THE EFFECT OF ANTIMICROBIAL INTERVENTIONS ON QUALITY AND SAFETY CHARACTERISTICS OF BLADE TENDERIZED BEEF, AND VEAL AND GOAT CARCASSES

by

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(Under the Direction of Alexander M. Stelzleni)

ABSTARCT

Meat safety is a major concern for the meat industry. Ruminant animals are natural reservoirs for pathogens such as Shiga toxin producing *Escherichia coli* (STEC), as such, non-intact beef and meat from small ruminants can be a major source of foodborne illness. The industry continuously seeks interventions that will improve the microbial safety of meat. The aim of the industry is to find effective antimicrobials to produce meat that is free from microbial hazards but do not cause adverse changes to the quality or organoleptic properties. The main objective of the current research was to evaluate the effects of antimicrobial interventions on the quality and safety characteristics of blade tenderized beef, and veal and goat carcasses. Results indicated that levulinic acid plus sodium dodecyl, an emerging intervention, is comparable to industry standard antimicrobial interventions such as lactic acid and peroxyacetic acid.

INDEX WORDS: Antimicrobial, Beef, Goat, Shiga toxin producing *Escherichia coli*, Blade tenderized, Veal

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DEDICATION

I would like to dedicate this work to my parents Wilton and Valrie Thomas, my siblings, Tavia and Lionel Thomas, and my extended family.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

In the United States (US) and other developed countries, meat and meat products comprise a significant portion of the human diet (Daniel et al., 2011), and are the centerpiece of most meals. In developing countries, the demand for meat continues to grow. According to data published by the US Department of Agriculture (USDA), in 2018 Americans were predicted to consume 100.8 kg/person of red meat and poultry, which will surpass the previous record of 100.6 kg/person, set in 2004 (USDA-ERS, 2017). Historically, as household income rises, so does the consumption of meat. As meat consumption increases, so will production, as livestock owners expand their herds with the objective of meeting consumer demands. This high demand of animal products further provokes intensive animal production and processing of products, which could lead to defective processing practices and an increased risk of contamination by foodborne pathogens at any point of the farm to fork chain (Heredia & Garcia, 2018).

Meat safety remains a major concern for the meat industry. Despite extensive scientific progress and technological developments over the last several decades, the safety of meat and meat products continues to be a global concern (Mead et al., 1999). Ruminant animals are natural reservoirs for pathogens such as Shiga toxin producing *Escherichia coli* (STEC), therefore, meat from ruminants can be a source of STEC and is known to causes foodborne illnesses. According to Sofos (2005) the contamination of animal carcasses and raw meat by microorganisms (spoilage and pathogenic bacteria) during processing, is

practically unavoidable. Specific sources of contamination on carcasses and fresh meat during slaughter and dressing include, hide, water, air, intestines, lymph nodes, processing equipment, utensils, and humans, with fecal contamination of carcasses as the primary avenue for contamination (Sofos, 2005). Pathogens such as STEC, *Salmonella*, and *Campylobacter* may reside in fecal material, both in gastrointestinal tract and on the exterior surfaces of the animal that can contaminate the carcass during slaughter. The presence of these microorganisms on meat products can have adverse effects on human health (Sofos, 2005). In addition, the presence of spoilage organisms on meat may lead to product and economic losses. Thus, there is a need to control microbial contamination in animal and animal products to enhance the quality and safety of fresh meat. With aims of meeting regulatory requirements, as well as to produce safe products for customers, the meat industry has employed and continues to seek new interventions that will reduce and ideally, eliminate contamination on meat.

The safety, shelf-life, and ultimately, profitability for the producers of meat and meat products are heavily reliant on the quality of innovation used in food processing and storage technologies (Ravensdale, Coorey, & Dykes, 2018). In spite of increasingly sophisticated hygienic measures, carcasses will inevitably be contaminated. The USDA has implemented and enforces the Hazard Analysis Critical Control Point system (HACCP). According to the International HACCP alliance, HACCP is a process control system that identifies where hazards might occur in the food production process and puts into place stringent actions to prevent the hazards from occurring. The HACCP program brought a more science-based approach to inspection using HACCP principles to monitor and verify safe processing conditions (Ricke et al., 2005) ensuring that meat processing

facilities were meeting food safety performance standard. Through the use of post-harvest interventions and other innovative treatments the meat industry has continually improved the microbial quality and safety of meat.

Numerous studies have validated the efficacy of various organic acids, oxidizers, and hot water among other intervention methods. Currently, lactic acid is the most commonly used organic acid in commercial practice (Koohmaraie et al., 2005). Beef processors commonly utilize a 'multiple-hurdle' intervention system, employing sequential interventions at various processing steps to ensure the safety of their products (Bacon et al., 2000). As antimicrobials are tested, their effects on meat quality must also be considered. Color is one of the most important fresh meat characteristics at the point of purchase (Font-i-Furnols & Guerrero, 2014), because consumers equate the color of meat as an indicator of spoilage and wholesomeness (Mancini, 2009). In addition, quality characteristics of the meat including tenderness, juiciness, and flavor, are highly correlated with the overall satisfaction of the eating experience, which are all important attributes that affect consumer purchasing decisions and willingness to pay (Ravensdale et al., 2018). Therefore, the objectives of this research was:

1. To evaluate the intervention strategies of pulse ultra-violet light, electrolyzing oxidizing water, and levulinic acid plus sodium dodecyl sulfate when applied to beef strip loin subprimals before blade tenderization to assess their effects on meat shelf life and quality characteristics when compared to lactic acid.

2. To quantify the efficacy of levulinic acid plus sodium dodecyl sulfate, acidified sodium chlorite, lactic acid, and peroxyacetic acid on surrogate *E.*

coli populations when inoculated on beef striploins intended for blade tenderization.

3. To validate the efficacy of lactic acid, peroxyacetic acid, and hot water, for their individual or combined ability to reduce STEC surrogates on bob veal carcasses pre- and post-chill, and through fabrication.

4. To evaluate lactic acid, peroxyacetic acid, a hydrochloric and citric acid blend, levulinic acid plus sodium dodecyl sulfate, for their efficacy in reducing STEC surrogates and their effect on carcass color from slaughter through 24 h chill.

CHAPTER 2

LITERATURE REVIEW

The meat and poultry industries are the two largest segments of U.S. agriculture. U.S. meat production totaled 52 billion pounds in 2017 (NAMI, 2017). With American meat companies producing 26.3 billion pounds of beef, 25.6 billion pounds of pork, 80.2 million pounds of veal and 150.2 million pounds of lamb and mutton. Increase of human population and urbanization, coupled with per capita income increase, has resulted in increased consumption of animal products (Dhama et al., 2013; Heredia & Garcia, 2018). This high demand of animal products provokes intensive animal production and processing of meat products (Heredia & Garcia, 2018).

Meat can be defined as the flesh, skeletal muscle and any attached connective tissue or fat excluding bone and bone marrow (Williams, 2007). Red meat contains a high biological value, is a good source of protein, essential fatty acids, minerals, and vitamins. However, meat is easily perishable because it provides a suitable medium for the growth of various microorganisms (Bantawa et al., 2017). Skeletal muscle from meat animals is normally considered sterile prior to slaughter, with the exception of lymph nodes (Huffman, 2002). However, meat can easily be contaminated during the slaughter and dressing process. Sources of contamination on carcasses and fresh meat during slaughter and dressing includes, hide, water, air, intestines, lymph nodes, processing equipment, utensils, and humans. These contaminations can readily cause a variety of biological, chemical, physical, and microbial food hazards (Ko et al., 2013). Depending on the extent

of microbial contamination and composition of the microbial flora, there can be adverse effects on the overall hygiene of the meat (Bantawa et al., 2017). The most important foodborne bacterial pathogens associated with meat are *Campylobacter* species*,* non-Typhi serotypes of *Salmonella enterica,* Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* (Bhandare et al., 2007). Additionally, *Pseudomonas* species are commonly associated with spoilage of meat, causing off-odors, off-flavors, discoloration, and gas production (Arnaut-Rollier, De Zutter, & Van Hoof et al., 1999).

Meat safety

For the last several decades, meat safety has been a major societal concern, and indications exist that challenges to meat safety will continue in the future (Sofos, 2008). Highly publicized outbreaks of foodborne disease in the U.S., caused by pathogenic bacteria such as *Escherichia coli* (*E. coli*) O157:H7 and *Listeria monocytogenes*, have brought meat safety and associated issues to the forefront of societal concerns. The majority of safety concerns and product recalls associated with fresh meat products are from *E. coli* O157:H7 and related enteric pathogens such as *Salmonella*, while the Gram-positive *L. monocytogenes* is the pathogen of concern in ready-to-eat meat and poultry products.

