DISSERTATION

EFFICACY OF SULFURIC ACID SODIUM SULFATE ON INOCULATED POPULATIONS OF SALMONELLA SPP. AND CAMPYLOBACTER SPP. ON PORK SUBPRIMALS, AND ITS EFFECTS ON NATURAL SPOILAGE MICROFLORA, LEAN DISCOLORATION AND OFF-ODORS

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ABSTRACT

EFFICACY OF SULFURIC ACID SODIUM SULFATE ON INOCULATED POPULATIONS OF SALMONELLA SPP. AND CAMPYLOBACTER SPP. ON PORK SUBPRIMALS, AND ITS EFFECTS ON NATURAL SPOILAGE MICROFLORA, LEAN DISCOLORATION AND OFF-ODORS

Salmonella and *Campylobacter* are pathogens commonly associated with foodborne illness. As these pathogens are often found in fresh pork, efforts to reduce or eliminate them is imperative to the pork industry. Additionally, fresh pork is highly perishable and maintenance of desirable attributes is imperative. So, extending shelf life of fresh pork is important to maintain profitability and desirability of product. Although a variety of attributes can determine pork shelf-life, reducing spoilage microflora is an important quality control point. Therefore, this study was conducted to determine efficacy of applying sulfuric acid sodium sulfate (SA) to reduce inoculated populations of *Salmonella* spp. and *Campylobacter* spp. on pork subprimals. Additionally, this study aimed to determine efficacy of SA application against inoculated populations of non-pathogenic *Escherichia coli* that could then serve as surrogates for *Salmonella* spp. and *Campylobacter* spp. on pork in in-plant trials (Experiment 1). This study also was conducted to determine effects of a SA spray on the natural spoilage microflora, off odor characteristics, and discoloration properties of pork subprimals during vacuum storage and simulated retail display (Experiment 2). And, SA was evaluated in a commercial pork in-plant system against the natural microflora and inoculated populations of a surrogate bacteria

(Experiment 3). For Experiment 1, vacuum packaged pork subprimals were obtained from a local retailer less than 10 days postmortem. Entire subprimals were cut into uniform sample pieces and assigned to one of the following treatments: 1.0 pH SA, 1.5 pH SA, water or an untreated control. Samples were inoculated to a target level of 6 logs CFU/g for *Salmonella* spp. and surrogate *E. coli*, or 5.5 logs CFU/g for *Campylobacter* spp., with cocktails before treatment. Surviving pathogen and non-pathogenic *E. coli* populations were determined at 5 minutes post-treatment and at 24 h post-treatment. For Experiment 2, boneless pork loins and bone-in backribs were obtained from a commercial pork processing facility and treated with a topical spray of SA at 1.5 pH, 1.0 pH, or an untreated control. After treatment, all samples were placed in dark, refrigerated storage for 14 d or 21 d, after which one half of the samples were removed from storage, overwrapped with polyvinyl chloride film, and placed into retail display cases maintained at 4°C (±2°C) for up to 96 h. At 12 h intervals for the duration of simulated retail display, trained panelists evaluated percent discoloration. Additionally, at 0, 48 and 96 h of display, trained panelists evaluated intensity of off odors and plated and enumerated populations of *Psychrotrophic*, *Pseudomonas*, Lactic acid bacteria and yeast and molds. For Experiment 3, 60 carcasses were railed off and market strategically with 5 x 10 cm^2 areas. Half the zones were inoculated with the surrogate bacteria, the other half remained uninocualted. Carcasses were then treated with the SA using a commercial application spray cabinet. For Experiment 1, application of 1.0 pH SA was the most effective ($P < 0.05$) at reducing inoculated populations of both *Salmonella* spp, and *Campylobacter* spp, compared to all other treatments. However, no difference ($P > 0.05$) was observed for *Campylobacter* and surrogate bacterial populations determined at 5 min versus populations at 24 h. Additionally, non-pathogenic *E. coli* strains were affected less by treatment than inoculated *Salmonella* spp. and *Campylobacter*

spp.populations and can, therefore, effectively serve as surrogates for *Salmonella* spp. and *Campylobacter* spp. For Experiment 2, after 14 and 21 d of dark storage, both boneless loins and backribs sprayed with 1.0 pH SA had lower ($P < 0.05$) *Psychrotrophic*, *Pseudomonas*, Lactic acid bacteria and yeast and mold populations than control or 1.5-pH treated samples at 0, 48 and 96 h of display. Percent discoloration of boneless loin chops increased over the duration of retail display for products stored for 14 and 21 d before simulated retail display. Boneless loin chops treated with 1.0 pH SA had a greater percent discoloration at each simulated retail display test time than untreated chops or those sprayed with 1.5 pH SA. For Experiment 3, SA proved to effectively lower ($P < 0.05$) both inoculated and uninoculated bacterial (TPC, EB, TCC, and ECC) populations on pork carcasses. However, treatment with 1.0 pH SA was more effective than treatment with 1.3 pH SA.

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CHAPTER I

INTRODUCTION

Bacterial pathogens are a major concern in fresh pork. Every year, foodborne pathogens are a leading source of human illness. The Centers for Disease Control and Prevention estimate that each year, 1 in 6 Americans get sick, 128,000 are hospitalized and 3,000 die because of foodborne diseases (Scallan et al., 2011a). While fresh pork does not have the highest association with foodborne illness compared to other meat sources, it is the most consumed meat in the world (Delgado et al., 2001). With the high demand for pork in the world, ensuring that pork products are safe is essential to the United States export market. Traditionally, the major pathogens of concern in the pork industry are: *Salmonella* spp., *Campylobacter* spp., *Trichinella spiralis*, *Toxoplasma gondii*, *Listeria monocytogenes*, and *Staphylococcus aureus* (Baer et al., 2013a). Keeping this in mind, *Salmonella* spp., *Campylobacter* spp. and *S. aureus* are also in the top five pathogens causing foodborne illness (Scallan et al., 2011a). While *S. aureus* is commonly caused further down on the food supply chain, mainly during food preparation; both *Salmonella* spp. and *Campylobacter* spp. can be reduced early along the food production chain (Warriner et al., 2002).

There are multiple technologies available along the production chain to help control bacterial pathogens such as *Salmonella* spp. and *Campylobacter* spp. (Bacon et al., 2000). One of the most commonly used pathogen control technologies in fresh meat production is the use of antimicrobial agents. Some of the more widely used antimicrobial agents include organic acids

such as lactic acid and citric acid; and chemical antimicrobials such as peroxyacetic. Other common interventions that can be used to reduce pathogens and the microbial load include thermal or steam pasteurization. Using more than one of these technologies throughout the food processing system are more effective than just using a single intervention (Bacon et al., 2000; Davidson and Harrison, 2002; Baer et al., 2013a). Pork production facilities are required in their regulatory HACCP plan to show validation of a system's critical control points. These critical control points or interventions must prove though scientific and in-plant validation that they reduce or eliminate pathogens classified as reasonably likely to occur. The purpose of these interventions is to reduce the overall burden of foodborne illness and improve public health.

While ensuring that the safety of fresh pork is at the forefront of pork industries efforts, fresh pork also is highly perishable and improving shelf life is also vital for both domestic purposes and export trade. Fresh pork is highly perishable because of its rich nutritional composition, pH, and water activity. Spoilage to an unacceptable level can be indicated by offensive off-odors, undesirable color deterioration and excessive microbial growth. Rate of spoilage is affected by numerous intrinsic and extrinsic factors and it is the goal of any production facility to control as many as these extrinsic factors as is possible to slow the rate of spoilage and extend shelf life. Large numbers of microorganisms are naturally present in fresh meat, but overtime, they grow exponentially and cause reduction in quality, leading to spoilage. In vacuum packaged fresh pork, growth of aerobic bacteria is suppressed, allowing anaerobic lactic acid bacteria (LAB) to multiply. However, once fresh pork is exposed to oxygen, growth of anaerobic bacteria will slow and growth of aerobic spoilage bacteria such as *Pseudomonas* (PSD) will proliferate.

Increased globalization of the world economy has resulted in a greater need to ship fresh meat products across the world. With more fresh meat products being shipped over excessive distances in foreign trade, there is a need to extend shelf life of meat and to ensure safety. Utilizing new and innovative technologies, such as antimicrobial spray treatments on fresh meat products to reduce and control pathogens and spoilage microflora, could extend shelf and storage life. These studies were conducted to determine effects of using sulfuric acid sodium sulfate (SA) to (1) validate that inoculants of non-pathogenic *E. coli* effectively serve as surrogates for *Salmonella* spp. and *Campylobacter* spp.; (2) compare effectiveness of an acid spray cabinet using SA on chilled pork subprimals to reduce inoculated populations of *Salmonella* spp. and *Campylobacter* spp.; (3) determine effects of SA application on natural spoilage microflora populations of *Pseudomonas,* lactic acid bacteria, and *Psychrotrophic* bacteria, as well as yeasts and molds; 4) determine effects of SA on fresh pork indicators of spoilage including color and off odor intensity across simulated retail display; and (5) determine effects of SA on a commercial harvest floor in a pork packing facility on hot carcasses.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The pork industry continually strives to improve safety of pork and pork products. Many bacterial pathogens are naturally harbored within the gastrointestinal tract and growing environment of live hogs. Food safety concerns and issues are always changing, but the expectation of ensuring safe products has and always will remain important. Therefore, new advances and improvements in food safety technologies must continually be researched and sought after to help ensure the wholesomeness of meat.

There are multiple points within the pork production system, both pre-harvest and post-harvest, where safety can be improved. However, throughout the production chain, *Salmonella* spp. and *Campylobacter* spp. are two of the major pathogens of concern. Every pork production facility, including harvest, fabrication, case-ready, and further processing, has regulatory food safety requirements. Currently, there are multiple intervention technologies available to control bacterial pathogens that are persistent throughout the post-harvest production chain such as different antimicrobials and thermal pasteurization.

While ensuring food safety is the foremost focus of the meat and pork industry, increasing shelf and storage life of products also is of major concern. The financial success of the pork industry is highly dependent on the ability to export. For pork to be eligible for export, certain food safety standards must be achieved and specific quality attributes must be met.

Spoilage indicators, such as the level of natural microflora present, off-odor and color, must be minimized. Pork must be as free as possible from bacterial pathogens, maintain an attractive reddish-pink color, and minimize any off-odor intensities.

Foodborne Illness

The purpose of the United States Department of Agriculture's Food Safety Inspection Service (USDA-FSIS) is to ensure that meat, poultry, processed egg products and catfish are safe, wholesome and correctly labeled to help reducing the burden of adulteration. Each year, it is estimated that 31 different pathogens cause 37.2 million U.S. illnesses, of which 36.4 million are acquired domestically (Scallan et al., 2011a). In 2011, Scallan et al. (2011a) determined that of 36.4 million domestically acquired illnesses, 9.4 million were of foodborne origins. Of these foodborne illnesses, 5.5 million (59%) were caused by viruses, 3.6 million (39%) by bacteria and 0.2 million (2%) by parasites (Scallan et al., 2011a). Overall, foodborne pathogens causing the most U.S. illnesses are norovirus (5.5 million, 58%), nontyphoidal *Salmonella* spp. (1.0 million, 11%), *C. perfringens* (1.0 million, 10%), and *Campylobacter* spp. (0.8 million, 9%) (Scallan et al., 2011a).

These 31 different pathogens also cause 228,744 hospitalizations annually and, of these, 55,961 were caused by contaminated food. Of the hospitalizations caused by foodborne illness, 64% were caused by bacteria, 27% by viruses, and 9% by parasites. Leading causes of hospitalization were nontyphoidal *Salmonella* spp. (35%), norovirus (26%), *Campylobacter* spp. (15%), and *T. gondii* (8%) (Scallan et al., 2011a). These 31 pathogens also caused 2,312 deaths, of which 1,351 were via contaminated food. Of all foodborne illness related deaths, 64% were caused by bacteria, 25% by parasites, and 12% by viruses; the leading causes of foodborne

illness related deaths were nontyphoidal *Salmonella* spp. (28%), *T. gondii* (24%), *L. monocytogenes* (19%), and norovirus (11%) (Scallan et al., 2011a).

Similar to findings of Scallen et al. (2011a), every year, the Centers for Disease Control and Prevention (CDC) estimates that one in six Americans (48 million) get sick from foodborne illness each year; 128,000 people are hospitalized and 3,000 people die from consuming contaminated food (Center for Disease Control and Prevention, 2016). There are several surveillance systems and networks for monitoring the burden of foodborne illness at the Federal, local, state, and regional levels. Although there are multiple surveillance programs, these systems only capture a small portion of U.S. foodborne illnesses due to infected people not seeking medical care and lack of reporting to the appropriate networks and authorities (McCabe-Sellers and Beattie, 2004).

Of all of the 9.6 million annual illnesses associated with a study (Painter et al., 2013) conducted between 1998 to 2008, approximately 51% were associated with produce or plant products, 42% to land animal commodities and 6% to aquatic animal commodities. Further broken down, fruits-nut and vegetables accounted for 46% of illnesses and meat and poultry products accounted for 22% of illnesses (Painter et al., 2013). Of those illnesses associated with bacterial contamination, 18% were associated with dairy products, 18% with poultry and 13% with beef (Painter et al., 2013). Of the land-animal commodity group, the highest proportion of illnesses were associated with *Campylobacter* spp., *Clostridium perfringens*, *Listeria* spp., *Salmonella* serotypes Enteritidis and Heidelberg, *Streptococcus* spp. group A, *Yersinia enterocolitica*, and *Trichinella* spp. (Painter et al., 2013).

A study by His et al. (2015) was conducted to determine the average likelihood of *Salmonella* spp and shiga-toxin producing *Escherichia coli* O157 illnesses that were associated

with the amount of beef, lamb, pork and poultry consumed annually. This study determined that, of all of the illnesses associated with *Salmonella* spp., median number of illnesses caused by poultry, beef, lamb, and pork were 208,400, 85,100, 700, 71,600, respectively (His et al., 2015). For shiga-toxin producing *Escherichia coli* O157 illnesses, 400 were associated with poultry, 15,500 with beef, 100 with lamb and 700 with pork (His et al., 2015).

The United States Pork Industry

According to the National Pork Producers Council (NPPC), there are 68,000 pork producers nationwide that market more than 110 million hogs each year (National Pork Producers Council, 2016). An estimated 2.2 million metric tons of pork and pork products are exported annually; this is more than 26% of total U.S. production. These exports, add more than $62 to the value of each hog sold and support approximately 110,000 job in the United States pork industry (National Pork Producers Council, 2016). Pork is the most consumed meat in the world. It is estimated that 28 kg per capita of pork are consumed each year in the developed world and 10 kg per capita in the developing world (Delgado et al., 2001). By 2020, total consumption of pork is estimated to be at 39 kg per capita (Delgado et al., 2001). However, 60.4% of the total US populations reported not consuming pork within a two-day span (Guenther et al., 2005). Within the U.S., Midwest populations reported eating 117.8% pork compared to the entire U.5. population (considered 100%) (Guenther et al., 2005). This same study also reported that households ranging in size between 2-3 individuals eat 113.2% of pork compared to the entire U.S. population, and Asian, Pacific Islander; American Indian, Alaskan Native, and other racial/ethnic groups consumed 179.7% (Guenther et al., 2005). Lastly, Guenther et al. (2005) showed that higher income households eat more pork than lower income households .and males eat more pork than females.

Food Safety in the Pork Industry

Pork production is a vertically-integrated industry, consisting of different phases such as farrowing, growing and packing. To effectively improve the safety of pork, understanding the nature of pathogens and their prevalence at each phase of pork production is essential (Baer et al., 2013b). Some pathogens of importance to the pork industry are: *Salmonella* spp., *Campylobacter* spp., *Trichinella spiralis*, *Toxoplasma*. *gondii*, *Listeria monocytogenes*, and *Staphylococcus aureus*. *Salmonella* spp., *Campylobacter* spp., and *Staphylococcus aureus* are relevant to the pork industry because they can be found throughout pork production systems and are among the top five pathogens causing foodborne illness and leading to hospitalization in the U.S. (Scallan et al., 2011b). Recently, concern for Methicillin-resistant *Staphylococcus aureus* (MRSA) has grown and one of the sources under scrutiny is live hogs, and it is important to understand its relation to pork production (Baer et al., 2013b).

Pork Pre-Harvest Food Safety

On the farm and in the live animal, *Salmonella* resides in the intestinal tract and can be shed to other animals on the operation. Pregnant sows have a greater prevalence of *Salmonella* than lactating or young sows (Funk et al., 2001; Jacob et al., 2003; Wilkins et al., 2010). One study determined that 51% of sows tested positive for *Salmonella*, along with 32% of nursery pigs and 38% of grower-finisher hogs tested (Wilkins et al., 2010). Multiple studies have shown that as hogs progress along the market hog supply chain, from nursery or weaned pigs to growing and finishing hogs, prevalence of *Salmonella* increases (Jacob et al., 2003; Dorr et al., 2009; Wilkins et al., 2010). However, some studies have shown that *Salmonella* decreases as the market hog supply chain progresses (Kranker et al., 2003). Floors and equipment inside of the hog operation can become contaminated with *Salmonella* and then spread to other animals (Rajić

et al., 2005). Hog feed also has been shown to be contaminated with *Salmonella* (Jacob et al., 2003; Davies et al., 2004; Farzan et al., 2006; Wilkins et al., 2010).

Pigs can become colonized with *Campylobacter* less than one week after birth, and sows have been shown to be the main source of contamination of piglets (Alter et al., 2005; Baer et al., 2013b). Throughout live hog production stages, *Campylobacter* prevalence generally increases (Farzan et al., 2010). Furthermore, when the hogs reach the finishing stage, most are positive for *Campylobacter* (Schuppers et al., 2005). Across all production stages of hogs, more *Campylobacter coli* is found than *Campylobacter jejuni*; more than 90% of hogs that were tested and found to be positive for *Campylobacter* spp. were positive for *Campylobacter coli* (Schuppers et al., 2005; Fosse et al., 2009; Farzan et al., 2010). The farm environment has shown not to contribute to *Campylobacter* prevalence within swine, but the organism still can persist in the environment (Alter et al., 2005).

Toxoplasma gondii is a protozoan parasite that forms intracellular cysts in the muscles brains and other organs (Guo et al., 2016). Prevalence of *Toxoplasma gondii* is highly variable along each stage in the hog production chain (Baer et al., 2013b). However, pork is associated with 41% of meatborne *Toxoplasma gondii* infections (Guo et al., 2016). The type of swine management operation has an effect on *Toxoplasma gondii* prevalence (Venturini et al., 2004; Baer et al., 2013b). Intensive hog production systems have lower *Toxoplasma gondii* prevalence than organic and free-range systems (van der Giessen et al., 2007; Hill et al., 2010). This is likely due to increased exposure to wildlife and cats that carry the parasite in those production systems (Smith et al., 1992; Dubey et al., 2002; Hill et al., 2010; Jiang et al., 2012). In addition to *Toxoplasma gondii*, another parasite of concern is *Trichinella spiralis*. However, prevalence of *Trichinella spiralis* has declined to extremely low levels across all hog production stages in

the U.S. (Davies et al., 1998; Gamble and Bush, 1999; van der Giessen et al., 2007) and is now a concern for wild game.

Pork Post-Harvest Food Safety

The USDA-FSIS has regularly tested for *Salmonella* in slaughter facilities since 1998 and, in 2010, they found that prevalence in market swine declined from 4.3% to 2.4% (Baer et al., 2013b). While prevalence of *Salmonella* at slaughter is low, there are a large number of hogs slaughtered in the U.S. and, therefore, a lot of product has potential to be contaminated. A study conducted by USDA-FSIS in a commercial slaughter plant found that 91% of pre-scalded carcasses tested positive for *Salmonella*. Of carcasses that tested positive for *Salmonella*, 37% were contaminated to a level of between 1 – 3.9 log CFU/100cm^2 (Schmidt et al., 2012). *Salmonella* has shown to cross-contaminate knives, polished machinery and other equipment (Baer et al., 2013b). Additionally, swine feces and lymph nodes can harbor *Salmonella* (Fosse et al., 2009). Thus, evisceration and head removal equipment are especially prone to contamination. After slaughter, final rinse, and carcass chilling, *Salmonella* prevalence was 3.7% at the harvest facility (Schmidt et al., 2012). However, up to 69% of *Salmonella* contamination on carcasses occur due to a contaminated slaughter environment (Duggan et al., 2010). Pork subjected to more extensive handling and processing have higher microbial populations (Duffy et al., 2001). Both intact and ground products have the potential to be contaminated with *Salmonella*, but ground products have proven to have an even greater risk (Duffy et al., 2001; Baer et al., 2013b). Ground pork was found to have a 12.5% prevalence of *Salmonella* (Duffy et al., 2001). The type of retail processing facility can also influence prevalence of *Salmonella*, and pork in butcher shops was reported to have a higher prevalence than pork in supermarkets (Hansen et al., 2010).

