2000). For hair removal, pork carcasses are singed, a process using open-flame gas burners, which will burn the remaining hairs. Proper singeing is performed at 1300-1500°C, which reduces of 1.5 to 3 log of the total bacterial count. (Bolton et al., 2002). The re-contamination of pork carcasses occurs during carcass polishing after singeing (James, 2007) because pathogens surviving singeing will be disseminated into polishing tools (Delhalle, 2008). However, Zwirzitz et al. (2019) reported that total bacterial count was reduced from 5 log during bleeding to 3 log after singeing and polishing pig carcasses. The chilling of pork carcass is another method of reducing the further growth of *Salmonella*. Chilling of the carcass at a temperature less than 7°C within 24 h can reduce the entry of pathogens on the carcass, even though the reduction in the total viable count is less (Savell et al., 2004). Temperature monitoring, sanitation of the surface, and carcass spacing should be appropriately monitored to avoid cross-contamination during chilling (Buncic et al.,2012). Carcass chilling or meat freezing prolongs the lag phase of the bacteria and reduces their proliferation on the carcass surfaces. Jay (2000) reported that chilling could cause permanent physical damage to bacteria due to chemical changes happened in their lipid bilayer because microbial activities are based on chemical and enzymatic reactions, which are limited at low temperatures. *Salmonella* contamination can also occur through contact with workers, knives, band saw, conveyor belt, surfaces, and among carcasses or tissues (Yang et al., 2017). Youssef et al. (2013) found a 1.4 log total bacterial count on knives and 1.6 log on the conveyor belt after the fabrication.

Therefore, in addition to best practices during slaughter and fabrication, decontamination technologies must be employed to further reduce the risk of *Salmonella*.

Decontamination technologies

Current decontamination methods to control pathogenic microorganisms include the use of antimicrobial agents, steam ultrasound, hot water wash, steam vacuuming, ultraviolet (UV) light, and carcass chilling (Loretz et al., 2011). Comparing different decontamination methods, washing with hot water is the second most cost-effective for *Salmonella* decontamination. The use of cold water only produces cosmetic effects and cannot be considered as an effective decontamination method for *Salmonella* contamination (Bolton et al., 2002). Other than hot water wash, the use of steam ultrasound has been practiced and is more cost-effective than hot water; however, it has lower efficacy in *Salmonella* reduction than hot water. In steam ultrasound, steam at 130ºC was directed to the carcass surface to kill bacteria. Lawson et al. (2009) found a reduction of 1 log CFU/cm^2 *Salmonella* using hot water at 80ºC and 0.8 log CFU/cm^2 using the steam method and suggested that steam ultrasound was cheaper than hot water. Ultraviolet light with a wavelength from 100 to 400 nm classified into three categories, UV-A (315-400 nm), UV-B (280-315 nm), and UV-C (200-280 nm) range (Lazaro et al., 2014) is another decontamination technology for carcasses. The UV-C at a wavelength of 250 to 260 nm is effective for decontaminating the surface of fresh products (Guo, Huang, &Chen, 2017). The photochemical effect of the UV will directly destroy the DNA helix and interferes with cell replication (Yeh et al., 2017). Similar to the UV light technique, several studies have been conducted on pulsed light method, a recent technique to decontaminate meat and contaminated surfaces in the meat processing facilities. In the pulsed light technique, full-spectrum lights from inert gases (200 to 1100 nm) are applied on the meat surface with high intensity for a short time

to destroy bacterial DNA (Barba et al., 2018). Yeh et al. (2018) found a reduction of 1.2 log of *Salmonella* using UV lights on ground beef. Koch et al. (2019) used pulsed light to obtain 1.7- and 3.6- log reduction in *Salmonella* on the pork loin and pork skin, respectively. The disadvantage of both UV and pulsed lights is that they cause damages to meat color due to oxidation of myoglobin and lipids (Mahendran et al., 2019). Phage therapy or bacteriophages are considered as therapeutic agents in the medical industry for a long time, and they were approved by the U.S. Food and Drug Administration (FDA) in 2006 as a decontaminating agent in the food industry (Mahony et al., 2011). Phages infect specific bacteria without causing harm to the microflora. Cocktails from phages are very effective in reducing various food pathogenic bacteria (Radford et al., 2017). Currently, many bacteriophage solutions are commercially available to be used in the food industry, such as ListShield^{TM}, the first commercial bacteriophage for ready-to-eat (RTE) meat products (USDA/FSIS, 2014). Commercial phages against *Salmonella* are SalmoFresh^{TM} and Salmonelex ^{TM}. Bacteriophages are classified into lytic (virulent) and lysogenic (temperate) phages (Enderson et al., 2014). The lytic bacteriophages have an antimicrobial property, and it is widely used in the food industry because they destroy the host bacteria without affecting the host genome, results in the rapid death of the bacteria with minimal development of resistance (FDA, 2006). Lysogenic bacteriophages, however, induce bacterial death under environmental stress by incorporating their DNA within the bacterial genome for replication, which can potentially result in antibacterial resistance (Grant et al., 2016). Hooton et al. (2011) found that *Salmonella*-specific bacteriophage application in pig skin produced a reduction of 1.2 log CFU/g in *Salmonella*. Similarly, Duc et al. (2018) reported a reduction of 1.8 log *Salmonella* on chicken meat using phage therapy. The disadvantage of phage

therapy is the limitation of their use in broad-spectrum protection against various bacterial pathogens and difficulty of phage delivery to the target site (Wei et al., 2019).

The critical control points (CCP) during pork slaughter are scalding, evisceration, singeing and chilling (FAO, 2004). The use of decontaminating agents is limited during hog slaughter, which may be the reason for an increase in bacterial load on pork and pork products. The application of antimicrobial agents on meat, such as beef and chicken, gives insights into potential applications in pork processing to reduce bacterial load on the final pork carcass (Buncic et al., 2012). Chemicals such as acetic acid, citric acid, and lactic acid (organic acids), hydrogen peroxide, acidified sodium chlorite, saponin, electrolyzed water, peroxy-acids, or trisodium phosphate in water are widely accepted as antimicrobial agents in the meat industry. In pig carcasses, the use of antimicrobial agents is limited to organic acids (Loretz et al., 2011). Usually, antimicrobial agents are applied during slaughter fabrication on carcass or meat surfaces as mist, fog, or small droplet rinse (Gutzmann et al., 2011). Organic acid treatments are economical, simple, quick, and more efficient than other antimicrobial agents (Hinton & Corry, 1999; Mir & Masoodi, 2018). The FDA designated organic acids as generally recognized as safe (GRAS) for meat (FDA, 2017). Organic acids are considered as weak acids. Only a few studies reported the decontamination property of organic acids on pork carcasses in commercial facilities. Spraying or dipping with lactic acid effectively reduced *Salmonella* load on carcasses and various meat cuts (Epling et al., 1993). Fu, Sebranek, and Murano (1994) reported a reduction of 0.7 to 1.7 log of *Salmonella* on pig carcasses after spraying them with 1.5% acetic or citric acid. Eggemberger-Solorzano et al. (2002) combined 1.8% acetic acid with hot water (82ºC) on hog carcasses and achieved a 2.3-log bacterial reduction. Christiansen et al. (2009) reported that spraying 2.5%

lactic acid at 80ºC for 15 s reduced *Salmonella* by 2.8 and 2.0 log CFU/cm^2 on pork skin and lean pork surfaces, respectively. Choi et al. (2009) used a combination of 3% lactic and 3% acetic acid on fresh pork and found a reduction of 2.0 to 2.6 log CFU/cm^2 on the total bacterial count. Other combinations, such as 5% lactic acid and 400 ppm peroxy-acetic acid, only reduced *Salmonella* by 0.6 log in beef trimming (Yeh et al., 2018). The authors suggested that such a low reduction could be explained by the ability of *Salmonella* cells to develop oxidative stress resistance during adverse conditions.

Pork trimming production is typically the final step in the fabrication, and it should be a critical control point to apply antibacterial intervention (Castelo et al., 2001); however, no antimicrobial is normally applied at this step. Due to extensive handling during fabrication and further processing of the carcasses, the probability of re-contamination of pork trimmings and pork cuts are inevitable. The effectiveness of antimicrobial interventions on the whole carcasses during slaughter is decreased in the fabricated trimming products. The potential source of contamination during pork fabrication is from worker hands and tools. Therefore, it is imperative to perform antimicrobial interventions to pork trim before grinding (Duffy et al., 2001). However, for trimming, harsh interventions such as heating the surface with steam cannot be applied because it can adversely impact the meat quality (Kang et al., 2001). Prendergast et al. (2008) studied the prevalence of *Salmonella* on pork cuts and found approx. 1.6 to 2.5 log CFU/g of *Salmonella* after fabrication on pork cuts. It has been reported that using a hurdle application reduced bacterial growth more efficiently. Kang et al. (2001) studied the efficacy of cold-water wash, followed by hot water wash, hot air, and 2% lactic acid spray in beef trimming and found greater efficacy than using individual treatments for the same duration. On the other hand, using 0.2%

buffered propionic acid, Badvela et al. (2016) reported a reduction of 1 log CFU/ml *Salmonella* Typhimurium in ground pork. Mohan and Pohlman (2016) used 0.2 g/L peroxyacetic acid or 30 g/L of other organic acids (pyruvic acid, fumaric acid, malic acid, and capric acid) on beef trimming for 15 s and found that organic acids (1.8-log reduction) had greater efficacy in than peroxyacetic acid (0.7-log reduction). The efficacy of organic acids depends on the time, concentration, and temperature (Snijders et al., 1985). Organic acids, such as acetic or citric acids, exhibit greater antibacterial activity at 35°C than at room temperature. Moreover, acidic treatment at an elevated temperature of 50 to 55°C has been reported to be even more efficient than at 35°C in reducing bacteria (Anderson et al., 1988). Therefore, understanding the modes of action of organic acids are important for improving pork safety while maintaining a clean-label approach to food safety.