A major outbreak of *E. coli* O157:H7 in 1993 associated with undercooked ground beef patties from a popular chain restaurant, which caused numerous illness and even deaths (Bell et al., 1994), led to major developments within the food industry. The most notable development was the Food Safety and Inspection Service (FSIS) implementation of the ‘zero tolerance’ policy for visible contamination and declaration of *E. coli* O157:H7 as an adulterant in fresh ground beef and other non-intact fresh beef (USDA-FSIS, 1993).

The enforcement of this rule resulted in several highly publicized recalls over the last several decades.

Recalls of meat products in the USA are regulated by the Federal Meat Inspection Act. According to FSIS, a food recall is a voluntary action by a manufacturer or distributor to protect the public from products that may cause health problems or possible death (USDA-FSIS, 2015). A recall is intended to remove food products from commerce when there is reason to believe that the products may be adulterated or misbranded. Recalls are classified based on the relative health risk, as follows: Class I; this recall involves a health hazard situation in which there is a reasonable probability that eating the food will cause health problems or death. Class II; involves a potential health hazard situation in which there is a remote probability of adverse health consequences from eating the food. Class III; involves a situation in which eating the food will not cause adverse health consequences (USDA-FSIS, 2015). Recalls are normally voluntary removal of a product from trade and consumer channels by manufactures with the purpose of protecting human health and well-being. However, recalls of contaminated meat products contribute directly to industry cost and have dramatically increased during the last two decades (Marsh, Schroeder, & Mintert, 2004). Product recalls directly impact the industrial sector and can adversely impact consumer demand. In 2018 alone, there were 20,552,911 lbs. of meat and poultry products recalled (USDA FSIS, 2019a) due to the contamination of various pathogenic bacteria. Consumers associate recall events with inadequate process controls and lack of hygienic control, which in turn induces consumers to substitute out meat products being recalled for other meat or non-meat products.

Pathogenic bacteria

Escherichia coli

Escherichia coli is the pathogen of most concern in regard to red meat safety. *Escherichia coli* is a Gram-negative, rod-shaped, facultative anaerobic bacterium within the family *Enterobacteriaceae*. Most *E. coli* strains are harmless and colonize the gastrointestinal tract of humans and animals as a normal flora. However, there are some pathogenic strains of *E. coli*. Pathogenic *E. coli* can be categorized based on serogroups, pathogenicity mechanisms, clinical symptoms, or virulence factors (Lim, Yoon, & Hovde, 2012). Strains of *E. coli* can be categorized serologically based on the detection of O (somatic), H (flagella), and K (capsule) antigens. However, for most *E. coli* strains the O and H antigens are sufficient to identify the strain. These antigens allow researchers to separate each strain into a distinct category. These categories include enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC) enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), and diffusely adhering *E. coli* (DAEC) (Doyle, Beuchat, & Montville, 2001).

Enterohemorrhagic *E. coli* contains the classification of bacteria known as STEC. This subgroup is comprised of two major types, O157 and non-O157 STEC. These pathogenic bacteria are responsible for multiple food outbreaks and deaths every year. Shiga toxin producing *E. coli* are estimated to cause 265,000 illnesses, 3,600 hospitalizations, and 30 deaths annually in the United States (CDC, 2012). It has been estimated that *E. coli* O157:H7 causes two thirds of the human EHEC infections in the US, with the other one-third of cases attributed to the non-O157 STEC population (Mead et al., 1999).

A common method for *E. coli* nomenclature is based on the antigens present. For example, *E. coli* O157:H7 expresses the 157th somatic (O) antigen identified and the 7th flagellar (H) antigen (Mead & Griffin, 1998). *Escherichia coli* O157:H7 was first identified as a human pathogen in 1982, after being implicated in two outbreaks caused from under cooked hamburgers in the states of Oregon and Michigan (Doyle, 1991; Doyle, Beuchat, & Montville, 2001; Laine et al., 2005). In 1993, there was another multistate outbreak of *E. coli* O157:H7 associated with under cooked hamburgers served at a popular chain restaurant. This outbreak led to a large number of hospitalizations (178) as well as development of Hemolytic uremic syndrome (HUS) by 56 people and the death of 4 children (Montville, Matthews, & Kniel, 2012). Because of this outbreak, in 1994, the USDA FSIS declared *E. coli* O157:H7 an adulterant. *Escherichia coli* O157:H7 causes an estimated 73,000 infections and 61 deaths annually in the United States (Laine et al., 2005). Cattle are the primary reservoir for *E. coli* O157:H7, although other ruminants such as sheep, goats, and deer can also be sources (Mead et al., 1999; Laine et al., 2005). *Escherichia coli* is found in the feces of these animals and can easily be transferred to meat and meat products.

Although *E. coli* O157:H7 is currently the predominant strain and accounts for ~ 75% of STEC infection worldwide, other non-O157 STEC serotypes can produce Shiga toxins and foodborne illnesses (US-FDA, 2012). The USDA FSIS has identified six serogroups (O26, O45, O103, O111, O121, and O145), often referred to as the "Big 6" within the non-O157 STEC that cause about 70% of non-O157 STEC foodborne illnesses (USDA FSIS, 2012).

Shiga toxin-producing *E. coli* are characterized by the production of Stx. There are two main Stx types, designated Stx1 and Stx2. Currently, there are 3 known Stx1 (Stx1a, Stx1c and Stx1d) and 7 known Stx2 (Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g) subtypes (Feng et al., 2014). However, some Stx subtypes are produced mostly by environmental or animal strains and have not affected humans, so, not all STEC strains appear to be human pathogens. Therefore, the production of Stx alone is deemed to be insufficient to cause severe human illness. However, both *E. coli* O157:H7 and non-O157 STEC carry other virulence factors as a primary way of causing disease. Most notable of these is the intimin protein that enables EHEC to attach to epithelial cells. Intimin is encoded by the *eae* gene that resides on a pathogenicity island called locus of enterocyte effacement (LEE). The LEE island contains two important genes necessary for attachment and effacing. These are the *eae* gene, which encodes intimin, and the *tir* gene, which encodes the Tir protein (Feng et al., 2014).

In humans, these microorganisms have been linked to diseases such as hemorrhagic colitis, and HUS. Hemorrhagic colitis is a type of gastroenteritis in which *E. coli* infect the large intestine and produce a Stx that causes bloody diarrhea and other serious complications. Hemolytic uremic syndrome (HUS), is associated abnormal lysis of red blood cells resulting in the destruction of blood platelets, a low red blood cell count (anemia) and kidney failure due to damage to the very small blood vessels of the kidneys and can be fatal.

New and established antimicrobial interventions used in the meat industry

The slaughter industry uses good manufacturing practices (GMPs) when processing meat carcasses and as a result, the major portion of a carcass surface remains intrinsically

clean during this process. However, unavoidable and inadvertent contamination of carcasses occurs despite GMPs, thus necessitating the use of effective antimicrobial intervention strategies (Koohmaraie et al., 2005). The meat industry currently utilizes numerous decontamination technologies, to reduce the microbial contamination of carcasses. These can be divided into three major types: (i) physical (e.g. hot water, steam, steam vacuuming), (ii) chemical (e.g. organic acids, chlorine, and acidified sodium chlorite) and (iii) biological (bacteriophages, bacteriocins). In this review, both physical and chemical decomposition methods will be further discussed.

Thermal intervention

Hot water

Washing with hot water as an intervention step on meat has been extensively researched and widely used around the world. Automated hot water wash cabinets for pre-evisceration and final carcass interventions are commonly used in processing plants in the U.S. (Wheeler, Kalchayanand, & Bosilevac, 2014). Hot water has been shown to be effective in removing physical or visible contaminants on carcasses and meat parts. Studies conducted on various meat types, and under different conditions, have reported between 1–3 log reductions of pathogenic and spoilage bacteria (Sofos & Smith, 1998). The effectiveness of hot water as a decontamination technique depends on both operational factors and factors related to the product itself. These include water temperature, pressure, flow rate, and target surface distance, method of application, the time or stage of application in the slaughtering sequence, and plant variation (Sofos & Smith, 1998). In addition, intrinsic and implicit factors such as; animal lots, type of meat tissue, surface temperature of products, initial microbial load, the type of the microbial ecology of the

product, and the time of exposure to contamination (which affects bacterial attachment), and biofilm formation are all factor influencing the effectiveness of hot water as a decontamination method (Hugas and Tsigarida, 2008).

