EFFICACY OF ANTIMICROBIAL INTERVENTIONS IN REDUCING *SALMONELLA*, SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI*, *CAMPYLOBACTER*, AND *ESCHERICHIA COLI* BIOTYPE I SURROGATES ON PORK, PORK BY-PRODUCTS, AND BEEF BY-PRODUCTS

A Dissertation

by

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ABSTRACT

Information regarding the effectiveness of antimicrobial interventions on pork carcasses, pork by-products, and beef by-products is limited. To determine the efficacy of various antimicrobial interventions on pork carcasses, hogs were harvested (n = 12) at the Texas A&M University Rosenthal Meat Science and Technology Center (College Station, TX), and skin-on and skinless pieces were designated as hot or chilled for antimicrobial application. Beef and pork head meat, cheek meat, hearts, and pork livers were collected from carcasses harvested at Texas A&M (College Station) and two commercial processing facilities. Warm pieces of all product types were transported to the Texas A&M Food Microbiology Lab where they were inoculated with *Salmonella,* STEC, *Campylobacter* or *Escherichia coli* biotype I surrogates. Following a 30 min microbial attachment period, inoculated pieces were assigned to one of six antimicrobial treatments: 2.5% room temperature lactic acid, 2.5% hot (55 °C) lactic acid, 5.0% room temperature lactic acid, 5.0% hot (55 °C) lactic acid, 400 ppm peroxyacetic acid, and hot (55 °C) water. For each inoculum type, samples were taken before applying the antimicrobial treatment to the warm pork and beef tissue, 30 min, and 24 h post-treatment. For chilled pork, samples were taken after 24 h chill and 30 min after treatment. Objective and trained panel color evaluations were performed on hot and chilled skinless pork, pork head meat, cheek meat, hearts, and livers to assess color attributes before and after antimicrobial application. Lactic acid at both concentrations and temperatures and peroxyacetic acid generally reduced ($P < 0.05$) counts of *Salmonella*, STEC, *E. coli* biotype I surrogates, and *Campylobacter*, whereas hot water did not have a similar impact. Further, greater reductions were seen across all treatments on beef and

pork hearts and pork livers. There were minimal negative impacts on product color, primarily as a result of lactic acid compared to peroxyacetic acid and hot water treatments. This study provides valuable information for beef and pork establishments to help support their food safety programs. Validation of commonly used antimicrobial interventions specific to these products will help the industry comply with regulatory requirements and trade expectations.

DEDICATION

I dedicate this dissertation to my parents, Jerry and Barbara Eastwood, and my grandparents, Eddy and Doris Wolfshohl. Mom, Dad, Meme, and Paw, I would not be the person I am today without the continued guidance and support from y'all. Thank you for always believing in me and pushing me when I didn't believe in myself. I am forever grateful that you have all been on this journey with me, and I couldn't have done it without you. I love you all more than you know.

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Contributors

This work was supervised by a dissertation committee consisting of Dr. Jeffrey W. Savell (co-chair), Dr. Kerri B. Gehring (co-chair), and Dr. Davey B. Griffin of the Department of Animal Science and Dr. T. Matthew Taylor, Graduate Faculty of Food Science and Technology. All work for the dissertation was completed independently by the student.

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CHAPTER I

INTRODUCTION

Improving food safety and reducing the risk of foodborne illness are targeted areas for improvement within the meat industry. It is estimated that about 48 million people become ill, 128,000 are hospitalized, and 3,000 people die from foodborne disease in the United States each year (Centers for Disease Control and Prevention, 2016b). Specifically, the non-typhoidal salmonellae are reported as the leading cause of domestically acquired foodborne illness resulting in hospitalization, followed by Norovirus, *Campylobacter* spp., *Toxoplasma gondii*, and *Escherichia coli* O157:H7, respectively (Centers for Disease Control and Prevention, 2016b). Therefore, research related to the improvement of food safety is necessary for advancement and can provide long-term economic advantages to the meat industry.

To enhance beef safety, antimicrobial interventions such as lactic acid and peroxyacetic acid (PAA) are commonly utilized on beef carcasses and trimmings at multiple points of beef processing (Bosilevac, Nou, Barkocy-Gallagher, Arthur, & Koohmaraie, 2006; Castillo, Lucia, Goodson, Savell, & Acuff, 1998a; Castillo, Lucia, Mercado, & Acuff, 2001; Chen, Ren, Seow, Liu, Bang, & Yuk, 2012; Ellebracht, Castillo, Lucia, Miller, & Acuff, 1999; Hamby, Savell, Acuff, Vanderzant, & Cross, 1987; Snijders, van Logtestijn, Mossel, & Smulders, 1985). However, research on the effectiveness of these interventions for specific pathogens of concern for pork carcasses, chilled pork, and edible pork and beef by-products such as head meat, cheek meat, hearts, and livers is more limited. Additional information related to the effectiveness of these interventions could prove beneficial.

Information from this study could help beef and pork establishments support their food safety programs. The United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) Compliance Guideline Hazard Analysis and Critical Control Point (HACCP) Systems Validation (USDA-FSIS, 2015b) states, "…the scientific support for raw meat and poultry products should also be sufficiently related to the process, product, and hazard identified in the hazard analysis… For example, in slaughter establishments, interventions such as lactic acid and peroxyacetic acid (PAA) have been found to perform differently for different pathogens (e.g., *Salmonella* and *E. coli* O157:H7) and different species (e.g., poultry vs. beef)…". Therefore, information specific to the product and pathogen of concern is necessary to ensure maximum efficacy and properly support decisions in a processor's food safety system.

Export markets are of great economic value to the U.S. meat industry, and it is equally important to supply safe products for export. Edible by-products from beef and pork are crucial for the profitability and longevity of international food products trade (USDA-ERS, 2011). With the validation of lactic acid and PAA for pork and beef by-products, the industry will be better equipped to comply with food safety-focused requirements and expectations set forth by trade regulations. Results from this research will be applied to both the U.S. beef and pork industries spanning both domestic and global markets. Information related to the efficacy of targeted interventions in pork products and beef and pork edible by-products could enhance the viability and sustainability of trade across the world.

CHAPTER II

REVIEW OF LITERATURE

2.1 Foodborne pathogens and human illness

Foodborne pathogens and resulting illnesses have long been a concern for the food industry. Pathogens are categorized as bacteria, viruses, and other microorganisms that can cause illness or disease. In 1820, German poet Justinus Kerner noted an illness, likely botulism, with a high fatality rate and described it as "sausage poisoning" (Jay, Loessner, & Golden, 2005). In addition, *Salmonella enteritidis* was isolated from meat in 1888 by Gaertner and caused 57 cases of illness (Jay et al., 2005). *E. coli* was documented as a foodborne pathogen after causing an outbreak of foodborne gastroenteritis from imported cheeses in the U.S. in 1971, with outbreaks in other countries occurring as early as the 1940s. Further, the first two outbreaks of foodborne *E. coli* O157:H7 hemorrhagic colitis were reported in Oregon and Michigan in 1982 (Riley et al., 1983). More recently, non-typhoidal *Salmonella* and *Campylobacter spp*. ranked among the top five pathogens contributing to domestically acquired foodborne illnesses in the U.S. (Centers for Disease Control and Prevention, 2016b). Because the food system is very complex and encompasses many multifaceted industries, attributing illnesses related to food often can be challenging. However, the Centers for Disease Control (CDC) has been able to characterize food categories and their association with foodborne illness. A study conducted by the CDC found that produce accounted for almost half of the reported illnesses, with meat and poultry being the more common sources of fatal infections, likely caused by *Salmonella* or *Listeria* (Centers for Disease Control and Prevention, 2016a). Despite advancements in food safety, illnesses from

foodborne pathogens remain at the forefront of the food industry's efforts for enhancing consumer safety.

2.2 *Escherichia coli*

E. coli was first discovered in 1885 by German bacteriologist Theodor Escherich through his research on the microflora of infant gastrointestinal tracts. *E. coli* is a gram-negative, non-spore forming, rod shaped, facultative anaerobe bacterium that colonizes in the intestines of humans and warm-blooded animals (Lim, Yoon, & Hovde, 2010; Robins-Browne & Hartland, 2002; Stenutz, Weintraub, & Widmalm, 2006). As members of the *Enterobacteriaceae* family, *E. coli* along with *Salmonella* spp., *Klebsiella* spp., *Shigella* spp., and *Yersinia* spp. have all been linked to illness in humans. Among the *Escherichia* genus, there are six species: *E. coli, E. albertii, E. fergusonii, E. hermannii, E. blattae*, and *E. vulneris* (Huys, Cnockaert, Janda, & Swings, 2003; Rice, Sowers, Johnson, Dunnigan, Strockbine, & Edberg, 1992). Because *E. coli* colonizes in the intestines of warm-blooded animals, livestock animals such as cattle, sheep, pigs, and poultry have been known to be carriers, with cattle being a primary carrier (Barkocy-Gallagher et al., 2003; Coia, 1998; Jay et al., 2005). Even though *E. coli* is a common inhabitant in the gastrointestinal tract of warm-blooded animals, it is characterized as a non-pathogenic commensal and is thus not generally harmful to its host (Nataro & Kaper, 1998; Stenutz et al., 2006).

E. coli has an optimum growth temperature of 35 - 37 °C, but has the ability to grow poorly at 7 °C and up to 46 °C (Doyle & Schoeni, 1984; Nataro & Kaper, 1998). In addition, optimum pH for growth ranges from 6.5 - 7.0, and requires a minimum a_w of 0.95 for growth (Jay et al., 2005). These bacteria are characterized by their ability to ferment glucose with production of acid and gas for most strains and does not possess great heat resistance with a D-

value of 60 °C for 0.1 min (Jay et al., 2005). Due to this organism's survival and growth properties, *E. coli* can easily survive on fresh meat products but is easily destroyed through proper cooking or heat processing.

For *E. coli* serotyping, there are three antigens which can be utilized to differentiate antigenic properties as first described by Kauffmann (1947). These are the somatic (O) antigen, flagellar (H) antigen, and capsular polysaccharide (K) antigen (Stenutz et al., 2006). There are over 200 O serogroups, whereas H and K serotypes are fewer for *E. coli* (Jay et al., 2005). Kauffmann (1947) noted that strains possessing the K antigen are more toxic. For O and H antigens, the O antigen denotes the serogroup and the H antigen identifies the serotype for a given strain (Meng, LeJeune, Zhao, & Doyle, 2013), and thus unique combinations of O and H groups describe specific pathogens (Nataro & Kaper, 1998; Stenutz et al., 2006). This nomenclature system is widely utilized to distinguish and differentiate pathogens based on their antigen properties (Kauffmann, 1947; Robins-Browne & Hartland, 2002).

2.3 Shiga toxin-producing *Escherichia coli* (STEC)

Some *E. coli* such as the generic *E. coli* are non-pathogenic organisms; however, certain types are well-known foodborne human pathogens. Types of pathogenic *E. coli* include the Enteropathogenic (EPEC), Enterotoxigenic (ETEC; common cause of traveler's diarrhea), Enteroinvasive (EIEC), Enterohemorrhagic (EHEC), Enteroaggregative (EAggEC), and Diffusely adherent (DAEC) *E. coli* (Jay et al., 2005; Nataro & Kaper, 1998; Robins-Browne & Hartland, 2002). EPEC cause watery diarrhea and was the first pathogroup identified, but do not produce a heat-labile (LT) or heat-stable (ST) toxins, whereas ETEC and EAggEC have been associated with diarrhea worldwide and have the ability to produce toxins (Meng et al., 2013). Specifically, ETEC are more prevalent in areas with poor sanitation and produce a toxin similar

to the cholera toxin, whereas EAggEC have the unique ability to produce aggregative adherence on HEp-2 cells and produce a LT toxin (Meng et al., 2013). EIEC cause diarrhea and dysentery but do not produce LT, ST or Shiga (Stx) toxin. *E. coli* O157:H7 falls within the EHEC and is categorized by its ability to produce Stx toxins, resulting in bloody diarrhea and hemolytic uremic syndrome (HUS) (Meng et al., 2013). EHEC possess virulence factors cytotoxic to Vero cells and are thus named verotoxins or Shiga toxins due to their similarity to toxins produced by *Shigella dysenteriae* encoded on shigellae prophages (Meng et al., 2013).

Two outbreaks in 1982 resulted in illness that was characterized by abdominal pain, watery followed by bloody diarrhea, and little to no fever in people that ate at the same fast-food chain (Riley et al., 1983). At the time Riley et al. (1983) described the illness as a rare serotype (O157:H7) of *E. coli* and noted the only other serotype isolation was from a sporadic incidence in 1975. *E. coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* such as O26, O45, O103, O111, O121, O145 (commonly referred to as the "Big Six") have caused serious illness including hemorrhagic colitis, hemolytic uremic syndrome (HUS), and even death (Smith, Fratamico, & Gunther IV, 2013). *E. coli* O157:H7 and non-O157 STEC foodborne illness is characterized by severe diarrhea that can be bloody, abdominal pain, cramps, fever, and vomiting (Smith et al., 2013). Hemorrhagic colitis or bloody diarrhea occurs in most patients with this illness, whereas HUS occurs in a smaller percentage of patients, often children, the elderly or immunocompromised (Smith et al., 2013). In more severe cases, HUS can result in destruction of red blood cells, acute kidney failure, and low platelet counts, whereas thrombotic thrombocytopenic purpura (TTP) can result in blood clots throughout the body (Smith et al., 2013). Susceptibility of infection depends on the vulnerability of a host and thus may require a lower number of organisms necessary to make an individual sick in those that are

immunocompromised (Lund & O'Brien, 2011). Onset of illness can occur within a few days after ingestion and can last several days, depending on severity (Bryan, Youngster, & McAdam, 2015).

EHEC strains are characterized by the presence of specific virulence factors by genes encoding intimin (*eae*), hemolysin (*hlyA*), and Shiga toxins (Stx1 and Stx2) (Fagan, Hornitzky, Bettelheim, & Djordjevic, 1999; Wieler et al., 1996). The *eae* gene aids in formation of the attaching and effacing (AE) lesion in enterocytes, but also requires a type III secretion system and many other genes (Wieler et al., 1996). Shiga toxin-producing *E. coli*, sometimes referred to as verotoxigenic (VTEC), have at least one Shiga like toxin encoded by either Stx1 or Stx2 (Bryan et al., 2015; Fagan et al., 1999). Both toxins are AB_5-type toxins that have the ability to disrupt protein synthesis in the host resulting in apoptotic cell death (Melton-Celsa, Mohawk, Teel, & O'Brien, 2011). In addition, toxins bind to the globotriaosylceramide (Gb_3) endothelial cell surfaces of host cells, thus causing damage to the endothelial cells in the intestinal lining (Melton-Celsa et al., 2011; Smith et al., 2013). Although both toxins cause illness, Stx2 is more commonly isolated from clinical samples (Bryan et al., 2015). Illness from *E. coli* O157:H7 can result from a relatively low infectious dose, causing illness with fewer than 100 cells (Jaeger & Acheson, 2000; Smith et al., 2013). STEC are a serious food safety risk and have been associated with hemorrhagic colitis and HUS, with *E. coli* O157:H7 being the most widely known and a leading cause of STEC-related foodborne illness.

2.4 *Salmonella*

Salmonella was named for veterinary surgeon Dr. Daniel Salmon. Theobald Smith, a scientist working under Salmon's direction, discovered *Salmonella enterica* during his studies of hog cholera in 1885. These bacteria were formerly named *Salmonella choleraesuis* because it

was believed to be the cause of hog cholera, but it was later discovered that hog cholera resulted from a viral infection (Schultz, 2008). *Salmonella*, like *E. coli*, is an inhabitant of the intestinal tract of animals and are small gram-negative, non-spore forming rods that are members of the *Enterobacteriaceae* family. The two bacteria are indistinguishable under the microscope and on ordinary nutrient media (Jay et al., 2005). Nontyphoidal *Salmonella* ranks among the top five pathogens causing the most foodborne illness, hospitalization, and deaths. Specifically, *Salmonella* ranks second behind Norovirus for pathogens contributing to domestically acquired foodborne illness (Centers for Disease Control and Prevention, 2016b). In addition, *Salmonella* ranks first among pathogens contributing to domestically acquired foodborne illness resulting in hospitalizations and domestically acquired foodborne illnesses resulting in death (Centers for Disease Control and Prevention, 2016b).

There are two species of *Salmonella*, *S. bongori* and *S. enterica* that are related to human illness, however, *S. enterica* is more commonly associated with foodborne illness (McClelland et al., 2001). Within *S. enterica* subsp. *enterica*, there are over 2,400 serovars, which include some of the most common foodborne illness culprits: *Salmonella* Typhimurium and *Salmonella* Enteriditis (Jay et al., 2005; McClelland et al., 2001). Serotyping of *Salmonella* is achieved by grouping species and serovars into A, B, C…groups based upon similarities of O antigens (Jay et al., 2005). In addition, classification using the H antigen is employed for further classification. Further, there are two types of antigens, specific or phase 1 and group or phase 2, with the group or phase 2 antigens being more widely distributed among species (Jay et al., 2005).