Prevalence of *Campylobacter* spp. found on the carcasses during harvest, is similar to the level that is found in and on live hogs (Baer et al., 2013b). However, *Campylobacter* spp. is reduced on the carcasses by scalding, singeing, and polishing (Pearce et al., 2003). Carcasses can be contaminated with *Campylobacter* during the harvest process. However, any *Campylobacter* on contaminated carcasses are often reduced in number during the chilling process because of sensitivity to drying and low temperatures (Pearce et al., 2003; Baer et al., 2013b). The overall prevalence of *Campylobacter* spp. in retail pork products has been reported to be 1.3% (Duffy et al., 2001).

Pork Bacterial Pathogens of Concern

While there are certainly multiple bacterial pathogens of concern in the pork industry, two of the most imperative pathogens to understand and control are *Salmonella* spp. and *Campylobacter* spp. (Baer et al., 2013b). Both *Salmonella* and *Campylobacter* are among the top pathogens causing foodborne illness, hospitalizations, and deaths (Scallan et al., 2015). In the U.S., disability-adjusted life years lost because of *Salmonella* and *Campylobacter* foodborne illness are 32,900 and 22,500, respectively (Scallan et al., 2015). Both of these pathogens persistent throughout the pork supply chain and are needed to be reduced or eliminated to decrease risk of foodborne illness.

Salmonella

Salmonella is a Gram-negative bacterium of the *Enterobacteriaceae* genus; like *Escherichia*, *Shigella*, *Yersinia* and many others. *Salmonella* spp. can grow between 5 to 45°C and a pH range from 4 to 9 (Baer et al., 2013b). The organism is very resilient and can survive for extended periods of time in low moisture foods (Podolak et al., 2010). There are two species of *Salmonella*: *Salmonella enterica* and *Salmonella bongori* (Montville and Matthews, 2008).

Of *Salmonella enterica*, there are six recognized subspecies and the majority of isolates associated with foodborne illness are *Salmonella enterica* subsp. *Enterica*. Underneath subspecies, strains can be further differentiated by serotyping over 1,400 serovars of *Salmonella enterica* subsp. *Enterica*. These serovars of *Salmonella enterica* subsp. *Enterica* are considered typhoidal or non-typhoidal (Montville and Matthews, 2008). Typhoidal *Salmonella* can cause symptoms such as fever, diarrhea, and abdominal cramps. Non-typhoidal *Salmonella* are by far the subtype serovars most common in food and they can affect humans and animals by causing gastroenteritis and symptoms such as diarrhea and abdominal pain (Montville and Matthews, 2008; Baer et al., 2013b). Severe infection with non-typhoidal *Salmonella* can cause Salmonellosis. *Salmonella* causes 1.2 million illnesses and 450 deaths in the U.S. annually (Scallan et al., 2011b). Children under the age of five are at the highest risk for *Salmonella* infection; they have higher rates of infection than any other age group (Scallan et al., 2011b).

Campylobacter

Similar to *Salmonella*, *Campylobacter* is a Gram-negative bacterium. However, it also is very different because it requires low levels of oxygen for growth. *Campylobacter* grows in the narrow temperature range of 30 to 47°C and requires a minimum pH of 5.8 for growth (Bolton and Coates, 1983; Doyle and Cliver, 1990; Kaakoush et al., 2007). *Campylobacter* lacks the enzyme 6-phosphofructokinase, which means it cannot use glucose as an energy source; but instead uses fumarate, nitrate or sulfite for energy (Bolton and Coates, 1983). The pH, microaerophilic oxygen levels, temperature and available energy sources make the intestinal tracks of mammals the ideal environment for growth of *Campylobacter* spp. (Anderson et al., 2009; Baer et al., 2013b). Additionally, it is possible for hogs to be asymptomatic carriers of both *Salmonella* and *Campylobacter* (Baer et al., 2013b). *Campylobacter* is the leading cause of

human gastrointestinal illness from an animal source, and 80% of the illnesses are foodborne (Baer et al., 2013b). Each case of foodborne *Campylobacter* illness accounts for 9% off all foodborne illnesses annually (Scallan et al., 2011b). Infection with *Campylobacter* can cause gastrointestinal illness with symptoms of diarrhea, bloody diarrhea, vomiting, fever and abdominal pain (Stern et al., 1985). Additionally, infection with *Campylobacter* can cause Guillain-Barre syndrome (Nachamkin et al., 1998). The majority of foodborne *Campylobacter* infection (campylobacteriosis) is caused by *Campylobacter jejuni*, which is not as common as *Campylobacter coli* in pork production (Schuppers et al., 2005; Baer et al., 2013b). However, *Campylobacter coli* can also cause illness in humans.

Pathogen Control Methods

Foodborne pathogens persist throughout the entire span of the pork production chain, and because of this it is imperative to have multiple pathogen control methods in place to assist in reducing or eliminating pathogens of concern. Use of multiple sequential interventions, also known as multiple hurdles technology, is considered the most effective method at reducing and controlling risk of pathogens on meat and meat products (Delmore et al., 1998; Stivarius et al., 2002; Koohmaraie et al., 2005; Scott et al., 2015). Currently, strategies such as physical and chemical interventions are commonly utilized in production facilities (Koohmaraie et al., 2005; Scott et al., 2015). Chemical intervention technologies proven to be effective as pathogen control measures include antimicrobial treatments, steam pasteurization, and hot water (Delmore et al., 1998; Stivarius et al., 2002; Koohmaraie et al., 2005; Scott et al., 2015). The USDA-FSIS requires proof that pathogen control methods put in place by a packing facility are operating effectively; this is called validation. Pathogens that are classified as 'reasonably likely to occur'

must have a critical control point in place that is validated to eliminate the pathogen or reduce its prevalence to acceptable levels.

Pre-Harvest Interventions

Salmonella is found and harbored in the intestinal tract of live swine and, therefore, intervention technologies can begin on the farm. Effective management and biosecurity practices can help reduce cross-contamination and spreading of pathogens (Baer et al., 2013b). Vaccinations, antibiotics and probiotics can be administered to live swine to reduce *Salmonella* prevalence levels (Baer et al., 2013b). One study showed that a *Salmonella* Choleraesuis vaccine administered to 3 to 16 week old piglets reduced the number of *Salmonella*-positive lymph nodes by 6.6% (Maes et al., 2001). This vaccination could also perhaps cross-protect live animals against other bacteria such as *Salmonella* Typhimurium (Maes et al., 2001). Vaccinations, administered orally or in water, have been demonstrated to reduce prevalence of *Salmonella* Typhimurium (Maes et al., 2001; Baer et al., 2013b).

Use of probiotics or prebiotics in the feed also decrease prevalence of *Salmonella* in live hogs. Probiotics or prebiotics used as a feed additive alters the gut microbiota of the animal causing a shift in levels of harmful bacteria (Baer et al., 2013b). Ferlac-2 and Flavomycin are probiotics administered to swine that have been shown to effectively decrease prevalence of *Salmonella* Typhimurium in lymph nodes, but were not effective at reducing shedding of the pathogen (Letellier et al., 2000). A SC54 live attenuated *Salmonella* choleraesuis vaccine given to swine seemed to reduce *Salmonella* Typhimurium in the gastrointestinal tract and feces of hogs. However, unlike probiotics, no reduction was observed in the lymph nodes because of the vaccination. A combination of probiotics and vaccination will give hogs the greatest possible

reduction of *Salmonella* by reducing prevalence of shedding and the presence in the lymph nodes (Letellier et al., 2000; Baer et al., 2013b).

Carbadox and copper sulfate are additives in feed commonly given to nursery swine as growth-promoting agents, and they have been shown to also effectively decrease shedding of *Campylobacter* in feces. However, at the same time, this combination also increased shedding of *Enterobacteriaceae* such as *Salmonella* (Wells et al., 2010; Baer et al., 2013b). Also, use of deaminase inhibitors, such as thymol or diphenyliodonium chloride inhibit amino acid catabolism and, therefore, decrease survival of *Campylobacter* (Anderson et al., 2009). Inhibition of fermentation with nitro-alcohols has been shown to be effective against *Campylobacter jejuni*. *Campylobacter* spp. were reduced by 1.16 $\log_{10}$ and 3.92 $\log_{10}$ with use of 2-nitro-1-proponal and nitroethane, respectively (Horrocks et al., 2007).

Post-Harvest Interventions

Eliminating all pathogens in live animals is impossible. No current pre-harvest method has been able to eliminate all pathogens found throughout the pork production chain. Therefore, a combination of both pre-harvest and post-harvest interventions in a multiple hurdles system is the best method for reducing and controlling pathogens. Organic acids are commonly used to effectively reduce *Salmonella* populations on meat products (Delmore et al., 1998; Pohlman et al., 2002; Koohmaraie et al., 2005; Baer et al., 2013; Scott et al., 2015). Weak acids, such as organic acids, utilized in food systems are neutrally charged molecules that cross the bacterial cell membrane (Brul and Coote, 1999). This undissociated acid, happens when the pH of the meat product is near or lower than the pKa of the organic acid. Bacterial pH is higher, which causes release of a proton from the neutral acid once it has crossed the cell membrane. The released proton can then inhibit growth of the bacteria by either disruption of the membrane,

metabolic reactions, or intracellular pH homeostasis (Brul and Coote, 1999; Mani-López et al., 2012; Baer et al., 2013b). Organic acids have been used as carcass spray washes to decrease a variety of pathogens including *Salmonella* (Epling et al., 1993a). Peroxyacetic and lactic acid carcass washes reduced *Salmonella* by 50% and 66% (Baer et al., 2013b). The combination of organic acids and hot water washing was shown to be more effective than just organic acids on pork skin (Niebuhr et al., 2002). Post-harvest interventions are not just applied to carcasses; they can also be applied to cuts of pork. Treatment with acetic acid and lactic acid or salt was effective at decreasing the prevalence of *Enterobacteriaceae* in anaerobically stored pork (Baer et al., 2013b). Use of carbon dioxide and lactic acid also have been shown to decrease *Salmonella* on boneless pork loins (Choi et al., 2009).

Typical slaughter procedures are effective at decreasing *Campylobacter* on pork skin, but additional methods should be utilized and explored to ensure *Campylobacter* reduction (Baer et al., 2013b). A 2% lactic acid spray decreased prevalence of *Campylobacter* spp. from 2% to 0% on the shoulder and from 6% to 1% on the ham (Epling et al., 1993a; Carpenter et al., 2011). Carcass chilling in pork production effectively controls *Campylobacter* (Baer et al., 2013b). Blast-chilling also is extremely effective at decreasing prevalence of *Campylobacter* (Chang et al., 2003). However, thermal treatment is the most common intervention to kill *Campylobacter*. Thermal inactivation occurs at 50°C and such temperatures are possible to apply on the harvest floor of packing facilities (Baer et al., 2013b). On retail pork cuts, antimicrobials are often used to reduce the *Campylobacter* bacterial load. Oils can be very effective antimicrobials against *Campylobacter jejuni*, including carrot seed, celery seed, marigold, ginger root, gardenia, orange bitter, patchouli, cedarwood, mugwort, spikenard, carvacrol, cinnamaldehyde, thymol, geranyl acetate, benzaldehyde, perillaldehyde, carvone R, eugenol, citral, and estragole (Friedman et al.,

2002). These oils also have been evaluated for effectiveness against other pathogens and *Campylobacter jejuni* was more sensitive to the antimicrobial effects of the oil agents than the other pathogens tested (Friedman et al., 2002). This is most likely due to the specific growth conditions of *Campylobacter jejuni* and its unique structure (Friedman et al., 2002; Baer et al., 2013b). Additionally, due to the low oxygen levels required for optimal *Campylobacter* growth, packaging methods can also greatly effect growth on retail cuts (Baer et al., 2013b).

Pork Spoilage Indicators

Meat and pork spoilage rate is a complex phenomenon. Ultimately spoilage occurs based off the meats internal pH as well as state and charge of the myoglobin molecule. The ferric or ferrous state of the iron at the center of the myoglobin molecule will determine the rate and extent of lipid oxidation as well as effects other intrinsic factors associated with meat spoilage. There are many intrinsic and extrinsic factors that influence pork spoilage rates, including microbial quality, pH moisture content, available nutrients and temperature (Casaburi et al., 2015). Intrinsic factors are nearly impossible to control, so in order to effectively control spoilage throughout the pork production system, the industry must control the extrinsic factors as much as possible. The effect of temperature on meat is one of the most effective and easiest ways to control spoilage (Koutsoumanis and Taoukis, 2005; Nychas et al., 2008). The industry should pay special attention to primary and secondary chilling of product. Primary chilling is the cooling of carcasses directly after slaughter from the hot temperature on the harvest floor to refrigerated temperatures. Rapid growth of both pathogenic and spoilage microorganisms can occur during primary chilling. Processing of product after primary chilling can raise the product temperature again, so secondary chilling is required (Nychas et al., 2008). Overall, spoilage is

characterized by a combination of multiple different indicators, including microbial quality, color or appearance, and off-odor development.

Spoilage Bacteria Effecting Pork

Microbial spoilage of meat occurs when populations reach 7 to 9 log CFU/g^{-1} (Gram et al., 2002). Several organisms such as *Enterbacteriacea*, lactic acid bacteria, *Pseudomonas*, and *Brochothrix thermosphacta* can contribute to spoilage of aerobically stored meat (Borch et al., 1996; Lambropoulou et al., 1996; Liu et al., 2006a). *Pseudomonas* has been implicated as one of the predominant bacteria associated with the spoilage of pork (Liu et al., 2006a). The predominate bacteria affecting vacuum packaged pork in high bacterial populations is *psychrotrophic* and lactic acid bacteria (Holley et al., 2004). At lower bacterial levels, spoilage often is caused by *Brochothrix thermosphacta*, *Shewanella* spp. or *Aeromonas* spp., and/ *Enterobacteriaceae* (Gill and Greer, 1993). Lower initial counts at the beginning of storage will result in a longer storage life (Holley et al., 2004). A 100-fold reduction in the initial starting bacterial counts has resulted in a two week longer storage life for pork when held at between 4 or -1°C (Mcmullen and Stiles, 1991). Lactic acid bacteria gradually grows in anaerobic storage and eventually becomes the predominant bacteria (Jiang et al., 2010). Microflora populations on vacuum stored pork has been widely studied and it is accepted that lactic acid bacteria are the predominant bacteria in the spoilage microflora (Boers et al., 1994; Jeremiah et al., 1995; Blixt and Borch, 2002; Holley et al., 2004).

Lactic acid has proven to be very effective at reducing *psychrotrophic* bacteria on pork (Gill and Greer, 1993). Treatment with organic acids can reduce cold tolerant spoilage bacteria on pork fat and lean (Greer and Dilts, 1995). Additionally, both lactic and acetic acid have been shown to extend the storage life of pork loins and pork chops (Stringer et al., 1983; Mendonca et

al., 1989). The combination of temperature and availability of oxygen can also suppress the growth of *Brochothrix thermosphacta*, *Enterobacteriaceae* and yeasts and molds (Blickstad et al., 1983). Yeasts and molds also have been shown to shorten storage life and contribute to spoilage. *Pseudomonas*, lactic acid bacteria, *psychrotrophic* bacteria, as well as yeast and mold populations all contribute to spoilage in pork products and, in order to extend storage life, their numbers must therefore be controlled.

Pork Color Stability

Retail shelf-life of meat products, including pork, is greatly limited by color stability and development of surface discoloration which can occur before microbial spoilage is reached due to mechanisms not associated with surface bacteria numbers. Spoilage by discoloration begins with the pork surface being exposed to oxygen. Therefore, methods to increase color stability and extend shelf-life need to be continually researched. Use of different packaging technologies and antioxidants can help stabilize and fix color to maintain a fresh appearance. Modified atmosphere packaging (MAP) often is used to help maximize meat color stability (Lambert et al., 1991; Gill, 1996; Luño et al., 2000; Jayasingh et al., 2001). Meat placed into MAP packaging is exposed to an artificial environment created using differing gas mixtures pumped into the packages; gases such as oxygen, nitrogen, carbon dioxide and carbon monoxide (Viana et al., 2005). Such gases bind to the myoglobin porphyrin ring responsible for the meat's red color. Pork packaged with 100% carbon dioxide has been shown to have improved color fixation or no change in color (Jeremiah et al., 1995; Sørheim et al., 1997). Maintaining a positive and attractive color appearance is important because color plays a major role in purchasing decisions (Sørheim et al., 1997; Luño et al., 2000; Viana et al., 2005). Enhanced MAP loin chops with higher pH values have greater color stability (Livingston et al., 2004). Throughout retail display,

surface discoloration of meat products occurs and therefore effects consumers purchasing decisions (Livingston et al., 2004). Therefore, it is imperative for the success of the pork industry to maximize color stability and minimize surface discoloration.

Treatment with antimicrobials that accelerate oxidation reactions associated with the iron porphyrin ring of myoglobin cannot only contribute to increased surface discoloration, but can also have negative impacts on instrument color measures of *CIE* L*, a* and b* values (Shrestha and Min, 2006). Lightness to darkness is measured by L*, red to green is measured with a* values and blue to yellow is measured using b* values. Treatment with lactic acid can result in decreased a* values on fresh pork (Arganosa and Marriott, 1989; Shrestha and Min, 2006) and greater L* values, and the effect is increased as the concentration of lactic acid is increased (Huang et al., 2005; Viana et al., 2005; Shrestha and Min, 2006). However, treatment with antioxidants can result in greater redness (a*) values (Sánchez-Escalante et al., 2001; Huang et al., 2005; Balentine et al., 2006). Fresh meat, including pork treated with organic acids such as citric or ascorbic acid, also had higher b* values then untreated meat (Huang et al., 2005; Viana et al., 2005).

Off-odors

Throughout extended display or storage, off-odors develop (Lambert et al., 1991; Brooks et al., 2008). Off-odor is often used as an indicator for spoilage. Even without excessive microbial growth and color deterioration, off-odors can still develop (Viana et al., 2005). However, when microbial population reach 7 to 8 log CFU/cm^2, off-odors have been shown to develop (Gill, 1983). Extensive growth of specific bacterial organisms can affect which off-odor is the most intense and detectable (Lambert et al., 1991). Excessive growth of *Brochothrix*

thermosphacta utilizes glucose for energy and creates acetic acid as a byproduct which causes a sweet off-odor (Lambert et al., 1991).

Volatile compounds develop over storage and display time and can intensify when exposed to different factors. Combinations of the meat biochemical status and bacterial populations can affect the volatile compounds present and ultimately what off-odors are detectable (Viana et al., 2005; Brooks et al., 2008). Off-odors also develop as a result of lipid oxidation, and both lipid oxidation and production of volatile compounds are associated with meat spoilage (Brooks et al., 2008). Meat in traditional PVC overwrap packages developed off-odors faster in storage than MAP packages containing carbon monoxide (Brooks et al., 2008). Glucose is often a precursor of many off-odors during storage as it is metabolized to acetate, acetoin, diacetyl, acetic acid, iso-butyric acid, iso-valeric acid, 2-methylbutyric acid, 3-methylbutanol, 2 methylpropanol and ethanol (Nychas et al., 1988; Nychas and Arkoudelos, 2007; Casaburi et al., 2015). These compounds are responsible for off-odors such as rancid, putrid and sour.