The mode of action of heat treatment such as hot water is mainly by inactivating the most sensitive vital enzymes (denaturation) for bacterial life as well as causing DNA strand breakage and RNA degradation (Ray, 2001; Wheeler et. al., 2014). Decontamination of red meat carcasses using hot water washes (70 to 96°C) has been shown to be an effective bacterial intervention method (Patterson, 1970; Barkate et. al., 1993; Dorsa et al., 1996). In these studies, hot water (>70°C) was determined to be superior to water at ambient temperatures for reducing general bacterial populations, including *E. coli* and *Salmonella* from beef or lamb carcasses. The effectiveness of hot water is strongly affected by the temperature of the water and the type of meat tissue (Hugas and Tsigarida, 2008). However, the effectiveness in the reduction in microbial numbers have been shown to increase by increasing the temperature of the water (Sofos & Smith, 1998; Hugas and Tsigarida, 2008). Castillo et. al., (1998) reported that hot water sprays provided mean reductions of initial counts for *E. coli* 0157:H7 and *S. typhimurium* of 3.7 and 3.8 log, APC reductions of 2.9 log, and coliform and thermotolerant coliform count reductions of 3.3 log on beef carcasses. According to Barkate et. al., (1993), spraying hot water at 95°C for 5 s at 24 psi reduced total coliforms, thermotolerant coliforms, *S. Typhimurium*, and *E. coli* O157:H7 up to 3 logs. Kalchayanand et al., 2012 reported that hot water (85°C) for 15 s at 15 psi reduced *E. coli* O157:H7 and the top six non-O157 STEC between 3.2 and 4.2 log CFU/cm^2 on inoculated surfaces of fresh beef.

Chemical intervention

Chemical decontamination treatment involves the application of a chemical substance at a given step during the slaughter process and is a common practice used within the US meat industry. Organic acids are the most common substances currently used for chemical decontamination of carcasses and meat parts and have been extensively studied. The efficacy of chemical decontamination methods depends on their ability to destroy the cellular membranes and other cellular constituents and pathways of the bacteria. Their action normally results in a decrease in numbers of the various types of bacterial flora. Decreased bacterial flora improves the microbiological quality and safety of products by a reduction in the number of pathogenic bacteria and, an improved shelf-through the destruction of spoilage bacteria (Hugas & Tsigarida, 2008).

Organic acids

Organic acid rinses are well documented as effective and economically viable carcass interventions (Castillo et. al. 2001). Numerous studies have evaluated the efficacy of organic acids for decontaminating red meat (Siragusa, 1995; Dorsa, 1997; Koohmaraie et al., 2005). The effectiveness of an organic acid in reducing populations of pathogenic or spoilage bacteria on red meat varies with the concentration of acid used, the temperature of the acid and carcass, the contact time, the spray application pressure, the point at which the sanitizer is used in the slaughtering and processing process, tissue type and composition, and the sensitivity of the target organism to the specific acid (De Martinez, Ferrer, & Salas, 2002).

According to Wheeler et al. (2014), the specific mode of action of organic acids as an antimicrobial is not known, but is likely a combination of actions of the undissociated

molecules and the dissociated ions, causing interference with the transmembrane proton gradients of the microbial cells, and interference with three-dimensional structures of cell surface, outer membranes, and cytoplasmic membrane. In the undissociated form, organic acids can penetrate the cell membrane lipid bilayer easier. Once inside the cell, the acid dissociates because the interior of the cell has a greater pH than the exterior. When dissociation occurs, the microorganism tries to maintain homeostasis to prevent conformational changes to the cell structural proteins, enzymes, nucleic acids, and phospholipids. To maintain homeostasis, the protons generated from the intercellular dissociation of the organic acids that acidify the cytoplasm must be extruded to the exterior. However, the cytoplasmic membrane is impermeable to protons, and they must be transported to the exterior. This proton extrusion creates an electrochemical potential across the membrane called the proton motive force. Since protons generated by the organic acids inside the cell must be extruded using energy (ATP), the constant influx of these protons will eventually deplete cellular energy (Doyle, Beuchat, & Montville, 2001), causing cell death. The cellular changes caused by organic acids can interfere with nutrient transport and energy generation and in turn growth. Additionally, the low pH of acids can cause both reversible and irreversible damage to cellular macromolecules, which can inflict sub-lethal and lethal injury to microbial cells (Wheeler et al., 2014). Organic acid treatments have been shown to be most effective when applied as a warm (50 - 55°C) carcass rinse (Acuff, 2005). However, the corrosive effects of organic acids on processing equipment increases as the temperature rises. Other limitations of organic acid treatments include product discoloration, organoleptic changes, and development of acid-resistant pathogens (Wheeler et al., 2014).

Lactic acid

Lactic acid is one of the most commonly used organic acids and antimicrobials in the US meat industry (Castillo et al., 2001; Koohmaraie et al., 2005). Lactic acid is approved for use at levels up to a 5% concentration (USDA-FSIS, 2019). The bactericidal properties of lactic acid have been well documented (Woolthuis & Smulders, 1985; De Martinez et. al., 2002; King et al., 2005). Lactic acid inhibits gram-negative bacteria, such as *E. coli,* through reducing the pH (Gill & Newton, 1981). Lowering the pH prevents metabolic activities such as proteolysis, and reduces bacterial growth (Doyle, Beuchat, & Montville, 2001). Different studies have shown that the temperature of the lactic acid solution can have a profound effect on the magnitude of reductions in bacterial counts on red meat (Castillo et al, 2001; Acuff, 2005). Lactic acid sprays have been shown to produce 1 to 3 logs of microbial reduction on the surface of hot beef carcasses (Wheeler, Kalchayanand, & Bosilevac, 2014) however, reports on decontamination of cold, fabricated beef, and veal carcasses indicated that lactic acid sprays up to 4% may not be as effective (Castillo et al. 2001; Sevart et al., 2016). Various studies (Acuff et al., 1987; Castillo et al., 2001; Thomas et al., 2019) have observed a continued antimicrobial effect during cold storage of meat after spraying lactic acid on hot carcass surfaces.

Citrilow

Citrilow, formerly known as Precure, is a proprietary blend containing citric and hydrochloric acids. Citrilow is a generally recognized as safe (GRAS) acid solution designed to rapidly produce and sustain a pH range that effectively eliminates pathogens. Citrilow is approved for use at a pH of 0.5 – 2.0 for meat carcasses, parts, trim, and organs (USDA-FSIS, 2019). Citrilow has been effective for reducing aerobic plate counts,

coliforms, and *E. coli* on inoculated fresh beef (Pohlman et al., 2010). Kalchayanand, (2014) reported that a 2% Citrilow spray was able to reduce *E. coli* O157:H7, non-O157 STEC including O26, O45, O103, O111, O121, and O145 serogroups, and *Salmonella* on surfaces of fresh beef by approximately 1.5 log. The efficacy of Citrilow was also evaluated for microbial control on chilled beef subprimals. Beers, Cook, & Coleman, (2011) showed a 1.8 log reduction of aerobic bacteria on sections of chilled beef brisket subprimals treated with a Citrilow solution at pH 1.5. Cook, Beers, & Coleman (2011), reported a 1.1 log reduction of *E. coli* 0157:H7 on chilled beef subprimals treated with Citrilow solution at pH 1.3. Sevart et al. (2016) reported only a 0.4 log CFU/cm^2 on pre-rigor veal carcasses that were inoculated with STEC surrogates and no additional reduction when applied to chilled carcasses.

Levulinic acid plus sodium dodecyl sulfate

The combination of levulinic acid (LVA) plus sodium dodecyl sulfate (SDS) reported by Zhao, Zhao, & Doyle (2009), is rapidly effective against bacterial foodborne pathogens on produce. The safety of levulinic acid for humans has been widely tested, and it has been designated by the U.S. Food and Drug Administration (FDA) as GRAS for direct addition to food as a flavoring substance or adjunct (21 CFR 172.515). The pKa of levulinic acid is between that of lactic and acetic acids (4.61, 3.88 and 4.76, respectively). Levulinic acid behaves similar to lactic acid in its effectiveness at reducing the environmental pH to a level where bacteria cannot survive (Carpenter, Smith, & Broadbent, 2011). According to Zhao et al. (2009), levulinic acid can be produced at low cost and in high yield from renewable feedstocks. Sodium dodecyl sulfate (SDS), has also been labeled as GRAS by the FDA as a food additive (21 CFR 172.822). The food additive SDS

enhances the antibacterial properties of organic acids by acting as a surfactant (Zhao et al., 2009; Elramady et al., 2013). The foamability and composition of these compounds is believed to extend its potential applications to decontamination of hard-to-reach surfaces and control of foodborne pathogens on food contact surfaces (Cannon et al., 2012). There are limited studies looking at the efficacy of LVA plus SDS on red meat. Stelzleni, Ponrajan, & Harrison (2013) applied 1.0% LVA plus 0.1% SDS to beef trim prior to grinding and reported a reduction of *Salmonella* populations by only 0.17 to 0.36 logs. However, Zhao et al. (2014) reported the use of 3% LVA plus 2% SDS when applied by spray application on inoculated beef trim for 1 to 5 minutes reduced *E. coli* O157:H7 by 1.5 log CFU/cm^2. Zhao et al., (2014) reported that LVA plus SDS for reducing STEC contamination on beef was effective but that many factors can interfere with their efficacy including the surface temperature of the target product.