Salmonella can cause two types of illness, typhoid (typhoid fever) and non-typhoidal enteritis (more common to foodborne illness). Salmonellosis is most often contracted from contaminated food products such as red meat, poultry, milk, vegetables, and eggs. Contamination

of meat and poultry may occur during the harvest process because *Salmonella* is an enteric pathogen. In addition, cross-contamination by consumers is often largely responsible for *Salmonella* transfer from one product to another, though this may be easily avoided. Non-typhoidal Salmonellosis is characterized by an incubation period of 6 - 48 hours, a duration of 4 - 7 days; a low infectious dose is possible (with as few as 1 cell causing illness), with symptoms including nausea, vomiting, diarrhea, abdominal cramping, fever, and headache (Kothary & Babu, 2001).

Salmonella enterica serovars possess pathogenicity islands (SPI-1 and SPI-2) which aid in their pathogenesis and ability to cause human illness (Jay et al., 2005; Marcus, Brumell, Pfeifer, & Finlay, 2000). The infection is initiated when the pathogen colonizes the Peyer's patches of the intestine and penetrates the intestinal barrier via M cells (Marcus et al., 2000; Van Der Velden, Bäumler, Tsolis, & Heffron, 1998). From there, *Salmonella* are endocytosed by a ruffle-like mechansim thus enclosing the bacterium and creating a vacuole (Hansen-Wester & Hensel, 2001; Marcus et al., 2000). The pathogen thus gains access to mesenteric lymph follicles and macrophages where they then begin to replicate (Hansen-Wester & Hensel, 2001; Marcus et al., 2000). The previously mentioned SPI-1 and SPI-2 pathogenicity islands contain large clusters of genes which contribute to the virulence of *Salmonella enterica* (Marcus et al., 2000). In addition, this pathogen contains a type III secretion system that allows *Salmonella* to inject virulence proteins into its host (Hensel et al., 1998; Marcus et al., 2000).

This organism can grow from approximately 10 - 45 °C with an optimum growth range of approximately 35 - 37 °C and has an optimum pH range for growth near neutrality, ranging from 6.6 - 8.2 (Jay et al., 2005). These bacteria are characterized by their ability to ferment glucose with the production of gas, but are generally unable to ferment sucrose or lactose (Jay et

al., 2005). *Salmonella* is also relatively sensitive to heat and is thus easily destroyed at milk pasteurization temperatures, but requires a minimum a_w of 0.94 for growth, with higher a_w necessary as pH decreases (Jay et al., 2005). However, *Salmonella* has the ability to adapt to stressors such as extreme temperatures, pH, and lack of nutrients, thus allowing it to survive for extended periods in drier environments (Podolak, Enache, Stone, Black, & Elliott, 2010). Therefore, the adaptability of this pathogen enhances its thermal resistance in foods with low moisture properties. *Salmonella* is a leading cause of domestically acquired bacterial foodborne illness, hospitalizations, and deaths in the U.S., and thus remains at the forefront of the meat industry's efforts to reduce prevalence and severity of this pathogen in the food supply (Centers for Disease Control and Prevention, 2016b).

2.5 *Campylobacter jejuni*

Campylobacter was first noted in 1886 by Theodor Escherich while he studied stool samples from children with diarrhea (Altekruse, Stern, Fields, & Swerdlow, 1999). Over time, researchers identified campylobacters from various sources, but it was not until the development of more selective media in the 1970s that allowed the isolation of *Campylobacter* from human stool samples (Altekruse et al., 1999; Jay et al., 2005). This development aided in the identification of *Campylobacter* spp. as a human pathogen. *Campylobacter* spp., like *Salmonella,* ranks among the top five pathogens contributing to foodborne illnesses in the U.S. (Centers for Disease Control and Prevention, 2016b). Specifically, *Campylobacter* spp. is the fourth leading cause of domestically acquired foodborne illness, whereas this pathogen ranks third and fifth for pathogens contributing to domestically acquired foodborne illness resulting in hospitalizations and death, respectively (Centers for Disease Control and Prevention, 2016b). In addition, this

pathogen is a leading cause of gastroenteritis worldwide (Altekruse et al., 1999; Jay et al., 2005; Kaakoush, Castaño-Rodríguez, Mitchell, & Man, 2015).

Campylobacter jejuni, like *E. coli* and *Salmonella*, is an enteric pathogen associated with warm-blooded animals, especially poultry (Altekruse et al., 1999; Jay et al., 2005). In the developed world, most often campylobacteriosis disease arises from contaminated poultry products, whereas in less developed areas, the water supply is a common source (Young, Davis, & DiRita, 2007). Unpasteurized milk is another common source of the pathogen. *Campylobacter* spp., from the *Campylobacteraceae* family of bacteria, are gram-negative spiral, rod-shaped bacterium that possess a flagellum at one or both ends of the cell (Jay et al., 2005; Kaakoush et al., 2015). This organism is microaerophilic; thus growth is inhibited when oxygen levels reach 21% (Jay et al., 2005). Growth conditions are best when CO_2 is present at approximately 5 - 10% and oxygen is at approximately 3 - 6% (Jay et al., 2005). In addition, the optimum temperature for growth is approximately 40 – 42 °C and grows best at a pH range of 5.5 – 8.0 (Jay et al., 2005). *Campylobacter jejuni* is also heat sensitive and thus easily destroyed when heat is applied. Several *Campylobacter* spp. can cause human gastroenteritis, *Campylobacter jejuni* is the most important foodborne pathogen among other species. While there is extensive genomic information about *Campylobacter* spp., there is less information about virulence factors. Compared with other well-known pathogens that cause gastroenteritis, *Campylobacter jejuni* does not possess numerous virulence factors (Dasti, Tareen, Lugert, Zautner, & Groß, 2010). *Campylobacter jejuni* is able to utilize N-linked glycosylation for colonization, adherence, and invasion of its host (Dasti et al., 2010). The pathogen then colonizes the intestines and produces a cytolethal distending toxin, which is necessary for the induction of host cell apoptosis and is the major pathogenicity virulence factor (Dasti et al., 2010).

Campylobacter illness is characterized by watery or bloody diarrhea, fever, and abdominal cramping, but severe cases of campylobacteriosis may lead to autoimmune conditions such as the Guillain-Barrè (GBS) and Miller Fisher syndrome (Dasti et al., 2010; Kaakoush et al., 2015). In addition, in a small number of patients, *Campylobacter* infection has been linked to extragastrointestinal complications such as bacteremia, lung infections, brain infections, and meningitis (Kaakoush et al., 2015). Infection can be caused by fewer than 1,000 cells; Hara-Kudo and Takatori (2011) noted that a dose as low as 360 CFU caused campylobacteriosis from raw beef liver. Onset of illness generally occurs within 24 – 72 h after ingestion and can last 2 – 10 d (Jay et al., 2005; Kaakoush et al., 2015). As a leading cause of gastrointestinal illness in the U.S. and across the globe, this enteric pathogen is of great concern for the food industry (Kaakoush et al., 2015).

2.6 Food safety regulation

As causes of foodborne illness in the U.S., STEC, nontyphoidal *Salmonella*, and *Campylobacter* spp. among other pathogens, require oversight from food safety governing bodies. According to the CDC 2015 FoodNet Surveillance Report, there were 20,098 laboratory-confirmed infections, 4,598 hospitalizations, and 77 deaths related to bacterial and parasitic pathogens (Centers for Disease Control and Prevention, 2017). Specifically, there were 465 laboratory-confirmed cases of O157 STEC, 807 cases of non-O157 STEC, 7,719 cases of *Salmonella,* and 6,289 cases of *Campylobacter* (Centers for Disease Control and Prevention, 2017). Of the "Big Six" non-O157 STEC, O26, O103, and O111 were the top three laboratory-confirmed non-O157 STEC causing infections (Centers for Disease Control and Prevention, 2017). However, there are limitations to these data, as many cases of foodborne illness go unreported, and thus the illnesses associated with these pathogens is likely greater. Therefore,

federal agencies such as the USDA-FSIS and the U.S. Food and Drug Administration (FDA) have implemented food safety regulation as a result of this foodborne illness burden.

As a result of the Jack-in-the-Box *E. coli* O157:H7 outbreak in 1993 from undercooked beef patties, four children died and hundreds of others became ill (Centers for Disease Control and Prevention, 2001). *E. coli* O157:H7 was announced as an adulterant by USDA-FSIS in 1994 and with the addition of the "Big Six" non-O157 STEC, which include O26, O45, O103, O111, O121, and O145 in 2011, there are now seven STEC considered adulterants in raw, non-intact beef products and raw, intact beef products intended for raw non-intact use (USDA-FSIS, 2012). After the declaration of O157:H7 as an adulterant in raw beef products, there were some other significant changes in the meat industry to shift food safety efforts from a system that was more preventative instead of reactive and based upon scientific evidence. In July 1996, the USDA-FSIS published the Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) System final rule to be implemented in meat and poultry establishments to reduce the occurrence and numbers of pathogens, thus improving food safety (Schlosser et al., 2000; USDA-FSIS, 1996). The new regulation, which were to be implemented over time based upon establishment size, required meat and poultry processors to do several things: implement written sanitation standard operating procedures (SSOPs), have regular microbial testing in slaughter facilities to verify process controls related to prevention and reduction of fecal contamination, comply with pathogen reduction standards for *Salmonella* for slaughter facilities and establishments producing raw ground products, and required all meat and poultry establishments to implement HACCP (USDA-FSIS, 1996). HACCP, as outlined by 9 CFR part 417, is a system by which meat and poultry establishments utilize 7 principles as outlined by the National Advisory Committee on Microbiological Criteria for Food (NACMCF) to evaluate their processes,

implement preventive controls, and verify that their system is operating according to their plan (FDA, 2017; USDA-FSIS, 1996). *Salmonella* performance standards also were set forth in an effort to reduce pathogen contamination of finished meat and poultry carcasses to a level below the national baseline (USDA-FSIS, 1996).

After the declaration of the "Big Six" as adulterants in raw, non-intact beef in 2011, USDA-FSIS announced in May 2012 that they would implement additional verification testing in raw beef trimmings for these additional STEC (USDA-FSIS, 2012). Further, in May 2015 USDA-FSIS required the labeling designation of mechanically tenderized, blade tenderized or needle tenderized on raw or partially cooked beef products (USDA-FSIS, 2015a). In addition, FSIS has performance standards for *Salmonella* and *Campylobacter* in raw chicken parts and not ready-to-eat (NRTE) comminuted poultry products (USDA-FSIS, 2017b). However, there are no current performance standards for *Campylobacter* in species other than poultry. These ever-evolving regulations are an important component for the meat and poultry industry to produce the safest products possible for consumers.

2.7 Source and prevalence of pathogenic bacteria

There has been extensive research related to the prevalence of pathogenic bacteria on cattle hides, as hide contamination can result in carcass contamination during slaughter (Arthur, Barkocy-Gallagher, Rivera-Betancourt, & Koohmaraie, 2002; Arthur et al., 2004; Bacon, Belk, Sofos, Clayton, Reagan, & Smith, 2000). Contamination of a carcass during slaughter is a result of fecal or gastrointestinal contamination that may be inherent to an animal, a result of lairage environments or from contact with other animals, and cross-contamination during processing (Arthur et al., 2010; Barkocy-Gallagher et al., 2003). In pigs at slaughter, contamination also can result from the skin or exterior surfaces of the carcass in addition to fecal or gastrointestinal

contamination (Berends, Van Knapen, Snijders, & Mossel, 1997). Berends et al. (1997) also estimated that 5 – 15% of all *Salmonella* carcass contamination during hog slaughter is a direct result of contaminated polishing equipment after singeing. In a study conducted by Arthur et al. (2002), carcass sponge samples were obtained from four large U.S. beef processors to determine the prevalence of O157:H7 and non-O157 STEC and they found 54% of samples were positive for non-O157 STEC when sampled before evisceration. In addition, 8% of samples were positive for non-O157 STEC after the application of an antimicrobial intervention (Arthur et al., 2002). Elder, Keen, Siragusa, Barkocy-Gallagher, Koohmaraie, and Laegreid (2000) noted that prevalence of O157 STEC at Midwestern U.S. processing plants was 28% from feces samples and 11% from hide samples. Sampling results from various processing steps indicate that 43% of samples were positive at pre-evisceration, 18% positive at post-evisceration (before antimicrobial intervention), and 2% positive at post-processing (Elder et al., 2000). Further, there was a positive correlation observed between O157 STEC prevalence in feces, hides, and carcass contamination (Elder et al., 2000). The prevalence of *E. coli* O157:H7 and *Salmonella* was evaluated at two geographically distant (one northern plant and one southern plant) beef processors, and researchers found the prevalence of both pathogens to be higher in hides sampled at the southern plant, thus showing geography-specific differences among plants (Elder et al., 2000). Arthur et al. (2004) also noted high (75.7%) prevalence of *E. coli* O157:H7 on cattle hides at slaughter, but showed lower prevalence rates pre-evisceration, post-evisceration, and post-intervention. Specifically, for both plants the overall prevalence rate was 14.7% pre-evisceration, 3.8% post-evisceration, and 0.3% post-intervention (Arthur et al., 2004). In addition, both plants surveyed in this study employed a multiple hurdle intervention system, which aided in less than detectable O157 populations on chilled carcasses (Arthur et al., 2004).

Letellier, Messier, and Quessy (1999) found 5.2% of fecal samples from finishing pigs were positive for *Salmonella* in Canadian processing plants. *Campylobacter* prevalence in swine at slaughter was studied and researchers found that 33% of post-exsanguination, 0% post-polishing, 7% pre-chill, and 0% post-chill carcasses were positive for *Campylobacter* (Pearce et al., 2003). *Campylobacter* also was recovered from all (100%) composite fecal samples (Pearce et al., 2003). Thakur and Gebreyes (2005) noted a higher prevalence of *Campylobacter* post-evisceration versus pre-evisceration and showed that carcass chilling reduced pathogen prevalence. Farzan, Friendship, Cook, and Pollari (2010) studied the pathogen occurrence from finishing pigs, sows, and weaned pigs using samples from manure storage tanks and fresh feces. *Salmonella* were isolated from 31.5% of samples, *Campylobacter* from 36.5% of samples, and *E. coli* O157 from 3.3% of samples, with manure storage tank samples being more likely detected for all pathogens (Farzan et al., 2010). In a Canadian national surveillance program, *Salmonella* were isolated from 17.5% of pork and 2.6% of beef carcass samples, whereas *Campylobacter* were isolated from 16.9% of pork and 22.6% of beef carcass samples (Lammerding et al., 1988).

Results from a study conducted by Koohmaraie et al. (2007) suggested that transportation and lairage environments can increase the prevalence of *Salmonella* and *E. coli* O157:H7 on cattle hides at slaughter. Some cattle have been characterized as super shedders, which are those animals that shed *E. coli* O157:H7 at higher ($> 10^4$ CFU/g) levels than other cattle in the population (Arthur et al., 2010; Omisakin, MacRae, Ogden, & Strachan, 2003). Omisakin et al. (2003) found 9% of feces samples positive for O157 were considered super shedders. In addition, seasonality plays a role in the prevalence of pathogenic bacteria. Barkocy-Gallagher et al. (2003) found that the prevalence of *E. coli* O157:H7 and *Salmonella* in feces was highest in the summer months and had a higher hide prevalence in both summer and fall months at

Midwest fed cattle slaughter plants. The roles of geographic differences, seasonality, and super-shedding animals are not fully understood, but certainly have an impact on the prevalence of pathogenic bacteria present at slaughter.

In a global review study, Hussein and Bollinger (2005) described a wide range of *E. coli* O157 prevalence in beef. This review noted prevalence rates of 0.01% to 43.4% for beef carcass samples obtained from packing plants and 0.1% to 54.2% from supermarket samples of ground beef (Hussein & Bollinger, 2005). Retail pork and beef in the Washington, DC area were assessed for the prevalence of *Campylobacter, E. coli* and *Salmonella* (Zhao et al., 2001). For the beef samples, 19.0%, 1.9%, and 0.5% of samples were positive for *E. coli, Salmonella,* and *Campylobacter*, respectively. Retail pork samples showed a similar trend of prevalence with 16.3% positive for *E. coli*, 3.3% positive for *Salmonella,* and 1.7% positive for *Campylobacter* (Zhao et al., 2001). USDA-FSIS conducted a nationwide microbiological baseline study from August 2010 – August 2011 on market hogs to determine the prevalence of *Salmonella*, *E. coli*, aerobic plate counts, *Enterobacteriaceae*, and total coliforms pre-evisceration and post-chill (USDA-FSIS, 2018). Results from this study showed a 69.6% *Salmonella* prevalence rate at pre-evisceration, whereas the post-chill prevalence was 2.7% (USDA-FSIS, 2018). In addition, *Enterobacteriaceae* prevalence was 98.1% pre-evisceration and 24.2% post-chill and generic *E. coli* prevalence was 95.8% pre-evisceration and 11.8% post-chill. A similar study on beef carcasses from August 2014 – December 2015 showed *Salmonella* positives post-hide-removal on 27.1% of carcasses, *E. coli* O157:H7 on 1.8%, and non-O157 STEC on 6.1% (USDA-FSIS, 2018). Pathogen prevalence varies among specie and pathogen, however, the fact that there is pathogen prevalence throughout the production chain illustrates the continued need for development and implementation of novel intervention strategies.