Antimicrobial modes of action of organic acids

Organic acids affect microbial activity by two mechanisms, either cytoplasmic acidification associated with uncoupling of energy production or the accumulation of dissociated acid anion in the microbial cytoplasm at a toxic level (Taylor et al., 2012). Freese, Sheu, & Galliers, (1978) stated that the general mechanism of action of organic acids is the uncoupling of electron transport from oxidative respiration. Organic acids cause injury to the bacterial cells by altering the transmembrane proton gradient of the microbial cells, resulting in changing the nutrient transportation and energy generation. Low pH also damages cellular macromolecules and induces cellular damages (Wheeler et al., 2004). The undissociated form of organic acid diffuse through the microbial cell membrane when the pH of the cellular cytoplasm is higher than that of the surrounding environment. Active transportation is required to efflux protons (H^+) to maintain internal pH, which requires more energy from the bacterial cells. Moreover, acidic pH in the

internal cell causes damages in genetic materials, enzymes, and protein structures, which leads to cellular death (Mani-López, 2012). The evidence for this mode of action is that *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Streptococcus thermophiles* produce lactic acid in food spoilage (FDA, 2015) and limit the growth of other bacteria because the environmental pH is altered (Jones et al., 2008). However, *Salmonella* can adjust to unfavorable environments, especially with an acidic environment, under stressful conditions. *Salmonella* increases the expression of genes, including *rpo*S, *nlp*D, and *clp*P, that help it develop acidic tolerance behavior (Burin, Silve Jr, and Nero, 2014). These genes protect *Salmonella* from acidic stress conditions (Foster, 2001). Some resistance can also occur when organic acids are used inappropriately because the efficacy of organic acids depends on their pH, concentration, and dissociation of anions. If the treatments are not optimal or below lethal conditions, bacteria can develop resistance by adapting to such conditions (Foster &Hall, 1990). *Salmonella,* when being exposed to an acid pH, can additionally produce approximately 50 acid shock proteins (ASP) to repair the damaged cells (Bearson & Foster, 1997; Foster, 1995). For example, *Salmonella* Typhimurium, during extreme pH conditions, produces several amino acids and proteins such as RpoS, Fur, and PhoP to maintain homeostasis in unfavorable pH conditions (Bearson & Foster, 1997). Compared to other strains of *Salmonella*, *Salmonella* Typhimurium exhibits more efficient adaptation to an acidic environment (Álvarez-Ordóñez et al., 2009). This ability of *Salmonella* is a great concern to the meat industry because it may be responsible for the development of acid tolerance response of *Salmonella* in normally a lethal stress environment (Tosun & Gonul, 2003). Peroxyacetic acid or peracetic acid is a combination of acetic acid, hydrogen peroxide, and water. This organic acid causes oxidation of the outer membrane of the *Salmonella* cells through the release of reactive oxygen species (Liberti & Notarnicola, 1999),

leading to the destruction of chemiosmotic functions of the lipoproteins in the bacterial cell membrane. This mode of action alters the transport of essential compounds and leads to the damages of the cell wall (Baldry & Fraser, 1988). However, King et al. (2005) found that the concentration of peroxyacetic acid up to 600 ppm did not reduce *Salmonella* when sprayed on beef carcass. In contrast, Ellebracht et al. (2005) found a reduction of 1 log of *Salmonella* when beef trimming was submerged in peroxyacetic acid of 200 ppm or 500 ppm. Acetic acid is another weak organic acid that is frequently used in the beef industry to minimize the risk of *E. coli* (Theron & Lues, 2007). The mechanism of action of acetic acid is similar to other organic acids in which undissociated acid molecules easily enter the cell cytoplasm and dissociate into anions and protons, both of which exert an inhibitory effect on the metabolism of bacteria. Acetic acid inhibits DNA synthesis in the bacterial cells that further causes cellular depletion due to the release of the undissociated acid molecule (Tan et al., 2015). Recent scanning and transmission electron microscopic images obtained in our laboratories indicated severe damages to the cellular membrane of *Salmonella* treated with 3% acetic acid at 50°C for 45 to 75 s. These organic acids and other treatments to reduce *Salmonella*, however, may have negative impacts on the quality attributes of pork, especially the trimmings. Therefore, an evaluation of meat quality is important to determine the applicability of these antimicrobial agents in pork production.

Effects of decontamination technologies on pork quality

The quality of a meat product is related to its color, eating satisfaction, and perceived freshness (Sionek et al., 2016). Meat color, water holding capacity, oxidative status, and fat content decides pork quality (Rosenvold & Anderson, 2003). Decontamination technologies discussed above are effective in reducing bacteria in both pre- and post-harvest operations; however, the impact of decontamination technologies on pork quality attributes needs to be evaluated. Quality

of meat not only depends on decontamination technologies but also on other factors such as feeding, stress during transport, handling, etc. (Dvorak et al., 2020). Both pH and temperature also have a significant role in meat quality development (Gardner et al., 2006). Low pH can lead to paler color, softer meat, and exudation of moisture. High temperature can lead to protein denaturation and loss of water-holding capacity (Deng et al., 2002). Unfortunately, low pH and warm temperatures are effective antimicrobial conditions. Scalding is a primary decontamination stage to reduce bacteria from pork carcasses. High-temperature scalding can drastically affect the quality of pork because of protein denaturation (Kauffman et al., 1969). However, Van der Wal et al. (1993) did not find any negative effects of scalding at 60ºC for 5.5 to 7.5 min on pork quality. These authors attributed such findings to the minimal thermal conductivity of subcutaneous fat on the pork carcasses. However, it is suggested to reduce the time of carcass in the scalding tank and monitor the temperature of scalding water for an effective scalding operation (Gardner et al., 2006). Monin et al. (1995) reported that the singeing of carcass also did not affect pork quality negatively. Carcass chilling is important to improve pork quality, in addition to improving pork safety. Chilling of carcasses as early as possible after slaughter reduces carcass temperature, which slows down the metabolic processes and reduces the rate of pH decline. Moreover, chilling improves the color and tenderness of meat (Huff-Lonergan et al., 2001). On the other hand, numerous studies suggested the use of ultraviolet light or pulsed light, although effective as antimicrobial agents for carcass decontamination, damages the meat quality by increasing oxidation of lipid and myoglobin and negatively affecting the oxidative stability, color, and structural characteristics of meat (Wambura & Verghese, 2011). The application of organic acid is effective in reducing the microbial population; however, the concentration, duration, and temperature are important factors to be considered while using organic acids to

protect the meat quality (Mohan et al., 2012). Organic acids are weak acids having low pH (Theron & Lues, 2007). Low pH can negatively affect meat quality, such as causing the hydrolysis of triglycerides and increasing lipid oxidation by liberating fatty acids from triglycerides through lipolysis, leading to hydrolytic rancidity of meat (Jamilah et al., 2008). In addition, a decline in pH less than the isoelectric point of meat (5.4) can cause a decrease in the repulsion of myofilaments that decreases the myofilament lattice, causing denaturation of proteins (Lin & Chuang, 2001). Denatured proteins are less soluble and hold less water that leads to a decrease in the water holding capacity (WHC) of meat (Li et al., 2018). Low pH affects meat color by inducing the denaturation of MMb, making it insensitive to the metmyoglobin reductases (Zhu & Brewer, 2002). Metmyoglobin reductases are essential for converting MMb to DMb for subsequent oxygenation of DMb to OMb, which provides bright red color to the meat. More MMb causes browning of the meat, which is unacceptable to consumers. Castelo et al. (2000) used a combination of hot water at 65ºC with 2% lactic acid for 120 s and found this method to be effective in decontaminating meat. However, for long exposure times, it negatively affected the color and emulsion stability of the ground pork. In contrast, Fu, Sebranek & Murano, (1994) sprayed 1.5% acetic, citric, or lactic acid on pork carcass. The authors found no difference in pH, lipid oxidation, color, and sensory attributes compared with the control samples after treatment. Similarly, Anthappan et al. (2001) observed no change in color or organoleptic characters in pork carcass when a combination of 3% lactic acid and propionic acid was sprayed. Shrestha & Min (2006) applied 4 or 6% lactic acid on fresh pork and found a significant decline in pH (0.4 unit less) and more lipid oxidation (0.6 mg MDA/kg) of the treated pork compared to control pork (0.4 mg MDA/kg). Similarly, Kim et al. (2004) observed greater lipid oxidation (0.36 mg MDA/kg) compared to control (0.04 mg MDA/kg) when a combination of 3 kGy

electron beam irradiation and 2% acetic acid applied on the pork loins. However, the authors found no difference in lipid oxidation when 2% of acetic acid sprayed on pork loins. Recently, Pohlman et al. (2019) sprayed beef trimmings with novel organic acids such as 3% octanoic, pyruvic, or malic acids along with 0.2% peroxyacetic acid as decontaminating agents. The treatment was effective in reducing bacteria without causing a difference in instrumental color compared to the control trimming.

Conclusion

The fundamental principles for controlling *Salmonella* in the pork industry are through maintaining proper sanitation and hygienic processing during slaughter and fabrication. However, a hurdle approach is recommended in commercial operations to minimize *Salmonella* risk, using the previously discussed decontamination technologies. Current industry practice places most interventions during animal harvest. Safety interventions for pork trimmings are limited. Hence, it is necessary to apply additional interventions during further processing. Organic acids are widely accepted as effective antimicrobial agents to reduce *Salmonella* in meat. To maximize efficacy and protect the pork quality, concentration, duration, and temperature of the organic acid application must be carefully controlled. It is challenging to develop a chemical decontamination agent that can be applied directly into meat products without negatively affecting the color and organoleptic properties.

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CHAPTER III

POST-HARVEST REDUCTION OF *SALMONELLA* IN PORK TRIMMING

Abstract

Pork trimming was dipped in 3% acetic acid to reduce *Salmonella*. For 15-s dipping and 5-log CFU/pork cube inoculation, only 0.2- to 0.3-log reduction was observed ($P \leq 0.026$), but it was 1.3-log reduction ($P = 0.001$) at 8-log CFU/pork cube inoculation for bioluminescence imaging. Acetic acid worked best at 75 s and 50°C, providing 1.4-log reduction ($P < 0.001$), damaging *Salmonella* cell membranes. Without acetic acid, heat shock alone was ineffective ($P > 0.200$). When an inoculated pork cube was placed at the geometrical center of 2.3-kg pork trimming, dipping at 50°C for 75 s only reduced *Salmonella* by 0.2 log ($P = 0.040$). Although dipping slightly increased lightness ($P < 0.001$) and decreased redness ($P \leq 0.008$) on the meat surface, no inside color change was detected ($P = 0.120$). Neither lipid oxidation (TBARS, $P = 0.644$), protein solubility ($P = 0.187$), nor water-holding capacity ($P = 0.076$) were affected by treatments.