Conclusion

Pork is a highly valuable protein enjoyed all around the world. While many consumers enjoy pork regularly, the industry must be diligent to ensure safety and quality. Foodborne illness affects thousands of people year in the United States. It is imperative to reduce or eliminate pathogens throughout the supply chain on pork destined for both domestic sale and international trade. Additionally, it is necessary for the industry to reduce and control spoilage bacteria and to reduce formation of off-odors and discoloration. There are multiple stages in both pre-harvest and post-harvest production systems where interventions can be put in place to help control quality and safety.

Both *Salmonella* spp. and *Campylobacter* spp. are two of the bacterial pathogens of concern, both causing foodborne illness and found inherently in the pork supply chain. Use of antimicrobial chemicals, such as organic acids, have proven to be effective in controlling bacterial pathogens. Other interventions such as thermal or steam pasteurization have also been effective at controlling bacterial pathogens. These control methods or interventions used during the post-harvest stage of the pork production supply chain must be validated to prove they are effectively reducing or eliminating pathogens that are classified as reasonably likely to occur in their regulatory HACCP plan.

In order for the pork industry to maximize profitability, storage and shelf life needs to be extended as long as is possible. Spoilage is a multifactorial, complex phenomenon that can be initiated by high microbial counts, off-odor development and surface discoloration caused by oxidation of muscle pigments. There are different technologies available to help control different spoilage indicators such as packaging method, antimicrobial treatment and use of antioxidants. Packaging method can reduce certain bacterial populations and help ensure color stability. Antimicrobial treatment can greatly reduce the bacterial populations present, but also can negatively impact color stability and discoloration rate. Antioxidants have shown positively impact color and off-odor development.

Continued research is needed to determine effects of different antimicrobial interventions throughout different post-harvest stages. Treatment with antimicrobial acids over different stages of production should be evaluated to determine their effect on both food safety and spoilage indicators to assist in ensuring safety, quality and profitability of the pork industry.

CHAPTER III

EFFICACY OF SULFURIC ACID SODIUM SULFATE ON INOCULATED POPULATIONS OF SALMONELLA SPP. AND CAMPYLOBACTER SPP. ON PORK SUBPRIMALS, AND ITS EFFECTS ON NATURAL SPOILAGE MICROFLORA, LEAN DISCOLORATION AND OFF-ODORS

Materials and Methods

Experiment 1: Inoculation and Surrogate Validation

Effects of sulfuric acid sodium sulfate (SA) on populations of *Salmonella* spp. and *Campylobacter* spp. inoculated onto chilled pork bone-in Boston-butt shoulders were evaluated, and inoculants of non-pathogenic *E. coli* surrogates for *Salmonella* spp. and *Campylobacter* spp on subprimals were assessed in an effort to validate their use in commercial plants. Shoulders where obtained from a local wholesale distributor less than 11 days postmortem and delivered to the Colorado State University, Center for Meat Safety and Quality's Meat Science Laboratory. Upon arrival to at the laboratory, each shoulder was separated into three equal portions using a band saw and then transported to the microbiology laboratory. The SA used to address objectives of this study was Titon™ (Zoetis, Florham Park, N. J.). Each pathogen inoculant strain was subjected to four treatments including: 1.0 pH SA, 1.5 pH SA, water or an untreated control (CON). Shoulder portions were then assigned to one of the four treatments under each bacterial inoculum type. A total of 18 shoulder samples or replicates (n = 18) were assigned to

each treatment for each inoculant strain (see Figure 1) over three days (6 subprimal samples treated/day, three for 0 hour samples and three for 24 hour samples).

As previously stated, this experiment utilized three inoculum cocktails, including: (i) a 6-strain mixture of *Campylobacter* spp, (3-coli and 3-jejini) with *C.jejuni* B3-55, *C.jejuni* HS-99-01-8-100, *C.jejuni* HS-99-10-11-12, *C.coli* B3-59, *C.coli* HS99-01-4-11s and *C.coli* HS99-01-9-30, (ii) a 6-strain mixture of *Salmonella* spp with *S.* anatum, *S.* schwartzengrund, *S.* montevideo, *S.* agona, *S.* derby and *S.* Tennessee, and (iii) a 5-strain mixture of non-pathogenic *E. coli* surrogates for *Salmonella* spp and *Campylobacter* spp. with TCC BAA-1427, ATCC BAA-1428, ATCC BAA-1429, ATCC BAA-1430, and ATCC BAA-1431. *Salmonella* and *Campylobacter* strains were obtained from the USDA-ARS and were all of pork origin. Non-pathogenic *E. coli* strains (surrogates) were grown to be rifampicin-resistant to allow for selection and differentiation of the inoculum from natural microflora associated with fresh pork. Xylose lysine deoxycholate (XLD) agar was used for selective enumeration of the *Salmonella* inoculum. Campy-Cefex agar (Becton Dickinson, Sparks, MD) was used for selective enumeration of the *Campylobacter* inoculum. Strains of *Salmonella* spp were activated and subcultured (35°C, 24 ± 2 h) in 10 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD). Strains of the non-pathogenic *E. coli* were activated and subcultured (35°C, 24 ± 2 h) in 10 ml of TSB supplemented with 50 µg/ml rifampicin (Sigma-Aldrich Inc., St. Louis, MO; for all rifampicin-resistant *E.coli* strains). Strains of *Campylobacter* spp. were activated and subcultured (42°C, 48 ± 2 h) in boltons broth infused with lysed horse blood (Hardy Diagnostics, Santa Maria, CA). Strains of each were individually cultured and subcultured (35°C, 24±2 h) in 10 ml of appropriate broths. Broth cultures (10 ml) of the strains belonging to the same inoculant type were combined, cells were harvested by centrifugation (3220 x *g*, 20 min, 4°C, Eppendorf model

5810 R, Brinkman Instruments Inc., Hamburg, Germany), washed with 10 ml phosphate buffered saline (PBS, pH 7.4; Sigma), re-centrifuged, and resuspended in PBS to the original volume resulting in a concentration of approximately 8 to 9 log CFU/ml for each inoculum type.

After each of the bacterial cocktails were centrifuged, washed and re-suspended, the shoulder samples were then inoculated. Pork shoulder samples were spot-inoculated and inoculum was then spread across the surface on both the top and bottom sides with 200 µl on each side to obtain a target inoculation level of approximately 6 log CFU/g before treatment application with water or SA. Inoculated samples were held for 15 min, after inoculation on each side, to allow for bacterial cell attachment before treatment application.

After samples were inoculated, they were treated using a custom-built spray cabinet (Chad Co., Olathe, KS) at the Colorado State University, Center for Meat Safety and Quality's microbiology lab. The spray cabinet was fitted with 12 flood-jet nozzels, (FloodJet, SS316) spraying at 0.14 gpm. There were four on top, two on each side and four on the bottom. Samples were then placed onto the conveyor belt and sprayed for approximately 11 seconds. Each treatment, except for the inoculated controls, were sprayed at 20 psi and had beginning and ending weights to ensure there was no excessive amount of pick-up (not to exceed 0.5%) after spray treatment.

Half the samples were immediately excised within five-minutes of treatment and the other half were aseptically placed in to vacuum bags and stored anaerobically for 24 hours before excision and plating. Samples were aseptically excised by taking 50 grams from each side (50 g from top + 50 g from bottom = 100 g total), a volume of 200 ml (1:1 ratio of excised pork and broth) of Dey Engley (Difco, Becton Dickinson) neutralizing broth (D/E) was added to a whirl-pack bag, and each was mechanically pummeled (Masticator, IUL Industries, Barcelona, Spain)

for two-minuets. Pummeled samples plus the D/E mixture were then serially diluted (10-fold) in 0.1% buffered peptone water (Difco, Becton Dickinson). Appropriate dilutions were surface-plated (0.1 ml or 1 ml) onto tryptic soy agar (TSA; Acumedia, Lansing, MI) to determine total bacterial populations, TSA with rifampicin (100 μg/ml; TSA + rif) to enumerate rifampicin-resistant non-pathogenic *E. coli* or surrogate populations, on XLD agar (Acumedia) for *Salmonella* spp., and on Campy-Cefex (Difco, Becton Dickinson) agar to enumerate *Campylobacter* spp. populations. Colonies were counted after incubation of plates at 35°C for 24 h (TSA + rif and XLD agar), 25°C for 72 h (TSA) or 42°C for 48 h (Campy-Cefex agar).

Experiment 2: Effects of Sulfuric Acid Sodium Sulfate on Spoilage Indicators

Bone-in backribs and boneless pork loins were used to determine effects of SA on spoilage of chilled pork subprimals. Backribs and loins were obtained from a commercial packing facility and transported to Colorado State University, Center for Meat Safety and Quality's microbiology laboratory. Subprimals were randomly collected during two-shifts on one production day to allow for a range in natural microflora. During transportation to Colorado State University, Center for Meat Safety and Quality's microbiology laboratory, subprimals were maintained at 4°C ± 2. Upon arrival, subprimals were portioned into approximately 10 x 10 cm^2 pieces based on the top surface and treated using the custom-built spray cabinet (Chad Co., Olathe, KS). The cabinet applied treatments as previously described for Experiment 1 and weights were monitored to ensure there was not an excessive amount of pick-up (not to exceed 0.5%) after spray treatment.

Samples were randomly assigned to one of three treatments: 1.0 pH SA, 1.5 pH SA or an untreated control (CON). Samples were then assigned to one of two dark anaerobic storage times: 14 days or 21 days. Following treatment, samples were vacuum packaged and placed into

dark storage (4°C ± 2) for the designated storage period. After samples were stored for their designated dark storage time, they were removed from the vacuum packages and overwrapped with polyvinyl chloride (PVC) on black Styrofoam trays (size 2P) and placed into simulated retail display (4°C ± 2) for up to 96 hours. The bulb-type used in the simulated retail display were warm fluorescent bulbs with a light intensity that remained between 1,612.5 to 2,152 lux and 150 – 200 foot-candles, measured using a light meter placed level with the meat surface. Samples from each treatment were also kept in dark anaerobic storage for up to 28 days post-treatment. One a week, on day 0, 7, 14, 21 and 28 rib and loin sections were sampled and analyzed.

After packages were placed into simulated retail display, instrument color CIE L*, a* and b* values were measured every 12-hours (0, 12, 24, 36, 48, 60, 72, 84, and 96 hours) using MiniScan EZ 4500L Spectrophometer (HunterLab Reston, VA). Additionally, a set of panelists (minimum of 6), previously trained to assess color and discoloration, evaluated the percent surface discoloration every 12 hours during retail display (0, 12, 24, 36, 48, 60, 72, 84, and 96 hours). Percent surface discoloration was evaluated using an electronic ballot (Qualtrics Provo, UT) with an unstructured line scale on touch screens. The line was anchored with two verbal descriptors, far left being 0 percent or no discoloration and far right indicating complete or 100 percent discoloration. Trained panelists would drag the curser to the percent of surface discoloration for each sample. Backribs and loin samples were evaluated for the level of natural spoilage microflora present and off-odor development every 48 hours of display (0 hour, 48 hours and 96 hours). At each sampling time, instrument color, subjective color panel and spoilage microflora enumeration, six replicates (n = 6) from each treatment and subprimal type

were evaluated (Table 1). Populations of of natural yeasts and molds (YM), *Psychrotropic* bacteria (PSY), *Pseudomonas* spp. (PSD) and Lactic acid bacteria (LAB) were enumerated.

Subprimal samples were excised by aseptically removing 50 g from each sample. After aseptic excision, 100-ml of D/E was added (1:2 ratio of excised pork sample to D/E) and mechanically pummeled (Masticator, IUL Industries, Barcelona, Spain) for two min. The pummeled sample and D/E mixture was then serially-diluted (10-fold) in 0.1% buffered peptone water (Difco, Becton Dickinson). Appropriate dilutions were surface-plated (0.1 ml or 1 ml) onto dichloran rose bengal chloramphenicol (DRBC, Difco, Becton Dickinson) agar for YM enumeration, *pseudomonas* agar supplemented with CFC (Oxoid, Lenexa, KS) for PSD enumeration, tryptic soy agar (TSA; Acumedia, Lansing, MI) for PSY enumeration and pour plated lactobacilli MRS agar (LAB, Difco, Becton Dickinson) for LAB enumeration. Plates were incubated following the manufactures instructions; PSD and LAB plates for 72 hours at 25°C ± 2, YM plates for 120 hours at 25°C ± 2 and PSY plates for 240 hours at 7°C ± 2. As samples were opened and excised for spoilage microflora sampling, subprimal samples also were evaluated for off-odor intensity by a set of trained panelists (minimum of 6 panelists/panel) using an electronic ballot (Qualtrics Provo, UT) with an unstructured line scale as previously described. Small pieces of pork were excised from the cut surface (approximately 1 x 1 cm cubes) and placed into glass jars with lids. Off-odors evaluated included acid/chemical, putrid, ranicd/oxidized and sour odor intensity using an unstructured line scale verbally anchored with five verbal descriptors equally spaced across the length of the line. The verbal anchors ranged from “not detectable” on the far left, to “mildly detectable,” “slightly intense,” “intense,” and “extremely intestine” on the far right. Panelists were trained to consider off odor intensity unacceptable at 50 percent of the way or half way across the unstructured line.

Experiment 3: In-plant Validation of Sulfuric Acid Sodium Sulfate on Pork Carcasses

Sixty pork carcasses (N = 60) were used to determine effects of SA application on carcass surface microbiological levels in a production system of a commercial packing facility. Populations of surrogate bacteria and natural microflora were enumerated before and after the SA spray intervention treatment, following a snap-chill cycle on hot pork carcasses, and after 15 hours post SA treatment in the holding coolers. Surrogate inoculum consisted of the same five-strain mixture of non-pathogenic *E. coli* (ATCC BAA-1427, ATCC BAA-1428, ATCC BAA-1429, ATCC BAA-1430, ATCC BAA-1431) previously assessed as an effective surrogate for *Salmonella* spp. and *Campylobacter* spp. in Experiment 1. Inoculant strains were individually cultured and subcultured (35°C, 24±2 h) in 10 ml of tryptic soy broth. Broth cultures (10 ml) of all five strains were then combined and cells harvested by centrifugation. Cell pellets where then washed with 10 ml PBS, re-centrifuged, and resuspended into the original volume in PBS to obtain a concentration of 8 to 9 log CFU/ml and allow for a target inoculation level of 6 log CFU/cm^2.

All carcasses were railed off to allow for ease of inoculation and to prevent cross-contamination. External surfaces of carcass' skin were marked with eight 5 x 10 cm^2 zones using carcass crayons (see Figure 2). Half of the zones were inoculated with the surrogate bacterial cocktail, and the other remained uninoculated to determine the effects of SA on both surrogate bacterial populations and the natural microflora present on the carcass surface. Inoculation was performed by using separate sampling sponges, hydrated with 10 ml of inoculum. Sponges were then used to sample the carcass surface within the marked zones. Separate inoculum-hydrated sponges were used for each of the 5 x 10 cm^2 zones. After

inoculation, carcasses were left stationary for a minimum of 10 minutes before SA treatment to allow for bacterial cell attachment.

Carcass sides were then assigned to one of 2 treatments consisting of 1.3 pH SA or 1.0 pH SA over two sampling days. Two carcass zones (one inoculated and one not inoculated) were then sampled at each intervention time point: 1) before SA spray, 2) after SA spray, 3) after SA spray and rapid chill cycle, and 4) after SA spray, rapid chill cycle and 15 hours post SA treatment. Zones were sampled by making 10 vertical and 10 horizontal passes within the 5 × 10 cm^2 area, using a 3M sampling sponge hydrated with 10 ml D/E neutralizing broth, with enough force to remove dried blood. Sample bags were then sealed and placed in a cooler and transported back to Food Safety Net Services (Amarillo, TX) to be plated and enumerated. Upon arrival, 15 ml of Butterfields broth was added to all sponge samples for a total of 25 ml of diluent (10 ml D/E + 15 ml Butterfields). Samples were then mechanically pummeled for 2 min and serially diluted (10-fold) in 0.1% buffered peptone water. Appropriate dilutions were plated in duplicate to enumerate aerobic plate counts (3M Petrifilm Aerobic Count Plates) and *Enterobacteriaceae* counts (3M Petrifilm Enterobacteriaceae Count Plates). Colonies on APC Petrifilm plates were enumerated following 48 hours incubation at 37°C. Colonies on EB Petrifilm plates were enumerated following 24 hours incubation at 37°C.

Statistical Analysis

Experiment 1, inoculation and surrogate validation portion of the study was repeated on three separate days (blocks) with three samples analyzed per treatment per hour (0 or 24) on each day (i.e., a total of nine samples per treatment level; n = 9 replicates). This experiment was conducted as a completely randomized block 4 x 2 factorial (SA treatment x hour) design with day as the block effect. Bacterial populations were expressed as least squares means for log

CFU/g, calculated under an assumption of a log-normal distribution of plate counts. Data were evaluated using the Mixed Procedure of SAS 9.3 (Cary, NC) with independent variables for each inoculum type included spray treatment (Untreated control, water, 1.5 pH SA and 1.0 pH SA), sampling hour (0 and 24) and the respective interactions. All differences were reported using a significance level of $\alpha = 0.05$.

Experiment 2, effects of SA on spoilage indicators had a total of 6 samples (n = 6/treatment/day or hour) to evaluate the effect of SA on spoilage indicators. In order to evaluate odor characteristics, 2 samples/cut/treatment/day (n = 2) were evaluated on each odor panel. The spoilage microflora enumeration and off-odor development determination for both the loins and backribs portion of this experiment was conducted as a 3 x 3 factorial (SA treatment x display hour) nested within dark anaerobic storage period. Instrument color and panel discoloration portion of this experiment was conducted as a longitudinal repeated measures comparison across time and SA treatment nested within dark anaerobic storage period. Data were evaluated using the Mixed Procedure of SAS 9.3 (Cary, NC) with independent variables within each cut and each day of dark storage times (14 days and 21 days) included spray treatment (untreated control, 1.5 pH SA and 1.0 pH SA), retail display hour and the respective interactions. For instrument color and subjective color panel analysis, time was analyzed as a longitudinal repeated measure. All differences were reported using a significance level of $\alpha = 0.05$.

Experiment 3, in-plant validation of SA effects on pork carcasses, had bacterial populations expressed as means log CFU/cm^2. This experiment was conducted as a complete randomized block design with repeated measure. Day served as the block effect and sampling point served at the repeated measure. Data were evaluated using the Mixed Procedure of SAS

9.3 (Cary, NC) with independent variables of SA treatment. All differences are reported at a significance level of alpha = 0.05.

Results and Discussion

Experiment 1: Inoculation and Surrogate Validation

After pork shoulder samples were surface-plated on each agar and incubated at the appropriate time and temperature, colonies were counted and converted to $\log_{10}$ CFU/g. For each inoculum type (*Salmonella* spp., *Campylobacter* spp. and Surrogate bacteria) and agar type (selective and non-selective for each bacteria type), there were no interactions ($P > 0.05$) between treatment (1.0 pH, 1.5 pH, Water and CON) and sampling time (0 hour and 24 hour), excluding *Salmonella* spp. plated on XLD agar ($P = 0.0168$) (Table 2). Table 2 shows the sulfuric acid sodium sulfate treatment by sampling time interaction for each bacteria type on each agar; shoulder samples inoculated with *Salmonella* spp. and sampled 24 hours post treatment with 1.0 pH SA and plated on XLD agar had the lowest ($P < 0.05$) log CFU/g than all other shoulder samples.

Table 3 shows the main effect of treatment for each inoculum and agar type across both sampling times. For each inoculum and agar type, samples treated with 1.0 pH SA had lower ($P < 0.05$) bacterial populations and the SA was more effective at reducing inoculated bacterial populations on pork shoulder samples, excluding *Salmonella* spp. populations plated on TSA agar. There was no difference ($P < 0.05$) between SA treatment levels at 1.5 pH and 1.0 pH on inoculated *Salmonella* spp. populations plated on TSA agar across both 0 and 24 hours sampling times. Additionally, the SA 1.5 pH and 1.0 pH treatments were more ($P < 0.05$) effective at reducing inoculated populations of ˆ*Salmonella* spp. and *Campylobacter* spp. on pork shoulder samples than samples treatment with water. However, log CFU/g on shoulder samples had the

same ($P > 0.05$) log CFU/g after treatment with water and 1.5 pH SA and plated on both TSA and TSA + Rif plates. Overall, treatment with SA, especially 1.0 pH SA, reduced inoculated populations of *Salmonella* spp., *Campylobacter* spp., and Surrogate bacteria on pork shoulders sampled at both 0 and 24 hours post treatment.