Oxidizer antimicrobials

Oxidizer antimicrobials are proposed to have multiple targets within a cell as well as in almost every biomolecule; these include peroxidation and disruption of membrane layers, oxidation of oxygen scavengers and thiol groups, enzyme inhibition, oxidation of nucleosides, impaired energy production, disruption of protein synthesis and, ultimately, cell death (Finnegan, 2010; Wheeler et al., 2014). When a stronger oxidant is used, the electrons are transferred to the microorganism much faster, causing the microorganism to be rapidly deactivated. Oxidizing agents are usually low molecular weight compounds and can pass easily through cell walls/membranes, making them able to react with internal cellular components, leading to apoptotic and necrotic cell death. Oxidizers can severely

damage microbial structures causing the release of intracellular components, which are then oxidized (Finnegan, 2010).

Peroxyacetic acid

Peroxyacetic acid, also known as peracetic acid, is approved by FSIS for use on beef carcasses and parts (FDA, 2003) and is widely used in the beef processing. The regulatory limits for peroxyacetic acid, have gone up in recent years, and depending on the product approval, are allowed up to 2000 parts per million (ppm) for applications on meat. Various studies have reported the efficacy of peroxyacetic acid treatment to reduce *E. coli* O157:H7 load on meat carcasses (Kalchayanand et al., 2012, King et al., 2005, Penney et al., 2007, Ransom et al., 2003). A study conducted by King et al. (2005) found that peroxyacetic acid (180 ppm; 43°C) reduced *E. coli* O157:H7 and *Salmonella Typhimurium* by 0.7 logs on hot carcass surfaces. Under laboratory conditions, peroxyacetic acid treatment produced a 1.0 to 1.4 log reduction of *E. coli* O157:H7 inoculated onto beef carcass tissue (Ransom et al., 2003). Ellebracht et al. (2005) found that dipping beef trimmings into 200 ppm PAA solutions for 15 seconds reduced *E. coli* O157:H7 and *Salmonella* Typhimurium by 0.6 and 1.01 log CFU/cm^2, respectively. Several experiments conducted by King et al. (2005) showed that applying peroxyacetic acid at low concentrations, such as 200-600 ppm, on chilled beef surfaces had minimal effect on inoculated *E. coli* O157:H7 when applied at varying temperatures. However, the application of 1000 ppm of peroxyacetic acid (55°C) resulted in reductions of 1.70 log CFU/cm^2.

Peroxyacetic acid functions well as an antimicrobial agent because of its high oxidizing potential. It destroys microorganisms by oxidation and subsequent disruption of

their cell membranes, causing cell lysis and, ultimately, death (Vandekinderen et al., 2009). Peroxyacetic acid is primarily used as a carcass rinse in beef processing plants. It may also be employed during spray-chilling of carcasses, with the assumption that it breaks down to safe and nonpolluting products (acetic acid and hydrogen peroxide) so that no unacceptable residues remain on the meat surface (Stopforth et al., 2004).

Acidified sodium chlorite

Acidified sodium chlorite (ASC; CFR 173.325) is approved for use in the U.S. at concentrations between 500 and 1200 ppm (USDA-FSIS, 2019). According to Chen et al., (2012) the antimicrobial effect of ASC is due to the oxidative effect of chlorous acid, which originates from the conversion of chlorite ion into its acid form under acidic conditions. The efficacy of ASC depends on the type of acid used, the method of application, and the contact time with the meat surface; all these factors play an integral role in the success of its antimicrobial capability. As a gaseous antimicrobial, the effects tend to be transient, providing no extended bactericidal or bacteriostatic effect after treatment. The primary reason is that these compounds are readily reactive with unsaturated bonds, thus quickly removing them from solution and negating further action against bacterial cells (Ricke et al. 2005).

Several studies (Ransom et al., 2003; Castillo et al., 1999; Kalchayanand et al., 2012) have reported the efficacy of ASC. Ransom et al. (2003) reported a 1.9 to 2.3 log reduction in *Salmonella* and *E. coli* O157:H7 on beef carcass tissues using a wash/spray of sodium chlorite acidified with citric acid. Castillo et al. (1999), in a laboratory trial, demonstrated up to 4.6 log reductions in *E. coli* O157:H7 and *Salmonella* resulting from a water wash followed by an ASC spray. Kalchayanand et al. (2012) showed reductions

ranging from 0.6 to 2.0 log CFU/cm^2 after applying ASC to fresh beef surfaces that was inoculated with the top six non-O157 STEC. However, other studies indicate limited success (Gill & Badoni, 2004). Gill and Badoni (2004) reported that ASC reported less than 1 log reduction of aerobic bacteria, nonpathogenic *E. coli*, *E. coli* O157:H7, or *S. Typhimurium* on inoculated beef carcass surfaces under laboratory conditions.

Electrolyzed oxidizing water

Electrolyzed oxidized (EO) water, has gained attention as a disinfectant for use in the food industry (Wheeler et al., 2014). Electrolyzed water is produced by electrolysis of a dilute salt (NaCl) solution in an electrolysis chamber where anode and cathode electrodes are separated by a membrane (Park et al., 2002). An EO water generator dissociates the salt solution into acidic electrolyzed water and alkaline electrolyzed water. Negatively charged ions move to the anode to produce oxygen gas (O_2), chlorine gas (Cl_2), hypochlorite ions (OCl-), hypochlorous acid (HOCl), and hydrochloric acid. The positive ions move to the cathode to become hydrogen gas (H_2) and sodium hydroxide (NaOH). On the anode side, acidic EO water is generated and has strong bactericidal effect on most known pathogenic bacteria, due to its low pH, and has a strong oxidation reduction potential (ORP; ca. 1100 mV) and the presence of hypochlorous acid (Park et al., 2002). One product of the reaction is sodium hydroxide (NaOH), and the other is hypochlorous acid, which has a low pH, contains active chlorine, and has a strong oxidation reduction potential. Chlorine is one of the most investigated chemical interventions for meat decontamination in the beef and poultry industries. The chlorine compound has several advantages, namely; ease of application, economical, and effectiveness against most microbial forms such as Gram-positive and -negative bacteria. The antimicrobial activity

of chlorine is mainly due to its strong oxidative effect on bacterial cell wall, causing the inactivation of enzymes and DNA cleavage. In the United States, use of chlorine at the concentration of 50 ppm has been approved in poultry washes/sprays, and permitted for decontamination of red meat carcasses (USDA-FSIS, 2019).

Electrolyzed oxidized water has been shown to reduce populations of *Campylobacter jejuni* on poultry carcasses by 4.9 logs (Park, Hung, & Brackett, 2002). A study conducted by Bosilevac et al. (2005) reported that EO water reduced total aerobic count on cattle hides by 3.5 logs, and *Enterobacteriaceae* counts by 0.9 log while reducing *E. coli* O157:H7 prevalence from 82% to 35%. However, a study conducted by Jadeja, Hung, & Bosilevac (2013), reported that *E. coli* O157:H7 is more resistant than the non-O157 STEC to EO water treatment and that the reductions of these organisms generally correlate with the increased levels of free chlorine in the EO water. Other studies have been less successful (Kalchayanand et al., 2008). Kalchayanand et al. (2008) reported less than 0.5 log CFU/cm^2 reduction of *E. coli* O157:H7 inoculated on surfaces of beef heads and cheek meat after the application of EO water.

Non-thermal interventions

Non-thermal processing technologies are alternative interventions that use no to low levels of heat to reduce microbial contamination while minimizing the quality and nutrient losses (Wheeler et al., 2014). Various non-thermal technologies such as electron beam, ultraviolet (UV) light and UV-ozone combination, cold atmospheric plasma, and high-pressure processing are currently being used or investigated as interventions in the meat industry.