Introduction

A growing number of outbreaks caused by human enteric pathogens is an increasing concern for pork processors and regulatory agencies because of their negative impacts on consumers' health and their trust in the U.S. food system (Self et al., 2017; Rostagno & Callaway, 2012). Despite both pre- and post-harvest critical control measures employed by commercial pork operations, approximately 240,858 kg of pork products (from the whole hog to pork trimming) were recalled

due to *Salmonella* contamination in 2016 (USDA/FSIS, 2016). The USDA/FSIS data from Raw Pork Products Exploratory Sampling Program Phase II from July 1, 2015 to June 30, 2017, showed a steady increase in *Salmonella* incidence rate in comminuted pork, from 21.24% in 2015 to more than 30.93% in 2017 (USDA/FSIS, 2017). These data indicate that additional antimicrobial treatment is needed to minimize post-harvest recurrence of *Salmonella* in pork trimming.

Decontamination methods in the meat industry include antimicrobials, steam pasteurization, hot water wash, steam vacuuming, singeing, ultraviolet light, and carcass chilling (Loretz et al., 2011). The pork industry mostly employs scalding, washing, and singeing (Rodríguez et al., 2018). Acid spraying or washing, have been extensively studied for both beef and poultry processing, whereas limited research has been conducted for pork processing (Britton, 2018). Most organic acids are designated by the FDA as generally recognized as safe (GRAS) for meat products (FDA, 2017). Both acetic and lactic acids are an inexpensive and effective intervention in the beef industry to reduce human enteric pathogens as applied on warm and cold carcass surfaces (Cutter and Rivera-Betancourt, 2000; Yoder et al., 2012). Antimicrobial interventions are normally applied during hog slaughter (Hendricks et al., 2018). Pork trimming production, typically the final step in pork fabrication, should be a critical control point of microbiological hazards (Castelo et al., 2001). Therefore, the objective of the current study was to determine the efficacy of 3% acetic acid in reducing *Salmonella* in pork trimming and the effects of such treatment on meat quality.

Materials and methods

Pork samples and reagents

Pork loin and pork trimming were purchased from a commercial purveyor. Pork loins were trimmed to remove the surrounding muscle, connective tissues, and external fat, leaving only the *longissimus* muscle. This muscle was then cut into 1.3-cm thick chops, which were further cut into 2.5 cm (L) × 2.5 cm (W) × 1.3 cm (H) cubes. The pork cubes were vacuum-packaged and stored in -20°C freezer until further experiments. Pork trimmings were further ground through a kidney plate to approximately 5-cm cubes and aliquoted into 2.3-kg chubs. The meat chubs were vacuum-packaged and stored similarly. These pork cubes and chubs were used in *Salmonella* reduction experiments. Additional pork loins were prepared similarly and cut into 14 1.3-cm thick chops. These chops were further cut laterally into 28 halves per loin to be used in meat quality experiments.

Distilled white vinegar (5% acetic acid; Great Value, Walmart, MS, USA) was purchased from a local grocery store and diluted with Millipore® filtered water to a concentration of 3% for further use in treatment application. *Salmonella enterica* serovar Typhimurium stock culture (ATCC®™ 14028™) and *Salmonella enterica* serovar Enteritidis stock culture (ATCC®™ 4931™) were purchased from Thermo Scientific™ (MA, USA). Various microbiological supplies were Luria–Bertani (LB) powder (BD, NJ, USA), tryptic soy agar powder (Fisher Scientific, NH, USA) nalidixic acid sodium salt (Sigma-Aldrich, MO, USA), and buffered peptone water (BPW) and XLD agar (Thermo Scientific™, MA, USA).

Chemical reagents purchased for testing meat quality attributes were 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, butylated hydroxytoluene (BHT), 1,1,3,3-tetramethoxypropane (TMP), thiobarbituric acid (TBA), methanol, ethanol (Sigma-Aldrich, MO, USA), trichloroacetic acid (TCA; Fisher Scientific, NH, USA), Coomassie blue reagent (Thermo Scientific™, Waltham, MA, USA), bovine serum albumin (BSA; Thermo Scientific™, Waltham, MA, USA). Radical ABTS+ was prepared by dissolving ABTS in water at 7 mM in 2.45 mM potassium persulfate and incubating solution for at least 6 h in the dark. This solution was further diluted to an absorbance of 0.8 to 0.85. Trichloroacetic acid (TCA) was prepared in 10% solution by adding 100 g of TCA in 1 L of Millipore® filtered water. Malondialdehyde standards were prepared by mixing 8.26 µL of 1,1,3,3-tetramethoxypropane in 10 mL of 10% TCA. This solution was further diluted to a series of working standards of 0, 2.5, 5, 7.5, 10, 15, 20, 25 µM by 10% TCA. Butylated hydroxytoluene was prepared to 15,000 ppm by adding 0.75 g of BHT to 50 mL of ethanol. Thiobarbituric acid (TBA) in 10% TCA was prepared to 0.02 M by adding 0.288 g of TBA and 10 g of TCA in 100 mL of warm Millipore® filtered water.

Salmonella **cultures and inocula**

Nalidixic acid-resistant *Salmonella enterica* serovar Typhimurium (ATCC 14028) and *Salmonella enterica* serovar Enteritidis (ATCC 4931) inoculum were prepared by successive overnight culture and selection in 10 mL TSB supplemented with sterile filtered (0.4 µm) nalidixic acid sodium salt in concentrations of 0 (control), 5, 15, 20, and 25 ppm until having a fully-grown culture (9 log CFU/mL) that is resistant to 25 ppm of nalidixic acid. Resistance was confirmed by streaking onto tryptic soy agar and XLD agar plates containing 25 ppm of nalidixic acid. Both nalidixic acid-resistant stock cultures were stored at -80°C freezer in TSB

supplemented with 20% glycerol. Prior to the experiment, the frozen culture was thawed, streaked onto XLD agar plates with 25 ppm of nalidixic acid, and incubated at 37°C for 24 h. An isolated colony from each culture was transferred to a culture tube containing 10 mL of TSB with 25 ppm of nalidixic acid and incubated at 37°C for 18 to 20 h. On the day of the experiment, *Salmonella* culture was serially diluted using 1% BPW to 6 log and used as inoculum. A cocktail *Salmonella* was also prepared by mixing *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis cultures in equal volumes in another culture tube. Both serotypes are the most isolated non-typhoidal *Salmonella* in pigs (Boyen et al., 2008). This cocktail culture was then serially diluted using 1% BPW to 6 log and used as inoculum.

Bioluminescent *Salmonella enterica* serovar Typhimurium (ATCC 14028) was constructed by electroporation of pXen5-luxCDABE (Caliper Life Sciences, Hopkinton, MA, USA) containing ampicillin-resistant gene into bacterial cells (Park et al., 2018). Colonies of successfully transformed *Salmonella* exhibiting bioluminescence were positively selected on LB-agar medium containing ampicillin (100 µg/mL). This bioluminescent *Salmonella enterica* serovar Typhimurium was cultured in LB broth at 37°C to 9 log CFU/mL and used as inoculum.

Pork sample inoculation

On the day of experiments, pork cubes were thawed at 2°C for 24 h. Pork cubes served as experimental units with one cube per treatment per replicate; therefore, the number of pork cubes were calculated as replicates × treatments for each experiment. Because bacteria proliferate, the pork cubes of the same replicates were inoculated quickly together, to minimize inoculum variation among treatments within a replicate that might be caused by the amount it took to

complete a round (replicate) of the experiments. Pork cubes were inoculated with 100 µL of 6-log CFU/mL *Salmonella* inoculum prepared as described previously by spreading this volume evenly on the 2.5-cm surface of the pork cubes, resulting in 5 log CFU/pork cube. These inoculated pork cubes were stored at 4°C for 1 h to ensure bacterial attachment. For IVIS imaging, 100 µL of 9-log CFU/mL lux-modified *Salmonella* inoculum was used, resulting in 8 log CFU/pork cube. To inoculate the 2.3-kg pork chub, a pork cube similarly inoculated with *Salmonella* cocktail (*Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis) at 5-log level was placed at the geometrical center of the chub and surrounded by non-inoculated pork pieces of the chub. The chub was placed in a perforated cylindrical canister (Better Homes & Gardens, Walmart, MS, USA) and shaken gently to ensure that meat pieces fit tightly together to create a simulation of a trimming bucket in the industry.

***Salmonella* enumeration and bioluminescent imaging**

After treatment, pork cubes were retrieved and placed in a sterile Whirlpak® bag with 50 ml BPW and homogenized by gentle massaging for 2 min. A volume of 20 µL of the BPW was plated in duplicate onto XLD agar plates with 25-ppm nalidixic acid, and the plates were incubated at 37° C for 24 h. Black colonies with metallic sheen were counted as *Salmonella* colony-forming units (CFU). For bioluminescent imaging, pork cubes were retrieved and placed in a tissue culture plate for analysis using the In Vivo Imaging System (IVIS; Lumina XRMS Series III system, Perkin Elmer, Waltham, MA, USA). Numbers of CFU (XLD plating method) or relative light units (RLU; IVIS imaging method) were converted to common logarithm (log). The difference by subtracting values of treated pork cubes from those of POS cubes were reported as log reduction. The NEG cubes were used to monitor background *Salmonella*.

Throughout all experiments, no background *Salmonella* was detected on 25-ppm nalidixic acid XLD agar plates.