The main effect of sampling time (0 hour versus 24 hour) for each agar type (selective and non-selective) under each bacteria type (*Salmonella* spp., *Campylobacter* spp., and Surrogates) are shown in Table 4. Overall, for each agar type, under each bacteria type, the 0 hour samples had higher ($P < 0.05$) log CFU/g than the 24 hour samples, excluding the surrogate bacteria on TSA plates. Therefore, pork shoulders inoculated with *Salmonella* spp., *Campylobacter* spp., and Surrogate bacteria, treated with SA and then stored in anaerobic packages had lower ($P < 0.05$) log CFU/g counts of *Salmonella* spp., *Campylobacter* spp., and Surrogate bacteria sampling at 24 hours than at 0 hours.

Results from a similar study showed that incidence of *Campylobacter jejuni* and *Campylobacter coli* to be similar across all retail products and was the least frequent pathogen found on retail pork (Duffy et al., 2001). Many other studies involving inoculation with *Campylobacter* spp. lack consistent results and recovery of the pathogen, probably due to its microaerophilic nature, sensitivity to water activity and masking of *Campylobacter* spp. by other organisms (Stern et al., 1985; Genta et al., 1995; Oosterom et al., 2016). Another study found that *Campylobacter* spp. is a common contaminate on many live hog operations, with 85% prevalence amongst piglets (Minveille et al., 2007). Minveille et al (2007) found that 100% of hogs were contaminated with high levels (40,000 CFU/g) of bacteria, 23% of carcasses were contaminated with low levels of bacteria before chilling. Presence of *Campylobacter* spp. on pork products on retail meat products is very low (Stern et al., 1985).

Salmonella spp. has been isolated on pork carcasses at different prevalence rates; 40-50%, 12.5%, and 5% (Epling et al., 1993b). While a study found a very low incidence of *Campylobacter* spp. in non-enhanced retail pork, they found a higher incidence (8.3%) of *Salmonella* spp. in samples of non-enhanced retail pork (Duffy et al., 2001). This same study also looked at the incidence of *Campylobacter* spp. and *Salmonella* spp. in harvest and fabrication facilities; *Salmonella* spp. was found in 7.5% of the surveyed facilities and no *Campylobacter* spp. was found (Duffy et al., 2001). These studies prove that there is a possibility for pork, specifically pork subprimals, to be contaminated with both *Salmonella* and *Campylobacter* which this study shows that sulfuric acid sodium sulfate has the ability to effectively reduce and control.

Experiment 2: Effects of Sulfuric Acid Sodium Sulfate on Spoilage Indicators

After pork backribs and loins were portioned, treated, and sampled at the assigned times, samples were surface plated to enumerate populations of specific spoilage microorganisms, as well as, determine the effect of SA on off-odor development, and color. Table 5 shows the effect of SA treatment and display hour on the natural spoilage microflora of PSY, LAB, PSD and YM on pork loins after 14 days and 21 days of dark storage. For each spoilage bacteria, no SA treatment by display hour interactions were evident ($P > 0.05$) on pork loins placed into simulated retail display following 14 and 21 days of dark anaerobic storage.

Table 6 shows the effect of SA treatment and display hour on the natural populations of PSY, LAB, PSD and YM on pork backribs placed into simulated retail display following 14 days or 21 days of dark storage. For PSY, LAB and PSD, populations, effect of SA treatment and display hour on backribs were not ($P > 0.05$) dependent on each other, there was no interaction after both 14 days or 21 days of dark storage. However, an interaction existed ($P = 0.0173$)

between the factors of display hour and SA treatment on YM populations for backrib samples placed in simulated retail display following 14 days of dark storage. Populations of YM on backrib samples treated with 1.5 pH and 1.0 pH SA and stored for 14 days before simulated retail display, were generally higher ($P < 0.05$) at 48 hours of display than both 0 hours of display and 96 hours of display. Backrib CON samples placed into simulated retail display after 14 days of dark storage showed YM populations to increase as display hour increased. Similarly, backrib samples stored for 21 days in the dark before simulated retail display were affected dependently by an interaction ($P = 0.0163$) between display hour and SA treatment on YM populations. Backrib samples treated with 1.5 pH SA and stored in the dark for 21 days before simulated retail display had higher ($P < 0.05$) YM populations at 48 hours of display than both 0 hours and 96 hours.

The main effect of SA treatment on PSY, PSD, LAB and YM populations on pork loin samples across 96 hours of simulated retail display after 14 and 21 days of dark anaerobic storage as well as over 28 days of dark anaerobic or vacuum storage, are shown in Table 7. Generally, loin samples treated with SA had lower ($P < 0.05$) PSY, PSD, LAB and YM populations across all 96 hours of simulated retail display after 14 and 21 days of dark anaerobic storage as well as over 28 days of vacuum storage compared to CON samples. However, in most cases, SA treatment of loins with 1.0 pH SA was the most ($P < 0.05$) effective at reducing populations of PSY, PSD, LAB and YM across 96 hours of simulated retail display after 14 or 21 days of dark anaerobic storage as well as over 28 days of vacuum storage.

The main effect of SA treatment on PSY, PSD, LAB and YM populations on pork backrib samples across 96 hours of simulated retail display after 14 days or 21 days of dark anaerobic storage as well as over 28 days of vacuum storage, are shown in Table 8. Overall,

backrib samples treated with SA had lower ($P < 0.05$) PSY, PSD, LAB, and YM populations than CON backrib samples, excluding PSY and PSD samples over 96 hours of dark anaerobic storage, which had no difference ($P > 0.05$).

The main effect of display hour after both 14 and 21 days of dark anaerobic storage across SA treatment, on PSY, PSD, LAB, and YM populations of loin and backrib samples are shown in Table 9. Log CFU/g of PSD, LAB, and YM on loin and backrib samples, as well as, PSY on backrib samples are the lowest ($P < 0.05$) at the beginning of display and grow as display continues for both subprimal types placed into simulated retail display after 14 and 21 days of dark anaerobic storage.; however, populations of PSY after 14 days of dark anaerobic storage are an exception. Dissimilar to populations of the other measured spoilage microorganisms, PSY bacteria on loin samples placed into simulated retail display after 14 days of dark anaerobic storage were the highest ($P < 0.05$) at 48 hours, not 96 hours.

The interaction between vacuum storage day and SA treatment on populations of PSY, PSD, LAB and YM on loin and backrib samples are shown in Table 10. There were no interactions ($P > 0.05$) between vacuum storage day and SA treatment on populations of PSY, PSD, LAB, and YM on loins, or on populations of PSY, PSD, and LAB on backribs. Conversely, there was an interaction ($P = 0.0284$) between vacuum storage day and SA treatment on populations of YM on backrib samples. The storage day by SA treatment interaction on backrib samples YM populations showed that samples treated with 1.0 pH SA tended grow at a slower rate across storage days, and remained lower than samples treated with 1.5 pH SA and CON samples until day 28 of vacuum storage, where all treatments were the same ($P > 0.05$).

Several microorganisums such as *Enterbacteriacea*, LAB, PSU, *Brochothrix thermosphacta*, will occur in meat placed into aerobic conditions (Borch et al., 1996; Blixt and

Borch, 2002; Liu et al., 2006a). One study found that *Brochothrix thermospacta* and coliforms to be the most predominant spoilage bacteria associated with pork exposed to the air and pork that was kept under artificial conditions (40% CO_2/59% N_2/1% O_2) at -2, 4 and 10°C (Liu et al., 2006b). However, the same study found PSD could dominate packages exposed to the air as well (Liu et al., 2006b). Correlations to shelf-life were found to be the highest between the initial cell number, of PSY, LAB, coliforms, PSD and *Brochothrix thermospacta* on microbial shelf-life (Liu et al., 2006b). Another study found PSD and *Salmonella* spp. were the predominant bacteria associated with pork spoilage (Liu et al., 2006a). As LAB and yeasts grew, microbial activity on Coliforms, PSD, *Brochothrix thermosphacta*, *Salmonella* spp. slowed. Yeasts and LAB grew at similar rates under similar conditions (Liu et al., 2006a). Yeasts and molds can be effectively controlled or suppressed by CO_2, however, LAB and PSY growth increased (Blickstad et al., 1983). CO_2 also inhibited the growth of total aerobic plate count, while allowing LAB to dominate (Enfors et al., 1979; Blickstad et al., 1981; Gill, 1983; Lambropoulou et al., 1996).

The interaction between display hour and SA treatment on off-odor development of loin samples placed into simulated display after 14 days of dark anaerobic storage are shown in Table 11. There was no treatment x display hour interaction ($P > 0.05$) for sour and acid/chemical off-odor development on loin samples placed into simulated retail display after 14 days of dark anaerobic storage. Table 11 also shows the treatment x display hour interaction ($P < 0.05$) on putrid and oxidized/rancid off-odor development on loin samples placed into simulated retail display after 14 days of dark anaerobic storage. Control loin samples at the beginning of display (0 hours) placed into simulated retail display after 14 days of dark anaerobic storage, had the highest ($P < 0.05$) off-odor intensity than all other SA treatments and display hours. There was

no difference ($P > 0.05$) among SA treatments at 0 and 48 hours of display for oxidized/rancid off-odor intensity development of loin samples placed into simulated retail display after 14 days of dark anaerobic storage, as well as CON samples at 96 hours of display; however, both 1.5 pH and 1.0 pH SA treated samples at 96 hours of display had higher ($P < 0.05$) oxidized/rancid off-odor intensities than all other samples at all other display hours.

The interaction between display hour and SA treatment on off-odor development of loin samples placed into simulated retail display after 21 days of dark anaerobic storage are also shown in Table 11. Acid/chemical and putrid off-odor development on loin samples placed into simulated retail display after 21 days of dark anaerobic storage had no interaction ($P > 0.05$) between SA treatment and display hour. Although there was no interaction ($P = 0.3170$) between display hour and SA treatment for acid/chemical off-odor development on loin samples placed into simulated retail display after 21 days of dark anaerobic storage, off-odor intensity values from loin samples of all treatments tended to decrease in intensity from 0 hours to 48 hours of display and then increase by 96 hours of display. Also, although there was no interaction ($P = 0.5315$) between display hour and treatment for putrid off-odor intensity development of loin samples placed into simulated retail display after 21 days of dark storage, off-odor intensity values from loin samples of all treatments also generally tended to increase from 0 to 96 hours of display.

Table 11 also showed the SA treatment by display day interaction ($P < 0.05$) of sour and oxidized/rancid off-odors from loin samples placed into simulated retail display after 21 days of dark anaerobic storage. The SA treatment by display day interaction ($P = 0.0004$) of sour off-odor intensities on loin samples placed into simulated retail display after 21 days of dark anaerobic storage indicated that CON loin samples off-odor intensities to increase ($P < 0.05$)

over display hour; however, both the 1.5 pH and 1.0 pH SA treated loin samples sour odor intensities decreased from 0 to 48 hours of display and then increased by 96 hours of display. 1.0 pH SA treated loin samples stored for 21 days in dark anaerobic storage prior to simulated retail display, had lower ($P < 0.05$) sour off-odor intensities than 1.5 pH SA and CON samples by 96 hours of display. The display day by SA treatment interaction of oxidized/rancid off-odor intensity development of loins placed into simulated retail display after 21 days of dark anaerobic storage indicated that CON samples oxidized/rancid off-odor intensity to increase ($P < 0.05$) from 0 to 48 hours of display and then decrease ($P < 0.05$) from 48 to 96 hours of display. Table 11 also showed oxidized/rancid off-odor intensity of 1.5 pH and 1.0 pH SA treated loin samples placed into simulated retail display after 21 days of dark storage to be similar, from 0 to 48 hours of display off-odor intensity values decreased ($P < 0.05$), then from 48 to 96 hours of display, values increased and where significantly more intense ($P < 0.05$) than CON samples at 96 hours of display.

Table 12 shows the interaction between display day and SA treatment on off-odor development on backrib samples placed into simulated retail display following 14 days of dark anaerobic storage. There were no teatment x display day interactions ($P > 0.05$) for sour, acid/chemical, putrid, and oxidized/rancid off-odor intensity development on backrib samples placed into simulated retail display following 14 days of dark storage. Additionally, off-odor intensity values of backrib samples placed into simulated retail display following 14 days of dark storage showed 1.0 pH SA treated samples to be less intense for putrid and oxidized/rancid odors than CON and 1.5 pH SA treated backrib samples. Table 12 also shows the interaction between display hour and SA treatment on off-odor intensity development of backribs placed into simulated retail display after 21 days of dark anaerobic storage. There were no ($P > 0.05$) SA

treatment by display hour interactions for acid/chemical and oxidized/rancid off-odor intensities on backrib samples placed into simulated retail display following 21 days of dark anaerobic storage.

Additionally, Table 12 shows interactions ($P < 0.05$) between display hour and SA treatment of sour and putrid off-odor intensity values on backrib samples placed into simulated retail display following 21 days of dark anaerobic storage. Sour off-odor intensity of backrib samples placed into simulated retail display following 21 days of dark anaerobic storage showed an interaction ($P = 0.0368$) between display hour and SA treatment, generally, backrib samples from all treatments were the least intense ($P < 0.05$) at 0 hours of display and by 96 hours of display, CON backrib samples had the most intense ($P < 0.05$) sour off-odor. Putrid off-odor intensity of backrib samples placed into simulated retail display following 21 days of dark anaerobic storage showed an interaction ($P < 0.0001$) between display hour and SA treatment, generally, samples from all treatments were the most intense by 96 hours of display, with 1.5 pH SA treated backrib sample putrid off-odor having the most intense off-odor than any other treatment, and any display hour at 96 hours.

Table 13 shows the main effect of SA treatment on off-odor intensity development on both loin and backrib samples placed into simulated display after 14 and 21 days of dark anaerobic storage. No difference ($P > 0.05$) was found in off-odor intensity development between treatments on loin samples that were placed into simulated retail display following 14 days of dark anaerobic storage. Conversely, CON loin samples placed into simulated retail display following 14 days of dark anaerobic storage were more putrid ($P = 0.0012$) than 1.5 pH and 1.0 pH SA treated loin samples. Dissimilar to putrid odor intensity of loin samples placed into simulated retail display following 14 days of dark anaerobic storage, intensity of

oxidized/rancid off-odors for CON loin samples were less ($P = 0.0269$) intense than the 1.0 pH SA treated loin samples but the same ($P > 0.05$) as 1.5 pH SA treated loin samples and the 1.5 pH SA treated loin samples were the same ($P > 0.05$) as the 1.0 pH SA treated loin samples.

Table 13 also showed no difference ($P = 0.6514$) for oxidized/rancid off-odor intensities between SA treatments of loin samples placed into simulated retail display following 21 days of dark anaerobic storage. However, there was a difference ($P < 0.05$) found in sour, acid/chemical, and putrid off-odor intensities between SA treatments on loin samples placed into simulated retail display following 21 days of dark anaerobic storage. Sour off-odor intensity of loin samples placed into simulated retail display following 21 days of dark storage indicated that 1.0 pH SA treated loin samples to be less intense ($P = 0.0006$) than CON and 1.5 pH SA treated loin samples. Acid/chemical off-odor intensities of loin samples placed into simulated retail display following 21 days of dark anaerobic storage showed 1.0 pH SA treated loin samples to be more intense ($P = 0.0467$) than 1.5 pH SA treated loin samples; as well as, CON loin samples to be the same as both 1.0 pH and 1.5 pH SA treated loin samples. Putrid off-odor intensities of loin samples placed into simulated retail display following 21 days of dark storage showed 1.5 pH SA treated samples to be the same as both CON and 1.0 pH SA treated loin samples; along with, CON loin samples being less intense ($P = 0.0230$) than 1.0 pH SA treated loin samples.

No difference ($P > 0.05$) was also found between SA treatments on sour, acid/chemical, and oxidized/rancid off-odor intensities on backrib samples placed into simulated retail display following 14 days of dark anaerobic storage. However, there was a difference ($P = 0.0316$) in putrid off-odor intensities of backrib samples placed into simulated retail display after 14 days of dark anaerobic storage; 1.0 pH SA treated backrib samples were less intense ($P < 0.05$) in putrid off-odors than both CON and 1.5 pH SA treated backrib samples. Similar to backrib samples

placed into simulated retail display following 14 days of dark anaerobic storage, backrib samples placed into simulated retail display after 21 days of dark storage had no difference ($P > 0.05$) between SA treatments for sour, acid/chemical, and oxidized/rancid off-odor intensities. Also similar to backrib samples placed into simulated retail display following 14 days of dark anaerobic storage, backrib samples placed into simulated retail display after 21 days of dark anaerobic storage had a difference ($P = 0.0007$) between SA treatments for putrid off-odor intensities; 1.0 pH SA treated backrib samples were less ($P < 0.05$) putrid than both CON and 1.5 pH SA treated backrib samples.

Other studies showed off-odors associated with spoilage increased during storage (Liu et al., 2006b). Other studies also show that spoilage can be indicated by development of off-odors; when carbohydrate supplies, amino acids are all utilized and there is production of volatile fatty acids, off-odors develop (Lambert et al., 1991). Previous research has shown that the formation of off-odors is associated with bacteria growth such as PSD after it has reached a certain level (Nychas et al., 2008). Conversely, total plate count, has not been found to be related to off-odor development (Blickstad et al., 1983). Glucose has been found to be a precursor to many off-odors (Nychas et al., 2008; Casaburi et al., 2015). Glucose is also the first substrate utilized by bacteria during storage or display under any packaging conditions (Gill, 1983; Nychas et al., 1988; Borch and Agerhem, 1992; Drosinos and Board, 1995; Casaburi et al., 2015).

Table 14 shows the interaction between SA treatment and display hour on the percent surface discoloration on loin and backrib samples placed into simulated retail display following 14 and 21 days of dark anaerobic storage. There was no SA treatment by display hour interaction ($P > 0.05$) on surface discoloration of loin samples placed into simulated retail display following 21 days of dark anaerobic storage as well as backrib samples placed into

simulated retail display following 14 and 21 days of dark anaerobic storage. However, there was a significant SA treatment by display hour interaction ($P < 0.0001$) on the surface discoloration of loin samples placed into simulated retail display following 14 days of dark storage. Overall, the SA treatment by display hour interaction of loin samples placed into simulated retail display following 14 days of dark anaerobic storage showed that at the beginning of display (0 hours) loin samples from all treatments (CON, 1.5 pH SA, and 1.0 pH SA) stared off with the same ($P > 0.05$) percent surface discoloration; however, loin samples treated with 1.5 pH and 1.0 pH SA discolored to a higher ($P < 0.05$) percent throughout display than CON loin samples.

Table 14 also shows that there is no significant SA treatment and display hour interaction ($P > 0.05$) on surface discoloration of loin samples placed into simulated retail display following 21 days of dark anaerobic storage, LS means of percent discoloration on CON loin samples tended to have minimal surface discoloration compared to 1.5 pH and 1.0 pH SA treated loin samples which had more extensive surface discoloration throughout all display hours. Conversely, backrib samples placed into simulated retail display after both 14 and 21 days of dark anaerobic storage showed regardless of SA treatment, all samples discolored at a similar rate and to a similar extent.

Table 15 shows the main effect of SA treatment on percent surface discoloration of loin samples placed into simulated retail display following 21 days of dark storage. Control loin samples placed into simulated retail display after 21 days of dark storage were less ($P < 0.0001$) discolored than 1.5 pH and 1.0 pH SA treated loin samples. Backrib samples placed into simulated retail display following 14 days of dark storage showed 1.5 pH SA treated backrib samples to have the same ($P > 0.05$) percent surface discoloration as both CON and 1.0 pH SA treated backrib samples; however, 1.0 pH SA treated backrib samples were more ($P < 0.05$)

discolored than CON backrib samples. Table 15 also showed no difference (P = 0.7906) between percent surface discoloration of SA treatments on backrib samples placed into simulated retail display following 21 days of dark storage.