Pulse ultraviolet light

Pulse ultraviolet (PUV) light involves applying a short-duration pulse of light within the range of 200 to 1100 nm, so that the pulse duration is no longer than 2 milliseconds (msec). The total cumulative treatment shall not exceed 12.0 Joules/square centimeter (21CFR179.41). Pulse ultraviolet light utilizes photo-dynamic effects (toxicity that is generated through light absorbing molecules), which gives PUV light its antimicrobial effectiveness (Chen et al., 2012). According to Kaess & Weidermann (1973) the effective wavelength for bactericidal activity is between 253.7 nm and 180 nm, wavelengths which produce ozone, enhancing the bactericidal effect. This technology is commonly used in hospitals and laboratories for decontamination of surfaces, air and water (Wheeler et al., 2014). Pulse UV treatment has been used for a number of years in water purification and research is ongoing into the application of PUV directly to foods (Chun et al., 2010, Sommers et al., 2009). This technology is currently being investigated in the meat industry and has the potential to improve microbiological quality and safety of meat products. Moreover, PUV light is a non-thermal processing technology that does not leave any chemical residues on products or cause any physical damage (Khadre, Yousef, & Kim, 2001).

The antimicrobial effect of PUV light comes from permanent cross-links that form in the microbial DNA, preventing the cell from carrying out its normal functions (Sastry, Datta, & Worobo, 2000). Kalchayanand et al. (2013) reported that when PUV light was applied to inoculated fresh beef for 75 s, there was approximately a 1.0 log reduction for *E. coli* O157:H7 and non-O157 STEC. Currently there is some commercial use of PUV on beef products that has been implemented in the industry (Wheeler et al., 2014). Hierro et

al. (2011) reported a 1 to 2 log CFU reduction of *L. monocytogenes* on cooked ham and bologna slices. Similarly, Keklik et al. (2010), and Paskeviciute, Buchovec, & Luksiene (2011) were able to reduce various foodborne pathogens including *Campylobacter jejuni*, *L. monocytogenes*, and *Salmonella* spp. by 1.0 to 2.5 log when PUV light was applied on the surface of chicken meat. Keklik et al. (2010) did report several factors including treatment time, intensity of PUV light, and treatment distance could influence the chemical and physical quality of meat products; therefore, it is necessary to explore an optimum condition ensuring microbial safety without deterioration before its successful commercialization.

Multiple hurdle technology

No particular antimicrobial intervention is 100% effective (without limitations), as such most beef processors now utilize a 'multiple-hurdle' intervention system. Multiple hurdle interventions consist of sequential interventions at various processing steps to ensure the safety of their products (Bacon et al., 2000; Koohmaraie et al., 2005). Various studies have evaluated the effectiveness of sequential, multiple hurdle intervention systems to improve beef safety (Arthur et al., 2004; Bacon et al., 2000). Results have validated that sequential multiple hurdle interventions reduce bacteria on meat better than any one intervention alone (Koohmaraie et al., 2005). These studies have shown that the use of two or more interventions in a sequence may achieve a synergistic effect, or at least an additive effect (Bacon et al., 2000, Sofos, 2005). Hurdle technology refers to the use of a combination of suboptimal growth conditions in which each hurdle factor alone is insufficient to prevent the growth of spoilage and pathogenic bacteria. According to Wheeler et al. (2014) the use of the multi-hurdle approach provides insurance against the

variation in contamination coming in on the hides of animals and minimizes the chance that the hide load and subsequent carcass load will exceed the capacity of the interventions.

Meat quality

The quality of meat is determined by the attractiveness of meat to consumers (Wood et. al., 1999). Luning, Marcelis, & Jongen (2002) defined quality as the features/properties of a product that resulted in satisfying consumer physiological and/or psychological needs. The major meat quality attributes are appearance, texture, juiciness, and flavor. Of these, the most important at the point of purchase have traditionally been appearance.

Microbial quality

Microbiological contamination of meat is a significant contributor to global food waste and illness (Ravensdale et al., 2018). In North America alone, microbial food spoilage directly costs manufactures and distributors US $7 billion/year in recalls and legal fees, and US $166 billion/year in product losses (Buzby & Hyman, 2012; Hussain & Dawson, 2013).

The shelf-life of meat and meat products is the storage time until spoilage. The point of spoilage may be defined by a certain maximum acceptable bacterial level, or an unacceptable off-odor, off-flavor, appearance, or combination of the aforementioned (Borch et. al., 1996). Meat spoilage is often a subjective judgement that can be influenced by cultural and economic considerations, individual background, sensory acuity of the individual, and the intensity of the change (Nychas et. al., 2008). Meat spoilage is a complex event and consist of a combination of biological and chemical activities that interact and render the product unacceptable for human consumption (Gram et al., 2002; Casaburi et al., 2015).

Apart from lipid oxidation and autolytic enzyme reaction, the greatest contribution to meat spoilage can be attributed to the microbial activity of a wide variety of microorganisms. Meat nutrient composition, pH (5.5-6.5), and high moisture content are all factors that allow for the growth and survival of a large range of microorganisms. Product shelf life is greatly impacted by the types of microorganisms, initially present and their subsequent growth. Commonly isolated spoilage bacteria for red meat includes *Lactobacillus* spp., *Pseudomona*s spp., *Moraxella* spp. and *Acinetobacter* spp. (Eisel, Linton, & Muriana, 1997). *Pseudomonas* spp. are most common on meat and in most cases, are responsible for spoilage of meat stored aerobically at different temperatures (−1 to 25 °C; Nychas et al., 2008).

The microbial quality of raw meat also depends on the physiological status of the animal at slaughter, during processing, transportation, preservation, as wells as the storage conditions (Nychas et al., 2008). During storage, temperatures and packaging atmospheres are the two most important factors that affect microbial growth and selection during storage of fresh meat. Meat spoilage is caused only by a fraction of species and strains of the initial microbial association. Refrigeration selects psychrotrophic species while further selection is introduced by the type of packaging. In particular, the availability of oxygen affects microbial growth and metabolism (Casaburi et. al., 2015). Therefore, depending on the affinity of each species for oxygen, bacteria differ in their competitive growth potential under aerobic or anaerobic conditions. Their spoilage potential depends on which groups or microorganisms will dominate the meat matrix, and on their ability to produce spoilage-associated compounds such as esters, ketones, aldehydes, sulfur compounds, amines and volatile fatty acids (Lambert, Smith, & Dodds, 1991).

Color

Apart from microbial quality, there are numerous other factors that contribute to the quality of meat. Meat color has been reported to be one of the most important fresh meat characteristics at the point of purchase (Font-i-Furnols & Guerrero, 2014). Consumers relate red-purple color with freshness and brown color with a lack of freshness (Carpenter et al., 2001). According to Mancini & Hunt (2005), meat purchasing decisions are influenced by color more than any other quality factor because consumers use discoloration as an indicator of freshness and wholesomeness. Therefore, as we aim to improve the microbial safety of meat products, it is critical to monitor and maintain color stability because it directly affects the shelf life of meat and meat products and contributes to the visual acceptability of products by consumers at the point of purchase (Font-i-Furnols & Guerrero, 2014).

Myoglobin is the principle protein responsible for meat color. Myoglobin is a sarcoplasmic protein that determines meat color via its centrally located heme iron (Kerth, 2013). Most of the striking differences in the color of meat surfaces arise from the chemical state of the myoglobin molecules (AMSA, 2012). Myoglobin consists of a protein portion called a globin, and a nonprotein portion called a heme ring. The iron within the heme ring has the ability to form six bonds. Four of these bonds connect iron to the porphyrin ring, one binding site is used to link the globin to the heme group, and the 6th binding site is able to freely interact with a number of chemical elements such as oxygen, carbon monoxide, water, and nitric oxide (Romans et al., 1985; Kerth, 2013). The ligand occupying the 6th coordination site and the chemical state of the iron within the heme ring determines meat color. Different colors commonly associated with meat are

deoxymyoglobin, oxymyoglobin, carboxymyoglobin, and metmyoglobin (Forrest et al., 1975; Kerth, 2013).

Deoxymyoglobin results in a dark purplish-red color and is typical of the interior of fresh meat and meat that is vacuum packaged. Deoxymyoglobin is a combination of ferrous (Fe2+) iron and an unoccupied 6th binding site. When deoxidized meat is exposed to oxygen, it results in a bright-red color. In this case, oxygen is attached to the 6th binding site of a ferrous iron (Fe2+). Similar to oxymyoglobin, carboxymyoglobin formation occurs when carbon monoxide attaches to the vacant 6th position of deoxymyoglobin, producing a stable bright-red color. Metmyoglobin is characterized by a tan to brown colored form of myoglobin and it contains ferric iron (Fe3+). Typically, metmyoglobin forms easily at low concentrations of oxygen. In this case, water is the ligand at the 6th position.