2.8 Antimicrobial interventions and their use in controlling foodborne pathogens in meat

Antimicrobial interventions are commonly used throughout beef and pork processing in an effort to enhance food safety. Through the use of various antimicrobial interventions and sanitary dressing procedures (i.e., trimming by knife, worker sanitary practices, equipment sanitation), processors have been able to mitigate the prevalence of pathogenic bacteria. However, because livestock species are known carriers of enteric pathogens such as *E. coli*, *Salmonella,* and *Campylobacter*, they continue to be possible contaminants throughout the slaughter process (Jay et al., 2005; Lim et al., 2010). Even though the muscle of livestock is considered sterile, some frequency of contamination during processing is likely inevitable despite best efforts (Dorsa, 1997; Ellebracht et al., 2005; Jimenez-Villarreal, Pohlman, Johnson, & Brown, 2003; Prasai, Acuff, Lucia, Hale, Savell, & Morgan, 1991; Prasai, Acuff, Lucia, Morgan, May, & Savell, 1992).

Antimicrobial interventions such as lactic acid and peroxyacetic acid are commonly employed as control measures throughout multiple points of beef harvest and product manufacture, with lactic acid arguably being the most widely utilized antimicrobial intervention (Bosilevac et al., 2006; Castillo et al., 1998a; Castillo et al., 2001; Chen et al., 2012; Ellebracht et al., 1999; Hamby et al., 1987; Snijders et al., 1985). Research has suggested that sequential multiple hurdle interventions reduce bacteria on beef carcasses more effectively than any one intervention alone (Bacon et al., 2000; DeGeer, Wang, Hill, Singh, Bilgili, & Bratcher, 2016; Delmore, Sofos, Schmidt, & Smith, 1998). In pork slaughter, although scalding and singeing are an inherent part of the slaughter, these processes may contribute to carcass decontamination through the addition of heat (Loretz, Stephan, & Zweifel, 2011). Nonetheless, cross-contamination from other carcasses and equipment may occur and antimicrobial interventions

are often applied throughout the process. Epling, Carpenter, and Blankenship (1993) determined a 2% lactic acid solution was effective when applied to fresh pork at reducing the prevalence of *Campylobacter* spp. and *Salmonella*. Similarly, reductions of *Salmonella* Typhimurium were observed by Van Netten, Mossel, and In't Veld (1995) on fresh pork carcass surfaces following a 2% or 5% lactic acid spray. Fabrizio and Cutter (2004) reported a 1.74 $\log_{10}$ CFU/cm^2 reduction in *Campylobacter* following a 15-second spray time using a 2% lactic acid after 2 d of storage. Ransom, Belk, Sofos, Stopforth, Scanga, and Smith (2003) demonstrated the effectiveness of 2% lactic acid sprayed at 55 °C on reducing *E. coli* O157:H7 on beef carcass tissue, and DeGeer et al. (2016) noted the efficacy of 4% lactic acid as a component in a hurdle intervention system when applied to fresh beef and pork. Eggenberger-Solorzano, Niebuhr, Acuff, and Dickson (2002) also showed the effectiveness of using hot water followed by an acetic acid spray as a multiple-hurdle-system to reduce *Enterobacteriaceae* populations on scalded and skinned pork carcasses. Bosilevac et al. (2006) showed that hot water reduced *E. coli* O157:H7 prevalence by 81% using a hot water treatment on post-evisceration beef carcasses, whereas lactic acid treatments reduced pathogen prevalence by 35% and both treatments combined reduced prevalence by 79%.

King, Lucia, Castillo, Acuff, Harris, and Savell (2005) evaluated the impact of peroxyacetic acid on beef carcasses post-chilling to control *E. coli* O157:H7 and *Salmonella*. Researchers found peroxyacetic acid to be generally ineffective on chilled carcass surfaces, but saw a 0.7 $\log_{10}$ CFU/cm^2 reduction on hot carcass surfaces when applied at 200 ppm (King et al., 2005). When sprayed at 200 ppm on chilled carcasses, there was no antimicrobial effect, but when sprayed at 1000 ppm, *E. coli* O157:H7 and *Salmonella* were reduced by up to 1.7 and 1.3 $\log_{10}$ CFU/cm^2, respectively (King et al., 2005). However, when the same product types were

sprayed with a 4% lactic acid intervention, reductions of 2.7 and 3.4 $\log_{10}$ CFU/cm^2 for *E. coli* O157:H7 and *Salmonella* were observed to occur (King et al., 2005). In a study looking at the impact of water (32 °C), acetic acid (1.5%), lactic acid (3.0%), and trisodium phosphate (12%) on the reduction of *E. coli* O157:H7 on chilled beef carcasses, aerobic plate counts were reduced by approximately 1.3 to 2.0 $\log_{10}$ CFU/cm^2 by all treatments (Dorsa, Cutter, & Siragusa, 1997). In addition, all treatments, except water, reduced initial inoculum levels to a less than detectable level and researchers found 3.0% lactic acid to be the most effective at reducing *E. coli* O157:H7 contamination (Dorsa et al., 1997). Hardin, Acuff, Lucia, Oman, and Savell (1995) also found lactic acid to be more effective at reducing *E. coli* O157:H7 than acetic acid when applied to beef carcasses. Beefixde®, a lactic and citric acid mixture, was validated as an effective intervention to reduce *E. coli* O157:H7 and *Salmonella* contamination on hot beef carcasses (Pond, Gardner, Chaney, Echeverry, Laury, & Brashears, 2010). Carpenter, Smith, and Broadbent (2011) evaluated the effect of spraying hot 2% levulinic acid, 2% lactic acid, 2% acetic acid, and water on reducing *E. coli* O157:H7 on beef plate. The lactic acid treatment was found to be most effective with a slight decontamination advantage over the water wash and the acetic acid treatment displayed residual inhibition of the pathogen (Carpenter et al., 2011). In a similar study, Ransom et al. (2003) validated the utility and effectiveness of lactic and acetic acid interventions for commercial use to minimize *E. coli* O157:H7 contamination on fresh beef carcasses. Cetylpyridinium chloride, though not approved for use within the beef industry, showed the greatest reductions, followed by 2% hot lactic acid, 0.02% acidified sodium chlorite, 2% acetic acid, 0.02% peroxyacetic acid, and water (Ransom et al., 2003).

Even though edible offal items are generally considered less valuable than other parts of the carcass, these items have the potential for added overall carcass value. Research suggests

edible by-products have poor microbiological quality, likely resulting from handling practices and due to the nature and location of these products within an animal (Hanna, Smith, Savell, McKeith, & Vanderzant, 1982; Sinell, Klingbeil, & Benner, 1984; Woolthuis, Mossel, Van Logtestijn, De Kruijf, & Smulders, 1984). Nonetheless, Delmore, Sofos, Schmidt, Belk, Lloyd, and Smith (2000) reported reductions in aerobic plate counts, total coliform counts, and *E. coli* counts on beef cheek meat and livers following different antimicrobial interventions, but the research did not address specific pathogens of concern.

In addition to antimicrobial interventions, other procedures such as trimming of visible contamination, spraying hot water or employing steam pasteurization as a decontamination effort have been used. Based upon USDA-FSIS regulation, livestock carcasses must be free of any visible feces, milk or ingesta contamination and this is generally performed before a final carcass wash and before and antimicrobial intervention (USDA-FSIS, 2011). Laster, Harris, Lucia, Castillo, and Savell (2012) evaluated the effectiveness of trimming exterior subprimal surfaces from subprimals during fabrication that were inoculated with *E. coli* biotype I surrogates. Researchers found trimming to be effective at reducing *E. coli* biotype I surrogates, but noted that the microorganisms were still spread to newly exposed cut surfaces during fabrication (Laster et al., 2012). Lemmons, Lucia, Hardin, Savell, and Harris (2011) noted similar results on beef top sirloins inoculated with rifampicin-resistant *E. coli* O157:H7, with full or partial surface trimming reducing pathogen counts. However, Gill and Landers (2003) determined that trimming, along with 2% lactic acid and vacuum-hot water cleaning treatments were generally ineffective in comparison to steam pasteurization and a hot water wash. Castillo, Lucia, Goodson, Savell, and Acuff (1999) noted that reductions of aerobic plate counts, *Enterobacteriaceae,* total coliforms, thermotolerant coliforms, and *E. coli* were seen by using

steam vacuuming, but reductions were smaller than those observed when combining steam vacuuming with a sanitizing treatment. Results from a study conducted by Castillo et al. (1998a) showed that a water wash or trim singularly was not effective at reducing *Salmonella* Typhimurium and *E. coli* O157:H7 contamination on beef carcass surfaces, whereas a multiple intervention strategy was more effective. Further, Hardin et al. (1995) determined washing followed by an organic acid intervention to be more effective than trimming or washing alone on beef carcass surfaces. There have been concerns related to water or hot water interventions and researchers have shown how these processes can spread contamination but validated the use of a subsequent antimicrobial intervention to reduce contamination (Castillo et al., 1998a, 1999; Hardin et al., 1995).

Greater information on the effectiveness of antimicrobial interventions could prove beneficial for pork processors and processors of edible pork and beef by-products. This study can provide valuable information for beef and pork establishments to help support their food safety programs by validating commonly used antimicrobial interventions, helping the industry comply with regulatory requirements and trade expectations.

2.9 Antimicrobial interventions and meat quality

Antimicrobial interventions, applied singularly or as a part of a multiple hurdle intervention system play an important role in the production of safe meat. However, as a result of numerous applications, increased concentrations, and increased temperatures of interventions, quality characteristics may be negatively impacted. Dias-Morse, Pohlman, McDaniel, Guidry, and Coffman (2013) noted the potential for negative sensory characteristics in ground beef. Specifically, Dias-Morse et al. (2013) described trimmings that were treated with 5% lauric arginate, 4% sodium metasilicate, 10% trisodium phosphate or 0.02% peroxyacetic acid could be

utilized as a pre-grinding intervention with little negative color impact, whereas 0.4% cetylpyridinium chloride produced ground beef with adverse color characteristics. In addition, Bosilevac, Shackelford, Fahle, Biela, and Koohmaraie (2004) found that beef trimmings treated with 600 ppm acidified sodium chlorite produced ground beef with undesirable color and odor characteristics, whereas the trimmings treated with 300 ppm acidified sodium chlorite had no negative implications. A study was conducted by Eastwood, Arnold, Miller, Gehring, and Savell (2018) to determine the impact of multiple antimicrobial interventions used throughout beef processing on ground beef quality characteristics. Hot water, 4.0 to 5.0% lactic acid, acidified sodium chlorite, and Beefixde® interventions were applied to hot carcasses, cold carcasses, and trimmings before the production of ground beef patties. Color was minimally affected, whereas both consumer and trained panel scores were impacted by treatment combinations (Eastwood et al., 2018). Specifically, consumer panel scores overall liking, flavor liking and beefy flavor liking were impacted by combined treatment effects, whereas only trained panel scores for fat-like and cardboardy were impacted by treatments (Eastwood et al., 2018).

Some antimicrobial interventions have positively affected meat quality and shelf-life characteristics. Research has demonstrated that potassium lactate, sodium metasilicate, peroxyacetic acid, and acidified sodium chlorite can improve beef quality characteristics when applied to trimmings before grinding (Quilo et al., 2009a; Quilo et al., 2009b). Patties that had been treated with 4% sodium metasilicate and 0.02% peroxyacetic acid had lower lipid oxidation than control patties (Quilo et al., 2009a). Jimenez-Villarreal et al. (2003) found trimmings treated with either 0.5% cetylpyridinium chloride followed by 10% trisodium phosphate, 200 ppm chlorine dioxide followed by 0.5% cetylpyridinium chloride, 200 ppm chlorine dioxide followed by 10% trisodium phosphate or 2% lactic acid followed by 0.5% cetylpyridinium chloride to

actually be more effective than control treated trimmings at improving sensory characteristics. Specifically, trimmings treated with 0.5% cetylpyridinium chloride followed by 10% trisodium phosphate, 200 ppm chlorine dioxide followed by 10% trisodium phosphate, and 2% lactic acid followed by 0.5% cetylpyridinium chloride produced ground beef patties that were redder during their shelf-life display (Jimenez-Villarreal et al., 2003). Pohlman et al. (2009) determined that beef trimmings treated with 3% potassium lactate, 4% sodium metasilicate, 0.1% acidified sodium chlorite or 0.02% peroxyacetic acid not only reduced counts of *E. coli*, coliforms, and aerobic plate counts, but also had minimal impacts on sensory characteristics. However, trimmings treated with acidified sodium chlorite and peroxyacetic acid had lower overall color scores compared to control trimmings (Pohlman et al., 2009). Antimicrobial interventions are necessary during processing to reduce microbiological contamination; however, some negative quality characteristics may result from their use. Thus, more research on the utility of different antimicrobials for varying product types is needed to mitigate any potential negative implications.

2.10 Pork and beef export markets

Export markets are a vital component for the viability of the domestic meat industry. These markets are especially crucial for pork and beef and pork by-products, as many countries around the world have a demand for products not generally consumed in the U.S. The U.S. pork industry relies heavily upon exports, as approximately 30% of the pork that is produced domestically is exported, whereas approximately 14% of total beef production is exported (USMEF, 2018). In addition, pork exports contribute approximately $55 USD to every hog slaughtered in the U.S. (USMEF, 2018). Some global markets sell U.S. imported beef and pork by-products for a premium when compared to muscle cuts. Based on data from the U.S. Meat

Export Federation (USMEF), the value of total beef exports (including variety meats) in 2016 was $6.3 billion (USMEF, 2017). Additionally, the total value of 2016 pork exports (including variety meats) was $5.9 billion, which was a 7% increase from 2015 (USMEF, 2017). Of these totals, variety meats were responsible for $902 (beef) and $999 (pork) million, respectively (USMEF, 2017). Edible by-products from beef and pork are crucial for profitability and longevity of agriculture trade. With the validation of lactic acid and PAA for beef and pork by-products, the industry will be better equipped to stay compliant with the ever-changing requirements and expectations set forth by trade regulations.

CHAPTER III

MATERIALS AND METHODS

3.1 Treatment design and product collection

Objective 1: To validate the efficacy of using antimicrobial interventions to reduce *Salmonella*, Shiga toxin-producing *E. coli* (STEC), *E. coli* biotype I (non-pathogenic *E. coli* strains functioning as surrogates for *E. coli* O157:H7 and *Salmonella)*, and *Campylobacter*. To mimic contamination on pork carcasses and chilled pork subprimals, hogs were harvested (n = 12 total; n = 3 each for inoculum type: 1) *Salmonella,* 2) STEC*,* 3) *Campylobacter,* and 4) *E. coli* biotype I surrogates) at the Texas A&M University Rosenthal Meat Science and Technology Center (College Station, TX). Hogs were harvested in accordance with Institutional Animal Care and Use Committee (IACUC) at Texas A&M University (AUP #2017-0203). Hot carcasses were portioned and warm pieces from each carcass were transported in insulated containers to the Texas A&M University Food Microbiology Lab (n = 108 total warm pork samples per inoculum type: 2 product types × 3 sampling times × 6 treatments × 3 reps; n = 72 total chilled pork samples per inoculum type: 2 product types × 2 sampling times × 6 treatments × 3 reps). Warm pork pieces were inoculated with *Salmonella,* STEC, *Campylobacter* or *E. coli* biotype I surrogates. Following a 30 min microbial attachment period, warm pork pieces were assigned to one of the 12 antimicrobial treatments listed below. For chilled product, inoculated warm pork was chilled (≈ 4 °C) for 24 h, and then also was assigned to one of the 12 antimicrobial treatments listed below. Applying antimicrobial interventions to warm tissues mimics antimicrobial treatments generally applied during harvest. For each inoculum type, samples were taken before applying the antimicrobial treatment to the warm pork tissue, 30 min after

treatment, and 24 h post-treatment. For chilled pork, samples were taken after the 24 h chill and 30 min after the treatment. Treatments were replicated three times each for both warm tissue and chilled tissue antimicrobial interventions.