Table 16 shows the main effect ($P < 0.0001$) of display hour on the percent surface discoloration of loin samples placed into simulated retail display following 21 days of dark storage as well as backrib samples placed into simulated retail display after both 14 and 21 days of dark storage. Surface discoloration of loin samples placed into simulated retail display following 21 days of dark storage as well as backrib samples placed into simulated retail display after both 14 and 21 days of dark storage, all showed that percent surface discoloration increased as display hour increased ($P < 0.05$).

Results from this study showing percent surface discoloration increasing over time are consistent with other display and storage studies (Livingston et al., 2004; Huang et al., 2005). Surface discoloration on loin samples placed into retail display increase rapidly over 20 days (Livingston et al., 2004). However, in this study treatment with SA caused increased surface discoloration on loin samples but not on backrib samples; another study found that treatment with lactic acid had no effect on pork carcass surface discoloration compared to control samples (Prasai et al., 1992). Storage of fresh pork under higher oxygen conditions or with exposer to the atmosphere showed significant discoloration after three days of storage or display (Sørheim et al., 1997; Viana et al., 2005).

Table 17 shows the SA treatment by display hour interaction of L*, a* and b* values on loin lean samples placed into simulated retail display following14 and 21 days of dark storage. There is no treatment by display hour interaction ($P > 0.05$) on any L*, a* and b* values of loin lean samples after both 14 and 21 days of dark storage. Table 18 shows the interaction between

SA treatment and display hour on *CIE* L*, a* and b* values of pork loin fat samples placed into simulated retail display following 14 and 21 days of dark anaerobic storage. There was no SA treatment by display hour interaction ($P > 0.05$) for L*, a* and b* values on pork loin fat samples placed into simulated retail display following 14 and 21 days of dark storage. Table 19 shows the interaction between SA treatment and display hour on L*, a* and b* values of lean on backrib samples placed into simulated retail display after 14 and 21 days of dark storage. There is no SA treatment by display hour interaction ($P > 0.05$) on L*, a* and b* values of pork backrib lean samples placed into simulated retail display following 14 and 21 days of dark anaerobic storage. Table 20 shows the interaction between SA treatment and display hour on L*, a* and b* values of fat on pork backrib samples placed into simulated retail display following 14 and 21 days of dark storage. There is no SA treatment by display hour interaction ($P > 0.05$) on L*, a* and b* values of pork backrib fat samples placed into simulated retail display following 14 and 21 days of dark storage.

Table 21 shows the main effect of SA treatment on L*, a*, and b* values of fat and lean on both loin and backrib samples treated with SA and then stored for 14 days prior to simulated retail display. There was a difference ($P < 0.05$) between treatments for L*, a*, and b* values of fat and lean on both loin and backrib samples treated with SA and then stored in dark anaerobic storage for 14 days prior to simulated retail display, excluding ($P > 0.05$) L* values of fat on backribs and a* values of lean on backribs. L* values of both lean and fat on loin samples indicated that CON samples were lighter ($P < 0.05$) than both 1.5 pH and 1.0 pH SA treated samples placed into simulated retail display following 14 days of dark anaerobic storage. Additionally, a* values of lean on CON loin samples were more red ($P < 0.05$) than both 1.5 pH and 1.0 pH SA treated loin lean samples placed into simulated retail display after 14 days of dark

anaerobic storage. Fat on both loin and backrib samples placed into simulated retail display following 14 days of dark anaerobic storage had lower ($P < 0.05$) a* values on samples treated with 1.5 pH SA than CON and 1.0 pH SA treated fat backrib and loin samples. Also, b* values of both fat and lean on loin samples indicated that 1.0 pH SA treated samples to be higher ($P < 0.05$) than CON and 1.5 pH SA treated lean and fat samples when placed into simulated retail display following 14 days of dark anaerobic storage. Additionally, b* values of lean on backrib samples indicated that those treated with 1.0 pH SA to have higher ($P < 0.05$) b* values than 1.5 pH SA treated backrib fat samples; while CON treated samples were the same as both 1.0 pH and 1.5 pH SA treated samples when placed into simulated retail display following 14 days of dark anaerobic storage. Fat on backrib samples treated with 1.0 pH SA had higher ($P < 0.05$) b* values than both, 1.5 pH SA and CON samples; as well as, 1.5 pH SA samples had lower b* values than both 1.0 pH SA and CON samples when placed into simulated retail display following 14 days of dark anaerobic storage.

Table 22 shows the main effect of SA treatment on L*, a*, and b* values of lean and fat on loin and backrib samples placed into simulated retail display following 21 days of dark storage. There was a difference ($P < 0.05$) in SA treatment for L*, a*, and b* values on lean and fat of loin and backrib samples treated with SA and then stored for 21 days in dark anaerobic storage prior to simulated retail display, excluding ($P > 0.05$) a* values of loin lean, backrib lean and backrib fat; as well as L* values of both lean and fat of backrib samples as well as b* values of lean on backrib samples. Both fat and lean of 1.0 pH SA treated loin samples had lower ($P < 0.05$) L* values than 1.5 pH and CON treated loin lean and fat samples; additionally, lean on CON samples had higher ($P < 0.05$) L* values than both 1.5 pH and 1.0 pH SA treated loin lean samples placed into simulated retail display following 21 days of dark anaerobic storage. Fat on

loin CON samples stored for 21 days prior to simulated retail display had lower ($P > 0.05$) a* values than 1.5 pH loin fat samples and 1.0 pH SA treated loin fat samples; and 1.0 pH SA treated loin fat samples had higher a* values than both CON and 1.5 pH SA treated samples. Moreover, b* values of CON loin lean samples placed into simulated retail display following 21 days of dark anaerobic storage, were lower ($P < 0.05$) than both 1.5 pH and 1.0 pH SA treated loin lean samples. Fat on loin samples placed into simulated retail display following 21 days of dark anaerobic storage indicated that samples treated with 1.0 pH SA had higher ($P < 0.05$) b* values than both CON and 1.5 pH SA treated loin fat samples; additionally, fat on CON loin samples had lower ($P < 0.05$) b* values than 1.5 pH and 1.0 pH SA treated samples. Fat on backrib samples placed into simulated retail display following 21 days of dark anaerobic storage indicated that those treated with 1.0 pH SA had higher ($P < 0.05$) b* values than both 1.5 pH and 1.0 pH SA treated backrib fat samples.

Treatment with ascorbic and citric acid in combination with packaging type had an effect on objective color measurements (Gill, 1983). Pork placed in high oxygen MAP packages and then placed into simulated display were lighter (higher *L** values) than pork placed in PVC packages in simulated display (Gill, 1983). Another study noted a decrease in lightness (lower *L** values) throughout storage of loins placed into PVC packages (Viana et al., 2005). Post-mortem aging time for eight days, in chill improved blooming of loins from both Duroc and Landrace pig, causing higher *L**, *a** and *b** values (Lindahl et al., 2006). Lower metmyoglobin concentrations are associated with lower *L** values in pork (Lindahl et al., 2001). The breed of the live hog may also have an effect on the color of the loin muscle. The *longissimus dorsi* from Hampshire breed of hogs are more yellow (higher *b** values) and more red (higher *a** values) in color than Swedish Landrace and Swedish Yorkshire breed (Lindahl et al., 2001). This same

study found, no instrument color differences between gilts and barrows with breeds (Lindahl et al., 2001). Most of the color variation (86-90%) seen in pork considered normal quality can be explained by pigment content, myoglobin forms and internal reflectance (Lindahl et al., 2001). Increased metmyoglobin content caused a decrease in redness of pork loins. The color of pork loins from Duroc hogs were darker (lower L^* values) and less yellow (lower b^* values) than Landrace hogs because of higher pigment content (Lindahl et al., 2006).

Table 23 shows the main effect of treatment across display hour or storage day for both loins and backribs either placed into simulated retail display after both 14 and 21 days of dark anaerobic storage or across 28 days of vacuum storage on pH. Overall, there was a treatment effect ($P < 0.05$) on pH, of both loin and backrib samples placed into simulated retail display following both 14 and 21 days of dark anaerobic storage and across 28 days of vacuum storage, excluding ribs over 28 days of vacuum storage ($P > 0.05$). The pH of 1.0 pH SA treated loin samples were lower ($P < 0.05$) than CON and 1.5 pH SA treated samples placed into simulated retail display following 14 days of dark storage and samples held in vacuum storage for 28 days. Similarly, 1.5 pH and 1.0 pH SA samples on loin samples stored for 21 days in dark anaerobic storage prior to simulated retail display had lower ($P < 0.05$) pH values than CON loin samples. Additionally, backrib samples stored for 21 days in dark anaerobic storage prior to simulated retail display indicated that CON backrib samples had higher ($P < 0.05$) pH values than both 1.5 pH and 1.0 pH SA treated samples. Also, backrib samples placed into simulated retail display following 14 days of dark anaerobic storage indicated that CON backrib samples had higher ($P < 0.05$) pH values than 1.0 pH SA treated backrib samples and 1.5 pH SA treated backrib samples to be the same ($P > 0.05$) as both CON and 1.0 pH SA treated backrib samples.

The initial pH of fresh loins ranged from 5.4 to 5.8 pH and it did not show strong variation within the population (Viana et al., 2005). Similar to this study, a previous study found that dipping pork in acid (ascorbic and citric) and placing it in PVC overwrap, causes the pH to be lower directly after treatment (Huang et al., 2005). Internal pH of pork, has a large effect on color stability. Enhanced MAP loin chops had higher pH values and therefore had greater color stability; but also had greater microbial growth than loins with a lower pH. Pork loins with a lower pH value, had lower microbial growth but discolored quicker over time (Livingston et al., 2004).

Experiment 3: In-plant Validation of Sulfuric Acid Sodium Sulfate on Pork Carcasses

Table 24 shows the effect of 1.3 pH and 1.0 pH SA on log CFU/cm^2 on the natural microflora and inoculated populations of surrogate bacteria on hot pork carcasses treated on the harvest floor in a commercial packing facility. Log CFU/cm^2 of inoculated and uninoculated sponge samples from pork carcasses treated with SA and plated on APC and EB petrifilms showed that there was a significant difference ($P < 0.0001$) across sampling points (before, after, after rapid chill and after 15 hours). There were higher ($P < 0.05$) log CFU/cm^2 on all before samples compared to after samples of both 1.3 pH and 1.0 pH SA treatments on both inoculated and uninoculated carcass zones. There were also higher ($P < 0.05$) reductions in log CFU/cm^2 from both before and after samples compared to after rapid chill cycle samples of both 1.3 pH and 1.0 pH SA treatments on both inoculated and uninoculated carcass zones, with the exception ($P > 0.05$) of EB bacteria on 1.0 pH SA uninoculated zones. There was likely no difference ($P > 0.05$) between the after, after rapid chill and after 15 hours sample's log CFU/cm^2 of EB bacteria for 1.0 pH SA uninoculated zones because of the high percentage of after, after rapid chill and after 15 hour samples that were below detection limit.

Enterobacteriaceae counts from inoculated carcass zones treated with 1.3 pH and 1.0 pH SA and sampled after 15 hours were the same ($P > 0.05$) as zones sampled directly after rapid chill. Similarly, APC counts from inoculated carcass zones treated with 1.3 pH and 1.0 pH SA and sampled after 15 hours were the same ($P > 0.05$) as zones sampled directly after rapid chill. However, APC counts from uninoculated carcass zones treated with 1.3 pH and 1.0 pH SA and sampled after rapid chill were lower ($P < 0.05$) than zones samples after 15 hours. *Enterobacteriaceae* counts from uninoculated carcass zones treated with 1.3 pH SA were no different ($P > 0.05$) when sampled after rapid chill and after 15 hours.

Another study, similar to the current study, showed that the application of a 2% lactic acid spray was effective at reducing populations of *Salmonella* spp. and *Campylobacer* spp. on pork carcasses at both 5 mins and 24 hours post treatment (Epling et al., 1993a). Both *Salmonella* spp. and *Campylobacter* spp. can be found on the harvest floor of multiple pork production operations (Baer et al., 2013b). Treating pork carcasses with steam pasteurization and lactic acid are effective methods at reducing surface microbial counts directly after treatment and can continue to slow microbial growth throughout storage (Pipek et al., 2006). Similarly, the results from this study agree with the results from the study conducted in 2006; treatment with an antimicrobial acid, reduced the total aerobic plate count on the carcass surface (Pipek et al., 2006).

Conclusions

Results of this study show the five-strain mixture of non-pathogenic *E. coli* are effective surrogate bacterial strains for both *Salmonella* spp. and *Campylobacter* spp. when sulfuric acid sodium sulfate is sprayed on pork products. The reductions of *Salmonella* spp. and *Campylobacter* spp inoculated on cold pork subrimals and treated with sulfuric acid sodium

sulfate were greater than the reductions seen in inoculated populations of the non-pathogenic E. coli strains. Therefore, if sulfuric acid sodium sulfate can reduce inoculated populations of surrogate bacteria, it will be effective against populations of *Salmonella* spp. and *Campylobacter* spp. which makes it an effective surrogate for both pathogens.

Additionally, when sulfuric acid sodium sulfate is applied adequately to pork and product is receiving full coverage, it can help reduce and control bacterial growth. The sulfuric acid sodium sulfate effectively reduced inoculated populations of *Salmonella* spp. and *Campylobacter* spp. on cold pork subprimals. This study showed significant statistical reductions in the bacterial pathogen populations when treated with 1.0 pH and 1.5 pH sulfuric acid sodium sulfate. Treatment with the 1.0 pH sulfuric acid sodium sulfate solution was more effective at reducing the pathogen microbial load than the 1.5 pH concentration; as well as, more effective then treatment with water. Additionally, sulfuric acid sodium sulfate treatment was more effective at reducing inoculated populations of *Campylobacter* spp. than *Salmonella* spp. This may be partially because *Campylobacter* spp. is a microaerophilic and serotypes of *Salmonella* spp. ranges from aerobic to facultatively anaerobic; because *Campylobacter* spp. is a microaerophilic, sulfuric acid sodium sulfate may possible be more effective against reducing populations.

After the non-pathogenic *Escherichia coli* were proven to be sufficient surrogates for both *Salmonella* spp. and *Campylobacter* spp. in Experiment 1, these surrogates were utilized in Experiment 3. Sulfuric acid sodium sulfate was shown to be an effective method at reducing both the natural microflora (APC) and surrogate bacteria on pork carcasses. Greater reductions were seen on carcasses that were treated with SA and then placed into a rapid chill cycle and then held in the cooler for 15 hours. Additionally, carcasses treated with 1.0 pH SA showed greater reductions than those treated with 1.3 pH SA. Sulfuric acid sodium sulfate is an effective

method at reducing natural microflora and inoculated surrogate bacterial populations on pork carcasses in a commercial plant setting; especially when a part of a multiple hurdle approach with other intervention technologies.

Overall, sulfuric acid sodium sulfate was effective at controlling some spoilage indicators, but was not effective on others. Sulfuric acid sodium sulfate was effecting and helping control spoilage bacterial populations of *Pseudomonas,* lactic acid bacteria*, Psychrotrophic* as well as yeasts and molds on bone-in and boneless cold pork subprimals. When bone-in and boneless cold pork subprimals were treated with 1.0 pH and 1.5 pH sulfuric acid sodium sulfate concentrations, they had lower natural populations of the *Pseudomonas,* lactic acid bacteria*, Psychrotrophic* as well as yeasts and molds microflora. Although all populations of *Pseudomonas,* lactic acid bacteria*, Psychrotrophic* as well as yeasts and molds microflora were lower when treated with sulfuric acid sodium sulfate, treatment with the 1.0 pH concentration was more effective than the 1.5 pH concentration and no sulfuric acid sodium sulfate treatment. Treatment with sulfuric acid sodium sulfate is an effective method at controlling natural populations of spoilage microflora such as *Pseudomonas,* lactic acid bacteria*, Psychrotrophic* as well as yeasts and molds on pork product.

However, while sulfuric acid sodium sulfate is effective against spoilage microflora, this study suggests that more research needs to be done on its effects pork color. This study suggests that boneless loins treated with sulfuric acid sodium sulfate have more extensive percent surface discoloration than those that remained untreated. Boneless loins treated with sulfuric acid sodium sulfate that were stored in dark anaerobic storage for 14 days prior to simulated retail display initially started off with minimal percent surface discoloration, similar to untreated loins, but throughout simulated retail display, became extensively discolored. Boneless loins treated

with sulfuric acid sodium sulfate that were stored in dark anaerobic storage for 21 days prior to simulated retail display initially started off with more extensive percent surface discoloration than untreated boneless loins. Conversely, backrib samples treated with sulfuric acid sodium sulfate, did not have more extensive percent surface discoloration than untreated backrib samples; the sulfuric acid sodium sulfate did not cause discoloration of bone-in pork subprimals.

This study also showed that more research needs to be conducted on the effects of sulfuric acid sodium sulfate on *CIE* L*, a* and b* values of pork lean and fat on cold pork subprimals. There were no substantial effects of sulfuric acid sodium sulfate on L*, a* and b* values of boneless loin lean samples placed into simulated retail display following 14 days of dark anaerobic storage. However, for boneless loin lean samples placed into simulated retail display following 21 days of dark storage, L* and b* values of sulfuric acid sodium sulfate treated lean on boneless loins were higher than untreated lean on boneless loins. Additionally, fat on boneless loins treated with sulfuric acid sodium sulfate had minimal effect on *CIE* L*, a* and b* values. Sulfuric acid sodium sulfate treated on fat of boneless loins placed into simulated retail display following 14 days of dark anaerobic storage, showed no major effects on L* and a* values, however sulfuric acid sodium sulfate at a concentration of 1.0 pH caused fat on boneless loins to have higher b* values than 1.5 pH concentration of sulfuric acid sodium sulfate and untreated fat on boneless loins. However, fat on boneless loins treated with sulfuric acid sodium sulfate and placed into simulated retail display following 21 days of dark anaerobic storage had higher a* and b* values than untreated fat on boneless loins. Conversely, lean and fat on backrib samples treated with sulfuric acid sodium sulfate and placed into simulated retail display following 14 and 21 days of dark anaerobic storage showed no effect on *CIE* L*, a* and b*

values. Overall, the effect of sulfuric acid sodium sulfate on lean and fat of boneless loins and backribs *CIE* L*, a* and b* values was minimal and needs to be studied further.

Additionally, the effect of sulfuric acid sodium sulfate on off-odor intensities including, sour, acid/chemical, putrid, and oxidized/rancid odor development on boneless loins and backribs is generally minimal. Sulfuric acid sodium sulfate treated on boneless loins and bone-in backribs placed into simulated retail display following 14 days of dark anaerobic storage, had no major effects on off-odor intensity development. However, sulfuric acid sodium sulfate treated on boneless loins and bone-in backribs placed into simulated retail display following 21 days of dark anaerobic storage minimized sour, putrid, and oxidized/rancid off-odor intensities.

Table 1. Spoilage indicator portion (Experiment 2) design and sample breakdown.

	Boneless Loins		Backribs	
	Days in Dark Anaerobic Storage			
	14 Days	21 Days	14 Days	21 Days
Display Hour				
0 Hours	n = 6/trt*	n = 6/trt*	n = 6/trt*	n = 6/trt*
12 Hours	n = 6/trt*	n = 6/trt*	n = 6/trt*	n = 6/trt*
24 Hours	n = 6/trt*	n = 6/trt*	n = 6/trt*	n = 6/trt*
36 Hours	n = 6/trt*	n = 6/trt*	n = 6/trt*	n = 6/trt*
48 Hours	n = 6/trt*	n = 6/trt*	n = 6/trt*	n = 6/trt*
60 Hours	n = 6/trt*	n = 6/trt*	n = 6/trt*	n = 6/trt*
72 Hours	n = 6/trt*	n = 6/trt*	n = 6/trt*	n = 6/trt*
84 Hours	n = 6/trt*	n = 6/trt*	n = 6/trt*	n = 6/trt*
96 Hours	n = 6/trt*	n = 6/trt*	n = 6/trt*	n = 6/trt

*trt = treatments: Total of three treatments – Untreated Control, 1.5 pH SA, and 1.0 pH SA

Table 2. Effect of sulfuric acid sodium sulfate treatment by sampling hour on Log CFU/g of *Campylobacter* spp., *Salmonella* spp., and Surrogate bacteria inoculated on pork Boston butt shoulder samples (n = 9/treatment/sampling hour).