Experiment 1: Effects of temperature on efficacy of 3% acetic acid in reducing *Salmonella*

To preliminarily determine the effects of temperature on the efficacy of 3% acetic acid in *Salmonella* reduction, either a negative control (NEG), positive control (POS), 3% acetic acid at room temperature (21°C; ACC), or 3% acetic acid at 50°C (ACH) treatment was randomly assigned to a pork cube. Two inocula, 5-log inoculum of nalidixic acid-resistant *Salmonella enterica* serovar Typhimurium and 8-log inoculum of lux-modified *Salmonella enterica* serovar Typhimurium as described previously, were used. The NEG pork cube was not inoculated. The POS was inoculated but was not treated. The ACC and ACH cubes were inoculated immediately after the POS. At approximately the same time, ACC and ACH cubes were dipped in 100 mL of 3% acetic acid in a plastic cup (500 mL; Home Sense, Kroger, MS, USA) of either room temperature or 50°C, respectively, for 15 s. Cubes were subsequently removed from the cup and placed on a metal wire rack (Aleko, Walmart, MS, USA) for 1 min for dripping. The NEG cube and POS, ACC, and ACH cubes inoculated with at 5 log were extracted in BPW and prepared for plating as described previously; whereas the NEG cube and POS, ACC, and ACH cubes inoculated at 8 log were transferred to a tissue cell culture plate for bioluminescent imaging. This experiment was replicated five times.

Experiment 2: Effects of heat shock in reducing *Salmonella*

Two experiments using either inoculated pork cubes or *Salmonella* culture were conducted to determine the effects of heat shock in *Salmonella* reduction. For the first experiment, two inocula were used at 5 log and 8 log for plating and IVIS imaging, as previously described. Either NEG,

POS, cold-to-hot heat shock (HSC; dipping in ice-cold water and subsequently in 50ºC water) or hot-to-cold heat shock (HSH; vice versa) treatment was randomly assigned to a pork cube. The HSC and HSH cubes were inoculated immediately after the POS cube. The HSC and HSH cubes were subsequently treated with 100 mL of ice-cold water and 100 mL of 50ºC water in a plastic cup, each for 15 s according to their designated treatments. The cubes were then placed on a metal wire rack for 1 min to drip. The NEG, POS, and treated pork cubes were extracted in BPW for plating or in tissue culture plate for IVIS imaging. For the second experiment, 200 µL of 6-log nalidixic acid-resistant *Salmonella enterica* serovar Typhimurium was pipetted into a sterile 2-mL microcentrifuge tube to serve as either POS, HSC, or HSH. Ice-cold water was used for cold dipping, whereas 93°C water was used for hot dipping. Each HSC or HSH was dipped in ice-cold or hot water for 2 min according to their designated treatments. For the culture to reach 50°C, the dipping time was 2 min at a heating rate of 11.1ºC/min. The temperature was monitored by a thermometer (Ematik, Walmart, MS, USA) placed in 200 µL of water. After the treatment, a volume of 20 µL from each tube was directly plated onto XLD agar. Both experiments were replicated five times. Samples of cold and warm water were also plated to evaluate the washout effect; however, most plates had no colonies and only a few plates had 1 to 5 colonies.

Experiment 3: Effects of temperature and dipping time on efficacy of 3% acetic acid in reducing *Salmonella*

In this experiment, pork cubes were treated with ACC and ACH for 15, 45, or 75 s, resulting in 8 treatments (ACC15, 45, and 75; ACH15, 45, and 75) including NEG and POS, which were randomly assigned to eight pork cubes prepared as described previously. Only a 5-log inoculum of nalidixic acid-resistant *Salmonella enterica* serovar Typhimurium was used. The ACC and

ACH cubes, as described previously, were inoculated and treated according to their designated treatments. The cubes were dipped in 100 mL of 3% acetic acid in a plastic cup of either room temperature (21°C) or 50°C, respectively, for 15, 45, or 75 s. They were then retrieved and allowed to drip on a metal wire rack for 1 min. After dripping, NEG, POS, and treated pork cubes were extracted in BPW for plating as described previously. This experiment was replicated ten times at three different trials, resulting in thirty replications.

Experiment 4: Efficacy of 3% acetic acid at 50°C in reducing *Salmonella* inoculated at the geometrical center of pork chubs

Either NEG, POS, 3% acetic acid dipping at 50°C without (ACH) or with shaking (ACHS) was randomly assigned to a pork chub prepared and inoculated as described previously. The *Salmonella* cocktail was used for inoculation. The ACH and ACHS chubs in perforated canisters were dipped into a plastic bag containing 8 L of 3% acetic acid at 50°C. During the dipping, the canisters were either submerged in acetic acid in the bag (ACH) or submerged and hand-shaken gently (ACHS) for 75 s. After treatment, the canisters were allowed to drip on a metal wire rack for 1 min. The NEG, POS, ACH, ACHS pork cubes at the geometrical center of the chubs were retrieved and placed in BPW for *Salmonella* extraction. Four pork pieces surrounding the center cube were also retrieved and analyzed for *Salmonella* to monitor potential cross-contamination and washout effects. This experiment was replicated ten times.

Experiment 5: Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) of *Salmonella* cells

Nalidixic acid-resistant *Salmonella serovar* Typhimurium culture was prepared at 9 log in TSB overnight, as described previously. One mL of this culture was aliquoted into a 2-mL microcentrifuge tube with a snap cap, and the tube was centrifuged at 3,500 × g for 5 min to

remove TSB leaving the pellets of *Salmonella* cells at the bottom. Three treatments, including positive control (POS), 3% acetic acid at 50°C (ACH45) for 45 s, or 3% acetic acid at 50°C (ACH75) for 75 s, were randomly assigned to three tubes of *Salmonella* pellets. The ACH45 and ACH75 tubes received 1 mL of 3 % acetic acid at 50° for 45 s and 75 s, respectively, whereas the POS tube received 1 mL of BPW. After the designated treatment duration (75 s for POS), acetic acid and BPW (for POS tube) were immediately removed, and the pellets in both POS and the treated tubes were twice washed with 1% BPW and centrifuged at 3,500 × g for 5 min to neutralize and remove the acetic acid residue. The POS, ACH45, and ACH75 pellets were incubated in 1 mL of fixative (2.5% glutaraldehyde, 2% paraformaldehyde in O.1M sodium cacodylate at pH 7.2) for 2 h at room temperature for further preparation of the cells for SEM and TEM. This experiment was replicated three times.

For SEM, fixed pellets were washed three times with sterile water and fixed in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer on coverslips. The pellets were then dehydrated with gradient concentrations of ethanol (Autosamdri®-931, Tousimis) and coated with 20 nm of platinum. The coverslips were analyzed on a scanning electron microscope (JEOL JSM-6500F Field Emission Scanning Electron Microscope, MA, USA). Three randomly selected areas were analyzed at different magnifications to elicit the overall structure of the cells. For TEM, fixed cells were washed and dried similarly and embedded in LR white medium grade resin. Ultra-thin sections were cut using a Riecher Jung Ultra cut microtome, placed on copper grids, and stained with uranyl acetate and lead citrate. The ultra-thin slices were analyzed on a transmission electron microscope (JEOL JSM-1230; Jeol USA, MA, USA) at 80 ky. Three randomly selected areas were analyzed at different magnifications to reveal structural changes in *Salmonella* cells.

These analyses were repeated on all three replications to ensure that the visual differences among treatments were confirmed.

Experiment 6: Effects of temperature and dipping time of 3% acetic acid on pork quality

Treatment application and sample collection

There was no *Salmonella* inoculation in this experiment. Either NEG, ACC15, 45, 75, or ACH15, 45, or 75 were randomly assigned to one of the 28 halves within a pork loin. The ACC and ACH chop halves were dipped in 100 mL of 3% acetic acid in a plastic cup at either room temperature (21°C) or 50°C, respectively, for 15 s, 45 s, or 75 s according to their designated treatments. The cubes were subsequently removed from the cup and allowed to sit on a metal wire rack for 1 min for dripping. Instrumental color (CIE L*, a*, b*) and reflectance spectra (400 to 700 nm) were recorded for the surface of the chop halves before and after treatment, as well as at half-height (approx. 0.65 cm) cross-sectional surface after treatment. Cross-sectional surfaces were allowed to bloom for 30 min before color measurement. The half portions of the chop halves used for color measurement were subsequently cubed, frozen in liquid nitrogen, pulverized to finely divided powder, and stored in -80 ºC for chemical analyses.

Surface color (L*, a*, and b*) and myoglobin composition

The surface color of each chop half was measured at the 2.54-cm surface before and after treatment and at the cross-sectional surface after treatment using a Hunter Lab MiniScan 4500L spectrophotometer (Hunter Associates Inc, Reston, VA, USA) in triplicate. Reflectance spectra of 400 to 700 nm by a 10-nm interval and CIE L*, a*, b* values (illuminant A, 10° angle, and 25-mm aperture size) were recorded. Hue angle and chroma were calculated from a* and b*,

whereas percentages of deoxymyoglobin (DMb), oxymyoglobin (OMb), and metmyoglobin (MMb) were calculated from the reflectance spectra (AMSA, 2012).

Trolox-equivalent antioxidant capacity (TEAC)

Antioxidant capacity was determined using an ABTS+ radical cation assay (Re et al., 1999). Antioxidants in 1-g powdered samples were extracted using 4 mL of methanol (Szydłowska-Czerniak et al., 2008). A volume of 10 µL of sample extract was added to 200 µl of ABTS+ radical solution. The reducing reaction was allowed to equilibrate for 5 min, and the final absorbance was measured at 734 nm using Spectral Max Plus 384 spectrophotometer (Molecular Devices, LLC, Sunnyvale, CA) against 10 µL of methanol in 200 µL of ABTS+ as blank. The antioxidant capacity was expressed as millimoles of trolox equivalence per kg of meat (mmol/kg).

Thiobarbituric acid reactive substances (TBARS)

Lipid oxidation was measured according to a method described by Holtcamp et al. (2019) with modifications. A 1-g powdered sample was heated at 90°C with 3 ml of 10% TCA and 30 µL butylated hydroxytoluene (BHT) in a water bath for 30 min and cooled quickly after heating. The solution was centrifuged at 10,000 × g for 10 min at 4°C. An aliquot of 100 µl of the supernatant was heated at 90°C with 200 µL of 0.02-M TBA solution for 30 min and cooled quickly after heating. The solution was then centrifuged at 10,000 ×g for 10 min at 25°C. A volume of 200 µL of the supernatant was pipetted into a 96 well plate, and the absorbance was measured at 532 nm against a blank and an MDA standard curve. The TBARS value was calculated as mg of MDA per kg of meat.