Antimicrobial treatments:

1) Skin-on, sprayed with warm (55 °C), 2.5% lactic acid
2) Skinless, sprayed with warm (55 °C), 2.5% lactic acid
3) Skin-on, sprayed with room temperature, 2.5% lactic acid
4) Skinless, sprayed with room temperature, 2.5% lactic acid
5) Skin-on, sprayed with warm (55 °C), 5% lactic acid
6) Skinless, sprayed with warm (55 °C), 5% lactic acid
7) Skin-on, sprayed with room temperature, 5% lactic acid
8) Skinless, sprayed with room temperature, 5% lactic acid
9) Skin-on, sprayed with peroxyacetic acid, 400 ppm
10) Skinless, sprayed with peroxyacetic acid, 400 ppm
11) Skin-on, sprayed with hot water (55 °C)
12) Skinless, sprayed with hot water (55 °C)

Objectives 2 and 3: To validate the efficacy of using approved antimicrobial interventions to reduce *Salmonella*, STEC, *Campylobacter* (pork only), and *E. coli* biotype I surrogate contamination, beef and pork head meat, cheek meat, livers (pork only), and hearts were collected from carcasses (n = 216 total pork samples per inoculum type: 4 product types × 3 sampling times × 6 treatments × 3 reps; n = 162 total beef samples per inoculum type: 3 product types × 3 sampling times × 6 treatments × 3 reps) harvested at the Texas A&M University Rosenthal Meat Science and Technology Center (College Station) and two commercial

processing facilities. To mimic contamination during harvest, warm products were inoculated with *Salmonella,* STEC, *Campylobacter* (pork only), and *E. coli* biotype I surrogates. After inoculation, there was a 30 min attachment time. Products were assigned to one of the following treatment groups:

1) Warm (55 °C), 2.5% Lactic acid
2) Room temperature, 2.5% Lactic acid
3) Warm (55 °C), 5% Lactic acid
4) Room temperature, 5% Lactic acid
5) Peroxyacetic acid, 400 ppm
6) Hot water (55 °C)

For each inoculum type, samples were taken before applying the antimicrobial treatment to the warm tissues, 30 min after each treatment, and 24 h post-treatment.

Objective 4: Due to the importance of pork color for consumer acceptability (Mancini & Hunt, 2005), color characteristics were analyzed before and after treatment application for products identified in Objectives 1 to 3. For pork products described in Objective 1, non-inoculated tissue samples were obtained from pork carcasses for this purpose (n = 36 samples for color). For each of the antimicrobial treatments identified for Objectives 2 and 3, pork products of each type (head meat, cheek meat, hearts, and livers), were obtained to allow for non-inoculated color analyses before and after each treatment (n = 18 of each product type). As outlined by the American Meat Science Association (2012), color was subjectively evaluated by a 6-member trained color panel. Lean color scales for pork by-products (hearts and livers) were not outlined by AMSA color guidelines (American Meat Science Association, 2012), and were developed internally (see appendix). Visual assessments of lean color (hot and chilled skinless,

pork head meat, and cheek meat: 1 = pale grayish-pink; 8 = very dark grayish-pink; pork hearts: 1 = pale red; 8 = very dark red; pork livers 1 = pale purple-red; 8 = extremely dark purple-red) and discoloration (all products: 1 = none; 5 = extreme) were conducted by trained panelists. Instrumental color measurements were taken in three different locations using a Hunter MiniScan EZ (Model 4500L; Hunter Labs, Inc. Reston, VA; 31.8 mm aperture, Illuminant D65, 10° observer) colorimeter and averaged to represent the value for each sample. For each measurement, CIE *L*, a**, and *b** CIE color space values were recorded. Hue angle and chroma values were calculated according to the American Meat Science Association (2012) Meat Color Measurement Guidelines.

3.2 Antimicrobial preparation and spraying

Antimicrobial interventions were prepared on each research day in the Texas A&M University Food Microbiology Laboratory. Lactic acid (L-lactic acid 88% F.G., Birko Corp., Henderson, CO) and Peroxyacetic acid (PAA; Promoat™, Safe Foods Corp., North Little Rock, AR) were mixed according to manufacturer's recommendations, followed by titration procedures to ensure desired concentrations were obtained. Mixed acids were transferred to plastic garden sprayers (Scotts® Multi-Use Sprayer, Model #190499, Marysville, OH), and for interventions applied at 55 °C, sprayers were placed in a hot water bath to reach the desired temperature. For hot and chilled skin-on and skinless pork products, interventions were applied for 3 to 5 s (approximately 15 to 25 ml); for head meat, cheek meat, hearts, and livers, interventions were applied for 1 to 2 s (approximately 5 to 10 ml). Temperature of each intervention was taken in the sprayer immediately before application (room temperature interventions were approximately 25 °C and hot interventions were approximately 54.5 °C). Interventions also were titrated at the end of each research day.

3.3 Microbiological analyses

3.3.1 *Salmonella,* STEC, and *E. coli* biotype I surrogates

Rifampicin-resistant strains of *Salmonella enterica* serovar Typhimurium strain LT2, STEC (O26, O111, O145, O157), and non-pathogenic *E. coli* biotype I surrogates (BAA-1427, BAA-1428, BAA-1430) were obtained from the Texas A&M University Food Microbiology Laboratory (College Station). STEC were previously obtained from USDA-Agricultural Research Service. *E. coli* O91 was obtained from the American Type Culture Collection (ATCC; Manassas, VA). These isolates were selected due to their relevance in beef and pork-related foodborne illness. Mutants of the aforementioned strains were selected based on resistance to 100 mg/L rifampicin by incubating and growing the microorganisms in the presence of the antibiotic, and then selecting for isolates demonstrating stable resistance (Rif+). Rifampicin-resistant *Salmonella*, STEC, and *E. coli* biotype I surrogates were revived by duplicate identical passages in sterile tryptic soy broth (TSB, Becton, Dickinson and Co., Sparks, MD), followed by incubation at 35±1 °C for 24 h. Rifampicin resistance was confirmed by streaking cultures onto tryptic soy agar (TSA, Becton, Dickinson and Co., Sparks, MD) petri plates containing 100 mg/L rifampicin (rif-TSA) and incubating at 35±1 °C for 24 h. Rif-TSA was prepared by adding a solution of 0.1 g of rifampicin filtered to sterilize and dissolved in 5 ml methanol to 1 liter of autoclaved, tempered (55 °C) TSA. Following revival completion, isolates were individually streaked onto selective media appropriate for *Salmonella* (XLT-4 agar base with XLT-4 supplement, Becton, Dickinson and Co., Sparks, MD), STEC, and *E. coli* biotype I surrogates (MacConkey agar and Levine's Eosin Methylene Blue agar, Becton, Dickinson and Co., Sparks, MD) for confirmation and evidence of typical colony morphology before use in an inoculum as outlined in the USDA-FSIS Microbiological Laboratory Guidebook (MLG) 4.09 (USDA-FSIS,

2017a) and 5.09 (USDA-FSIS, 2015c). Well-isolated colonies were picked with a sterile needle and slants were prepared on TSA for later use during experimental trials. Slants were layered with sterile mineral oil and stored at 5±1 °C.

3.3.2 *Campylobacter*

Campylobacter jejuni isolates 29428, 33291, and BAA-374, all isolated from human feces, were obtained from the American Type Culture Collection (ATCC; Manassas, VA). Following revival in 3M *Campylobacter* Enrichment Broth (3M, St. Paul, MN) at 41.5±1°C (24 to 48 h), isolates, bearing known antimicrobial resistance characteristics, were streaked onto surfaces of laked horse blood (Innovative Research, Novi, MI)-supplemented Columbia Blood agar (Sigma-Aldrich, St. Louis, MO) and Campy-Cefex Agar (CCA, Neogen Corp., Lansing, MI) supplemented with antimicrobials for confirmation and evidence of typical colony morphology before use in an inoculum as outlined in the USDA-FSIS Microbiological Laboratory Guidebook (MLG) 41.04 (USDA-FSIS, 2016). Inoculated plates were incubated micro-aerobically (5% O_2, 5% CO_2, 90% N_2, Conroe Welding Supply, Conroe, TX) at 41.5±1 °C for 24 to 48 h. Well-isolated colonies were picked with a sterile needle and frozen stock prepared using Brain Heart Infusion broth (Becton, Dickinson and Co., Sparks, MD) with 50% glycerol and supplemented with 5% laked horse blood (Innovative Research, Novi, MI).

3.4 Inoculum preparation and microbiological sampling

Inoculum preparation began 48 h (*Salmonella*, STEC, and *E. coli* biotype I surrogates) or 96 h (*Campylobacter*) before each research day. For *Salmonella*, STEC, and *E. coli* biotype I surrogates, a disposable sterile plastic loop was used to aseptically transfer isolates from TSA slants into 10 ml TSB and incubated aerobically at 35±1 °C for 18 to 24 h. Following incubation, each isolate was again aseptically transferred into a new bottle of TSB (quantity dependent on

amount of inoculum needed) and incubated at 35±1 °C for 18 to 24 h. For *Campylobacter*, isolates were aseptically transferred using a disposable sterile plastic loop from frozen stock into 10 ml 3M *Campylobacter* Enrichment Broth and incubated aerobically at 41.5±1 °C for 24 to 48 h. Following incubation, each isolate again was aseptically transferred into a new bottle of 3M *Campylobacter* Enrichment Broth (quantity dependent on amount of inoculum needed) and incubated at 41.5±1 °C for 24 to 48 h. For all inoculum types, a cocktail was created by combining equivalent volumes of chosen strains via centrifugation and washing (3500 RCF, 15 min, 25 °C) in sterile peptone water. Product inoculation was achieved using a sterilized handheld plastic sprayer (The Bottle Crew, Model E3212, Farmington Hills, MI). Hot and chilled, skin-on and skinless pork were sprayed 6 to 7 times (approximately 4 to 5 ml) and head meat, cheek meat, hearts, and livers were sprayed 3 to 4 times (approximately 2 to 3 ml).
For hot and chilled, skin-on and skinless pork, two 10-cm^2 (2 mm in depth) surface excisions were removed using a sterile, scalpel, forceps, and stainless-steel borer. For beef and pork head meat, cheek meat, hearts, and pork livers, one 10-cm^2 area (2 mm in depth) was removed from each product surface using a sterile, scalpel, forceps, and stainless-steel borer. Each sample then was placed in a sterile stomacher bag, and diluent (50 mL buffered peptone water, Becton, Dickinson and Co.) added, and stomached for 1 min. Enumeration for each sample was conducted by preparing decimal dilutions in 0.1% peptone diluent and plating onto rif-TSA followed by 18 to 24 h incubation at 35±1 °C (*Salmonella*, STEC, and *E. coli* biotype I surrogate samples), whereas for each sample taken from *Campylobacter* inoculated products, counts were obtained after preparing decimal dilutions in 0.1% peptone diluent and plating on CCA petri plates incubated micro-aerobically for 24 to 48 h at 41.5±1 °C.

3.5 Statistical analyses

Microbiological count data CFU/cm^2 were transformed into logarithms (base 10) before data analysis. When plates had counts below the limit of detection, a number (0.4 $\log_{10}$ CFU/cm^2 for hot and chilled, skin-on and skinless pork and 0.7 $\log_{10}$ CFU/cm^2 for beef and pork head meat, cheek meat, hearts, and livers) between 0 and the lowest detection limit was used to facilitate data analysis. Data were analyzed using using JMP® Pro, Version 12.0.1 (SAS Institute Inc., Cary, NC). The fit model function was used for analysis of variance (ANOVA), and least squares means comparisons were conducted when appropriate using Student's t-test with an alpha-level 0.05.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Objective 1 – Hot and chilled, skin-on and skinless pork

Least squares means for counts from hot skin-on pork inoculated with *Salmonella,* STEC, surrogates, and *Campylobacter* stratified by sampling time and treatment main effects are shown in Table 1. Hot skin-on pork had lower ($P < 0.001$) counts 24 h post-treatment compared to both pre-treatment and 30 min post-treatment sampling times for product inoculated with *Salmonella*. However, there were no treatment-specific differences ($P = 0.123$) for hot skin-on pork inoculated with *Salmonella*. For STEC-inoculated product, counts were lower ($P = 0.013$) 30 min post-treatment, whereas counts 24 h post-treatment were not different than counts pre-treatment or 30 min post-treatment. There was no treatment main effect for hot skin-on pork inoculated with STEC ($P = 0.051$); however, 5.0% hot lactic acid treated product had the lowest numerical counts among treatments. Hot skin-on pork inoculated with *E. coli* biotype I surrogates and *Campylobacter* showed lower ($P < 0.001$) counts 30 min post-treatment and 24 h post treatment compared to pre-treatment samples, but there was no difference ($P = 0.056$ for surrogates; $P = 0.338$ for *Campylobacter*) among treatments.

Counts from hot skinless pork inoculated with *Salmonella,* STEC, surrogates, and *Campylobacter* were impacted by sampling time ($P < 0.001$) and treatment main effects (Table 2). For hot skinless pork inoculated with *Salmonella,* STEC, and surrogates, counts were lower 30 min post-treatment and 24 h post-treatment compared to pre-treatment and for *Campylobacter*-inoculated product, counts were lowest 24 h post treatment compared to other sampling times. In addition, all inoculum types were impacted by treatment main effect.

Specifically, *Salmonella*- and STEC-inoculated product showed lower ($P = 0.005$ for *Salmonella*; $P < 0.001$ for STEC) counts for all lactic acid treated products compared to hot water. In addition, for STEC-inoculated products, 2.5% room temperature lactic acid and both 5.0% lactic acid treatments were more effective ($P < 0.001$) than peroxyacetic acid. Hot skinless pork had lower ($P < 0.001$) counts of pathogen surrogates and *Campylobacter* following lactic acid treatment compared to hot water. In addition, for both inoculum types, products treated with 2.5% room temperature lactic acid and both 5.0% lactic acid treatments resulted in lower counts than peroxyacetic acid treated product.

Least squares means for counts from chilled skin-on pork inoculated with *Salmonella,* STEC, surrogates, and *Campylobacter* stratified by sampling time and treatment main effects are shown in Table 3. All four inoculum types were impacted by sampling time main effect. Chilled skin-on pork inoculated with *Salmonella*, STEC, surrogates, and *Campylobacter* had lower ($P < 0.001$ for *Salmonella,* surrogates, and *Campylobacter*; $P = 0.001$ for STEC) counts 30 min post-treatment compared to pre-treatment samples. *Salmonella*-inoculated product was not impacted by treatment ($P = 0.928$), whereas products inoculated with STEC, surrogates, and *Campylobacter* were impacted by treatment. For chilled skin-on pork inoculated with STEC, all lactic acid treatments along with peroxyacetic acid reduced ($P = 0.002$) counts more than the hot water treatment. Lactic acid treatments were more effective at reducing surrogates contamination on chilled skin-on pork than hot water ($P = 0.001$). In addition, 5.0% room temperature lactic acid treated products resulted in lower counts than product treated with peroxyacetic acid or hot water. *Campylobacter*-inoculated products had lower ($P = 0.047$) counts when treated with 2.5% hot lactic acid when compared to products treated with 2.5% room temperature lactic acid, 5.0% room temperature lactic acid, and hot water.

Table 4 shows least squares means for counts from chilled skinless pork inoculated with *Salmonella,* STEC, surrogates, and *Campylobacter* stratified by sampling time and treatment main effects. All four inoculum types were impacted by sampling time main effect. Chilled skinless pork inoculated with *Salmonella*, STEC, surrogates, and *Campylobacter* had lower ($P < 0.001$ for *Salmonella* and *Campylobacter*; $P = 0.003$ for STEC; $P = 0.001$ for surrogates) counts 30 min post-treatment compared to pre-treatment samples. STEC-inoculated products were not impacted ($P = 0.233$) by treatment main effect, whereas products inoculated with *Salmonella,* surrogates, and *Campylobacter* were impacted by treatment main effect. Specifically, *Salmonella* counts were lower ($P = 0.016$) when treated with both 2.5% lactic acid treatments, 5.0% room temperature lactic acid, and peroxyacetic acid compared to hot water. Further, 5.0% room temperature lactic acid produced lower counts than chilled skinless pork treated with 5.0% hot lactic acid and hot water. For surrogates-inoculated product, both hot lactic acid treatments had lower ($P = 0.030$) counts than both peroxyacetic acid and hot water treated products. Chilled skinless pork inoculated with *Campylobacter* had lower ($P = 0.022$) counts when treated with 5.0% room temperature lactic acid when compared to 2.5% hot lactic acid, peroxyacetic acid, and hot water.