	***Campylobacter* spp.**			
	Cefex[5]		**TSA[2]**	
	0 Hour	**24 Hour**	**0 Hour**	**24 Hour**
CON[6]	5.63	5.23	5.44	5.22
Water	5.18	4.93	5.08	4.79
1.5 pH SA[7]	4.82	4.52	4.77	4.55
1.0 pH SA[7]	4.51	4.17	4.41	4.16
SEM	0.10120		0.09427	
***P* Value**	0.9116		0.9629	

	***Salmonella* spp.**			
	XLD[4]		**TSA[2]**	
	0 Hour	**24 Hour**	**0 Hour**	**24 Hour**
CON[6]	6.46[a]	6.28[b]	6.62	6.54
Water	5.87[c]	5.51[e]	6.09	6.37
1.5 pH SA[7]	5.70[d]	5.66[de]	5.92	6.09
1.0 pH SA[7]	5.60[e]	5.28[f]	5.92	6.08
SEM	0.05356		0.1041	
***P* Value**	0.0168		0.1891	

	Surrogate[1]			
	TSA + Rif[3]		**TSA[2]**	
	0 Hour	**24 Hour**	**0 Hour**	**24 Hour**
CON[6]	6.60	6.40	6.65	6.48
Water	6.07	5.93	6.18	6.24
1.5 pH SA[7]	6.11	5.92	6.29	6.07
1.0 pH SA[7]	5.91	5.83	5.96	5.97
SEM	0.05328		0.08871	
***P* Value**	0.4503		0.0609	

[a-e]Superscripts within agar type, within bacteria type differ at an alpha level of 0.05

[1]Surrogate bacteria includes a 5-strain mixture of non-pathogenic Escherichia coli.

[2]TSA = Tryptic Soy Agar, non-selective agar

[3]TSA + Rif = Tryptic Soy Agar supplemented with Rifampicin, selective for Surrogate inoculum

[4]XLD = Xylose lysine deoxycholate agar, selective for *Salmonella* spp.

[5]Cefex = Campy Cefex Agar, selective for *Campylobacter* spp.

[6]CON = Untreated Control Treatment

[7]SA = Sulfuric Acid Sodium Sulfate

Table 3. Main effect of sulfuric acid sodium sulfate treatment on Log CFU/g of *Campylobacter* spp., *Salmonella* spp., and Surrogate bacteria inoculated on pork boston butt shoulder samples across 0 and 48 hours sampling times (n= 18/trt/agar).

	Campylobacter	
	Cefex[4]	**TSA**[2]
CON[5]	5.43[a]	5.33[a]
Water	5.06[b]	4.93[b]
1.5 pH SA[6]	4.67[c]	4.66[c]
1.0 pH SA[6]	4.34[d]	4.28[d]
SEM	0.07159	0.07471
***P* Value**	<0.0001	<0.0001

	Salmonella
	TSA[2]
CON[5]	6.58[a]
Water	6.23[b]
1.5 pH SA[6]	6.00[c]
1.0 pH SA[6]	6.00[c]
SEM	0.08686
***P* Value**	<0.0001

	Surrogate[1]	
	TSA + Rif[3]	**TSA**[2]
CON[5]	6.50[a]	6.56[a]
Water	6.00[b]	6.21[b]
1.5 pH SA[6]	6.01[b]	6.18[b]
1.0 pH SA[6]	5.87[c]	5.97[c]
SEM	0.04391	0.07815
***P* Value**	<0.0001	<0.0001

[a-d]Superscripts within agar type, within bacteria type differ at an alpha level of 0.05

[1]Surrogate bacteria includes a 5-strain mixture of non-pathogenic Escherichia coli.

[2]TSA = Tryptic Soy Agar, non-selective agar

[3]TSA + Rif = Tryptic Soy Agar supplemented with Rifampicin, selective for Surrogate inoculum

[4]Cefex = Campy Cefex Agar, selective for *Campylobacter* spp.

[5]CON = Untreated Control Treatment

[6]SA = Sulfuric Acid Sodium Sulfate

Table 4. Main effect of sampling hour across sulfuric acid sodium sulfate treatment on Log CFU/g of *Campylobacter* spp., *Salmonella* spp., and Surrogate bacteria inoculated on pork boston but shoulder samples (n = 27/sampling hour/agar).

		0 Hour	24 Hour	SEM	*P* Value
***Campylobacter* spp.**					
	Cefex[4]	5.04[a]	4.71[b]	0.05062	<0.0001
	TSA[2]	4.92[a]	4.68[b]	0.06289	<0.0001
***Salmonella* spp.**					
	TSA[2]	6.14[a]	6.27[b]	0.07699	0.0271
Surrogate[1]					
	TSA + Rif[3]	6.17[a]	6.02[b]	0.03838	<0.0001
	TSA[2]	6.27	6.19	0.07230	0.0595

[a,b] Superscripts within agar type, within bacteria type differ at an alpha level of 0.05

[1]Surrogate bacteria includes a 5-strain mixture of non-pathogenic *Escherichia* coli.

[2]TSA = Tryptic Soy Agar, non-selective agar

[3]TSA + Rif = Tryptic Soy Agar supplemented with Rifampicin, selective for Surrogate inoculum

[4]Cefex = Campy Cefex Agar, selective for *Campylobacter* spp.

Table 5. Effect of sulfuric acid sodium sulfate treatment by display hour on log CFU/g on the natural microflora of *Psychrotrophic*, *Pseudomonas*, yeasts and mold, and lactic acid bacteria on pork loin subprimals placed into simulated retail display following 14 and 21 days in dark anaerobic storage (n = 6/treatment/sampling hour).

	14 Days of Dark Anaerobic Storage						21 Days of Dark Anaerobic Storage					
		Treatment						Treatment				
Spoilage Bacteria	**Display Hour**	**CON[1]**	**1.5 pH SA[2]**	**1.0 pH SA[2]**	**SEM**	***P* Value**	**Spoilage Bacteria**	**Display Hour**	**CON[1]**	**1.5 pH SA[2]**	**1.0 pH SA[2]**	**SEM**	***P* Value**
Psychrotrophic	**0 Hour**	7.20	6.98	6.55	0.2804	0.0914	***Psychrotrophic***						
	48 Hour	8.00	7.84	7.50				**48 Hour**	7.67	7.23	7.37	0.2794	0.5653
	96 Hour	7.56	7.32	5.77				**96 Hour**	8.56	7.67	7.69		
Lactic Acid							**Lactic Acid**						
Bacteria	**0 Hour**	6.07	5.99	5.43	0.3292	0.6770	**Bacteria**	**0 Hour**	7.64	7.59	7.05	0.2511	0.5856
	48 Hour	6.60	6.29	5.78				**48 Hour**	7.91	7.53	7.21		
	96 Hour	7.42	6.55	5.91				**96 Hour**	8.51	7.67	7.78		
Pseudomonas							***Pseudomonas***						
	0 Hour	4.50	5.01	3.29	0.4456	0.1507		**0 Hour**	5.58	5.67	4.70	0.3372	0.4430
	48 Hour	4.89	4.34	4.43				**48 Hour**	5.67	5.79	5.60		
	96 Hour	6.24	4.97	4.55				**96 Hour**	6.85	6.19	5.76		
Yeast and Molds							**Yeast and Molds**						
	0 Hour	3.05	3.02	1.87	0.2607	0.4203		**0 Hour**	3.83	3.92	2.64	0.2913	0.4898
	48 Hour	3.46	2.81	2.36				**48 Hour**	4.09	4.20	3.75		
	96 Hour	4.82	4.89	4.28				**96 Hour**	5.48	5.63	5.12		

[1]CON = Untreated control treatment
[2]SA = Sulfuric acid sodium sulfate

Table 6. Effect of sulfuric acid sodium sulfate treatment by display hour on log CFU/g on the natural microflora of *Psychrotrophic*, *Pseudomonas*, yeasts and mold, and lactic acid bacteria on pork backrib subprimals placed into simulated retail display following 14 and 21 days in dark anaerobic storage (n = 6/treatment/sampling hour).

	14 Days of Dark Anaerobic Storage						21 Days of Dark Anaerobic Storage					
		Treatment						Treatment				
Spoilage Bacteria	Display Hour	CON[1]	1.5 pH SA[2]	1.0 pH SA[2]	SEM	*P* Value	Spoilage Bacteria	Display Hour	CON[1]	1.5 pH SA[2]	1.0 pH SA[2]	SEM	*P* Value
Psychrotrophic	**0 Hour**	5.96	6.02	4.81	0.3055	0.8208	***Psychrotrophic***	**0 Hour**					
	48 Hour	6.61	6.27	5.56				**48 Hour**	8.53	8.36	8.48	0.1748	0.1472
	96 Hour	8.75	8.84	8.12				**96 Hour**	8.76	8.85	8.25		
Lactic Acid							**Lactic Acid**						
Bacteria	**0 Hour**	7.51	7.19	6.51	0.1708	0.6427	**Bacteria**	**0 Hour**	8.28	8.25	8.02	0.1307	0.8704
	48 Hour	7.95	7.91	7.20				**48 Hour**	8.66	8.40	8.32		
	96 Hour	8.72	8.85	8.20				**96 Hour**	8.60	8.61	8.37		
Pseudomonas							***Pseudomonas***						
	0 Hour	6.59	5.94	5.39	0.3069	0.6519		**0 Hour**	6.94	6.22	6.41	0.2862	0.3697
	48 Hour	6.92	6.84	5.98				**48 Hour**	7.35	7.04	6.77		
	96 Hour	7.24	7.46	6.74				**96 Hour**	7.31	7.68	6.93		
Yeast and Molds							**Yeast and Molds**						
	0 Hour	3.89^{abc}	3.58^{bc}	1.82^{e}	0.2553	0.0173		**0 Hour**	4.29^{ab}	4.04^{b}	3.12^{c}	0.2694	0.0163
	48 Hour	4.05^{ab}	3.71^{abc}	3.41^{cd}				**48 Hour**	4.56^{ab}	4.45^{ab}	3.16^{c}		
	96 Hour	4.42^{a}	3.40^{cd}	2.83^{d}				**96 Hour**	4.93^{a}	4.27^{ab}	4.81^{a}		

[a-d] Superscripts within bacteria type, within storage period differ at an alpha level of 0.05

[1]CON = Untreated control treatment

[2]SA = Sulfuric acid sodium sulfate

Table 7. Main effect of sulfuric acid sodium sulfate treatment on the log CFU/g of natural microflora of *Psychrotrophic*, *Pseudomonas*, yeasts and mold, and lactic acid bacteria on pork loin subprimals placed into simulated retail display after 14 and 21 days of dark anaerobic storage and loin samples kept in dark anaerobic storage over 28 days (for samples placed in retail display following storage n = 18/storage time/treatment and for sample stored over 28 days, n = 24/treatment).

	Psychrotrophic		
	14 Days of Dark Storage	**21 Days of Dark Storage**	**28 Days of Vacuum Storage**
CON[1]	7.59[a]	8.12[a]	5.53[a]
1.5 pH SA[2]	7.38[a]	7.45[b]	5.26[ab]
1.0 pH SA[2]	6.61[b]	7.53[b]	4.98[b]
SEM	0.1639	0.1976	0.1323
***P* Value**	<0.0001	0.0445	0.0166

	Lactic Acid Bacteria		
	14 Days of Dark Storage	**21 Days of Dark Storage**	**28 Days of Vacuum Storage**
CON[1]	6.70[a]	8.02[a]	5.85[a]
1.5 pH SA[2]	6.28[b]	7.60[b]	5.65[a]
1.0 pH SA[2]	5.71[b]	7.35[b]	5.24[b]
SEM	0.1921	0.1450	0.1280
***P* Value**	0.0026	0.0076	0.0042

	Pseudomonas		
	14 Days of Dark Storage	**21 Days of Dark Storage**	**28 Days of Vacuum Storage**
CON[1]	5.21[a]	6.03[a]	4.61[a]
1.5 pH SA[2]	4.77[ab]	5.88[ab]	4.47[a]
1.0 pH SA[2]	4.09[b]	5.35[b]	3.76[b]
SEM	0.2574	0.1947	0.1437
***P* Value**	0.0123	0.0428	0.0001

	Yeast and Molds		
	14 Days of Dark Storage	**21 Days of Dark Storage**	**28 Days of Vacuum Storage**
CON[1]	3.77[a]	4.47[a]	3.11[a]
1.5 pH SA[2]	3.57[a]	4.58[a]	2.97[a]
1.0 pH SA[2]	2.84[b]	3.84[b]	2.46[b]
SEM	0.1505	0.1682	0.1283
***P* Value**	0.0001	0.0073	0.0016

[a,b] Superscripts differ between treatments within bacteria type, within storage time at an alpha level of 0.05.
[1]CON = Untreated control treatment
[2]SA = Sulfuric acid sodium sulfate

Table 8. Main effect of sulfuric acid sodium sulfate treatment on the log CFU/g of natural microflora of *Psychrotrophic*, *Pseudomonas*, yeasts and mold, and lactic acid bacteria on pork backrib subprimals placed into simulated retail display after 14 and 21 days of dark anaerobic storage and backrib samples kept in dark anerobic storage over 28 days (for samples placed in retail display following storage n = 18/storage time/treatment and for sample stored over 28 days, n = 24/treatment).

	Psychrotrophic		
	14 Days of Dark Storage	**21 Days of Dark Storage**	**28 Days of Vacuum Storage**
CON[1]	7.11[a]	8.64	5.77[a]
1.5 pH SA[2]	7.04[a]	8.61	5.76[a]
1.0 pH SA[2]	6.16[b]	8.37	5.05[b]
SEM	0.1764	0.1236	0.1289
***P* Value**	0.0005	0.2661	0.0001

	Lactic Acid Bacteria		
	14 Days of Dark Storage	**21 Days of Dark Storage**	**28 Days of Vacuum Storage**
CON[1]	8.06[a]	8.51[a]	6.93[a]
1.5 pH SA[2]	7.98[a]	8.42[ab]	6.80[a]
1.0 pH SA[2]	7.30[b]	8.24[b]	6.31[b]
SEM	0.0997	0.0763	0.0740
***P* Value**	<0.0001	0.0454	<0.0001

	Pseudomonas		
	14 Days of Dark Storage	**21 Days of Dark Storage**	**28 Days of Vacuum Storage**
CON[1]	6.92[a]	7.20	5.59[a]
1.5 pH SA[2]	6.75[a]	6.98	5.36[a]
1.0 pH SA[2]	6.03[b]	6.70	4.88[b]
SEM	0.1772	0.1652	0.1084
***P* Value**	0.0021	0.1133	<0.0001

	Yeast and Molds		
	14 Days of Dark Storage	**21 Days of Dark Storage**	**28 Days of Vacuum Storage**
CON[1]	4.12[a]	4.59[a]	3.49[a]
1.5 pH SA[2]	3.56[b]	4.25[a]	3.23[a]
1.0 pH SA[2]	2.69[c]	3.70[b]	2.48[b]
SEM	0.1474	0.1556	0.1067
***P* Value**	<0.0001	0.0007	<0.0001

[a-c] Superscripts differ between treatments within bacteria type, within storage time at an alpha level of 0.05.

[1]CON = Untreated control treatment

[2]SA = Sulfuric acid sodium sulfate

Table 9. Main effect of display hour on log CFU/g of natural microflora of *Psychrotrophic*, *Pseudomonas*, yeasts and mold, and lactic acid bacteria on pork loin and backrib subprimal samples placed into simulated retail display following 14 and 21 days of dark anaerobic storage across treatments (n = 18/bacteria type/subprimal type/storage type/display hour).

	Psychrotrophic			
	Loins		**Backribs**	
	14 Days of Dark Storage	**21 Days of Dark Storage**	**14 Days of Dark Storage**	**21 Days of Dark Storage**
Display Hour				
0 Hour	6.91[b]		5.60[c]	
48 Hour	7.78[a]	7.43[b]	6.14[b]	8.46
96 Hour	6.88[b]	7.97[a]	8.57[a]	8.62
SEM	0.1643	0.1614	0.1764	0.1010
***P* Value**	0.0002	0.0233	<0.0001	0.2618

	Lactic Acid Bacteria			
	Loins		**Backribs**	
	14 Days of Dark Storage	**21 Days of Dark Storage**	**14 Days of Dark Storage**	**21 Days of Dark Storage**
Display Hour				
0 Hour	5.83[b]	7.43[b]	7.07[c]	8.18[b]
48 Hour	6.22[ab]	7.55[b]	7.68[b]	8.46[a]
96 Hour	6.63[a]	7.98[a]	8.59[a]	8.52[a]
SEM	.1921	0.1450	0.0997	0.0763
***P* Value**	0.0212	0.0243	<0.0001	0.0078

	Pseudomonas			
	Loins		**Backribs**	
	14 Days of Dark Storage	**21 Days of Dark Storage**	**14 Days of Dark Storage**	**21 Days of Dark Storage**
Display Hour				
0 Hour	4.27[b]	5.32[b]	5.97[c]	6.52[b]
48 Hour	4.56[ab]	5.69[b]	6.58[b]	7.05[a]
96 Hour	5.25[a]	6.26[a]	7.15[a]	7.30[a]
SEM	0.2573	0.1947	0.1772	0.1652
***P* Value**	0.0251	0.0049	0.0001	0.0056

	Yeast and Molds			
	Loins		**Backribs**	
	14 Days of Dark Storage	**21 Days of Dark Storage**	**14 Days of Dark Storage**	**21 Days of Dark Storage**
Display Hour				
0 Hour	2.65[b]	3.46[c]	3.09[b]	3.82[b]
48 Hour	2.87[b]	4.01[b]	3.73[a]	4.05[b]
96 Hour	4.67[a]	5.41[a]	3.55[a]	4.67[a]
SEM	0.1505	0.1682	0.1474	0.1556
***P* Value**	<0.0001	<0.0001	0.0117	0.0010

[a-c] Superscripts differ between display hour within bacteria type, within suprimal, within storage time at an alpha level of 0.05.

Table 10. Effect of sulfuric acid sodium sulfate treatment by storage day on log CFU/g on natural microflora of *Psychrotrophic*, *Pseudomonas*, yeasts and mold, and lactic acid bacteria on pork loin and backrib subprimals kept in dark anaerobic storage for 28 days (n = 6/treatment/storage day/subprimal).