There are many intrinsic and extrinsic factors that affect meat color. Intrinsic factors such as pH, muscle type, areas within a muscle, muscle fiber composition, myoglobin concentration, disruption of various subcellular components related to meat color chemistry, water holding capacity, microbial load, and temperature all impact meat color. These factors ultimately affect how the meat uses oxygen and the meat's ability to reduce metmyoglobin. Extrinsic factors, such as animal genetics, gender, age, diet energy density, time on feed, seasonality, antemortem stress, carcass weight, postmortem conditions, postmortem processing and packaging methods, time and temperature of storage, extent of exposure to oxygen and the number of cycles meat goes through the color cycle (metmyoglobin reducing ability), and especially postmortem age of the product, all influence meat color by influencing the intrinsic factors of meat color. These extrinsic

factors affect the rate and extent of postmortem pH decline, amount of protein denaturation when muscle is converted to meat, the concentration of antioxidants in meat, biochemical intermediates available to modulate meat color, and the quantity of unsaturated fatty acids and hence affects the color of meat (AMSA, 2012).

Discoloration is a result of oxidation of both ferrous myoglobin derivatives to ferric iron (Mancini & Hunt, 2005). Discoloration is often referred to as the amount of surface area covered by metmyoglobin, however, subsurface myoglobin forms also play a role in product appearance. The metmyoglobin beneath the surface gradually thickens and moves towards the surface. The formation of metmyoglobin depends on numerous factors including oxygen partial pressure, temperature, pH, meat's reducing activity, and in some cases, microbial growth (Mancini & Hunt, 2005). Meat color is a major driver of retail meat case sales and profitability. The discoloration of meat is one of the leading causes of lost retail sales stemming from products being discarded or devalued due to discoloration (Mckenna et. al., 2005).

Flavor

Flavor is an important part of the eating quality of meat (Wood et. al., 1999). Flavor just like color, depends on intrinsic and extrinsic factors such as species, genetics, sex, feeding regimen, and management practices (Maughan & Martini, 2012; Melton, 1990). According to Mottram (1998), meat flavor is very complex, and it is created mainly when meat is treated thermally, because raw meat has only a bloody taste and very little aroma. When cooked, lipids and water-soluble components form several volatile compounds, mainly by means of lipid degradation and Maillard reactions or through reactions between their products. These volatile compounds are the main contributors to meat flavor (Font-i-

Furnols and Guerrero, 2014). Sensory enjoyment of meat is related to several traits including, visual appearance, in-mouth perception of both texture and flavor and aroma.

Tenderness

Tenderness is one of the main components of eating quality according to consumer studies (Wood et al., 1999). Meat tenderness is influenced by three main components: (1) the sarcomere contractile state, (2) the extent of integrity/degradation of the structural myofibrillar proteins (proteolysis), and (3) the connective tissue content/ composition (Koohmaraie et al., 2002; Kerth, 2013; Bolumar & Toepfl, 2016). The relative contribution of these three components to the ultimate meat tenderness varies considerably. Factors such as genetics, muscle type, preslaughter factors (such as diet, handling, temperament, and stress), early postmortem events (pH and temperature of muscles), as well as duration and temperature at which the product is stored postmortem can all have a significant effect on beef tenderness.

Tenderness variation arises mainly through changes to the myofibrillar protein structure of muscle in the period between animal slaughter and meat consumption (Wood et. al., 1999). Rigor formation causes permanent cross-bridges between myosin and actin filaments in the sarcomere (actomyosin), and ultimately affects meat tenderness. In addition, the biochemical environment inside the muscle fiber during the process of rigor can affect the final state of sarcomere contraction. In addition, during the storage of fresh meat, there are subtle changes that results in improved tenderness of the muscle. Alteration in the structure of the myofilaments and their cross bridges during aging causes the loss of Z-disk and sarcomere integrity. Loss of integrity is largely a result of proteolytic degradation of numerous cytoskeletal proteins (troponin, titin, α-actinin, and nebulin).

Degradation of these proteins results in a weakening of the myofibril structured matrix, resulting in tenderization.

The endogenous enzymes that are known to work on myofibrillar proteins are the cathepsins and the calpains. Cathepsins are acid proteases located in the lysosomes. They may be liberated into both the cytoplasm and the intracellular spaces as a consequence of lysosomal disruption occurring after cell death due to a pH fall (Chéret et al., 2007). As for calpain, it is widely thought that the level of calcium in postmortem muscle is not high enough to activate m-calpain, so μ-calpain is the primary enzyme responsible for protein degradation postmortem (Kerth, 2013). However, it's important to note, that calpastatin is found in the sarcoplasm and is a key component in myofibrillar protein turnover and meat tenderness. Calpastatin are known to reduce the activity of calpains and may reduce the proteolysis required to increase meat tenderness (Kemp et al, 2010).

When considering collagen or connective tissue as it relates to tenderness, there are some basic properties that must be taken into consideration: such as total collagen concentration, types of collagen present, and cross-linking of the collagen matrix. The type of connective tissue is related to its chemical makeup or structure and is an indicator of its strength and solubility. Total collagen varies between muscles depending on their skeletal location and function in the live animal, and partially explains tenderness between muscles. Muscles that are used for locomotion tend to have greater quantities of collagen, while muscles used for support or for very finely adjusted movements tend to have less collagen. There is also a possible link between meat tenderness and meat composition, with attention being focused on fat content. As fatness increases in the animal it does so in several body locations simultaneously, which could be important for tenderness. First, it accumulates in

subcutaneous and intermuscular sites, then intramuscular, which could provide insulation for muscles against the effects of refrigeration as the carcass cools. (Wood et. al., 1999).

Blade tenderization

Improvements in tenderness are desirable to consumers and have become a consumer-driven trend. A common consensus from various Beef Customer Satisfaction studies (Neely et al., 1999; Lorenzen 1999; Savell et al., 1999) concluded that tenderness is the leading contribution factor in customers' perception of taste. Consumers are willing to pay more for a guaranteed tender product. Currently there are two types of mechanical treatments used to improve palatability of meat. These are blade tenderization and moisture enhancement via needle-injection (Heller et al., 2007). Blade tenderizers work by inserting small needles or double-edged blades into the meat. This technique physically disrupts the connective tissue and myofibrillar contractile system of the meat (Pietrasik & Shand, 2004). The primary issue with mechanical tenderization is the potential of introducing pathogens into the interior of the meat, which is otherwise sterile. Pathogens that are translocated to the interior of mechanically tenderized products, present a public health threat if the product is not thoroughly cooked (Heller et al., 2007). Several of studies have addressed internalization of surface microflora into the deeper tissues of mechanically tenderized beef (Phebus et al., 2000; Hajmeer et al., 2002, Gill & McGinnis, 2004; Johns et al., 2011). Phebus et al. (2000), Sporing (1999), and Luchansky et al. (2008), reported that 3 to 4% of surface-inoculated *E. coli* O157:H7 organisms were transferred from the surface into the geometric center of a beef top sirloin subprimal.

The US has experienced several *E. coli* O157: H7 outbreaks and two recalls associated with mechanically tenderized products. In 2000, illnesses related to *E. coli*

O157:H7 associated with the consumption of mechanically tenderized products were reported in Michigan and Oregon (Lim, Yoon, & Hovde, 2010). In 2003, five illnesses prompted a voluntary recall of 335,506 kg of frozen, raw, mechanically tenderized steaks. The following year, 184,545 kg of mechanically tenderized and ground beef products that may have been contaminated with *E. coli* O157:H7 were recalled. As such, the USDA-FSIS required establishments producing mechanically tenderized beef products to reassess their HACCP plan (FSIS Directive 10,0101.2; USDA-FSIS, 2015a) and specifically address whole muscle non-intact beef for concerns associated with O157 and non-O157 STEC and adequately address biological hazards, including any interventions for *E. coli* O157:H7 applied to the products' source materials (USDA, 2005).

Conclusion

As the importance of meat safety remains a central focus to the industry, it is important that interventions continuously be tested for their combined safety and quality impact on meat. The effectiveness of antimicrobial interventions depends on various factors, namely; the methodology used for application, type and composition of meat tissue, microbial ecology of the product and initial microbial load, the ability of bacteria to attach to the product and to produce biofilm and the operational factors such as temperature, pH of the solution, time and stage of application in the process. Nonetheless, these interventions provide a means to improve the meat safety. However, disadvantages of using these interventions does exist. These include potential problems with disproportionate reliance on the decontamination step, consequent reduction of the process hygiene, and limited reduction rates (especially on chilled meat) to name a few. These intervention methods could impact the quality of meat, by negatively impacting color,

flavor, and aroma. As antimicrobial interventions are developed and tested it is critical that their effects on meat quality are evaluated.