Similar to the current study, Van Netten et al. (1995) noted reductions of *Salmonella* Typhimurium on hot pork carcasses following a cold or hot 2% or 5% lactic acid spray. Another study by Epling et al. (1993) determined a 2% lactic acid solution applied to hot pork carcasses was effective in reducing *Salmonella* prevalence when compared to controls. Epling et al. (1993) also determined that when compared to control, a 2% lactic acid solution applied to hot pork carcasses was effective at reducing the prevalence of *Campylobacter* spp. Further, Fabrizio and Cutter (2004) reported a 1.74-$\log_{10}$ CFU/cm^2 reduction in *Campylobacter* following a 15-s spray

time using a 2% lactic acid after 2 d of storage. In addition, Eggenberger-Solorzano et al. (2002) noted the added efficacy of applying an organic acid spray after a hot water wash to further improve microbiological quality of pork carcasses instead of a singular hot water treatment. While there is limited information about the efficacy of antimicrobial interventions on pork products in comparison to beef, there has been extensive research on antimicrobial interventions such as lactic acid at reducing pathogen contamination on beef carcasses and subsequent cuts or trimmings (Bosilevac et al., 2006; Castillo et al., 1998a; Castillo, Lucia, Goodson, Savell, & Acuff, 1998b; Castillo et al., 2001; Dorsa et al., 1997; Ellebracht et al., 1999; Ransom et al., 2003). Results from the current study suggest that lactic acid and peroxyacetic acid interventions can reduce *Salmonella*, STEC, surrogate, and *Campylobacter* contamination on hot and chilled, skin-on and skinless pork products, especially compared to hot water treatments. However, skin-on pork tended to have higher counts across inoculum types compared to skinless pork 30 min post-treatment, and therefore pork carcass interventions may be more effective when applied to skinless product. These differences may be a result of the varying product surfaces (i.e. skin versus lean and fat tissues). In instances in which room temperature or hot 2.5% lactic acid resulted in similar counts to room temperature or hot 5.0% lactic acid, processors may decide to employ a lesser concentration from an economic standpoint.

4.2 Objectives 2 and 3 – Pork and beef head meat, cheek meat, hearts, and pork livers

Table 5 shows least squares means for counts from pork head meat inoculated with *Salmonella,* STEC, surrogates, and *Campylobacter* stratified by sampling time and treatment main effects. All four inoculum types were impacted by sampling time main effect. Pork head meat inoculated with *Salmonella*, STEC, surrogates, and *Campylobacter* had lower ($P < 0.001$ for *Salmonella* and *Campylobacter*; $P < 0.001$ for STEC; $P = 0.007$ for surrogates) counts both

30 min post-treatment and 24 h post-treatment compared to pre-treatment samples. Further, counts were lowest for *Campylobacter*-inoculated pork head meat at 24 h post-treatment when compared to other sampling times. Head meat inoculated with *Salmonella* and surrogates was not impacted by treatment main effect ($P = 0.233$ for *Salmonella;* $P = 0.309$ for surrogates). However, STEC-inoculated products had lower ($P < 0.001$) counts when treated with both 5.0% lactic acid treatments compared to 2.5% hot lactic acid, peroxyacetic acid, and hot water. In addition, *Campylobacter* counts were lowest ($P = 0.001$) when treated with 5.0% room temperature lactic acid compared to all other treatments. Further, 2.5% room temperature lactic acid and 5.0% hot lactic acid produced lower counts than head meat treated with hot water.

Least squares means for counts from pork cheek meat inoculated with *Salmonella,* STEC, surrogates, and *Campylobacter* stratified by sampling time and treatment main effects are shown in Table 6. All inoculated pork cheek meat products were impacted ($P < 0.001$ for *Salmonella* and *Campylobacter*; $P < 0.001$ for STEC; $P < 0.001$ for surrogates) by sampling time main effect, with all counts being lower 30 min post-treatment and 24 h post-treatment compared to pre-treatment samples. In addition, cheeks inoculated with *Campylobacter* also had lower counts 24 h post-treatment compared to other sampling times. STEC products were not impacted ($P = 0.328$), whereas all other inoculum types were impacted by treatment. Cheeks inoculated with *Salmonella* produced lower ($P < 0.001$) counts when treated with 2.5% room temperature lactic acid and both 5.0% lactic acid treatments compared to 2.5% hot lactic acid and hot water, with all treatments being more effective at reducing counts than hot water. Pork cheek meat inoculated with surrogates had counts that were lower ($P = 0.050$) when treated with 2.5% hot lactic acid than products treated with peroxyacetic acid and hot water. For *Campylobacter*-inoculated pork cheeks, both 5.0% lactic acid interventions produced the lowest ($P < 0.001$)

counts among all treatments. Further, all lactic acid treatments were more effective at lowering counts than hot water.

Pork hearts inoculated with *Salmonella,* STEC, surrogates, and *Campylobacter* were impacted by sampling time main effect ($P < 0.001$; Table 7). In addition, pork hearts were impacted by treatment main effect when inoculated with *Salmonella* ($P < 0.001$), STEC ($P < 0.001$), and *Campylobacter* ($P = 0.001$), whereas counts from surrogates-inoculated product were not impacted ($P = 0.058$) by treatment main effect. *Salmonella* counts were lower on pork hearts when treated with both 5.0% lactic acid treatments compared to peroxyacetic acid and hot water. In addition, all treatments produced lower *Salmonella* counts than hot water treated pork hearts. For pork hearts inoculated with STEC, 5.0% hot lactic acid was more effective at reducing counts than 2.5% room temperature lactic acid and hot water, however, all organic acids reduced STEC counts more than hot water. *Campylobacter* counts were lower when treated with 2.5% and 5.0% hot lactic acid compared to peroxyacetic acid and hot water treatments, whereas all organic acid treatments had lower counts than hot water-treated products.

Pork livers inoculated with *Salmonella,* STEC, surrogates, and *Campylobacter* were impacted by sampling time and treatment main effects (Table 8). All inoculum types had lower ($P < 0.001$) counts 30 min post-treatment and 24 h post-treatment compared to pre-treatment samples. Pork liver inoculated with *Salmonella* had lower ($P < 0.001$) counts when treated with both 5.0% lactic acid interventions than peroxyacetic acid and hot water. Further, all treatments produced lower counts than hot water treated livers. Five percent hot lactic acid produced lower ($P < 0.001$) counts in STEC-inoculated pork livers than 2.5% and 5.0% room temperature lactic acid and peroxyacetic acid, but all organic acid treatments had lower counts than hot water treated pork livers. For pork livers inoculated with surrogates, 5.0% hot lactic acid had the lowest

($P = 0.001$) counts among treatments, and all other treatments did not differ. *Campylobacter* counts were lower ($P = 0.015$) on pork livers when treated with 2.5% hot lactic acid, both 5.0% lactic acid treatments, and peroxyacetic acid than hot water treated livers.

Beef head meat inoculated with *Salmonella,* STEC, and surrogates was impacted by sampling time main effect ($P < 0.001$; Table 9). All inoculum types had lower counts 30 min post-treatment and 24 h post-treatment compared to pre-treatment samples. Treatment did not have an impact for counts of *Salmonella* ($P = 0.241$), STEC ($P = 0.078$), and surrogates ($P = 0.294$) on beef head meat.

Least squares means for counts from beef cheek meat inoculated with *Salmonella,* STEC, and surrogates stratified by sampling time and treatment main effects are shown in Table 10. *Salmonella*-inoculated beef cheek meat was impacted ($P < 0.001$) by sampling time and had lower counts 30 min post-treatment and 24 h post-treatment compared to the pre-treatment samples. STEC-inoculated products also had a sampling time main effect ($P < 0.001$) with lower counts 30 min post-treatment compared to the pre-treatment samples, however, 24 h post-treatment counts were higher than 30 min post-treatment counts. Surrogates-inoculated beef cheek meat had lower ($P < 0.001$) counts 30 min post-treatment compared to pre-treatment and 24 h post-treatment counts were lower than both previous sampling times. *Salmonella*-inoculated beef cheeks were not impacted ($P = 0.181$) by treatment main effect, whereas products inoculated with STEC and surrogates were impacted by treatment. For STEC, beef cheek meat had lower ($P < 0.001$) counts with all treatments compared to hot water. In addition, 2.5% room temperature lactic acid and 5.0% hot lactic acid had lower counts than peroxyacetic acid and hot water treatments. Surrogates counts were lower ($P = 0.025$) when treated with both 2.5% lactic

acid treatments, 5.0% room temperature lactic acid, and peroxyacetic acid when compared to hot water interventions.

Table 11 shows least squares means for counts from beef hearts inoculated with *Salmonella,* STEC, and surrogates stratified by sampling time and treatment main effects. All inoculum types were impacted ($P < 0.001$) by sampling time main effect. Both *Salmonella*- and STEC-inoculated beef hearts had lower counts 30 min post-treatment and 24 h post-treatment compared to pre-treatment samples. Surrogate-inoculated beef hearts had lower counts 30 min post-treatment compared to pre-treatment and had the lowest counts 24 h post-treatment compared to other sampling times. *Salmonella* counts were lower ($P < 0.001$) for beef hearts treated with 5.0% room temperature lactic acid compared to both 2.5% lactic acid treatments, 5.0% hot lactic acid, and hot water. Further, all organic acid treatments with the exception of 2.5% hot lactic acid, produced lower counts than hot water. For STEC-inoculated beef hearts, peroxyacetic acid produced lower ($P = 0.001$) counts than 2.5% room temperature lactic acid and hot water but was not different from the other lactic acid treatments. Further, all organic acid treatments had lower ($P = 0.029$) surrogate counts than the hot water intervention.

Delmore et al. (2000) reported reductions in aerobic plate counts, total coliform counts, and *E. coli* counts on beef cheek meat and livers following different antimicrobial interventions. In addition, Kalchayanand et al. (2008) saw similar results to the current study when using hot water and lactic acid as interventions on beef heads. Researchers found hot water and lactic acid to reduce *E. coli* O157:H7 contamination on beef heads by 1.7 and 1.5 log CFU/cm^2, respectively (Kalchayanand et al., 2008). Schmidt, Bosilevac, Kalchayanand, Wang, Wheeler, and Koohmaraie (2014) demonstrated the efficacy of immersion of beef cheek meat in hot water, Beefxide®, 2.5% and 5.0% lactic acid, and 220 ppm PAA to reduce STEC and *Salmonella*

contamination. Woolthuis et al. (1984) found a 0.20% (v/v) lactic acid immersion to be more effective at reducing total colony counts on pork livers than a hot water immersion for 15 s. Further, King, Miller, Castillo, Griffin, and Hardin (2012) found a 2.0% hot lactic acid spray to be more effective than a water wash at reducing *Salmonella, Yersinia,* and *Campylobacter coli* when applied to pork livers, intestines, hearts, and stomachs. Patterson and Gibbs (1979) also noted a reduction in microbial load on beef hearts and tongues when using hot water, 1.0% lactic acid or 100 ppm chlorine immersion interventions. Chung, Dickson, and Grouse (1989) evaluated the attachment and proliferation of several bacteria on fresh lean muscle and fat tissues and they found no differences in number of cells that attached on the two product types. However, researchers noted bacteria proliferation was greater on the lean tissue compared to the fat tissue (Chung et al., 1989). Though there is little information on microbial attachment and survival on pork and beef by-products such as hearts and livers, attachment on these products may differ from other well-studied tissues. For instance, in the current study, pork and beef hearts and pork livers tended to have lower counts both pre-treatment and 30 min post-treatment. Thus, information about the attachment and proliferation of pathogens on different tissue surfaces may allow researchers to better understand and eliminate pathogen contamination on by-products. Nonetheless, our research shows that organic acids can reduce *Salmonella*, STEC, surrogate, and *Campylobacter* contamination in edible and pork and beef by-products. Similar to results seen in hot and chilled, skin-on and skinless pork, when room temperature or hot 2.5% lactic acid resulted in similar counts to room temperature or hot 5.0% lactic acid, using a lesser concentration may provide economic incentive for a processor of these products.

4.3 Objective 4 – Pork color evaluation

4.3.1 Hot and chilled skinless pork

Least squares means for color space values and color attributes (*L*, a*, b**, hue, chroma, ΔE) are shown in Tables 12 and 13 for hot and chilled skinless pork, respectively. Both hot and chilled pork had higher ($P = 0.017$ for hot; $P = 0.036$ for chilled) *L** values, lower ($P < 0.001$ for hot; $P = 0.005$ for chilled) *b** values, and a lower ($P < 0.001$ for hot; $P = 0.002$ for chilled) chroma after the treatments were applied. Lower chroma values detected post-treatment indicate lighter, less intense color, consistent with visual observations. Chilled pork also had lower ($P = 0.002$) *a** values post-treatment. There was no treatment main effect ($P > 0.05$) seen across objective color values for either product type (Tables 12 and 13). While there is abundant information about the impacts of antimicrobial interventions on beef color, information regarding the impacts of these interventions for pork is much more limited. Castelo, Kang, Siragusa, Koohmaraie, and Berry (2001a) noted a similar decrease in *a** values after combinations of hot water, hot air, and lactic acid were applied to pork trim. In another study by Castelo, Koohmaraie, and Berry (2001b), researchers found decreased *a** values and increased *L** values in ground pork treated with water, lactic acid, and hot air, which is similar to results seen in our study for hot and chilled skinless pork before and after treatments were applied. However, our study did result in lower *b** values post-treatment for both hot and chilled skinless pork. Color differences in the two studies are likely driven by differences in product types and varying antimicrobial interventions used.

Least squares means for trained panel attributes for sampling time and treatment main effects in hot and chilled skinless pork are shown in Tables 14 and 15, respectively. Trained panelists noted a lower ($P < 0.001$) lean color score post-treatment for both product types,

indicating a lighter color. For hot pork, 5.0% room temperature lactic acid produced a lower ($P <$ 0.001) mean color score than 2.5% hot lactic acid, 5.0% hot lactic acid, peroxyacetic acid, and hot water, signifying a lighter, paler, and less desirable color (Table 14). In chilled pork, product treated with 2.5% room temperature lactic acid had the lowest ($P = 0.002$) lean color score among treatments and panelists noted a greater ($P < 0.001$) amount of discoloration post-treatment (Table 15). Further, discoloration scores were higher for both 5.0% lactic acid treatments compared to peroxyacetic acid and hot water treatments ($P = 0.008$). A sampling time × treatment interaction was determined for hot skinless pork discoloration scores (Table 16). All lactic acid treatments had a greater ($P < 0.001$) amount of discoloration than the peroxyacetic acid or hot water treatments. Additionally, panelists noted 2.5% hot lactic acid had less ($P <$ 0.001) discoloration than other lactic acid treatments. While these interventions are effective at reducing pathogens, there were some lean color and discoloration differences among treatments noted by trained panelists. Generally, color evaluations on hot and chilled skinless pork indicated a lighter, less intense color post-treatment. Nonetheless, mean discoloration scores noted by panelists did not reach a score above 3 (small amount of discoloration). Castelo et al. (2001b) noted that pork trim treated with water and lactic acid resulted in a darker red color upon visual observation, whereas our study noted the opposite. Differences are likely driven by the varying product types and interventions used among studies however, researchers have acknowledged that lactic acid sprays not exceeding 1.0% (v/v) resulted in acceptable color attributes on beef tissues (Smulders, Barendsen, Van Logtestijn, Mossel, & Van Der Marel, 1986; Snijders et al., 1985).

4.3.2 Pork head meat, cheek meat, hearts, and livers

There was a sampling time × treatment interaction for *a** in pork head meat and pork hearts (Table 17). All treatments resulted in lower ($P = 0.025$) mean *a** values after treatments were applied to pork head meat, except for hot water. In pork hearts, all treatments lowered ($P = 0.047$) *a** values, however, the hot water treatment produced the least numerical change when compared to other treatments. Least squares means for color space values and color attributes (*L*, a*, b**, hue, chroma, ΔE) are shown in Tables 18, 19, 20, and 21 for pork head meat, cheek meat, hearts, and livers, respectively. Pork head meat had lower ($P < 0.001$) *a**, higher ($P < 0.001$) hue value, and lower ($P < 0.001$) chroma values 30 min post-treatment (Table 18). In addition, *a** values, hue, and chroma were impacted ($P < 0.05$) by treatment, with 5.0% room temperature lactic acid lowering ($P < 0.001$) *a** values the most. Pork cheek meat had lower *a** ($P < 0.001$) and chroma ($P = 0.003$) values after the treatment, whereas hue values were higher ($P < 0.001$) post-treatment (Table 19). Both 5.0% lactic acid treatments produced higher ($P = 0.016$) hue values than 2.5% room temperature lactic acid, peroxyacetic acid, and hot water treatments (Table 19). Pork hearts had higher ($P < 0.001$) *L**, higher ($P = 0.014$) hue value, and lower ($P < 0.001$) *a**, *b*,* and chroma values after treatments were applied (Table 20). Color attributes were not impacted ($P > 0.05$) by treatments, but ΔE was the highest ($P = 0.013$) when 2.5% hot lactic acid was applied to pork hearts (Table 20). Hue angle indicates the true red of a color measurement and chroma indicates the color saturation (Rentfrow, Linville, Stahl, Olson, & Berg, 2004). These results indicate that interventions produced lighter, less red, and less saturated color attributes after treatment application. Pork livers showed a similar trend to pork hearts with higher ($P < 0.001$) *L**, lower *a** ($P < 0.001$), lower ($P = 0.013$) *b*,* and lower ($P = 0.001$) chroma values after treatments were applied (Table 21). Hue values were the only color

attribute impacted ($P = 0.003$) by treatment main effect, with lactic acid treatments producing colors further from true red in comparison to pork livers treated with peroxyacetic acid (Table 21).