	Loins Across 28 Days in Anaerobic Storage						Backribs Across 28 Days in Anaerobic Storage					
		Treatment						Treatment				
Spoilage Bacteria	Storage Day	CON[1]	1.5 pH SA[2]	1.0 pH SA[2]	SEM	*P* Value	Spoilage Bacteria	Storage Day	CON[1]	1.5 pH SA[2]	1.0 pH SA[2]	SEM	*P* Value
Psychrotrophic	**Day 0**	3.11	2.91	2.60	0.2646	0.9970	***Psychrotrophic***	**Day 0**	3.10	2.85	2.55	0.2573	0.3792
	Day 7	3.95	3.49	3.37				**Day 7**	5.51	5.26	4.61		
	Day 14	7.20	6.98	6.55				**Day 14**	6.00	6.02	4.81		
	Day 21							**Day 21**					
	Day 28	7.87	7.68	7.39				**Day 28**	8.46	8.93	8.23		
Lactic Acid Bacteria							**Lactic Acid Bacteria**						
	Day 0	3.84	3.47	3.14	0.2862	0.9989		**Day 0**	5.25	4.97	4.41	0.1653	0.1924
	Day 7	3.82	3.47	3.14				**Day 7**	5.26	4.96	4.42		
	Day 14	6.07	5.99	5.43				**Day 14**	7.51	7.19	6.51		
	Day 21	7.64	7.59	7.05				**Day 21**	8.28	8.25	8.02		
	Day 28	7.84	7.72	7.42				**Day 28**	8.36	8.65	8.19		
Pseudomonas							***Pseudomonas***						
	Day 0	3.06	2.86	2.26	0.3212	0.2346		**Day 0**	2.72	2.86	2.45	0.2422	0.1263
	Day 7	3.09	2.64	2.82				**Day 7**	4.46	4.11	3.25		
	Day 14	4.50	5.01	3.29				**Day 14**	6.53	5.94	5.39		
	Day 21	5.58	5.67	4.70				**Day 21**	6.94	6.22	6.41		
	Day 28	6.85	6.19	5.76				**Day 28**	7.31	7.68	6.93		
Yeast and Molds							**Yeast and Molds**						
	Day 0	2.02	2.14	1.68	0.2869	0.1397		**Day 0**	2.23^{ef}	2.08^{efg}	1.56^{g}	0.2387	0.0284
	Day 7	2.16	1.73	1.95				**Day 7**	2.60^{de}	2.25^{ef}	1.84^{gf}		
	Day 14	3.05	3.02	1.87				**Day 14**	3.85^{abc}	3.58^{cd}	1.82^{gf}		
	Day 21	3.83	3.92	2.64				**Day 21**	4.29^{a}	4.04^{ab}	3.12^{cd}		
	Day 28	4.49	4.07	4.18				**Day 28**	4.48^{a}	4.23^{ab}	4.08^{ab}		

[a-g] Superscripts differ between display hour within bacteria type, within suprimal, within storage day at an alpha level of 0.05.

[1]CON = Untreated control treatment

[2]SA = Sulfuric acid sodium sulfate

Table 11. Effect of sulfuric acid sodium sulfate treatment by display hour on sour, acid/chemical, putrid, and randic/oxidized off-odor intensities on pork loin subprimals placed into simulated retail display following 14 and 21 days of dark anaerobic storage (n = 2/treatment/display hour/storage time).

	14 Days of Dark Anaerobic Storage						21 Days of Dark Anaerobic Storage					
		Treatment						Treatment				
Off-Odor	Display Hour	CON[1]	1.5 pH SA[2]	1.0pH SA[2]	SEM	*P* Value	Off-Odor	Display Hour	CON[1]	1.5 pH SA[2]	1.0pH SA[2]	SEM	*P* Value
Sour	**0 Hour**	9.52	8.90	7.84	3.33	0.1866	**Sour**	**0 Hour**	13.84^{c}	29.46^{b}	23.65^{bc}	6.53	0.0004
	48 Hour	13.52	4.68	9.81	2.84			**48 Hour**	29.58^{b}	20.35^{bc}	12.96^{c}	6.26	
	96 Hour	5.49	5.82	10.32	3.18			**96 Hour**	60.32^{a}	52.68^{a}	20.90^{bc}	6.90	
Acid/Chemical	**0 Hour**	10.79	8.66	5.10	3.48	0.6244	**Acid/Chemical**	**0 Hour**	9.15	8.21	16.21	4.19	0.3170
	48 Hour	9.73	9.36	9.57	2.89			**48 Hour**	7.77	4.05	4.32	4.02	
	96 Hour	4.88	9.44	8.12	3.29			**96 Hour**	11.86	4.21	17.36	4.43	
Putrid	**0 Hour**	13.71^{a}	1.21^{b}	3.84^{b}	2.05	0.0007	**Putrid**	**0 Hour**	8.30	13.74	12.05	5.69	0.5315
	48 Hour	1.70^{b}	2.61^{b}	1.49^{b}	1.74			**48 Hour**	9.35	11.68	16.74	5.50	
	96 Hour	4.39^{b}	1.61^{b}	4.05^{b}	1.96			**96 Hour**	7.86	17.29	25.51	5.96	
Oxidized/Rancid	**0 Hour**	3.15^{b}	4.59^{b}	4.28^{b}	3.66	0.0353	**Oxidized/Rancid**	**0 Hour**	16.87^{bc}	23.05^{abc}	13.99^{c}	5.86	0.0204
	48 Hour	2.29^{b}	2.17^{b}	3.04^{b}	3.14			**48 Hour**	23.89^{abc}	10.94^{c}	11.05^{c}	5.58	
	96 Hour	9.06^{b}	20.00^{a}	25.17^{a}	3.50			**96 Hour**	14.61^{c}	32.46^{a}	31.96^{ab}	6.23	

$^{a-c}$ Superscripts within storage time and off odor type, across display hour and treatment differ at an alpha level of 0.05.

[1]CON = Untreated control treatment

[2]SA = Sulfuric acid sodium sulfate

Table 12. Effect of sulfuric acid sodium sulfate treatment by display hour on sour, acid/chemical, putrid, and rancid/oxidized off-odor intensities on pork backrib subprimals placed into simulated retail display following 14 and 21 days of dark anaerobic storage (n = 2/treatment/display hour/storage time).

	14 Days of Dark Anaerobic Storage						21 Days of Dark Anaerobic Storage					
		Treatment						Treatment				
Off-Odor	Display Hour	CON[1]	1.5 pH SA[2]	1.0pH SA[2]	SEM	*P* Value	Off-Odor	Display Hour	CON[1]	1.5 pH SA[2]	1.0pH SA[2]	SEM	*P* Value
Sour	**0 Hour**	4.71	0.96	6.84	4.26	0.1026	**Sour**	**0 Hour**	27.10^{bc}	20.66^{c}	15.97^{c}	6.84	0.0368
	48 Hour	16.07	13.78	6.28	3.56			**48 Hour**	24.67^{bc}	24.95^{bc}	38.50^{ab}	6.55	
	96 Hour	12.64	18.14	17.69	4.04			**96 Hour**	48.18^{a}	26.39^{bc}	28.47^{bc}	7.24	
Acid/Chemical	**0 Hour**	13.66	8.66	12.91	5.95	0.5113	**Acid/Chemical**	**0 Hour**	9.48	6.54	13.29	3.84	0.8278
	48 Hour	15.80	14.97	10.01	5.55			**48 Hour**	3.11	4.28	6.11	3.66	
	96 Hour	13.78	15.11	14.78	5.82			**96 Hour**	6.42	0.56	8.42	4.06	
Putrid	**0 Hour**	1.36	1.77	0.14	4.40	0.0573	**Putrid**	**0 Hour**	42.32^{b}	5.45^{e}	11.51^{e}	7.16	<0.0001
	48 Hour	10.69	16.11	4.57	3.69			**48 Hour**	29.98^{bcd}	18.98^{cde}	14.14^{de}	6.78	
	96 Hour	22.55	11.82	6.10	4.18			**96 Hour**	48.26^{b}	86.12^{a}	34.83^{bc}	7.63	
Oxidized/Rancid	**0 Hour**	1.56	1.56	4.62	4.80	0.5336	**Oxidized/Rancid**	**0 Hour**	30.21	18.96	13.90	7.30	0.4191
	48 Hour	14.90	15.36	8.07	4.14			**48 Hour**	32.71	16.09	14.87	6.99	
	96 Hour	21.15	18.04	15.43	4.60			**96 Hour**	36.43	40.07	37.71	7.71	

[a-e] Superscripts within storage time and off odor type, across display hour and treatment differ at an alpha level of 0.05.

[1]CON = Untreated control treatment

[2]SA = Sulfuric acid sodium sulfate

Table 13. Main effect of sulfuric acid sodium sulfate treatment on off-odor intensities on loins and backribs placed into simulated retail display following both 14 and 21 days of dark anaerobic storage (n = 6/treatment).

Loins After 14 Days of Dark Storage

	Sour	Acid/ Chemical
CON[1]	9.51	8.47
1.5 pH SA[2]	6.47	9.15
1.0 pH SA[2]	9.32	7.59
SEM	2.2352	2.1024
***P* Value**	0.2610	0.8037

Loins After 21 Days of Dark Storage

	Acid/ Chemical	Putrid
CON[1]	9.59[ab]	8.50[b]
1.5 pH SA[2]	5.49[b]	14.24[ab]
1.0 pH SA[2]	12.63[a]	18.20[a]
SEM	3.0543	4.50
***P* Value**	0.0467	0.0230

Ribs After 14 Days of Dark Storage

	Sour	Acid/ Chemical	Putrid	Oxidized/Rancid
CON[1]	11.14	14.41	10.63[a]	12.54
1.5 pH SA[2]	10.96	12.91	9.90[a]	11.65
1.0 pH SA[2]	10.27	12.56	3.60[b]	9.37
SEM	2.6692	5.1364	2.7687	3.34
***P* Value**	0.9482	0.7296	0.0316	0.5278

Ribs After 21 Days of Dark Storage

	Acid/ Chemical	Oxidized/Rancid
CON[1]	6.34	33.11
1.5 pH SA[2]	3.79	25.04
1.0 pH SA[2]	9.27	22.16
SEM	2.7273	5.3387
***P* Value**	0.1284	0.0760

[a,b] Superscripts within off-odor type, storage time and subprimal type differ at an alpha level of 0.05

[1]CON = Untreated control treatment

[2]SA = Sulfuric acid sodium sulfate

Table 14. Effect of sulfuric acid sodium sulfate treatment and display hour on the percent surface discoloration of pork loin and backrib subprimal samples placed into simulated retail display following either 14 or 21 days of dark anaerobic storage (n = 6/treatment/display hour).

Loins

14 Days of Dark Anaerobic Storage

	0 Hour	12 Hours	24 Hours	36 Hours	48 Hours	60 Hours	72 Hours	84 Hours	96 Hours	SEM	*P* Value
Treatment											
CON[1]	2.61^{fg}	0.24^{g}	2.56^{fg}	0.03^{g}	3.35^{fg}	4.90^{fg}	1.99^{fg}	6.38^{efg}	6.92^{efg}	4.3204	<0.0001
1.5 pH SA[2]	4.86^{fg}	6.58^{efg}	15.01^{de}	26.22^{bc}	28.64^{bc}	33.73^{ab}	31.53^{abc}	34.77^{ab}	35.36^{ab}		
1.0 pH SA[2]	4.19^{fg}	11.51^{ef}	22.18^{cd}	33.38^{ab}	34.12^{ab}	42.84^{a}	40.24^{a}	40.33^{a}	40.89^{a}		

21 Days of Dark Anaerobic Storage

	0 Hour	12 Hours	24 Hours	36 Hours	48 Hours	60 Hours	72 Hours	84 Hours	96 Hours	SEM	*P* Value
Treatment											
CON[1]	5.20	1.36	3.98	1.88	6.36	10.24	10.69	12.13	14.16	5.3175	0.7484
1.5 pH SA[2]	25.65	30.44	35.90	36.24	38.14	43.44	47.69	44.10	54.58		
1.0 pH SA[2]	35.31	40.49	37.54	41.91	48.08	42.94	49.38	43.53	48.58		

Backribs

14 Days of Dark Anaerobic Storage

	0 Hour	12 Hours	24 Hours	36 Hours	48 Hours	60 Hours	72 Hours	84 Hours	96 Hours	SEM	*P* Value
Treatment											
CON[1]	0.55	1.12	1.30	0.36	0.72	1.49	2.87	6.90	10.88	1.5566	0.8635
1.5 pH SA[2]	2.67	1.78	2.15	1.51	1.71	4.22	4.57	8.13	7.95		
1.0 pH SA[2]	2.42	1.54	1.68	1.82	3.35	5.51	6.31	7.07	11.30		

21 Days of Dark Anaerobic Storage

	0 Hour	12 Hours	24 Hours	36 Hours	48 Hours	60 Hours	72 Hours	84 Hours	96 Hours	SEM	*P* Value
Treatment											
CON[1]	6.67	4.18	8.42	9.35	18.74	23.42	26.96	25.21	31.27	3.7229	0.3338
1.5 pH SA[2]	5.25	6.82	11.67	8.65	17.22	15.62	33.02	18.07	28.44		
1.0 pH SA[2]	2.89	9.41	15.97	12.74	18.72	19.27	22.49	22.64	23.27		

[a-g] Superscripts within storage time, within subprimal type differ at an alpha level of 0.05.

[1]CON = Untreated control treatment

[2]SA = Sulfuric acid sodium sulfate

Table 15. Main effect of sulfuric acid sodium sulfate treatment on the percent surface discoloration of pork loin and backribs placed into simulated retail display following either 14 or 21 days of dark anaerobic storage (n = 18/treatment/storage time).

	Loins After 21 Days of Dark Storage	Backribs After 14 Days of Dark Storage	Backribs After 21 Days of Dark Storage
CON[1]	7.33[b]	2.91[b]	17.14
1.5 pH SA[2]	39.57[a]	3.85[ab]	16.08
1.0 pH SA[2]	43.08[a]	4.56[a]	16.38
SEM	3.3963	0.9054	1.8802
***P* Value**	<0.0001	0.0209	0.7906

[a,b] Superscripts within storage time and subprimal differ at an alpha level of 0.05.

[1]CON = Untreated control treatment

[2]SA = Sulfuric acid sodium sulfate

Table 16 Main effect of display hour on percent surface discoloration on pork loin and backrib subprimal samples placed into simulated retail display following either 14 or 21 days of dark anaerobic storage (n=18/storage period).

	0 Hour	12 Hour	24 Hour	36 Hour	48 Hour	60 Hour	72 Hour	84 Hour	96 Hour	SEM	*P* Value
Loins After 21 Days of Dark Storage	22.06^{f}	24.10^{ef}	25.81^{cdef}	26.67^{def}	30.86^{bcde}	32.21^{abcd}	33.25^{abc}	35.92^{ab}	39.10^{a}	4.0206	<0.0001
Ribs After 14 Days of Dark Storage	1.88^{de}	1.48^{e}	1.71^{de}	1.23^{e}	1.93^{de}	3.74^{cd}	4.58^{c}	7.37^{b}	10.04^{a}	1.1332	<0.0001
Ribs After 21 Days of Dark Storage	4.94^{d}	6.80^{cd}	12.02^{cd}	10.25^{c}	18.22^{b}	19.43^{b}	27.49^{ab}	21.97^{a}	27.66^{a}	2.2391	<0.0001

[a-f] Superscripts within row differ at an alpha level of 0.05.

Table17 Effect of sulfuric acid sodium sulfate treatment by display hour on *CIE* L*, a* and b* values of pork loin subprimal lean samples placed into simulated retail display following 14 and 21 days of dark anaerobic storage (n = 6/treatment/display hour).

	14 days of Dark Anaerobic Storage										
	L* Values										
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	39.18	40.30	47.16	44.27	41.39	44.97	41.67	41.33	42.47	1.7522	0.9917
1.5 pH SA[2]	45.69	45.46	54.90	46.08	45.56	47.71	46.93	45.68	47.91		
1.0 pH SA[2]	46.40	45.09	53.13	48.03	44.85	48.49	46.70	44.62	47.06		
	a* Values										
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	10.37	4.99	5.16	5.77	5.06	4.57	4.49	5.87	4.63	1.2347	0.7824
1.5 pH SA[2]	4.20	4.78	4.22	4.24	3.70	3.68	4.04	4.19	3.36		
1.0 pH SA[2]	4.72	4.77	4.30	5.34	4.45	4.67	4.19	5.40	4.10		
	b* Values										
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	4.10	4.92	5.17	5.45	5.07	4.75	4.46	5.89	4.65	0.6584	0.9716
1.5 pH SA[2]	5.00	5.60	5.99	5.07	5.24	5.32	5.91	6.29	5.03		
1.0 pH SA[2]	5.94	5.78	6.20	6.70	6.18	7.63	6.29	7.59	6.22		
	21 Days of Dark Anaerobic Storage										
	L* Values										
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	41.89	42.24	41.60	41.99	42.38	42.92	43.79	45.06	42.61	0.9392	0.8843
1.5 pH SA[2]	44.90	46.55	46.29	45.93	45.89	46.12	47.12	47.48	45.93		
1.0 pH SA[2]	48.73	46.52	47.12	47.70	46.77	47.73	47.58	47.87	47.20		
	a* Values										
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	5.02	4.09	4.31	3.88	3.97	3.73	4.03	3.40	3.59	0.4917	0.9999
1.5 pH SA[2]	5.12	4.45	4.51	4.05	4.24	3.82	3.68	3.20	2.91		
1.0 pH SA[2]	5.54	4.61	4.93	4.05	4.76	4.17	3.94	3.89	3.53		
	b* Values										
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	4.82	4.36	4.36	3.57	4.45	4.19	4.48	4.27	4.15	0.7674	0.9999
1.5 pH SA[2]	6.29	6.47	6.95	6.46	6.57	6.30	6.10	6.10	5.32		
1.0 pH SA[2]	7.42	6.85	7.25	6.43	7.27	7.00	6.44	6.80	6.94		

[1]CON = Untreated control treatment

[2]SA = Sulfuric acid sodium sulfate

Table 18 Effect of sulfuric acid sodium sulfate treatment by display hour on *CIE* L*, a* and b* values of pork loin subprimal fat samples placed into simulated retail display following 14 and 21 days of dark anaerobic storage (n = 6/treatment/display hour).

	14 days of Dark Anaerobic Storage										
	L* Values										
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	71.88	71.24	84.55	77.93	72.98	76.87	73.34	74.61	75.20	1.8576	0.9916
1.5 pH SA[2]	68.25	71.27	81.55	73.28	70.25	74.22	70.02	71.17	74.33		
1.0 pH SA[2]	68.87	69.56	78.10	73.30	68.03	70.90	69.86	70.30	70.35		
	a* Values										
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	5.57	6.22	5.81	6.41	5.39	5.88	5.05	6.17	5.09	1.8951	0.5524
1.5 pH SA[2]	4.96	4.67	5.08	5.71	5.27	5.08	3.77	4.59	3.75		
1.0 pH SA[2]	6.87	17.06	8.24	8.73	7.65	8.07	6.48	7.40	7.15		
	b* Values										
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	8.08	8.90	9.25	9.14	8.25	8.77	7.98	9.41	8.23	1.0981	0.9999
1.5 pH SA[2]	8.30	8.10	9.44	9.69	8.93	9.13	7.80	8.92	8.48		
1.0 pH SA[2]	12.26	13.66	14.29	14.17	12.70	13.28	12.29	12.66	12.76		
	21 Days of Dark Anaerobic Storage										
	L* Values										
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	71.43	70.06	72.22	71.74	71.77	72.07	73.08	73.23	71.94	1.4327	0.9793
1.5 pH SA[2]	69.77	70.42	69.09	70.99	72.88	69.82	71.61	71.90	71.72		
1.0 pH SA[2]	68.54	69.84	70.07	68.57	68.61	69.22	69.14	70.15	69.99		
	a* Values										
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	3.77	3.57	2.80	3.74	2.41	3.20	2.38	3.18	3.03	0.7096	0.9561
1.5 pH SA[2]	4.62	4.86	3.70	3.85	3.88	3.49	4.35	3.33	2.98		
1.0 pH SA[2]	7.18	6.15	6.90	6.36	6.43	5.95	5.15	5.56	5.54		
	b* Values										
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	5.75	5.60	4.82	5.76	4.64	5.55	4.64	5.97	5.87	0.9496	0.9906
1.5 pH SA[2]	8.10	8.72	7.47	7.39	8.47	7.61	7.79	7.63	7.14		
1.0 pH SA[2]	12.36	11.22	11.88	11.59	12.07	11.68	10.26	11.00	11.19		

[1]CON = Untreated control treatment

[2]SA = Sulfuric acid sodium sulfate

Table 19 Effect of sulfuric acid sodium sulfate treatment by display hour on *CIE* L*, a* and b* values of pork backrib subprimal lean samples placed into simulated retail display following 14 and 21 days of dark anaerobic storage (n = 6/treatment/display hour).