Protein solubility

Protein solubility was determined using the Bradford protein assay (Joo, Kauffman, Kim, & Park, 1999). A 1-g powdered sample was mixed with 10 mL of Millipore® filtered water, vortexed vigorously for 5 min, and centrifuged at 15,000 × g for 15 min. The supernatant was diluted 10 times using Millipore® filtered water. A volume of 10 µL of the diluted supernatant was mixed with 300 µL of Coomassie blue reagent in a 96-well plate (Costar® 3370, Corning Inc., Corning, NY, USA) and incubated for 10 min at room temperature. The absorbance was measured using a spectrophotometer at 595 nm and compared with an external calibration curve of BSA. Protein solubility was expressed as mg of soluble protein per g of meat (mg/g).

Water-holding capacity (WHC)

Water holding capacity was measured by centrifugation as expressible juice (Jauregui, Regenstein, & Baker, 1981). Weighs of the insert (with a polypropylene mesh and 0.2-µm nylon membrane filter at the bottom) and the housing were recorded. A 0.5-g of powdered meat sample was weighed into an insert of a centrifugal device. The insert with meat was placed back into the housing, and the device was centrifuged at 15,000 × g for 30 min. After centrifugation, the insert with meat and the housing with expressible juice were removed and weighed. The WHC was calculated as the percentage of expressible juice in meat samples.

pH

A 1-g powdered sample was mixed with 9 mL of Millipore® filtered water and vortexed vigorously for 5 min and pH of the supernatant was recorded by a pH meter with temperature-compensation probe (Accument®, model 13-620-631; Fisher Scientific, Waltham, MA), calibrated by pH 4, 7, and 10 standards.

Statistical analysis

A completely randomized design was used for experiments 1 to 5. Experiment 3 had a factorial arrangement of temperature and time. Experiment 6 was a randomized block design with repeated measurement of color on the same chop halves at the surface before and after treatment and at the cross-section after treatment, nested within each block. For *Salmonella* reduction data, the POS and NEG data were used for calculation and background *Salmonella* monitoring, therefore, not included in the statistical analysis. *Salmonella* and meat quality data were analyzed in a generalized linear mixed model with acetic acid treatments, time (when applicable), and the interaction (when applicable) serving as fixed effects and replicate serving as a random effect. Pork quality data were analyzed in a similar model with treatment as a fixed effect and pork loin as a random block effect. Pork color data analysis also had surface and their interaction with treatment as fixed effects and pork cube within a pork loin and a treatment as a random effect. The selection of the appropriate covariance structure for the repeated measurement (pork color only) was based on three default Information Criteria that were calculated by SAS in the smaller-is-better format (AIC, Akaike's Information Criteria; AICC, AIC Corrected; and BIC, Bayesian Information Criteria; (Kincaid, 2005)), resulting in a compound symmetry structure being used for all color variables. Analysis of variance was performed by the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, USA). Means, if differing, were separated by a protected t-test using the LSMEANS/PDIFF/SLICEDIFF (color data) statement of the GLIMMIX procedure. Actual probability values were reported.

Results and Discussion

Salmonella reduction by 3% acetic acid

In preliminary experiment 1 (Fig. 1), when using plating method at a 5-log inoculation, there was 0.2-log reduction in *Salmonella* count with ACC treatment ($P = 0.026$) and 0.3-log reduction with ACH treatment ($P = 0.003$) and both treatments did not differ ($P = 0.320$). When using an 8-log inoculation of lux-modified *Salmonella*, IVIS imaging revealed a similar ($P = 0.370$) 1.3-log reduction ($P = 0.001$) for both ACC and ACH. In preliminary experiment 2 (Fig. 2), there was no reduction of *Salmonella* on pork cubes for both HSC and HSH regardless of whether plating or IVIS imaging was used ($P \geq 0.200$). When *Salmonella* culture was used, there was a similar ($P = 0.823$) reduction ($P \leq 0.003$) of 0.4 log for HSC and HSH.

In experiment 3, there was a 2-way treatment × time interaction ($P = 0.030$; Fig. 3). For 15-s dipping, ACH reduced *Salmonella* by 0.7 log ($P < 0.001$), 0.5 log greater than ACC ($P < 0.001$). For 45-s dipping, ACH reduced *Salmonella* by 1.0 log ($P < 0.001$), 0.7 log greater than ACC ($P < 0.001$). For 75-s dipping, the *Salmonella* reduction by ACH treatment was 1.4 log ($P < 0.001$), whereas the reduction was less for ACC treatment ($P = 0.004$), at only 0.5 log ($P < 0.001$). In experiment 4, ACH treatment reduced *Salmonella* in the geometrically centered cubes by 0.2 log ($P = 0.040$). The ACHS treatment similarly ($P = 0.198$) reduced *Salmonella* 0.3 log ($P = 0.010$; Fig. 4). Most surrounding pork pieces, including those of the POS chubs, had no *Salmonella* counts; only a few pieces had 1 to 3 CFU/plate.

In experiment 1, 15-s dipping duration resulted in a similar reduction between ACC or ACH treatments; both yielded an average of 0.3 log of *Salmonella* reduction. This might be explained

by the short dipping duration rather than the ineffectiveness of higher temperature. This duration was experimented by Anderson & Marshall (1989). These authors dipped inoculated beef *semitendinosus* muscle in 1, 2, or 3% acetic acid at 25, 40, 55, or 70 ºC for 15 s and found that 3% acetic acid at 70°C for 15 s was most effective, reducing *Salmonella* by 1.5 log. However, at 3% concentration and 25, 40, and 55ºC for 15 s, a similar reduction of 0.9 to 1.1 log of *Salmonella* was observed for the three temperatures. The authors suggested that the similar reduction at these temperatures was caused by a short dipping duration of 15 s, which was the same duration used in the current study. However, in the current study, only a 0.3-log reduction was observed at 3% concentration and 15-s dipping. *Salmonella* used in the current study was developed to be resistant to nalidixic acid at 25 ppm; whereas the strain used by Anderson & Marshall (1989) was not. Antibiotic-resistant bacteria have less outer membrane permeability which is mediated by porins (San Martin et al., 2005). This reduced permeability causes cross-resistance with antimicrobial agents (Bower et al., 1999). In experiment 2, the purpose was to examine whether temperature alone could have an impact on *Salmonella*. However, although there was a 0.4-log reduction *in vitro* (*Salmonella* culture), no reduction was observed on inoculated pork. Most *Salmonella* strains possess thermal tolerance that enhances their resistance to desiccation and starvation (Dawoud et al., 2017). Heat shock proteins are also produced under acidic or alkaline conditions, which protect them against thermal stress (Foster, 1991; Taglicht et al., 1987). Therefore, not all bacteria subjected to a stressor are killed. However, sub-lethal heat shock at 60ºC for 10 to 45 min induced injury to *Salmonella* Typhimurium (Wuytack et al., 2003). The preliminary experiments indicated that 15-s dipping duration might be too short to produce a meaningful reduction of *Salmonella* by 3% acetic acid. Moreover, heat shock may need to be combined with acetic acid to maximize efficacy of acidity. Most pork trimmings are

kept at refrigeration temperature to suppress bacterial growth (Nastasijević, Lakićević, & Petrović, 2017). This instant change from cold storage temperature to warm temperature of acetic acid may create an effective heat shock.

Experiment 3 produced a similar 0.2- to 0.3-log reduction to those in experiment 1 at 15 s and room temperature. However, as dipping time increased, acetic acid was more effective at 50°C than at room temperature, producing a 1.4-log reduction. Cutter and Rivera-Betancourt (2000) sprayed 2% acetic acid at 35°C on the surface of beef short plates at 862 kPa (125 psi) and produced 3.6-log reduction immediately after treatment and 2.4- to 3.3-log reduction from d 2 to d 35 on *Salmonella* count. Yoder et al. (2012) performed similar experiments at 276 kPa (40 psi) and 21 to 26°C (room temperature) for 15 s, allowed acetic acid to remained on the surface for 5 min and found a 3.5-log reduction on a pathogen cocktail. Similarly, Eggemberger-Solorzano et al. (2002) combined hot-water washing (25 psi, 82°C, 5 s) and 1.8% acetic acid rinse (25 psi, 3 s) and reduced *Escherichia coli* by 2 log. All these studies employed pressurized spraying of acetic acid, which potentially caused a wash-out effect in addition to bactericidal effects. Therefore, yielding a 1.4-log reduction in *Salmonella* count using acetic acid at 50 ºC for 75-s duration in the current study is reasonable, especially obtained from 30 replications. It is worth noting that most studies on acetic acid efficacy in reducing *Salmonella* have been conducted on beef cuts and carcasses, in which spraying duration could be longer and the acetic acid was allowed to reside on beef cuts or carcasses for a few days without negative impacts on meat quality. A few researchers, such as Kang et al. (2003) who sprayed 0.5, 1, 1.5, or 2% acetic acid on pork loins for 15 s at 30 °C and found that spraying acetic acid at 2% was most effective and was able to

reduce *Salmonella* by 1.5 log after 24 h storage. A similar reduction of *Salmonella* (1.4 log reduction) was observed in the current study even before the storage of the pork cubes.

In experiment 4, the goal was to test the penetration of antimicrobial solution into the center of a bulk of pork trimming, simulating how pork trimming is collected, stored, transported, and received at further processing plants. Only 0.3-log reduction was observed. A greater reduction at 1.5 log with 5% acetic acid was reported by Stivarius et al. (2002) in a benchtop study on beef trimming after 3-min tumbling. This finding is similar to a 1.4-log reduction observed in the current study because the beef trimming was tumbled, which allowed a much greater penetration of acetic acid. Similarly, Harris et al. (2006) sprayed 2% or 4% acetic acid on inoculated beef trimmings when it was being moved by a conveyor belt and found a 1.5-log reduction of *Salmonella* at both 2 and 4% concentrations. These observations together with the findings in the current study indicated that penetration of antimicrobial solutions is important for the efficacy of such solutions. Poor penetration of acetic acid into a large bulk of pork trimming might be the major reason why acetic acid was not effective at reducing *Salmonella* on the inoculated pork cube at the center of the bulk. Therefore, acetic acid treatment must be applied to a spread-out layer of pork trimming, similar to what was experimented by Harris et al. (2006) on beef trimming.