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CHAPTER 3

THE EFFECTS OF ANTIMICROBIALS ON QUALITY AND SENSORY CHARACTERISTICS OF BLADE TENDERIZED BEEF STRIPLOINS[1]

[1] Thomas, C.L., Y. Hung, M. Rigdon, R.W. McKee, and A.M. Stelzleni. Submitted to *LWT - Food Science and Technology*, 01/16/19.

Abstract

The beef industry utilizes blade tenderization (BT) to enhance tenderness; however, foodborne outbreaks have been associated with BT beef. Therefore, antimicrobials that reduce the risks associated with BT while maintaining quality are desired. The objective of this study was to assess the effects of pulse ultra-violet light (PUV), 5% v/v levulinic acid + 0.5% w/v sodium dodecyl sulfate (LVA+SDS), and electrolyzed oxidizing water (EOW; 50 mg/L) on beef strip loins before BT for their impact on quality and sensory characteristics compared to 4.5% v/v lactic acid (LA) and no antimicrobial interventions (CON). Beef strip loins were independently subjected to treatments prior to BT. After BT, two steaks were removed from the anterior end for sensory and Warner-Bratzler shear force analysis, and six roasts were assigned to retail display for determination of shelf life characteristics. Aerobic plate count bacteria were lower ($P \leq 0.05$) for LVA+SDS and LA compared to CON, EOW, and PUV. Treatment did not affect color, cooking, or sensory characteristics ($P > 0.05$). Lipid oxidation showed no difference among treatments ($P > 0.05$) until day 7 of display ($P \leq 0.05$). These results suggest that LVA+SDS could be an alternative to LA without impacting quality and shelf life.

Keywords: Antimicrobial intervention; Blade tenderized; Beef; Shelf life; Tenderness

Introduction

Pathogen outbreaks associated with contaminated food, including non-intact beef products (Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005), continue to be a primary concern within the beef industry. Since 2000, the U.S. Centers for Disease Control and Prevention has investigated several major outbreaks of foodborne illness that were specifically attributed to mechanically tenderized beef products prepared in restaurants and/or by consumers (Jones and Vega, 2015; Luchansky et al, 2009). According to Jones and Vega (2015), there were more *Escherichia coli* O157:H7 outbreaks reported during 2003-2012 than during the previous 20 years.

Tenderness is a primary contributor to palatability and ultimately the overall perception of quality in beef (Miller, Carr, Ramsey, Crockett, & Hoover, 2001). Therefore, processors utilize blade tenderization (BT) to help ensure a tender product and increase consumer satisfaction. Blade tenderization involves the penetration of the muscle with thin knives to sever and disrupt the integrity of muscle fibers and connective tissues, rendering the meat more tender (USDA-FSIS, 2002). However, BT can translocate spoilage bacteria and pathogens from the surface of intact beef cuts to subsurface (USDA-FSIS, 2002). Pathogens that are translocated during BT may be protected from the lethal effects of heat during cooking, especially in under cooked products. Therefore, the United States Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) mandated that plants utilizing BT and similar processes to manufacture non-intact beef had to reassess their hazard analysis and critical control point (HACCP) plans to specifically address hazards associated with whole muscle non-intact beef (USDA-FSIS, 2002; 2015).

Since the USDA-FSIS reassessment directive, there has been a focus to ascertain the prevalence of foodborne pathogens in BT products and identify effective interventions. Currently, the beef industry is seeking new intervention alternatives that will reduce microbial contamination without having adverse effects on quality characteristics and extend the shelf life of the product (Quilo et al., 2009). Some intervention technologies that have shown promise for use on meat surfaces include pulse ultra-violet light (Elmnasser et al., 2007), electrolyzed oxidizing water (Al-Haq, Sugiyama, & Isobe, 2005; Park, Hung, & Chung, 2004), and levulinic acid with sodium dodecyl sulfate (Liu, Duan, & Su, 2006; Zhoa, Zhoa, & Doyle, 2009).

Therefore, the objective of this study was to evaluate the intervention strategies of pulse ultra-violet light, electrolyzing oxidizing water, and levulinic acid plus sodium dodecyl sulfate when applied to beef strip loin subprimals before blade tenderization to assess their effects on meat shelf life and quality characteristics when compared to lactic acid.

Materials and methods

Meat procurement and treatment assignment

Whole USDA Choice beef boneless strip loins (Institutional Meat Purchase Specifications 180; N = 75) were purchased (FPL Foods LLC, Augusta, GA) across three replications 4 days postmortem and transported (161 km; 0 ± 2°C) to the University of Georgia Meat Science Technology Center (Athens, GA; UGA-MSTC) and stored (0 ± 1°C) for 3 days. The strip loins were randomly selected from the days fed beef production and were deemed by plant standards to be free of quality defects. On day 7 postmortem, the strip loins were randomly assigned to one of the five treatment groups: (1) pulse ultra-

violet light (PUV), (2) electrolyzed oxidizing water [EOW; pH 6.4 and oxidation-reduction potential (ORP) of 800 – 850; 50 mg/L Cl], (3) levulinic acid (LVA; 5% v/v) + sodium dodecyl sulfate (SDS; 0.5% w/v), (4) lactic acid (LA; 4.5% v/v), or (5) no intervention (CON).

Treatment groups

On each trial day, the five strip loins randomly assigned to PUV were transported (0 ± 2°C) to the University of Georgia Griffin Campus (83 km), where ultra violet light (Xenon Corporation, Wilmington, MA) pulse was applied. The strip loins were unpackaged and individually treated for 15 s at 5.754 J/cm^2 at distance of 6 ± 2 cm from the quartz window, ensuring all surfaces were treated. Following treatment, the subprimals were immediately vacuum packaged and transported (0 ± 2°C) back to UGA-MSTC where they were held (0 ± 2°C) while treatments were applied to the remainder of the strip loins. Electrolyzed oxidizing water was produced using an electrolyzed oxidizing water generator (ROX-20TA-U, Hoshizaki Electric, Japan) by electrolysis of a NaCl solution according to Park, Hung, & Brackett (2002). The morning of each replication, a 9.4% w/v salt solution (CAS # 7647-14-5, J.T Baker, Center Valley, PA) and deionized water were simultaneously pumped through the generator at approximately 10 volts and 18 amps, producing acidic and alkaline water. The electrolyzed oxidizing alkaline water (pH 11, ORP ~ -800 mV) from the cathodic side and the acidic water (pH = 2.3 - 2.7, ORP +1100 mV) from the anodic side were collected in separate sealable containers. Immediately before use, measured amounts of alkaline and acidic water portions were mixed to produce 37.85 L of acidic EOW with a pH of 6.2 - 6.5 and an ORP between +800 to +850 mV. Free chlorine concentration and ORP were determined using a pH meter and an ORP single

junction ion electrode (model WD-35649-50, Oakion Instruments, Vernon Hills, IL). Free chlorine content was determined following the Hach DPD-FEAS (diethyl-*p*-phenylenediamine - 0.00564 *N*-ferrous ethylenediammonium sulfate) titration method (Hach CO., Loveland, CO). To produce the LVA+SDS, 98% concentrated levulinic acid (CAS #123-76-2, Acros Organics, Fair Lawn, NJ) and 95% concentrated sodium dodecyl sulfate (CAS #151-21-3, Sigma Aldrich, St. Louis, MO) were measured out and mixed together in a 50 mL beaker. Immediately before use, the weighted sample of levulinic acid + sodium dodecyl sulfate was brought to volume (37.85 L) to create a 5% v/v LVA + 0.5% w/v SDS solution. A 4.5% v/v lactic acid solution was prepared by diluting 88% concentrate lactic acid (Birko Company, Henderson, CO) with water and bringing it to volume (37.85 L).

The treatments (EOW, LVA+SDS, and LA) were applied to subprimals within their respective groups using an automated six-nozzle sanitizing cabinet (Chad Co., Olathe, KS). The automatic premixed spray was used to treat all sides of the subprimal, with nozzles located above and below the subprimal at a flow rate of 0.42 L/nozzle•min^{-1} at 275.79 kPa. Between treatments, the spray cabinet and holding tank were thoroughly rinsed and flushed with hot potable water (55°C) for 2 min and allowed to cool to room temperature (3 ± 1°C) before applying the following treatment. After each treatment application, subprimals were transferred to the blade tenderizer (model TC700MC, Ross manufacturing, Midland, VA) conveyor belt (1.0 m/min) and made a single pass, lean side up. The tenderizer blade head consisted of seven alternating angled rows containing 32 perpendicular blades (3 mm wide) set 10 mm apart between rows and columns. After the subprimals from all other treatment

groups were blade tenderized, the PUV treated subprimals were then blade tenderized. The blade tenderizer was also cleaned thoroughly between each treatment groups.