Least squares means of trained panelists' scores for pork color attributes for sampling time × treatment interaction for pork hearts and livers are shown in Table 22. Pork hearts and livers received lower ($P = 0.028$ for pork hearts; $P < 0.001$ for pork livers) post-treatment lean color scores from panelists for all lactic acid-treated product, whereas PAA and hot water treated products were not different before and after treatment application. Discoloration also was impacted ($P < 0.001$ for pork hearts; $P < 0.001$ for pork livers) by lactic acid, with greater discoloration seen in pork hearts and livers post-treatment compared to pre-treatment. In pork livers, in addition to the detrimental impact of lactic acid, peroxyacetic acid also received higher discoloration scores after treatment application. These trained panelist color evaluations for edible pork by-products indicate that organic acid interventions have a more negative visual color and discoloration impact on hearts and livers compared to head meat and cheek meat. Woolthuis et al. (1984) also noted a negative discoloration impact in pork livers that were immersed in a lactic acid solution for 5 min or hot water for 15 s, but acknowledged that discoloration went away over time after opening the vacuum packaging. Thus, in future studies, re-evaluating color 24 h post-treatment may be beneficial to determine if product color can recover from intervention discoloration after a period of time.

Least squares means for trained panel attributes for sampling time and treatment main effects are shown in Tables 23 and 24 for pork head meat and cheek meat, respectively. For these product types, panelists noted lower ($P < 0.001$ for head meat; $P = 0.038$ for cheek meat) lean color scores post-treatment. Head meat was impacted ($P < 0.001$) by treatment with 2.5% hot

lactic acid receiving lower lean color scores than 5.0% hot lactic acid, peroxyacetic acid, and hot water (Table 23), whereas lean color scores for cheek meat were not affected ($P = 0.443$) by treatment (Table 24). There was a sampling time × treatment interaction for discoloration in pork head meat and pork cheek meat (Table 25). In pork head meat, all lactic acid treatments yielded higher ($P < 0.001$) discoloration scores from panelists post-treatment compared to peroxyacetic acid and hot water treatments. Pork cheeks showed a similar trend as pork head meat with lactic acid treated product producing higher ($P < 0.001$) discoloration scores post-treatment than peroxyacetic acid and hot water (Table 25). Lean color and discoloration differences showed that antimicrobial interventions, namely lactic acid, produced products with less intense or less saturated color, values that indicated color further from red (a^* and hue values), products that appeared lighter in color (L^*), and became visually discolored after treatments were applied. Although there were lean color and discoloration differences among sampling times and treatments, the overall negative impact to product color was minimal and could be minimized by applying interventions at lower concentrations and temperatures. Consumers often base meat-buying decisions on color attributes and can associate discoloration with freshness and wholesomeness (Mancini & Hunt, 2005). Therefore, balancing the use of antimicrobial interventions for food safety while also mitigating potential negative sensory characteristics is imperative.

CHAPTER V

CONCLUSION

Antimicrobial interventions are an effective means of enhancing the safety of pork and edible pork and beef by-products. Lactic acid at 2.5% and 5.0% concentrations and applied warm (55 °C) and at room temperature along with 400 ppm peroxyacetic acid interventions can be effective at reducing pathogen contamination on a variety of beef and pork by-products, especially beef and pork hearts and pork livers. However, hot water (55 °C) was not an effective intervention on all product types. In addition, minimal negative impacts from lactic acid interventions were seen for product color. For pork hearts and livers, peroxyacetic acid and hot water treatments did not negatively impact product color, however, lactic acid treatments were generally more effective at reducing contamination than peroxyacetic acid or hot water. Results from this research can be applied to both the U.S. beef and pork industries, spanning domestic and export markets. Information from this study related to the efficacy of targeted interventions in pork products and beef and pork edible by-products could enhance the viability and sustainability of trade across the world, while also enhancing food safety.

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APPENDIX

Table 1
Least squares means for counts (log_{10} CFU/cm^2) of rifampicin-resistant *Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* biotype I surrogates, and *Campylobacter jejuni* survival stratified by sampling time[a] and treatment[b] main effects for hot skin-on pork sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment

Main effects	Counts	SEM[c]
Salmonella		
Sampling time		
Pre-treatment	6.34a	
Post 30 min	5.88b	
Post 24 h	5.21c	
P-value	< 0.001	0.07
Treatment		
2.5% lactic acid, room temperature	5.87	
2.5% lactic acid, 55 °C	5.92	
5.0% lactic acid, room temperature	5.69	
5.0% lactic acid, 55 °C	5.60	
400 ppm peroxyacetic acid	5.86	
Water, 55 °C	5.92	
P-value	0.123	0.10
STEC		
Sampling time		
Pre-treatment	6.46a	
Post 30 min	5.88b	
Post 24 h	6.19ab	
P-value	0.013	0.13
Treatment		
2.5% lactic acid, room temperature	6.25	
2.5% lactic acid, 55 °C	6.34	
5.0% lactic acid, room temperature	6.18	
5.0% lactic acid, 55 °C	5.62	
400 ppm peroxyacetic acid	6.22	
Water, 55 °C	6.45	
P-value	0.051	0.18
E. coli biotype I surrogates		
Sampling time		
Pre-treatment	5.78a	
Post 30 min	4.93b	
Post 24 h	4.82b	
P-value	< 0.001	0.08
Treatment		
2.5% lactic acid, room temperature	5.03	
2.5% lactic acid, 55 °C	5.21	
5.0% lactic acid, room temperature	5.20	
5.0% lactic acid, 55 °C	5.04	
400 ppm peroxyacetic acid	5.06	
Water, 55 °C	5.52	
P-value	0.056	0.12
Campylobacter jejuni		
Sampling time		
Pre-treatment	4.50a	
Post 30 min	3.65b	
Post 24 h	3.51b	
P-value	< 0.001	0.06
Treatment		
2.5% lactic acid, room temperature	3.99	
2.5% lactic acid, 55 °C	3.78	
5.0% lactic acid, room temperature	3.99	
5.0% lactic acid, 55 °C	3.79	
400 ppm peroxyacetic acid	3.84	
Water, 55 °C	3.92	
P-value	0.338	0.09

Means within a pathogen and main effect lacking a common letter (a-c) differ ($P < 0.05$).

[a] Products ($n = 3$ reps per product type) were sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment.

[b] Treatments (verified by titration) were applied to hot skin-on pork. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[c] SEM = standard error of the mean.

Table 2
Least squares means for counts (log_{10} CFU/cm^2) of rifampicin-resistant *Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* biotype I surrogates, and *Campylobacter jejuni* survival stratified by sampling time[a] and treatment[b] main effects for hot skinless pork sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment

Main effects	Counts	SEM[c]
Salmonella		
Sampling time		
Pre-treatment	6.21a	
Post 30 min	4.74b	
Post 24 h	5.01b	
P-value	< 0.001	0.16
Treatment		
2.5% lactic acid, room temperature	5.26bc	
2.5% lactic acid, 55 °C	5.17bc	
5.0% lactic acid, room temperature	4.85c	
5.0% lactic acid, 55 °C	4.91c	
400 ppm peroxyacetic acid	5.66ab	
Water, 55 °C	6.06a	
P-value	0.005	0.23
STEC		
Sampling time		
Pre-treatment	6.56a	
Post 30 min	5.50b	
Post 24 h	5.43b	
P-value	< 0.001	0.09
Treatment		
2.5% lactic acid, room temperature	5.73c	
2.5% lactic acid, 55 °C	5.84bc	
5.0% lactic acid, room temperature	5.06d	
5.0% lactic acid, 55 °C	5.68c	
400 ppm peroxyacetic acid	6.12b	
Water, 55 °C	6.55a	
P-value	< 0.001	0.12
E. coli biotype I surrogates		
Sampling time		
Pre-treatment	5.87a	
Post 30 min	3.92b	
Post 24 h	3.89b	
P-value	< 0.001	0.15
Treatment		
2.5% lactic acid, room temperature	4.17cd	
2.5% lactic acid, 55 °C	4.62bc	
5.0% lactic acid, room temperature	3.67d	
5.0% lactic acid, 55 °C	4.15cd	
400 ppm peroxyacetic acid	5.15ab	
Water, 55 °C	5.59a	
P-value	< 0.001	0.21
Campylobacter jejuni		
Sampling time		
Pre-treatment	4.72a	
Post 30 min	3.27b	
Post 24 h	2.47c	
P-value	< 0.001	0.16
Treatment		
2.5% lactic acid, room temperature	3.28cd	
2.5% lactic acid, 55 °C	3.60bc	
5.0% lactic acid, room temperature	2.83d	
5.0% lactic acid, 55 °C	2.78d	
400 ppm peroxyacetic acid	4.01ab	
Water, 55 °C	4.43a	
P-value	< 0.001	0.23

Means within a pathogen and main effect lacking a common letter (a-d) differ ($P < 0.05$).

[a] Products ($n = 3$ reps per product type) were sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment.

[b] Treatments (verified by titration) were applied to hot skinless pork. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[c] SEM = standard error of the mean.

Table 3
Least squares means for counts (log_{10} CFU/cm^2) of rifampicin-resistant *Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* biotype I surrogates, and *Campylobacter jejuni* survival stratified by sampling time[a] and treatment[b] main effects for chilled skin-on pork sampled pre-treatment (24 h post-inoculation) and 30 min post-treatment

Main effects	Counts	SEM[c]
Salmonella		
Sampling time		
Pre-treatment	6.08a	
Post 30 min	5.45b	
P-value	< 0.001	0.06
Treatment		
2.5% lactic acid, room temperature	5.72	
2.5% lactic acid, 55 °C	5.78	
5.0% lactic acid, room temperature	5.72	
5.0% lactic acid, 55 °C	5.79	
400 ppm peroxyacetic acid	5.73	
Water, 55 °C	5.85	
P-value	0.928	0.10
STEC		
Sampling time		
Pre-treatment	6.63a	
Post 30 min	6.18b	
P-value	0.001	0.08
Treatment		
2.5% lactic acid, room temperature	6.25b	
2.5% lactic acid, 55 °C	6.19b	
5.0% lactic acid, room temperature	6.10b	
5.0% lactic acid, 55 °C	6.50b	
400 ppm peroxyacetic acid	6.37b	
Water, 55 °C	7.05a	
P-value	0.002	0.15
E. coli biotype I surrogates		
Sampling time		
Pre-treatment	5.62a	
Post 30 min	5.11b	
P-value	< 0.001	0.07
Treatment		
2.5% lactic acid, room temperature	5.31bc	
2.5% lactic acid, 55 °C	5.28bc	
5.0% lactic acid, room temperature	5.01c	
5.0% lactic acid, 55 °C	5.20bc	
400 ppm peroxyacetic acid	5.54ab	
Water, 55 °C	5.84a	
P-value	0.001	0.12
Campylobacter jejuni		
Sampling time		
Pre-treatment	3.83a	
Post 30 min	3.03b	
P-value	< 0.001	0.08
Treatment		
2.5% lactic acid, room temperature	3.57ab	
2.5% lactic acid, 55 °C	3.13c	
5.0% lactic acid, room temperature	3.56ab	
5.0% lactic acid, 55 °C	3.36abc	
400 ppm peroxyacetic acid	3.19bc	
Water, 55 °C	3.75a	
P-value	0.047	0.14

Means within a pathogen and main effect lacking a common letter (a-c) differ ($P < 0.05$).
[a] Products ($n = 3$ reps per product type) were sampled pre-treatment (24 h post-inoculation) and 30 min post-treatment.
[b] Treatments (verified by titration) were applied to chilled skin-on pork. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.
[c] SEM = standard error of the mean.

Table 4
Least squares means for counts (log_{10} CFU/cm^2) of rifampicin-resistant *Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* biotype I surrogates, and *Campylobacter jejuni* survival stratified by sampling time[a] and treatment[b] main effects for chilled skinless pork sampled pre-treatment (24 h post-inoculation) and 30 min post-treatment

Main effects	Counts	SEM[c]
Salmonella		
Sampling time		
Pre-treatment	5.86a	
Post 30 min	5.13b	
P-value	< 0.001	0.09
Treatment		
2.5% lactic acid, room temperature	5.33bc	
2.5% lactic acid, 55 °C	5.47bc	
5.0% lactic acid, room temperature	5.10c	
5.0% lactic acid, 55 °C	5.65ab	
400 ppm peroxyacetic acid	5.41bc	
Water, 55 °C	6.01a	
P-value	0.016	0.16
STEC		
Sampling time		
Pre-treatment	6.51a	
Post 30 min	5.83b	
P-value	0.003	0.14
Treatment		
2.5% lactic acid, room temperature	6.76	
2.5% lactic acid, 55 °C	5.95	
5.0% lactic acid, room temperature	6.15	
5.0% lactic acid, 55 °C	5.93	
400 ppm peroxyacetic acid	6.05	
Water, 55 °C	6.15	
P-value	0.233	0.25
E. coli biotype I surrogates		
Sampling time		
Pre-treatment	5.69a	
Post 30 min	5.06b	
P-value	0.001	0.12
Treatment		
2.5% lactic acid, room temperature	5.42ab	
2.5% lactic acid, 55 °C	5.09b	
5.0% lactic acid, room temperature	5.43ab	
5.0% lactic acid, 55 °C	4.83b	
400 ppm peroxyacetic acid	5.73a	
Water, 55 °C	5.76a	
P-value	0.030	0.21
Campylobacter jejuni		
Sampling time		
Pre-treatment	4.10a	
Post 30 min	2.87b	
P-value	< 0.001	0.13
Treatment		
2.5% lactic acid, room temperature	3.33ab	
2.5% lactic acid, 55 °C	3.93a	
5.0% lactic acid, room temperature	2.80b	
5.0% lactic acid, 55 °C	3.38ab	
400 ppm peroxyacetic acid	3.59a	
Water, 55 °C	3.88a	
P-value	0.022	0.23

Means within a pathogen and main effect lacking a common letter (a-c) differ ($P < 0.05$).
[a] Products ($n = 3$ reps per product type) were sampled pre-treatment (24 h post-inoculation) and 30 min post-treatment.
[b] Treatments (verified by titration) were applied to chilled skinless pork. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.
[c] SEM = standard error of the mean.

Table 5
Least squares means for counts (log_{10} CFU/cm^2) of rifampicin-resistant *Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* biotype I surrogates, and *Campylobacter jejuni* survival stratified by sampling time[a] and treatment[b] main effects for pork head meat sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment

Main effects	Counts	SEM[c]
Salmonella		
Sampling time		
Pre-treatment	6.47a	
Post 30 min	5.76b	
Post 24 h	5.80b	
P-value	< 0.001	0.08
Treatment		
2.5% lactic acid, room temperature	5.92	
2.5% lactic acid, 55 °C	6.07	
5.0% lactic acid, room temperature	5.98	
5.0% lactic acid, 55 °C	5.81	
400 ppm peroxyacetic acid	6.13	
Water, 55 °C	6.16	
P-value	0.233	0.11
STEC		
Sampling time		
Pre-treatment	6.71a	
Post 30 min	6.46b	
Post 24 h	6.27b	
P-value	< 0.001	0.07
Treatment		
2.5% lactic acid, room temperature	6.46bc	
2.5% lactic acid, 55 °C	6.55ab	
5.0% lactic acid, room temperature	6.22cd	
5.0% lactic acid, 55 °C	6.13d	
400 ppm peroxyacetic acid	6.70ab	
Water, 55 °C	6.81a	
P-value	< 0.001	0.10
E. coli biotype I surrogates		
Sampling time		
Pre-treatment	6.10a	
Post 30 min	5.71b	
Post 24 h	5.39b	
P-value	0.007	0.15
Treatment		
2.5% lactic acid, room temperature	5.90	
2.5% lactic acid, 55 °C	5.83	
5.0% lactic acid, room temperature	5.29	
5.0% lactic acid, 55 °C	5.82	
400 ppm peroxyacetic acid	5.66	
Water, 55 °C	5.89	
P-value	0.309	0.21
Campylobacter jejuni		
Sampling time		
Pre-treatment	4.57a	
Post 30 min	3.75b	
Post 24 h	3.48c	
P-value	< 0.001	0.09
Treatment		
2.5% lactic acid, room temperature	3.90b	
2.5% lactic acid, 55 °C	4.13ab	
5.0% lactic acid, room temperature	3.47c	
5.0% lactic acid, 55 °C	3.88b	
400 ppm peroxyacetic acid	3.94ab	
Water, 55 °C	4.27a	
P-value	0.001	0.12

Means within a pathogen and main effect lacking a common letter (a-d) differ ($P < 0.05$).
[a] Products ($n = 3$ reps per product type) were sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment.
[b] Treatments (verified by titration) were applied to pork head meat. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.
[c] SEM = standard error of the mean.