Structural damages of *Salmonella* cells by 3% acetic acid

The scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images (Fig. 5 and 6) indicated damages in the cell membrane of the *Salmonella* cells treated with 3% acetic acid at 50°C for 45 and 75 s. The SEM images showed a less rigid surface of treated cells than the POS cells, especially for the 75-s treatment. The treated cells had a smoother surface

with less rigid structural grooves. Moreover, the TEM images clearly revealed structural damages inside the treated *Salmonella* cells and the disappearance of the cell membrane of the treated cells, especially for 75-s dipping. Some *Salmonella* cells treated for 75 s appeared to be dead and had no cell membrane.

Tan et al. (2015) suggested that the mode of action of acetic acid on *Salmonella* was to cause cellular ATP depletion and altered cellular DNA synthesis. The authors found that acetic acid-stressed *Salmonella* had a greater ADP/ATP ratio, especially at neutral pH. They also suggested that undissociated acetic acid molecules actively participated in ATP depletion and is important because the meat medium is pH-neutral by buffering action of proteins. However, the authors also concluded that membrane disruption was not one of the mechanisms through which acetic acid kills *Salmonella*. The author reached this conclusion by using SEM images. Scanning electron microscopy, however, does not provide strong evidence of cellular damages in the case of acetic acid treatment because SEM images are produced by the reflected electrons and only provides the external appearance of bacteria through three-dimensional imaging. The TEM images, however, are produced from electron beams passing through thin sections of the bacterial cells, which form shadow-like images on a fluorescent screen and capture cellular structure (Kaláb, Yang, & Chabot, 2008). In the current study, the SEM images showed an intact membrane in both positive control and treated *Salmonella* cells, except for a smoother and less groovy surface of the treated cells. Once TEM was used to analyze the cross-sectional appearance of *Salmonella* cells, it was clear that the treated cell membrane was disappearing, and the integrity of the treated cells was compromised (black areas). This phenomenon was also observed by Jung et al. (2009), who used TEM to observe acetic acid-stressed *Salmonella*. The

authors provided similar images of damaged cells. Some cells, however, were able to repair the damages and recovered.

Effects of temperature and dipping time of 3% acetic acid on pork quality

Treatment had an overall effect on lightness (L*; $P < 0.001$; Fig. 7a). Treatment ACC45, ACH15, and ACH75 had slightly greater L* value (61.6 to 61.7) than NEG (60.3; $P \leq 0.008$). Across all treatments, the surface before treatment and cross-section surface had similar L* values (60.1 to 60.6; $P = 0.120$; Fig. 7b), which was less than the L* of surface after treatment (62.1; $P < 0.001$). There was a 2-way treatment × surface interaction for redness (a*; $P = 0.002$; Fig. 8). Before treatment, no difference in a* value was found ($P \geq 0.076$). After treatment, all treatment cubes had 2.2 to 2.9 units less in redness than the NEG cubes (17.2 to 18.8 vs. 19.1; $P \leq 0.037$). At the cross-sections after treatment, most treatment cubes had similar redness to that of the NEG cubes ($P \geq 0.154$), except for ACC15 cubes, which were 2.2 units greater ($P < 0.001$).

There was 2-way treatment × surface interaction for OMb ($P = 0.051$) and DMb ($P = 0.003$; Fig. 9). Before treatment, the OMb and DMb values similarly ranged from 65.9 to 66.6% and 5.1 to 8.3%, respectively ($P \geq 0.053$). However, after treatment, the OMb and DMb values of the NEG cubes remained at 66.6% (3 to 5% less; $P \leq 0.037$) and 7.3% (2.0 to 3.8% more; $P \leq 0.051$). No treatment difference in OMb was found at cross-sectional surfaces ($P \geq 0.244$). For DMb, only ACC15 and ACH15 had 2.6 to 3.1% more than NEG ($P \leq 0.032$). The NEG cubes had approximately 1.6 to 2.0 % more MMb than ACC15, ACC45, and ACH15 ($P \leq 0.021$) but were similar to other treatments ($P \geq 0.342$). Before-treatment surface had 1.7% more MMb than after-treatment surface ($P < 0.001$) but 1.8% less than cross-sectional surface ($P < 0.001$).

The pH of the NEG was 0.1 to 0.2 units greater than that of all treatment cubes (5.7 vs. 5.5 to 5.6; $P \leq 0.014$; Table 1), except for ACC15, which was similar to NEG ($P = 0.132$). Antioxidant capacity of the NEG was 0.8 mmol/kg trolox equivalence, 0.1 mmol/kg more than that of ACH45 ($P = 0.018$) and 0.12 mmol/kg less than that of ACC15 ($P \leq 0.040$; Table 1). The NEG cubes had a similar TEAC value to those of the other treatments ($P \geq 0.385$). No treatment difference was found for TBARS value (0.53 to 0.63 mg MDA/kg meat; $P = 0.644$;Table 1), protein solubility (30.92 to 33.72 mg/g of meat; $P = 0.187$; Table 1), and WHC as expressible moisture percentage (1.24 to 1.68%; $P = 0.076$ Table 1).

A slight increase in lightness after treatment could be explained by the acidity of acetic acid, resulting in more reflection of incident light and a brighter surface (Lopez et al., 2004). A similar increase in lightness was reported by Lin & Chuang (2006), who dipped pork loin chops in 2% acetic acid for 30 s to improve the pork shelf life. On the contrary, redness decreased slightly after treatment. However, OMb percentage of the pork cubes, which is usually positively correlated with redness (Salueña et al., 2019), increased slightly (3 to 5%). Acidity of acetic acid in the current study allows greater diffusion of oxygen that shifts the myoglobin oxygen dissociation curve towards more saturation state, i.e. oxygenation, and produces more OMb (Brewer, Novakofski, & Freise, 2006). In addition, Zhu & Brewer (2002) reported that low pH induces denaturation of MMb, making it insensitive to the metmyoglobin reductases. The reduction of MMb to DMb is important for the subsequent oxygenation of DMb to OMb (Friedrich et al., 2008). At temperatures from 25 to 50ºC, however, these authors found that there was no difference in MMb denaturation across all pH. At 50ºC, there was approximately 1 to 2%

more denatured MMb at pH of 5.3 than at pH of 5.6. This finding coincides with approximately 2% difference in surface MMb between NEG and treatment pork cubes. In the current study, the pH of the treatment pork cubes was 5.5 to 5.6, slightly less than 5.7 of the NEG cubes. Metmyoglobin reductase activity also decreases at the pH less than 5.6 (Andrews et al., 2007), which also decreased the redness. However, the only significant decrease in reductase activity was at the pH of less than 5.4. Therefore, the redness of pork cubes in the current could be affected by neither MMb denaturation nor reductase activity. Such a small decline (2 units over a total of 20 units) in redness could only be explained by the changes in light reflectance, similar to the changes in lightness, giving the short dipping duration and slight pH difference between NEG and treatment pork cubes. Although there were slight changes in lightness and redness of the meat surface after treatment, the analysis of the cross-sectional surface indicated that such a change did not occur inside the meat pieces. Surface changes in lightness and redness were less than 10%, and the changes on the surface do not alter the color of the entire pork cubes as well as the pork trimming in a meaningful way. A similar finding was reported by Jimenez-Villarreal et al. (2003) after the authors applied 5% lactic acid to ground beef and tumbled it for 3 min.

Low pH can cause the hydrolysis of triglycerides and increase lipid oxidation by liberating of fatty acids from triglycerides through lipolysis, leading to hydrolytic rancidity of meat (Jamilah et al., 2008). Isoelectric point of myosin (abundant protein of myofibrillar protein) is 5.4 (Huff-Lonergan, & Lonergan, 2005). A decline in pH from 5.7 to 5.4 results in less negative charge on the myofibrillar proteins, less repulsion of myofilaments, and less myofilament lattice, causing denaturation of myofibrillar proteins (Lin & Chuang, 2001). Denatured proteins are less soluble and hold less water (Westphalen et al., 2005; Li et al., 2018). The lack of negative impacts on the

overall quality of the treated pork cubes might be explained by the short dipping duration (75 s). Only a slight decrease in the pH of the treated cubes was observed because acetic acid is a weak organic acid (Kundukad et al., 2020) that remains undissociated on the meat surface (Jensen et al., 2003). Moreover, acetic acid dissociation at 50ºC is similar to that at room temperature (Kahyarian et al., 2017). Meat proteins have great buffering capacity (Puolanne, & Kivikari, 2000). Only a pH close to the isoelectric pH of meat proteins, specifically myosin, at 5.4 can drastically decrease WHC and protein solubility because the total net charge and myofilament repulsion, respectively, at such a low pH is minimal (Lin & Chuang, 2001). A 0.1 to 0.2 difference in pH in the current study did not induce such changes. These findings coincide with the discussion above regarding MMb and reductase denaturation, suggesting that only when the meat pH declines below 5.4, the properties of meat proteins will be altered greatly. The slight decrease in pH of the treatment pork cubes also did not increase lipid oxidation, as similarly reported by Fu, Sebranek, & Murano (1994), who sprayed 1.5 % acetic acid on pork carcasses. Slightly greater TEAC value of ACC15 and slightly lesser values of ACH45 than that of the NEG can be explained by either an increase in solubility or denaturation of a small number of redox enzymes. Jin et al. (2011) reported that only prolonged duration under low pH can decrease activity of such enzymes. Overall, no meaningful change in meat quality was observed in the current study.