	14 days of Dark Anaerobic Storage										
						L* Values					
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	35.46	32.53	44.35	36.96	34.75	37.28	39.34	37.46	36.18	2.4293	0.9485
1.5 pH SA[2]	35.64	31.03	40.09	34.41	31.87	35.82	35.74	30.29	34.77		
1.0 pH SA[2]	36.75	39.10	42.81	38.39	37.03	41.84	39.79	34.23	39.55		
						a* Values					
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	11.40	11.97	13.33	13.16	11.56	11.70	10.42	13.09	10.20	2.3882	0.9303
1.5 pH SA[2]	10.26	11.25	10.75	11.43	10.35	9.46	9.54	11.58	9.00		
1.0 pH SA[2]	9.41	10.11	19.92	11.00	9.76	11.16	11.58	12.46	10.47		
						b* Values					
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	8.09	8.41	11.11	9.72	8.16	8.81	8.21	10.98	7.66	2.2659	0.8875
1.5 pH SA[2]	7.32	8.07	8.16	8.19	6.86	7.24	7.67	8.98	6.83		
1.0 pH SA[2]	7.48	8.48	19.17	9.05	9.76	10.23	10.29	11.41	9.41		
	21 Days of Dark Anaerobic Storage										
						L* Values					
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	38.18	37.38	39.71	37.32	40.57	36.87	42.73	37.34	38.32	2.3860	0.9997
1.5 pH SA[2]	40.04	39.32	40.97	40.00	39.33	37.52	42.22	41.19	41.98		
1.0 pH SA[2]	39.28	39.17	39.42	40.31	40.66	38.53	41.85	41.49	41.13		
						a* Values					
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	10.98	10.11	9.69	9.56	8.66	8.14	7.80	7.96	7.02	1.3061	0.9999
1.5 pH SA[2]	10.53	9.60	9.58	9.11	8.91	8.86	8.32	8.22	7.43		
1.0 pH SA[2]	9.82	8.73	9.04	7.93	9.17	8.26	7.06	7.35	6.44		
						b* Values					
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	8.46	16.48	7.92	7.84	7.33	6.59	7.39	6.97	6.62	1.7321	0.4372
1.5 pH SA[2]	8.33	8.03	8.05	8.03	7.25	6.60	7.29	6.45	6.19		
1.0 pH SA[2]	8.19	7.60	7.81	7.50	8.60	7.93	7.09	7.61	7.23		

[1]CON = Untreated control treatment
[2]SA = Sulfuric acid sodium sulfate

Table 20 Effect of sulfuric acid sodium sulfate treatment by display hour on *CIE* L*, a* and b* values of pork backrib subprimal fat samples placed into simulated retail display following 14 and 21 days of dark anaerobic storage (n = 6/treatment/display hour).

	14 days of Dark Anaerobic Storage										
						L* Values					
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	71.02	69.11	85.50	74.17	70.09	74.00	70.64	71.45	71.06	2.6373	0.4131
1.5 pH SA[2]	60.13	69.17	77.93	74.08	68.97	72.06	70.94	71.09	70.26		
1.0 pH SA[2]	68.90	71.69	74.06	73.35	69.74	72.45	68.74	70.54	71.57		
						a* Values					
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	11.84	11.06	10.31	11.82	10.09	10.53	9.98	9.91	9.38	1.0419	0.9345
1.5 pH SA[2]	8.40	8.08	8.97	8.86	8.47	8.21	8.18	8.66	9.02		
1.0 pH SA[2]	9.42	10.46	10.53	11.01	10.62	11.32	11.31	11.65	11.09		
						b* Values					
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	13.00	12.35	12.92	13.25	11.83	12.51	11.77	12.23	11.97	0.9535	0.9966
1.5 pH SA[2]	11.22	10.92	11.86	11.46	10.87	11.01	10.69	11.76	11.81		
1.0 pH SA[2]	13.86	14.33	14.62	14.77	14.50	16.07	14.50	15.37	14.72		
	21 Days of Dark Anaerobic Storage										
						L* Values					
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	67.64	68.85	69.17	70.43	69.38	59.31	70.60	70.78	68.66	3.2702	0.9835
1.5 pH SA[2]	68.46	69.10	67.49	70.12	68.85	61.27	67.76	72.34	70.05		
1.0 pH SA[2]	68.30	67.64	69.10	68.17	67.05	68.76	68.83	69.94	68.99		
						a* Values					
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	11.79	9.28	9.28	9.10	8.66	7.97	7.86	7.27	5.88	2.2315	0.5695
1.5 pH SA[2]	8.38	9.39	7.73	19.02	7.22	7.71	6.92	7.31	7.46		
1.0 pH SA[2]	8.70	10.06	7.97	9.34	8.01	8.20	7.73	8.12	8.15		
						b* Values					
	0 Hour	**12 Hour**	**24 Hour**	**36 Hour**	**48 Hour**	**60 Hour**	**72 Hour**	**84 Hour**	**96 Hour**	**SEM**	***P* Value**
CON[1]	13.25	11.18	11.43	11.33	11.33	10.84	11.16	10.52	9.31	0.9366	0.9468
1.5 pH SA[2]	10.49	11.40	9.84	10.82	9.69	10.42	9.67	9.82	10.30		
1.0 pH SA[2]	13.15	14.37	12.59	13.66	12.37	12.88	12.18	13.06	12.92		

[1]CON = Untreated control treatment
[2]SA = Sulfuric acid sodium sulfate

Table 21 Main effect of sulfuric acid sodium sulfate treatment on *CIE* L*, a* and b* values of pork loin and backrib subprimal samples placed into simulated retail display following 14 days of dark anaerobic storage (n = 18/treatment/tissue/subprimal).

	Loin Lean Color			Backrib Lean Color		
	L*	**a***	**b***	**L***	**a***	**b***
CON[1]	42.52[a]	5.66[a]	4.94[b]	37.14[a]	11.87	9.02[ab]
1.5 pH SA[2]	47.32[b]	4.04[b]	5.50[b]	34.41[b]	10.40	7.70[b]
1.0 pH SA[2]	47.15[b]	4.66a[b]	6.50[a]	38.83[a]	11.76	10.59[a]
SEM	0.5841	0.4116	0.2195	0.8098	0.7961	0.7553
***P* Value**	<0.0001	0.0225	<0.0001	0.0007	0.3509	0.0283
	Loin Fat Color			**Backrib Fat Color**		
CON[1]	75.40[a]	5.73[b]	8.67[b]	73.00	10.55[a]	12.42[b]
1.5 pH SA[2]	72.70[b]	4.76[b]	8.75[b]	70.51	8.54[b]	11.29[c]
1.0 pH SA[2]	71.03[b]	8.63[a]	13.12[a]	71.23	10.82[a]	14.75[a]
SEM	0.6192	0.6317	0.3660	0.8791	0.3473	0.3178
***P* Value**	<0.0001	<0.0001	<0.0001	0.1228	<0.0001	<0.0001

[a,b] Superscripts within tissue, within subprimal, differ at an alpha level of 0.05.
[1]CON = Untreated control treatment
[2]SA = Sulfuric acid sodium sulfate

Table 22 Main effect of sulfuric acid sodium sulfate treatment on *CIE* L*, a* and b* values of pork loin and backrib subprimal samples placed into simulated retail display following 21 days of dark anaerobic storage (n = 18/treatment/tissue/subprimal).

	Loin Lean Color			Backrib Lean Color		
	L*	**a***	**b***	**L***	**a***	**b***
CON[1]	42.72[a]	4.00	4.29[b]	38.71	8.88	8.39
1.5 pH SA[2]	46.25[b]	4.00	6.29[a]	40.29	8.95	7.36
1.0 pH SA[2]	47.47[c]	4.38	6.93[a]	40.20	8.20	7.73
SEM	0.3131	0.1639	0.2558	0.7953	0.4354	0.5774
***P* Value**	<0.0001	0.1744	<0.0001	0.2930	0.4040	0.4429
	Loin Fat Color			**Backrib Fat Color**		
CON[1]	71.95[a]	3.12[c]	5.40[c]	68.31	8.56	11.15[b]
1.5 pH SA[2]	70.91[a]	3.90[b]	7.81[b]	68.38	9.02	10.27[b]
1.0 pH SA[2]	69.35[b]	6.13[a]	11.47[a]	68.53	8.47	13.02[a]
SEM	0.4776	0.2365	0.3165	1.1255	0.7680	0.3224
***P* Value**	0.0009	<0.0001	<0.0001	0.9903	0.8668	<0.0001

[a-c] Superscripts within tissue, within subprimal, differ at an alpha level of 0.05.

[1]CON = Untreated control treatment

[2]SA = Sulfuric acid sodium sulfate

Table 23 Main effect of sulfuric acid sodium sulfate across display hour on pH of pork loin and backrib subprimal samples either placed into simulated retail display following 14 or 21 days of dark anaerobic storage or over 28 days of dark anaerobic storage (for subprimals placed into display following 14 and 21 days of dark storage, n = 18/treatment, and for subprimals stored for 28 days, n = 30/treatment).

	Loins		
	14 Days of Dark Storage	**21 Days of Dark Storage**	**28 Days of Anaerobic Storage**
CON[1]	5.78[a]	5.77[a]	5.63[a]
1.5 pH SA[2]	5.77[a]	5.62[b]	5.67[a]
1.0 pH SA[2]	5.57[b]	5.57[b]	5.46[b]
SEM	0.05228	0.04612	0.0433
***P* Value**	0.0071	0.0072	0.0015
	Backribs		
	14 Days of Dark Storage	**21 Days of Dark Storage**	**28 Days of Anaerobic Storage**
CON[1]	6.36[a]	6.28[a]	6.20
1.5 pH SA[2]	6.27[ab]	6.05[b]	6.10
1.0 pH SA[2]	6.12[b]	6.05[b]	6.07
SEM	0.62160	0.05894	0.04625
***P* Value**	0.0317	0.0090	0.1121

[a,b] Superscripts within storage time, within subprimal, differ at an alpha level of 0.05.

[1]CON = Untreated control treatment

[2]SA = Sulfuric acid sodium sulfate

Table 24. Effect of sulfuric acid sodium sulfate at a concentration of 1.0 pH and 1.3 pH on LS means of log CFU/cm^2 on the natural microflora and inoculated populations on non-pathogenic *Escherichia coli* on pork carcasses (n = 30/treatment).

Enterobacteriaceae

	Inoculated						Uninoculated					
	Before[2]	After[3]	After Rapid Chill[4]	After 15 Hours[5]	SEM	*P* Value	Before[2]	After[3]	After Rapid Chill[4]	After 15 Hours[5]	SEM	*P* Value
1.3 pH SA[1]	6.74[a]	5.85[b]	4.31[c]	4.43[c]	0.3284	<0.0001	1.20[a]	0.59[b]	-0.04[c]	0.06[c]	0.1312	<0.0001
1.0 pH SA[1]	6.08[a]	4.77[b]	3.31[c]	3.28[c]	0.1106	<0.0001	1.21[a]	0.05[b]	-0.33[b]	-0.12[b]	0.1132	<0.0001

Aerobic Plate Count

	Inoculated						Uninoculated					
	Before[2]	After[3]	After Rapid Chill[4]	After 15 Hours[5]	SEM	*P* Value	Before[2]	After[3]	After Rapid Chill[4]	After 15 Hours[5]	SEM	*P* Value
1.3 pH SA[1]	7.02[a]	6.17[b]	4.59[c]	4.62[c]	0.2128	<0.0001	3.24[a]	2.38[b]	0.90[c]	2.19[b]	0.4440	<0.0001
1.0 pH SA[1]	6.19[a]	4.98[b]	3.56[c]	3.47[c]	0.1470	<0.0001	3.34[a]	1.52[b]	0.52[c]	1.50[b]	0.2257	<0.0001

[a-c] Superscripts within inoculated or uninoculated, within *Enterobacteriaceae* or Aerobic plate count, differ within row at an alpha level of 0.05.

[1] SA = Sulfuric acid sodium sulfate

[2] Before = Carcass zones sampled before sulfuric acid sodium sulfate treatment

[3] After = Carcass zones sampled after sulfuric acid sodium sulfate treatment

[4] After Rapid chill = Carcass zones sampled after sulfuric acid sodium sulfate treatment and after rapid chill

[5] After 15 Hours = Carcass zones sampled 15 hours after sulfuric acid sodium sulfate treatment and rapid chill

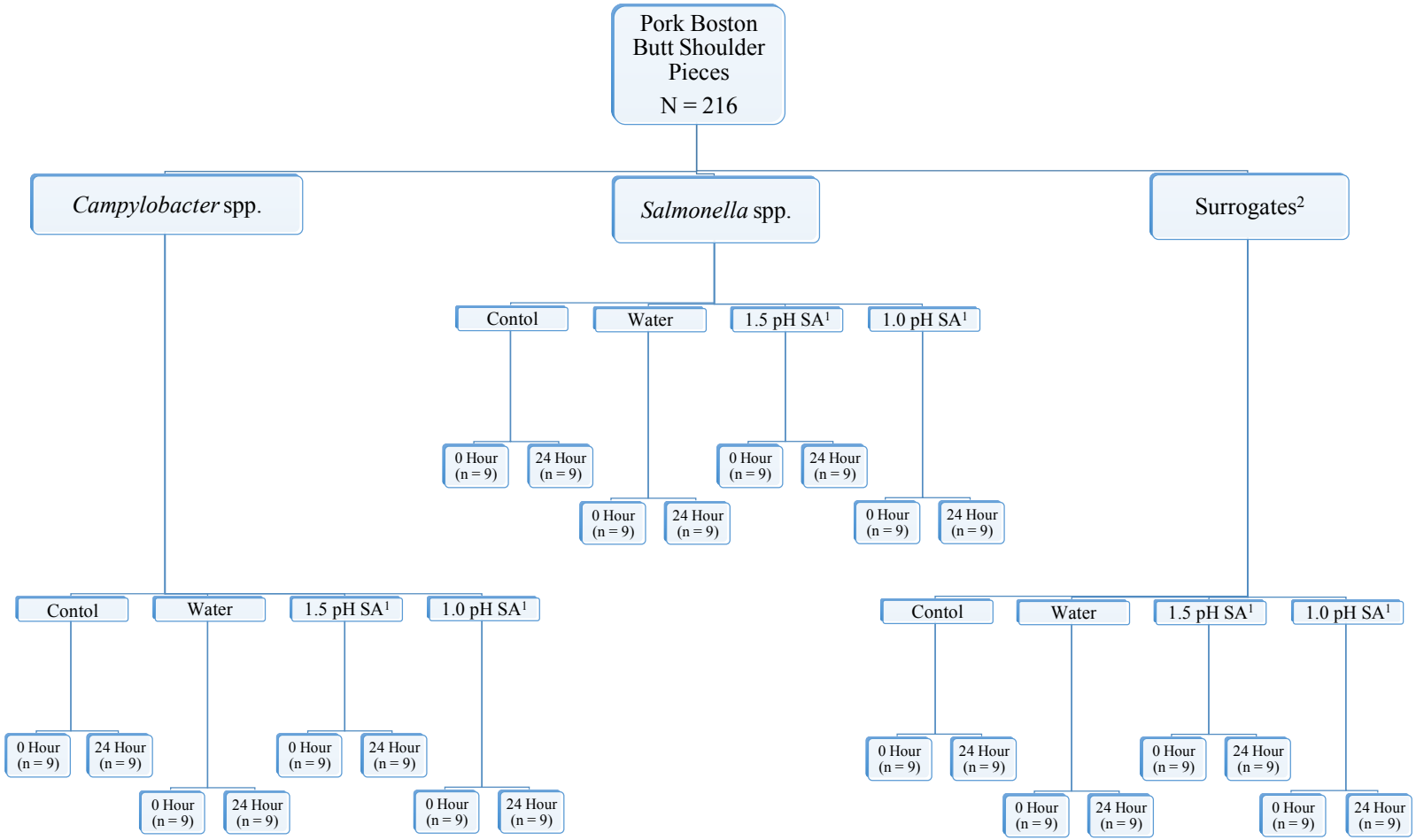

Figure 1. Inoculation and surrogate validation portion (Experiment 1) sample design and breakdown.
[1]Sulfuric Acid Sodium Sulfate
[2]Surrogate bacteria includes a 5-strain mixture of non-pathogenic Escherichia coli.

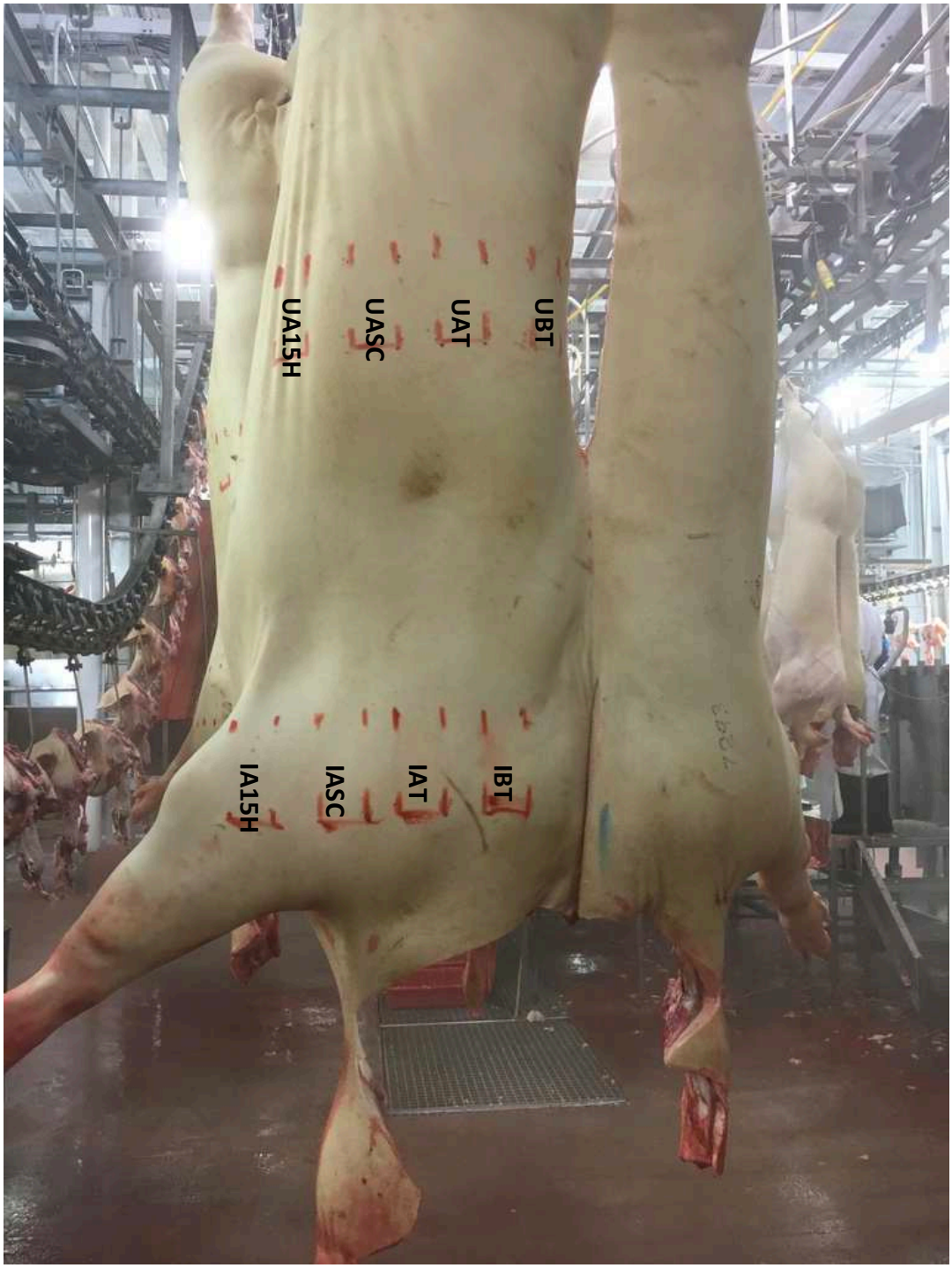

Figure 2. In-plant validation of sulfuric acid sodium sulfate on pork carcasses (Experiment 3) sample zoneing map.
*UBT = Uninoculated Before Treatment, IBT = Inoculated Before Treatment, UAT = Uninoculated After Treatment, IAT = Inocualted After Treatment, UASC = Uninoculated After Rapid chill Cycle, IASC = Inoculated After Rapid chill Cycle, UA15H = Uninoculated After 15 Hour, and IA15H = Inoculated After 15 Hour

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