Table 6
Least squares means for counts (log_{10} CFU/cm^2) of rifampicin-resistant *Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* biotype I surrogates, and *Campylobacter jejuni* survival stratified by sampling time[a] and treatment[b] main effects for pork cheek meat sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment

Main effects	Counts	SEM[c]
Salmonella		
Sampling time		
Pre-treatment	6.55a	
Post 30 min	5.85b	
Post 24 h	5.72b	
P-value	< 0.001	0.07
Treatment		
2.5% lactic acid, room temperature	5.90cd	
2.5% lactic acid, 55 °C	6.18b	
5.0% lactic acid, room temperature	5.89cd	
5.0% lactic acid, 55 °C	5.67d	
400 ppm peroxyacetic acid	6.06bc	
Water, 55 °C	6.56a	
P-value	< 0.001	0.10
STEC		
Sampling time		
Pre-treatment	6.73a	
Post 30 min	6.36b	
Post 24 h	6.15b	
P-value	< 0.001	0.09
Treatment		
2.5% lactic acid, room temperature	6.41	
2.5% lactic acid, 55 °C	6.33	
5.0% lactic acid, room temperature	6.24	
5.0% lactic acid, 55 °C	6.36	
400 ppm peroxyacetic acid	6.49	
Water, 55 °C	6.66	
P-value	0.328	0.13
E. coli biotype I surrogates		
Sampling time		
Pre-treatment	6.52a	
Post 30 min	5.99b	
Post 24 h	5.87b	
P-value	< 0.001	0.11
Treatment		
2.5% lactic acid, room temperature	6.14abc	
2.5% lactic acid, 55 °C	5.79c	
5.0% lactic acid, room temperature	5.96bc	
5.0% lactic acid, 55 °C	6.09abc	
400 ppm peroxyacetic acid	6.37ab	
Water, 55 °C	6.41a	
P-value	0.050	0.15
Campylobacter jejuni		
Sampling time		
Pre-treatment	4.55a	
Post 30 min	3.66b	
Post 24 h	3.17c	
P-value	< 0.001	0.10
Treatment		
2.5% lactic acid, room temperature	3.94b	
2.5% lactic acid, 55 °C	3.89b	
5.0% lactic acid, room temperature	3.41c	
5.0% lactic acid, 55 °C	3.11c	
400 ppm peroxyacetic acid	4.06ab	
Water, 55 °C	4.36a	
P-value	< 0.001	0.15

Means within a pathogen and main effect lacking a common letter (a-d) differ ($P < 0.05$).
[a] Products ($n = 3$ reps per product type) were sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment.
[b] Treatments (verified by titration) were applied to pork cheek meat. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.
[c] SEM = standard error of the mean.

Table 7
Least squares means for counts (log_{10} CFU/cm^2) of rifampicin-resistant *Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* biotype I surrogates, and *Campylobacter jejuni* survival stratified by sampling time[a] and treatment[b] main effects for pork heart meat sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment

Main effects	Counts	SEM[c]
Salmonella		
Sampling time		
Pre-treatment	5.83a	
Post 30 min	3.98b	
Post 24 h	3.75b	
P-value	< 0.001	0.14
Treatment		
2.5% lactic acid, room temperature	4.60bc	
2.5% lactic acid, 55 °C	4.44bcd	
5.0% lactic acid, room temperature	3.88d	
5.0% lactic acid, 55 °C	4.07cd	
400 ppm peroxyacetic acid	4.70b	
Water, 55 °C	5.43a	
P-value	< 0.001	0.20
STEC		
Sampling time		
Pre-treatment	5.99a	
Post 30 min	4.56b	
Post 24 h	4.29b	
P-value	< 0.001	0.17
Treatment		
2.5% lactic acid, room temperature	5.10b	
2.5% lactic acid, 55 °C	4.84bc	
5.0% lactic acid, room temperature	4.57bc	
5.0% lactic acid, 55 °C	4.19c	
400 ppm peroxyacetic acid	4.89bc	
Water, 55 °C	6.11a	
P-value	< 0.001	0.24
E. coli biotype I surrogates		
Sampling time		
Pre-treatment	5.83a	
Post 30 min	4.15b	
Post 24 h	3.97b	
P-value	< 0.001	0.17
Treatment		
2.5% lactic acid, room temperature	4.31	
2.5% lactic acid, 55 °C	4.73	
5.0% lactic acid, room temperature	4.79	
5.0% lactic acid, 55 °C	3.87	
400 ppm peroxyacetic acid	4.55	
Water, 55 °C	4.77	
P-value	0.058	0.23
Campylobacter jejuni		
Sampling time		
Pre-treatment	3.69a	
Post 30 min	1.47b	
Post 24 h	1.38b	
P-value	< 0.001	0.10
Treatment		
2.5% lactic acid, room temperature	2.27bc	
2.5% lactic acid, 55 °C	1.88cd	
5.0% lactic acid, room temperature	1.98bcd	
5.0% lactic acid, 55 °C	1.85d	
400 ppm peroxyacetic acid	2.33b	
Water, 55 °C	2.76a	
P-value	0.001	0.15

Means within a pathogen and main effect lacking a common letter (a-d) differ ($P < 0.05$).
[a] Products ($n = 3$ reps per product type) were sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment.
[b] Treatments (verified by titration) were applied to pork heart meat. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.
[c] SEM = standard error of the mean.

Table 8
Least squares means for counts (log_{10} CFU/cm^2) of rifampicin-resistant *Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* biotype I surrogates, and *Campylobacter jejuni* survival stratified by sampling time[a] and treatment[b] main effects for pork liver meat sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment

Main effects	Counts	SEM[c]
Salmonella		
Sampling time		
Pre-treatment	5.78a	
Post 30 min	3.48b	
Post 24 h	3.63b	
P-value	< 0.001	0.16
Treatment		
2.5% lactic acid, room temperature	3.97bcd	
2.5% lactic acid, 55 °C	4.39bc	
5.0% lactic acid, room temperature	3.75cd	
5.0% lactic acid, 55 °C	3.56d	
400 ppm peroxyacetic acid	4.43b	
Water, 55 °C	5.69a	
P-value	< 0.001	0.23
STEC		
Sampling time		
Pre-treatment	6.02a	
Post 30 min	3.90b	
Post 24 h	3.64b	
P-value	< 0.001	0.16
Treatment		
2.5% lactic acid, room temperature	4.71b	
2.5% lactic acid, 55 °C	4.20bc	
5.0% lactic acid, room temperature	4.57b	
5.0% lactic acid, 55 °C	3.68c	
400 ppm peroxyacetic acid	4.54b	
Water, 55 °C	5.43a	
P-value	< 0.001	0.22
E. coli biotype I surrogates		
Sampling time		
Pre-treatment	5.42a	
Post 30 min	4.40b	
Post 24 h	4.11b	
P-value	< 0.001	0.17
Treatment		
2.5% lactic acid, room temperature	4.99a	
2.5% lactic acid, 55 °C	5.07a	
5.0% lactic acid, room temperature	4.85a	
5.0% lactic acid, 55 °C	3.58b	
400 ppm peroxyacetic acid	4.48a	
Water, 55 °C	4.87a	
P-value	0.001	0.25
Campylobacter jejuni		
Sampling time		
Pre-treatment	3.79a	
Post 30 min	1.59b	
Post 24 h	1.23b	
P-value	< 0.001	0.13
Treatment		
2.5% lactic acid, room temperature	2.34ab	
2.5% lactic acid, 55 °C	2.03b	
5.0% lactic acid, room temperature	1.91b	
5.0% lactic acid, 55 °C	1.94b	
400 ppm peroxyacetic acid	2.21b	
Water, 55 °C	2.80a	
P-value	0.015	0.18

Means within a pathogen and main effect lacking a common letter (a-d) differ ($P < 0.05$).
[a] Products ($n = 3$ reps per product type) were sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment.
[b] Treatments (verified by titration) were applied to pork liver meat. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.
[c] SEM = standard error of the mean.

Table 9
Least squares means for counts (log_{10} CFU/cm^2) of rifampicin-resistant *Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC), and *E. coli* biotype I surrogate survival stratified by sampling time[a] and treatment[b] main effects for beef head meat sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment

Main effects	Counts	SEM[c]
Salmonella		
Sampling time		
Pre-treatment	6.57a	
Post 30 min	6.21b	
Post 24 h	6.09b	
P-value	< 0.001	0.06
Treatment		
2.5% lactic acid, room temperature	6.21	
2.5% lactic acid, 55 °C	6.42	
5.0% lactic acid, room temperature	6.37	
5.0% lactic acid, 55 °C	6.17	
400 ppm peroxyacetic acid	6.25	
Water, 55 °C	6.33	
P-value	0.241	0.08
STEC		
Sampling time		
Pre-treatment	6.91a	
Post 30 min	6.44b	
Post 24 h	6.42b	
P-value	< 0.001	0.08
Treatment		
2.5% lactic acid, room temperature	6.62	
2.5% lactic acid, 55 °C	6.66	
5.0% lactic acid, room temperature	6.54	
5.0% lactic acid, 55 °C	6.33	
400 ppm peroxyacetic acid	6.58	
Water, 55 °C	6.82	
P-value	0.078	0.11
E. coli biotype I surrogates		
Sampling time		
Pre-treatment	6.68a	
Post 30 min	6.26b	
Post 24 h	6.03b	
P-value	< 0.001	0.09
Treatment		
2.5% lactic acid, room temperature	6.22	
2.5% lactic acid, 55 °C	6.24	
5.0% lactic acid, room temperature	6.27	
5.0% lactic acid, 55 °C	6.23	
400 ppm peroxyacetic acid	6.36	
Water, 55 °C	6.60	
P-value	0.294	0.13

Means within a pathogen and main effect lacking a common letter (a-b) differ ($P < 0.05$).
[a] Products ($n = 3$ reps per product type) were sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment.
[b] Treatments (verified by titration) were applied to beef head meat. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.
[c] SEM = standard error of the mean.

Table 10
Least squares means for counts (log_{10} CFU/cm^2) of rifampicin-resistant *Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC), and *E. coli* biotype I surrogate survival stratified by sampling time[a] and treatment[b] main effects for beef cheek meat sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment

Main effects	Counts	SEM[c]
Salmonella		
Sampling time		
Pre-treatment	6.70a	
Post 30 min	6.21b	
Post 24 h	6.18b	
P-value	< 0.001	0.09
Treatment		
2.5% lactic acid, room temperature	6.21	
2.5% lactic acid, 55 °C	6.30	
5.0% lactic acid, room temperature	6.26	
5.0% lactic acid, 55 °C	6.38	
400 ppm peroxyacetic acid	6.37	
Water, 55 °C	6.67	
P-value	0.181	0.13
STEC		
Sampling time		
Pre-treatment	6.93a	
Post 30 min	6.41c	
Post 24 h	6.66b	
P-value	< 0.001	0.08
Treatment		
2.5% lactic acid, room temperature	6.44c	
2.5% lactic acid, 55 °C	6.62bc	
5.0% lactic acid, room temperature	6.56bc	
5.0% lactic acid, 55 °C	6.39c	
400 ppm peroxyacetic acid	6.81b	
Water, 55 °C	7.19a	
P-value	0.001	0.11
E. coli biotype I surrogates		
Sampling time		
Pre-treatment	6.57a	
Post 30 min	5.82b	
Post 24 h	5.54c	
P-value	< 0.001	0.08
Treatment		
2.5% lactic acid, room temperature	5.91b	
2.5% lactic acid, 55 °C	5.81b	
5.0% lactic acid, room temperature	5.81b	
5.0% lactic acid, 55 °C	6.04ab	
400 ppm peroxyacetic acid	5.97b	
Water, 55 °C	6.31a	
P-value	0.025	0.11

Means within a pathogen and main effect lacking a common letter (a-c) differ ($P < 0.05$).
[a] Products ($n = 3$ reps per product type) were sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment.
[b] Treatments (verified by titration) were applied to beef cheek meat. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.
[c] SEM = standard error of the mean.

Table 11
Least squares means for counts (log_{10} CFU/cm^2) of rifampicin-resistant *Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC), and *E. coli* biotype I surrogate survival stratified by sampling time[a] and treatment[b] main effects for beef heart meat sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment

Main effects	Counts	SEM[c]
Salmonella		
Sampling time		
Pre-treatment	5.78a	
Post 30 min	3.89b	
Post 24 h	4.00b	
P-value	< 0.001	0.15
Treatment		
2.5% lactic acid, room temperature	4.33c	
2.5% lactic acid, 55 °C	5.02ab	
5.0% lactic acid, room temperature	3.69d	
5.0% lactic acid, 55 °C	4.53bc	
400 ppm peroxyacetic acid	4.17cd	
Water, 55 °C	5.60a	
P-value	< 0.001	0.21
STEC		
Sampling time		
Pre-treatment	6.14a	
Post 30 min	4.35b	
Post 24 h	4.07b	
P-value	< 0.001	0.22
Treatment		
2.5% lactic acid, room temperature	5.20b	
2.5% lactic acid, 55 °C	4.72bc	
5.0% lactic acid, room temperature	4.32bc	
5.0% lactic acid, 55 °C	4.62bc	
400 ppm peroxyacetic acid	4.16c	
Water, 55 °C	6.11a	
P-value	0.001	0.31
E. coli biotype I surrogates		
Sampling time		
Pre-treatment	5.70a	
Post 30 min	4.15b	
Post 24 h	3.39c	
P-value	< 0.001	0.18
Treatment		
2.5% lactic acid, room temperature	4.28b	
2.5% lactic acid, 55 °C	4.16b	
5.0% lactic acid, room temperature	4.14b	
5.0% lactic acid, 55 °C	4.20b	
400 ppm peroxyacetic acid	4.44b	
Water, 55 °C	5.26a	
P-value	0.029	0.25

Means within a pathogen and main effect lacking a common letter (a-d) differ ($P < 0.05$).
[a] Products ($n = 3$ reps per product type) were sampled pre-treatment (30 min post-inoculation), 30 min post-treatment, and 24 h post-treatment.
[b] Treatments (verified by titration) were applied to beef heart meat. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.
[c] SEM = standard error of the mean.

Table 12

Least squares means of CIE color space values (*L*, a*, b**), hue, chroma, and ΔE for sampling time[a] and treatment[b] main effects for hot skinless pork

Main effects	*L**	*a**	*b**	Hue angle	Chroma	ΔE
Sampling time						
Before treatment	48.1b	6.1	11.1a	61.1	12.7a	-
After treatment	52.0a	5.2	8.7b	59.0	10.2b	-
P-value	0.017	0.125	< 0.001	0.345	< 0.001	
SEM[c]	1.06	0.38	0.37	1.58	0.42	
Treatment						
2.5% lactic acid, room temperature	50.9	6.0	9.9	58.5	11.6	5.9
2.5% lactic acid, 55 °C	51.7	4.9	10.5	65.0	11.8	6.0
5.0% lactic acid, room temperature	53.5	5.0	9.8	62.5	11.1	7.5
5.0% lactic acid, 55 °C	49.1	5.0	8.2	58.7	9.6	6.1
400 ppm peroxyacetic acid	46.6	7.0	10.5	56.3	12.7	5.0
Water, 55 °C	48.6	6.1	10.3	59.4	12.0	5.1
P-value	0.155	0.169	0.154	0.296	0.109	0.680
SEM[c]	1.84	0.65	0.65	2.74	0.72	1.15

Means within the same main effect and column lacking a common letter (a-b) differ ($P < 0.05$).

[a] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.

[b] Treatments (verified by titration) were applied to hot skinless pork. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[c] SEM = standard error of the mean.

Table 13

Least squares means of CIE color space values (*L*, a*, b**), hue, chroma, and ΔE for sampling time[a] and treatment[b] main effects for chilled skinless pork

Main effects	*L**	*a**	*b**	Hue angle	Chroma	ΔE
Sampling time						
Before treatment	51.7b	11.3a	16.6a	56.4	20.2a	-
After treatment	56.6a	8.1b	13.5b	58.6	15.8b	-
P-value	0.036	0.002	0.005	0.192	0.002	
SEM[c]	1.54	0.66	0.70	1.12	0.90	
Treatment						
2.5% lactic acid, room temperature	55.4	8.2	13.7	59.6	16.1	15.9
2.5% lactic acid, 55 °C	55.1	10.7	16.0	56.3	19.2	8.9
5.0% lactic acid, room temperature	54.8	11.1	16.5	56.4	19.9	7.0
5.0% lactic acid, 55 °C	53.3	10.7	14.9	54.6	18.4	8.6
400 ppm peroxyacetic acid	51.4	10.1	15.1	56.0	18.2	6.3
Water, 55 °C	54.8	7.5	14.0	62.2	16.0	7.2
P-value	0.894	0.165	0.586	0.105	0.407	0.210
SEM[c]	2.66	1.15	1.22	1.95	1.56	2.65

Means within the same main effect and column lacking a common letter (a-b) differ ($P < 0.05$).

[a] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.

[b] Treatments (verified by titration) were applied to chilled skinless pork. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[c] SEM = standard error of the mean.

Table 14

Least squares means of trained panelists' scores for pork color attributes[a] stratified by sampling time[b] and treatment[c] main effects for hot skinless pork

Main effects	Lean Color
Sampling time	
Before	4.3a
After	3.8b
P-value	< 0.001
SEM	0.09
Treatment	
2.5% lactic acid, room temperature	3.8bc
2.5% lactic acid, 55 °C	4.1b
5.0% lactic acid, room temperature	3.5c
5.0% lactic acid, 55 °C	4.0b
400 ppm peroxyacetic acid	4.7a
Water, 55 °C	4.1b
P-value	< 0.001
SEM	0.16

Means within a main effect lacking a common letter (a-c) differ ($P < 0.05$).