Conclusions

Acetic acid at 3% and 50°C provided a meaningful reduction of *Salmonella* by 1.4-log at 75 s. Heat-shock treatment without acetic acid on inoculated pork cubes did not reduce *Salmonella* efficiently. However, the heat-shock application revealed that there was no wash-out effect after *Salmonella* attached to the surface of meats. Dipping large bulks of pork trimming in acetic acids

was not effective in reducing *Salmonella* because acetic acid might not be able to penetrate large bulks of meat. The SEM and TEM images confirmed that acetic acid damaged bacterial cell membrane and caused damages in cellular structure. In addition, 3% acetic acid at 50°C did not cause any meaningful impact on the quality attributes of pork trimming. The surface color was slightly paler; however, no significant change in color in the whole meat pieces according to the analysis of cross-sections. Therefore, it is recommended to allow acetic acid to contact all meat pieces, such as spreading trimming on conveyor for a dipping or spraying application or loosening the trimming bulks to allow for adequate penetration by acetic acid.

Acknowledgement

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Tables and Figures

Table 1 Chemical quality of pork longissimus muscle treated with 3% acetic acid at room temperature (21°C; ACC) or 50ºC (ACH) for 15, 45, or 75 s compared with negative control (NEG).

Quality attributes	Treatments							SE[1]	*P value*[2]
	NEG	ACC15	ACH15	ACC45	ACH45	ACC75	ACH75		
pH	5.72[a]	5.64[ab]	5.60[bc]	5.58[bc]	5.53[cd]	5.52[cd]	5.45[d]	0.10	0.001
TEAC (mmol/kg)	0.76[a]	0.92[b]	0.75[a]	0.81[a]	0.67[c]	0.86[a]	0.74[a]	0.07	0.001
TBARS (mg MDA/kg)	0.56	0.56	0.55	0.61	0.63	0.53	0.59	0.10	0.644
WHC (% expressible juice)	1.61	1.25	1.50	1.37	1.68	1.68	1.60	0.17	0.076
Protein solubility (mg/g)	33.72	31.43	30.93	32.37	31.34	32.17	32.64	2.13	0.182

[1] Pooled standard error

[2]Probability value of type I error for the Fisher test of null hypothesis
[abc] Within a row, means without common letters differ.

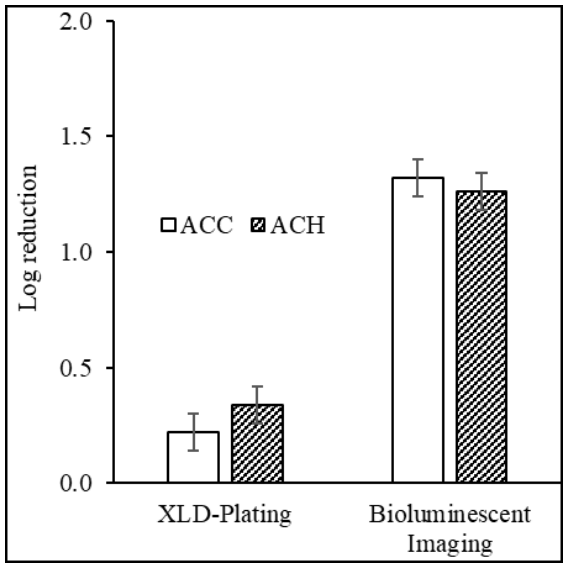

Figure 1 Log reduction of nalidixic acid-resistant *Salmonella* (5-log inoculation; XLD-plating) and lux-modified *Salmonella* (8-log inoculation; bioluminescent imaging) on pork cubes treated by dipping in 3% acetic acid at room temperature (21°C; ACC) and 50ºC (ACH) for 15 s.

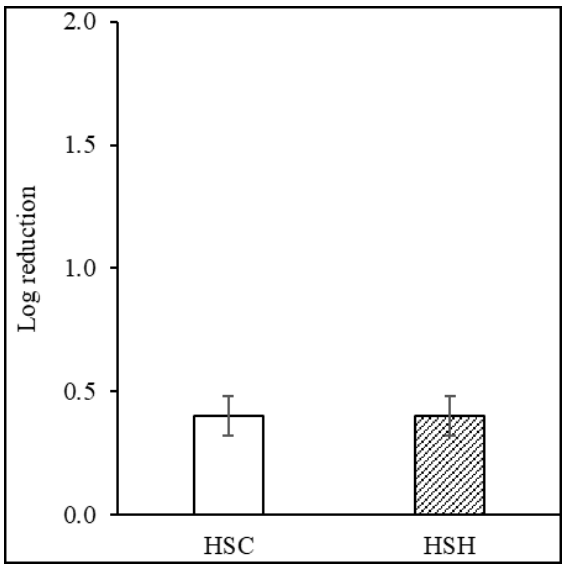

Figure 2 Log reduction of nalidixic acid-resistant *Salmonella* culture in microcentrifuge tube (200 µL, 6 log/mL; XLD-plating) by dipping culture tubes in ice-cold water (0°C) and 50ºC water in either cold-to-hot (HSC) or hot-to-cold (HSH) order. Application time was 2 min for culture to reach 50ºC at 11.1ºC/min.

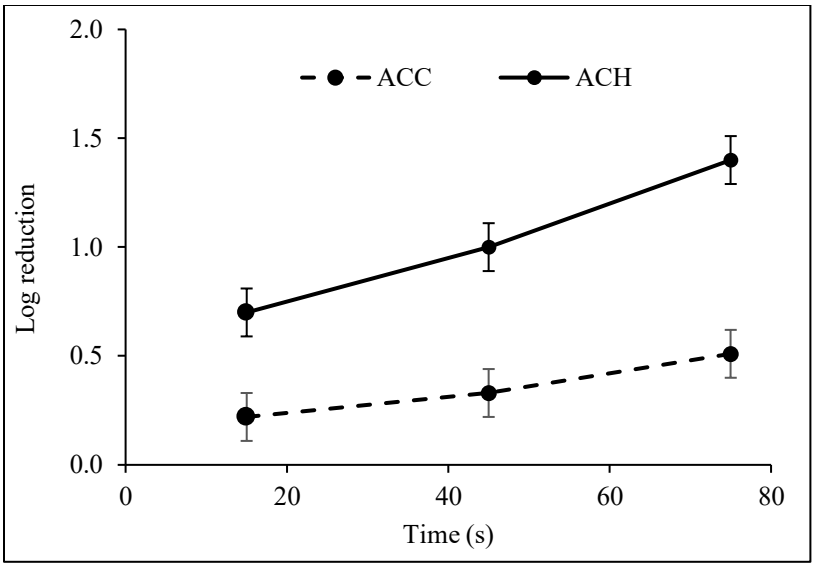

Figure 3 Log reduction of nalidixic acid-resistant *Salmonella* (5-log inoculation; XLD-plating) on pork cubes treated by dipping in 3% acetic acid at room temperature (21°C; ACC) or 50ºC (ACH) for 15, 45, or 75 s.

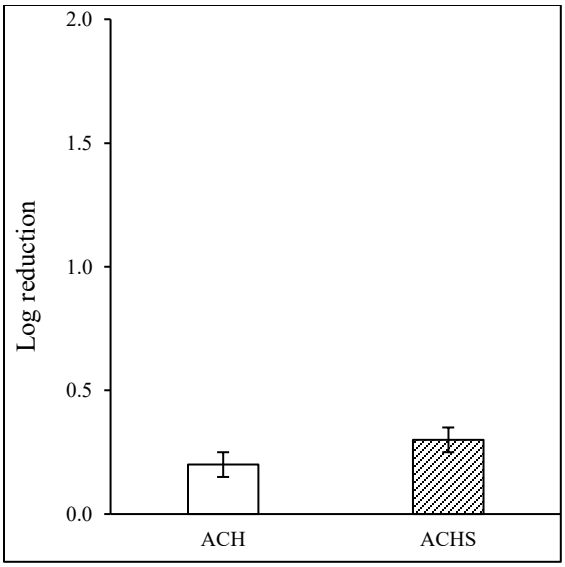

Figure 4 Log reduction of nalidixic acid-resistant *Salmonella* (5-log inoculation; XLD-plating) on pork cubes placed at geometrical center of 2.3-kg pork chubs treated by dipping in 3% acetic acid at 50ºC for 75 s without (ACH) or with handshaking (ACHS).

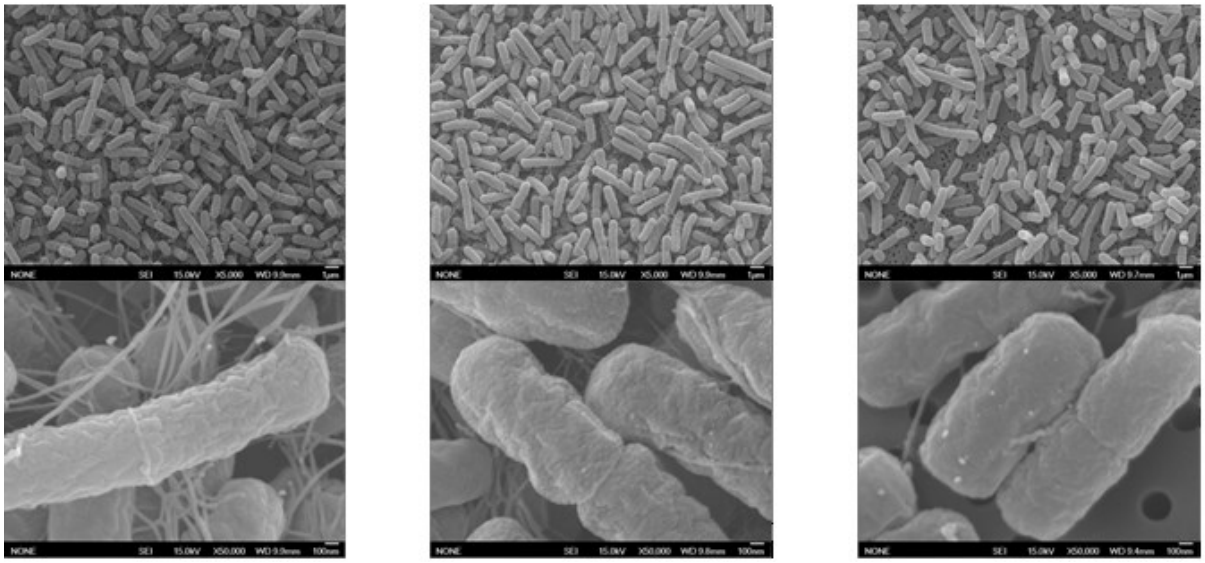

Figure 5 Scanning electron microscopy (SEM) images (top – 5,000X; bottom – 50,000X) of *Salmonella* cells for positive control (left), 45-s (center), and 75-s (right) 3% acetic acid treatment at 50°C.

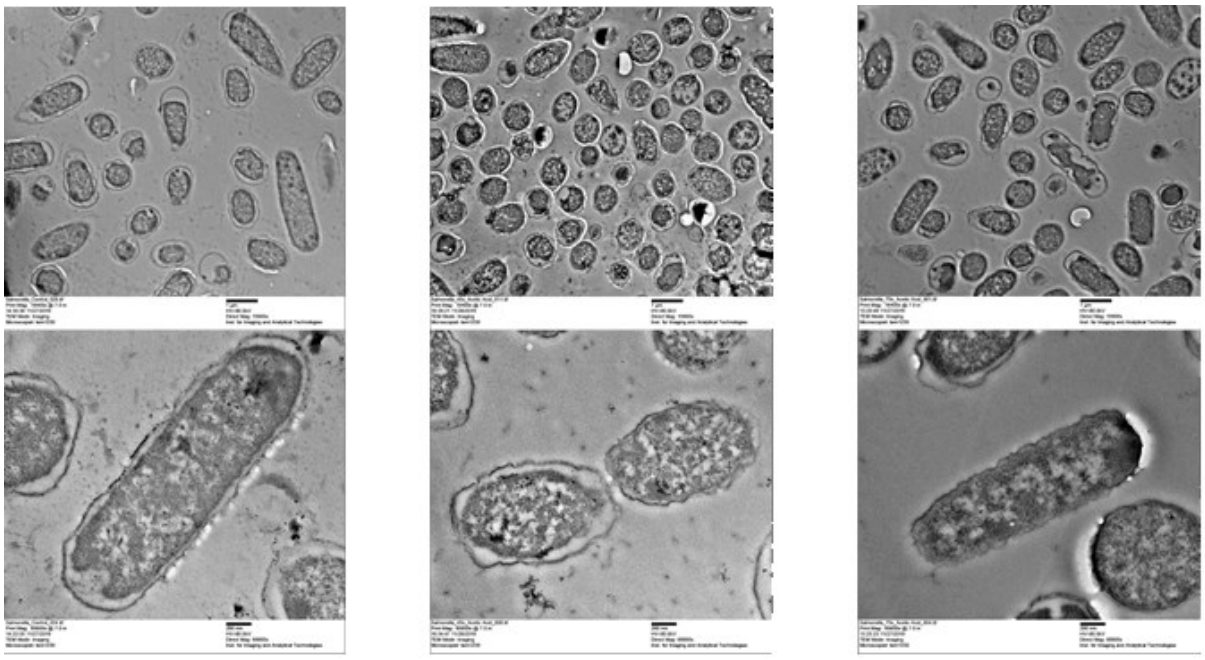

Figure 6 Transmission electron microscopy (TEM) images (top – 5,000X; bottom – 50,000X) of *Salmonella* cells for positive control (left), 45-s (center), and 75-s (right) 3% acetic acid treatment at 50°C.

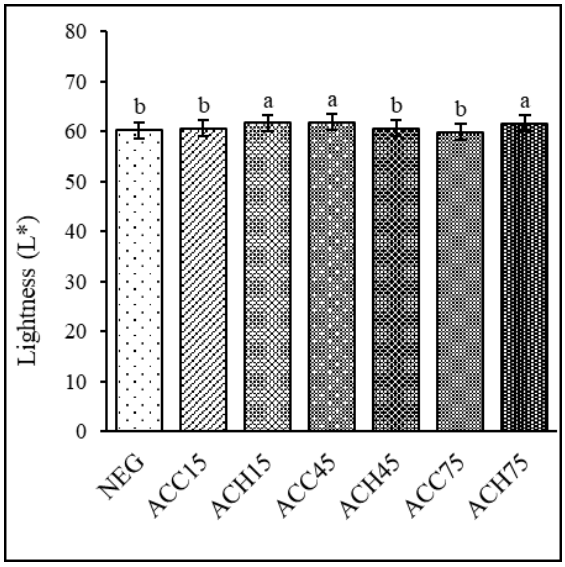

Figure 7a Lightness (L*) of pork *longissimus* muscle serving as negative control (NEG) or treated with 3% acetic acid at room temperature (21°C; ACC) or 50ºC (ACH) for 15, 45, or 75 s, averaged across before- and after-treatment surfaces and cross-sectional surfaces.

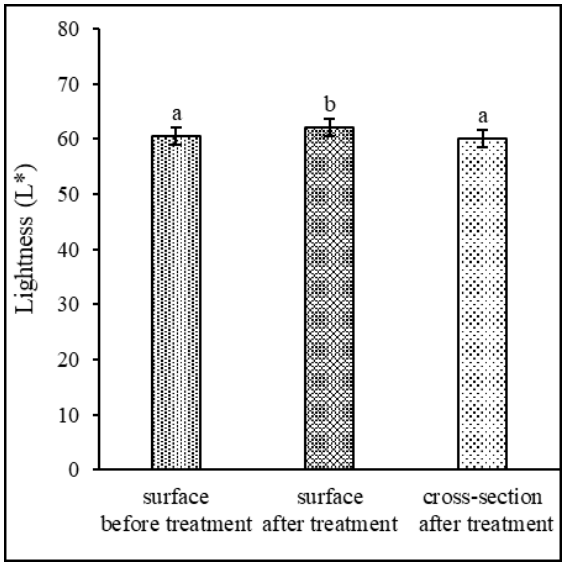

Figure 7b Lightness (L*) before- and after-treatment surfaces and cross-sectional surfaces, averaged across negative control (NEG) and six treatments of 3% acetic acid dipping at room temperature (21°C; ACC) or 50ºC (ACH) for 15, 45, or 75 s.

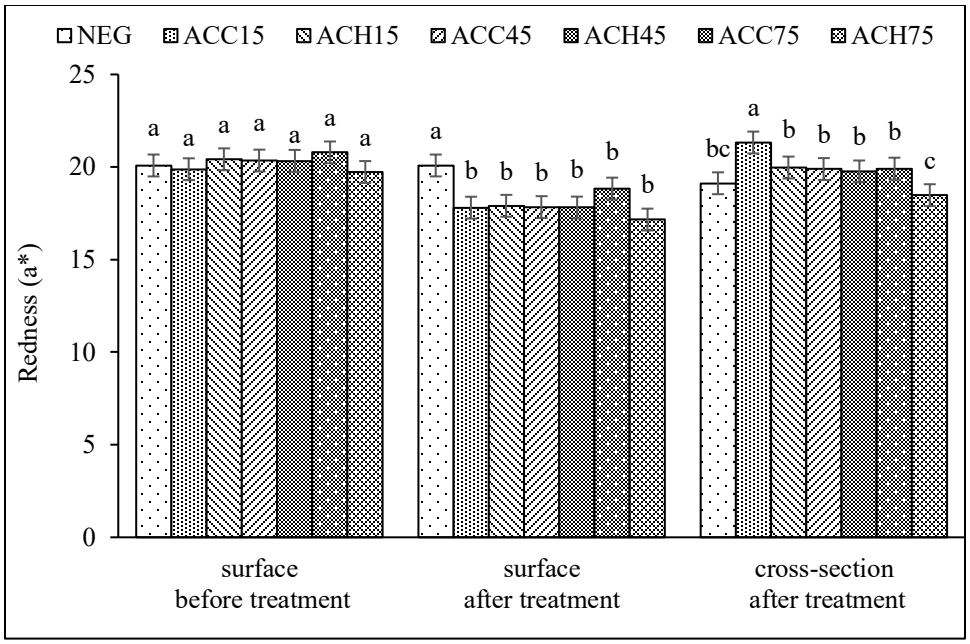

Figure 8 Redness (a*) of pork *longissimus* muscle serving as negative control (NEG) or treated with 3% acetic acid at room temperature (21°C; ACC) or 50ºC (ACH) for 15, 45, or 75 s, on before- and after-treatment surfaces and post-treatment cross-sectional surfaces.

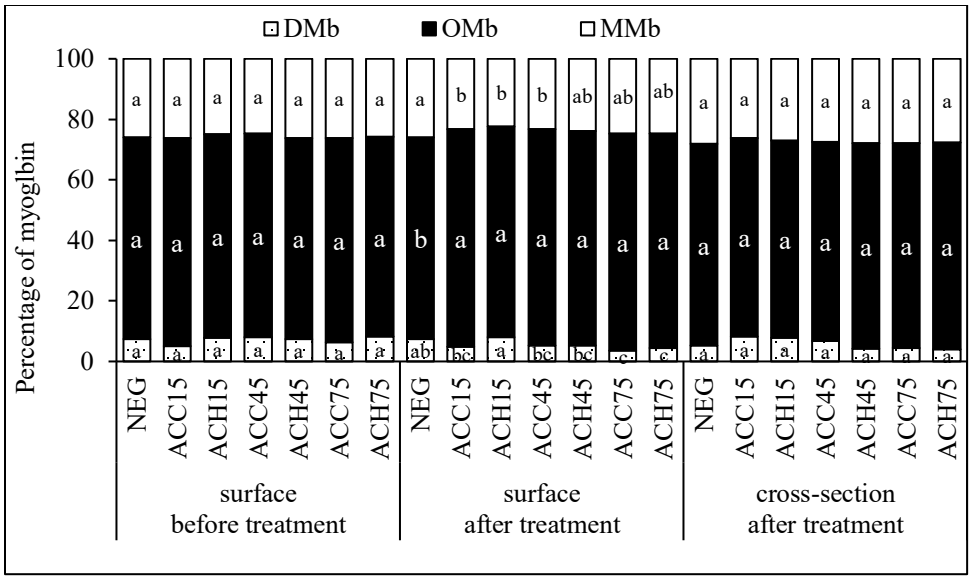

Figure 9 Myoglobin composition (%) of pork *longissimus* muscle serving as negative control (NEG) or treated with 3% acetic acid at room temperature (21°C; ACC) or 50ºC (ACH) for 15, 45, or 75 s, on before- and after-treatment surfaces and post-treatment cross-sectional surfaces.

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