[a] Panelists used the following scale: lean color (1 = pale grayish-pink; 8 = very dark grayish-pink).

[b] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.

[c] Treatments (verified by titration) were applied to hot skinless pork. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[d] SEM = standard error of the mean.

Table 15

Least squares means of trained panelists' scores for pork color attributes[a] stratified by sampling time[b] and treatment[c] main effects for chilled skinless pork

Main effects	Lean Color	Discoloration
Sampling time		
Before	3.4a	1.0b
After	2.9b	1.4a
P-value	< 0.001	< 0.001
SEM	0.07	0.04
Treatment		
2.5% lactic acid, room temperature	2.7c	1.2ab
2.5% lactic acid, 55 °C	3.1b	1.3ab
5.0% lactic acid, room temperature	3.1ab	1.4a
5.0% lactic acid, 55 °C	3.3ab	1.3a
400 ppm peroxyacetic acid	3.4a	1.1b
Water, 55 °C	3.1ab	1.1b
P-value	0.002	0.008
SEM	0.13	0.07

Means within a main effect and color panel attribute lacking a common letter (a-c) differ ($P < 0.05$).

[a] Panelists used the following scales: lean color (1 = pale grayish-pink; 8 = very dark grayish-pink), discoloration (1 = none; 5 = extreme).

[b] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.

[c] Treatments (verified by titration) were applied to chilled skinless pork. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[d] SEM = standard error of the mean.

Table 16

Least squares means of trained panelists' scores for pork discoloration[a] for sampling time[b] × treatment[c] interaction for hot skinless pork

	Discoloration	
Product type	Before	After
Hot skinless pork		
2.5% lactic acid, room temperature	1.1c	2.5a
2.5% lactic acid, 55 °C	1.1c	1.8b
5.0% lactic acid, room temperature	1.1c	2.4a
5.0% lactic acid, 55 °C	1.1c	2.8a
400 ppm peroxyacetic acid	1.1c	1.3c
Water, 55 °C	1.1c	1.2c
P-value < 0.001		
SEM[d] = 0.13		

Means lacking a common letter (a-c) differ ($P < 0.05$).

[a] Panelists used the following scale: discoloration (1 = none; 5 = extreme).

[b] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.

[c] Treatments (verified by titration) were applied to hot skinless pork. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[d] SEM = standard error of the mean.

Table 17

Least squares means of CIE color space values (a^*) for sampling time[a] × treatment[b] interaction for pork head meat and pork hearts

	a^*	
Product type	Before	After
Pork head meat		
2.5% lactic acid, room temperature	16.2a	10.4ef
2.5% lactic acid, 55 °C	16.6a	11.4cde
5.0% lactic acid, room temperature	13.5bc	8.7f
5.0% lactic acid, 55 °C	16.5a	10.8def
400 ppm peroxyacetic acid	15.7ab	13.1cd
Water, 55 °C	16.6a	15.8a
P-value 0.025		
SEM[c] = 0.79		
Pork hearts		
2.5% lactic acid, room temperature	14.6b	9.1de
2.5% lactic acid, 55 °C	17.6a	7.7e
5.0% lactic acid, room temperature	15.8ab	7.7e
5.0% lactic acid, 55 °C	14.5b	7.5e
400 ppm peroxyacetic acid	13.4bc	8.1e
Water, 55 °C	14.6b	11.4cd
P-value 0.047		
SEM[c] = 1.00		

Means within the same product type lacking a common letter (a-f) differ ($P < 0.05$).

[a] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.

[b] Treatments (verified by titration) were applied to hot pork head meat and hot pork hearts. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[c] SEM = standard error of the mean.

Table 18

Least squares means of CIE color space values (*L*, a*, b**), hue, chroma, and ΔE for sampling time[a] and treatment[b] main effects for pork head meat

Main effects	*L**	*a**	*b**	Hue angle	Chroma	ΔE
Sampling time						
Before treatment	45.8	15.8a	14.4a	42.2b	21.4a	-
After treatment	48.3	11.7b	13.0b	48.2a	17.5b	-
P-value	0.054	<0.001	0.061	<0.001	<0.001	
SEM[c]	0.85	0.32	0.51	0.85	0.52	
Treatment						
2.5% lactic acid, room temperature	49.6	13.3b	13.9	46.8ab	19.3abc	9.6
2.5% lactic acid, 55 °C	48.0	14.0b	14.3	45.9abc	20.1ab	6.5
5.0% lactic acid, room temperature	48.6	11.1c	12.5	49.3a	16.8c	6.0
5.0% lactic acid, 55 °C	43.2	13.7b	13.0	43.9bc	19.0bc	7.5
400 ppm peroxyacetic acid	46.7	14.4b	13.8	43.7bc	19.9ab	4.7
Water, 55 °C	46.2	16.2a	14.5	41.7c	21.7a	8.5
P-value	0.073	< 0.001	0.628	0.021	0.027	0.572
SEM[c]	1.48	0.56	0.89	1.47	0.91	1.99

Means within the same main effect and column lacking a common letter (a-c) differ ($P < 0.05$).

[a] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.

[b] Treatments (verified by titration) were applied to hot pork head meat. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[c] SEM = standard error of the mean.

Table 19

Least squares means of CIE color space values (*L*, a*, b**), hue, chroma, and ΔE for sampling time[a] and treatment[b] main effects for pork cheek meat

Main effects	*L**	*a**	*b**	Hue angle	Chroma	ΔE
Sampling time						
Before treatment	41.3	13.0a	10.8	39.2b	17.0a	-
After treatment	42.7	10.4b	9.8	43.6a	14.3b	-
P-value	0.287	< 0.001	0.130	<0.001	0.003	
SEM[c]	0.91	0.40	0.42	0.59	0.55	
Treatment						
2.5% lactic acid, room temperature	40.3	12.3	10.4	40.1bc	16.1	6.5
2.5% lactic acid, 55 °C	44.0	11.4	10.4	42.7ab	15.5	6.5
5.0% lactic acid, room temperature	42.1	11.4	10.6	43.4a	15.7	7.6
5.0% lactic acid, 55 °C	43.5	10.6	10.0	43.1a	14.7	5.7
400 ppm peroxyacetic acid	42.4	12.3	10.5	40.0bc	16.2	4.2
Water, 55 °C	39.9	12.2	9.8	38.9c	15.7	5.8
P-value	0.369	0.445	0.973	0.016	0.871	0.866
SEM[c]	1.57	0.69	0.73	1.02	0.95	1.86

Means within the same main effect and column lacking a common letter (a-c) differ ($P < 0.05$).

[a] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.

[b] Treatments (verified by titration) were applied to hot pork cheek meat. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[c] SEM = standard error of the mean.

Table 20

Least squares means of CIE color space values (*L*, a*, b**), hue, chroma, and ΔE for sampling time[a] and treatment[b] main effects for pork hearts

Main effects	*L**	*a**	*b**	Hue angle	Chroma	ΔE
Sampling time						
Before treatment	39.0b	15.1a	12.8a	40.3b	19.8a	-
After treatment	48.4a	8.6b	8.7b	44.7a	12.3b	-
P-value	<0.001	<0.001	<0.001	0.014	<0.001	
SEM[c]	1.02	0.41	0.54	1.16	0.61	
Treatment						
2.5% lactic acid, room temperature	41.5	11.9	9.7	38.9	15.3	10.2b
2.5% lactic acid, 55 °C	44.4	12.7	11.9	45.0	17.5	19.2a
5.0% lactic acid, room temperature	43.9	11.8	10.5	42.0	15.9	12.8b
5.0% lactic acid, 55 °C	44.0	11.0	10.2	43.0	15.1	13.4b
400 ppm peroxyacetic acid	44.2	10.7	10.0	42.9	14.7	9.7b
Water, 55 °C	44.1	13.0	12.2	43.0	17.9	10.4b
P-value	0.862	0.204	0.319	0.445	0.211	0.013
SEM[c]	1.77	0.71	0.94	2.00	1.06	1.57

Means within the same main effect and column lacking a common letter (a-b) differ ($P < 0.05$).

[a] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.

[b] Treatments (verified by titration) were applied to hot pork hearts. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[c] SEM = standard error of the mean.

Table 21

Least squares means of CIE color space values (*L*, a*, b**), hue, chroma, and ΔE for sampling time[a] and treatment[b] main effects for pork livers

Main effects	*L**	*a**	*b**	Hue angle	Chroma	ΔE
Sampling time						
Before treatment	29.1b	13.5a	12.2a	40.9	18.3a	-
After treatment	37.7a	8.5b	8.9b	45.4	12.5b	-
P-value	< 0.001	< 0.001	0.013	0.073	0.001	
SEM[c]	1.43	0.77	0.87	1.68	1.10	
Treatment						
2.5% lactic acid, room temperature	35.3	9.6	9.3	43.6ab	13.5	14.7
2.5% lactic acid, 55 °C	36.6	11.1	12.5	48.5a	16.8	13.0
5.0% lactic acid, room temperature	34.1	11.7	13.6	50.4a	18.1	14.2
5.0% lactic acid, 55 °C	37.4	10.5	10.7	45.9a	15.2	16.0
400 ppm peroxyacetic acid	28.0	12.3	9.2	35.1c	15.4	11.9
Water, 55 °C	28.9	10.8	8.1	35.3bc	13.6	8.3
P-value	0.052	0.787	0.118	0.003	0.501	0.410
SEM[c]	2.47	1.33	1.51	2.90	1.91	2.55

Means within the same main effect and column lacking a common letter (a-c) differ ($P < 0.05$).

[a] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.

[b] Treatments (verified by titration) were applied to hot pork livers. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[c] SEM = standard error of the mean.

Table 22
Least squares means of trained panelists' scores for pork color attributes[a] for sampling time[b] × treatment[c] interaction for pork hearts and livers

	Color score		Discoloration	
Product type	Before	After	Before	After
Pork hearts				
2.5% lactic acid, room temperature	4.9a	3.7b	1.0b	2.3a
2.5% lactic acid, 55 °C	4.8a	3.7b	1.0b	2.7a
5.0% lactic acid, room temperature	4.6a	3.4b	1.1b	2.7a
5.0% lactic acid, 55 °C	4.9a	3.6b	1.0b	2.8a
400 ppm peroxyacetic acid	4.7a	4.5a	1.1b	1.4b
Water, 55 °C	4.9a	4.7a	1.1b	1.5b
P-value	$P = 0.028$		$P < 0.001$	
SEM[d]	0.22		0.20	
Pork livers				
2.5% lactic acid, room temperature	5.6ab	2.8d	1.4bc	4.0a
2.5% lactic acid, 55 °C	4.3c	2.6d	1.6bc	3.8a
5.0% lactic acid, room temperature	5.1bc	2.8d	1.4bc	3.9a
5.0% lactic acid, 55 °C	5.0bc	2.8d	1.3bc	3.9a
400 ppm peroxyacetic acid	6.2a	5.8ab	1.1c	1.9b
Water, 55 °C	5.8ab	5.8ab	1.0c	1.6bc
P-value	$P < 0.001$		$P < 0.001$	
SEM[d]	0.31		0.23	

Means within a color panel attribute lacking a common letter (a-d) differ ($P < 0.05$).
[a] Panelists used the following scales: heart lean color (1 = pale red; 8 = very dark red), liver lean color (1 = pale purple-red; 8 = extremely dark purple-red), discoloration (1 = none; 5 = extreme).
[b] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.
[c] Treatments (verified by titration) were applied to hot pork hearts and livers. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.
[d] SEM = standard error of the mean.

Table 23

Least squares means of trained panelists' scores for pork color attributes[a] stratified by sampling time[b] and treatment[c] main effects for pork head meat

Main effects	Lean Color
Sampling time	
Before	4.6a
After	4.1b
P-value	< 0.001
SEM [d]	0.09
Treatment	
2.5% lactic acid, room temperature	4.1bc
2.5% lactic acid, 55 °C	4.0c
5.0% lactic acid, room temperature	4.2bc
5.0% lactic acid, 55 °C	4.7a
400 ppm peroxyacetic acid	4.5ab
Water, 55 °C	4.8a
P-value	< 0.001
SEM [d]	0.15

Means within a main effect lacking a common letter (a-c) differ ($P < 0.05$).

[a] Panelists used the following scale: lean color (1 = pale grayish-pink; 8 = very dark grayish-pink).

[b] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.

[c] Treatments (verified by titration) were applied to hot pork head meat. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[d] SEM = standard error of the mean.

Table 24

Least squares means of trained panelists' scores for pork color attributes[a] stratified by sampling time[b] and treatment[c] main effects for pork cheek meat

Main effects	Lean Color
Sampling time	
Before	5.4a
After	5.1b
P-value	0.038
SEM [d]	0.11
Treatment	
2.5% lactic acid, room temperature	5.4
2.5% lactic acid, 55 °C	5.1
5.0% lactic acid, room temperature	5.4
5.0% lactic acid, 55 °C	5.0
400 ppm peroxyacetic acid	5.4
Water, 55 °C	5.1
P-value	0.443
SEM [d]	0.18

Means within a main effect lacking a common letter (a-b) differ ($P < 0.05$).

[a] Panelists used the following scale: lean color (1 = pale grayish-pink; 8 = very dark grayish-pink).

[b] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.

[c] Treatments (verified by titration) were applied to hot pork cheek meat. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.

[d] SEM = standard error of the mean.

Table 25
Least squares means of trained panelists' scores for pork discoloration[a] for sampling time[b] × treatment[c] interaction for pork head meat and cheek meat

	Discoloration	
Product type	Before	After
Pork head meat		
2.5% lactic acid, room temperature	1.1cd	2.3b
2.5% lactic acid, 55 °C	1.1cd	2.4b
5.0% lactic acid, room temperature	1.0d	2.8a
5.0% lactic acid, 55 °C	1.0d	2.8a
400 ppm peroxyacetic acid	1.1cd	1.4c
Water, 55 °C	1.0d	1.3cd
P-value < 0.001		
SEM[d] = 0.14		
Pork cheek meat		
2.5% lactic acid, room temperature	1.0e	1.8cd
2.5% lactic acid, 55 °C	1.2e	1.9bc
5.0% lactic acid, room temperature	1.0e	2.2ab
5.0% lactic acid, 55 °C	1.0e	2.4a
400 ppm peroxyacetic acid	1.1e	1.2e
Water, 55 °C	1.0e	1.4de
P-value < 0.001		
SEM[d] = 0.15		

Means within the same product type lacking a common letter (a-e) differ ($P < 0.05$).
[a] Panelists used the following scale: discoloration (1 = none; 5 = extreme).
[b] Products ($n = 3$ reps per product type) were sampled pre-treatment and 30 min post-treatment.
[c] Treatments (verified by titration) were applied to hot pork head meat and hot pork cheek meat. Lactic acid was prepared (88% L-lactic acid F.G., Birko Corp., Henderson, CO) to a 2.5% and 5.0% ± 0.1% concentration and applied at room temperature and 55 °C. Peroxyacetic acid was prepared (Promoat™, Safe Foods Corp., North Little Rock, AR) to 400 ppm±15 ppm.
[d] SEM = standard error of the mean.

Lean color and discoloration scales used by trained panelists to evaluate pork color

Overall Lean Color – Hot and chilled skinless pork
1 = Pale grayish-pink
2 = Slightly pale grayish-pink
3 = Moderately light grayish-pink
4 = Bright grayish-pink
5 = Slightly dark grayish-pink
6 = Moderately dark grayish-pink
7 = Dark grayish-pink
8 = Very dark grayish-pink
**Panelists can record scores to the nearest 0.5 point.*

Overall Lean Color – pork head meat and cheek meat
1 = Pale grayish-pink
2 = Slightly pale grayish-pink
3 = Moderately light grayish-pink
4 = Bright grayish-pink
5 = Slightly dark grayish-pink
6 = Moderately dark grayish-pink
7 = Dark grayish-pink
8 = Very dark grayish-pink
**Panelists can record scores to the nearest 0.5 point.*

Overall Lean Color – pork hearts
1 = Pale red
2 = Slightly pale red
3 = Moderately light red
4 = Bright red
5 = Slightly dark red
6 = Moderately dark red
7 = Dark red
8 = Very dark red
**Panelists can record scores to the nearest 0.5 point.*

Overall Lean Color – pork livers
1 = Pale purple-red
2 = Slightly pale purple-red
3 = Moderately light purple-red
4 = Purple-red
5 = Slightly dark purple-red
6 = Moderately dark purple-red
7 = Dark purple-red
8 = Extremely dark purple-red
**Panelists can record scores to the nearest 0.5 point.*

Discoloration – all product types
1 = None
2 = Slight
3 = Small
4 = Moderate
5 =Extreme
**Panelists can record scores to the nearest 0.5 point.*