Following treatment application, subprimals were vacuum packaged (30 to 50 mL of O2/m^2/24 h; 101,325 Pa; 23°C; B-620 series, Cryovac Sealed Air Corporation, Duncan, SC), boxed, and held in cold dark storage (0 ± 1°C) for an additional 7 days to simulate transportation and storage. After 7 days, subprimals were unpackaged, squared at the anterior edge and fabricated anterior to posterior into two steaks (2.54 cm) and six roasts (5.08 cm). The two steaks were designated for Warner-Bratzler shear force (WBSF) and sensory analysis, and the six roasts were randomly assigned to 0, 1, 2, 3, 5, or 7 days of display for shelf life analysis. Roasts were placed on absorbent pads (Dri-Loc AC-40, Cryovac Sealed Air, Duncan, SC) in Styrofoam trays (2S; Genpak LLC, Charlotte, NC), wrapped with oxygen permeable polyvinylchloride (PVC) overwrap (O2 transmission=23, 250 mL/m^2/24 h, 72 gauge; Pro Pack Group, Oakland, NJ), and placed in retail display in an open-topped coffin style display case (0 ± 1.5°C, with two defrost cycles every 24 h; M1X-E, Hussmann, Bridgeton, MO) with 24 h continuous lighting (1850 lux; Octron/ECO; 30000K; F032/830/ECO; Sylvania Company, Versailles, KY). Roasts were rotated daily within the case, and the case temperature was monitored and recorded by continuous data loggers (TR-50U2, T&D Corp., Japan) placed at package height. Steaks designated for WBSF and sensory analysis were vacuum packaged and stored (-20°C) until further analysis. The sensory steaks from PUV were not analyzed due to the pulse UV apparatus being housed in a bio-safety level 2 laboratory.

Retail display color

Objective color was measured each day (15:00 ± 1 h) on day 7 samples with a Hunter-Lab Mini Scan XE (Hunter Associates Laboratory, Reston, WV) using illuminant A with a 10º viewing angle, standardized using white and black calibration tiles and a working standard before each use. Three readings were taken for each sample day, and the average was recorded for *Commission Internationale de l'Eclairage* (CIE) L* [black (0) to white (100)], a* [green (-60) to red (+60)], and b* [blue (-60) to yellow (+60)] color values. Hue angle [$\tan^{-1}$ (b*/a*)], chroma [$(a^{*2} + b^{*2})^{0.5}$], and ΔE {$[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$} were also calculated. Spectral data was collected, and myoglobin state was calculated via reflectance ratios 474, 525, 572, and 610 nm described by AMSA (2012) and McKenna et al. (2005).

Bacterial growth

Following the USDA-FSIS Microbiology Laboratory Guidebook 3.01 method (USDA-FSIS, 1998), total aerobic bacteria plate counts (APC) were conducted on days 0, 1, 2, 3, 5, and 7 on roasts. To sample each roast, a sterile 5 x 5 cm^2 metal template was used to create an impression on the treated lean surface of the roast before cutting. The 5 x 5 cm^2 surface area of the roast was aseptically excised to a depth of approximately 0.2 cm using a sterile scalpel handle and blade. Each sample was then placed in a sterile stomacher bag with 90 mL of 0.1% peptone (Difco Laboratories, Detroit, MI) and stomached for 2.5 min at 230 rpm. All utensils were sterilized by flame, rinsed with diluted chlorine and wiped free of residual chlorine. Serial dilutions were made for all samples using 9 mL of 0.1% peptone. One milliliter of each sample dilution was plated on 3M Petrifilm (3M

Manufacture, St. Paul, MN) following the manufacturer's instructions. Petrifilm plates were incubated at 35 ± 1ºC for 48 ± 2 h, counted, and reported as log CFU/cm^2.

Lipid oxidation

Thiobarbituric acid reactive substance analysis (TBARS) was conducted according to AMSA, 2012; Buege and Aust, 1978; and Sinnhuber and Yu, 1958. Samples were thawed (4 ± 1°C; 24 h), finely minced, and 0.5 g of each sample was weighed in duplicate and placed in disposable glass test tubes (13 x 100 mm). Thiobarbituric acid stock solution (0.375% thiobarbituric acid, 15% thichloracetic acid and 0.25 N HCL; 2.5 mL) was added to each sample and vortexed. Samples were heated (90°C) for 10 min in a water bath in loosely covered tubes and then cooled for 10 min in a tap water (20°C) bath. The tubes were then centrifuged at 2550 x *g* (CR 312, Jouan INC., Winchester, VA) for 10 min at 4°C to obtain the supernatant. The supernatant was separated for spectrophotometric analysis (model V-630, Jasco Analytical Instruments, Easton, MD) using a pipette. The absorbance of the supernatant was measured at 532 nm. Lipid oxidation values were recorded, averaged, and expressed as mg of malonaldehyde (MDA) per kg of meat.

Sensory analysis

Steaks for sensory analysis were thawed (4 ± 1°C) for 18 h. The following day, steaks were cooked to an internal temperature of 71°C on preheated clamshell grills (George Formen, Saltotn Inc., Miramar, FL). Internal temperature was monitored using a Digi-Sense 12-Channel Scanning thermometer with copper-constantan thermocouples inserted into the geometric center of each steak. After cooking, steaks were served in warmed yogurt makers (Euro Cuisine, Inc., Los Angeles, CA) to an eight-member trained sensory panel according to AMSA (2016). Panelists evaluated two cubes per steak (1.27

cm^3) and evaluated eight steaks per session, with two sessions per day. Samples from each treatment were randomly given to panelists. Loaded yogurt makers were passed through a breadbasket door from the sensory kitchen to the sensory analysis room. The sensory analysis room was equipped with positive pressure ventilation and eight individual booths with red lighting to minimize panelist influence and mask differences in cooked steak color. Panelists evaluated each sample for initial and sustained tenderness (8 = Extremely tender, 7 = Very tender, 6 = Moderately tender, 5 = Slightly tender, 4 = Slightly tough, 3 = Moderately tough, 2 = Very tough, and 1 = Extremely tough), beef intensity flavor (8 = Extremely intense, 7 = Very intense, 6 = Moderately intense, 5 = Slightly intense, 4 = Slightly bland, 3 = Moderately bland, 2 = Very bland, and 1 = Extremely bland), overall juiciness (8 = Extremely juicy, 7 = Very juicy, 6 = Moderately juicy, 5 = Slightly juicy, 4 = Slightly dry, 3 = Moderately dry, 2 = Very dry, and 1 = Extremely dry), and off-flavor (6 = Extreme off-flavor, 5 = Very strong off-flavor, 4 = Moderate off-flavor, 3 = Slight off-flavor, 2 = Threshold off-flavor, and 1 = None detected).

Warner-Bratzler shear force

Steaks assigned to WBSF were thawed and cooked following the methods described for sensory analysis (AMSA, 2016). Following cooking, steaks were placed on trays, wrapped with PVC and cooled (2 ± 2°C) for 18 h. Six cores (1.27 cm diameter) were obtained parallel to the longitudinal orientation of the muscle fibers from each steak utilizing a hand-held coring device. Cores were sheared perpendicular to the longitudinal orientation of the muscle fibers using a Universal Testing Machine (Instron Dual Column Model 3365, Instron corp., Norwood, MA) equipped with a Warner-Bratzler shear head (51 kgf load cell; cross head speed of 25 cm/min). The peak shear force (kgf) for each core

was recorded (Bluehill software, Instron Corp., Norwood, MA) and averaged for the shear force value of each steak.

Statistical analysis

Data were analyzed using Proc Mixed of SAS (version 9.3; Cary, NC) as a completely randomized split-plot, where subprimal was the whole-plot and the roast or steak within subprimal was the sub-plot. Subprimal within replicate by treatment was included as the random variable. Subprimal was considered the experimental unit and roast or steak was considered the observational unit. Main effects and all treatment by day interactions were tested when applicable. If an interaction occurred, data was reanalyzed and separated by day. Objective color, lipid oxidation, and APC values were analyzed for the main effects of antimicrobial treatment, sampling points, and their interaction. Objective color was further analyzed as a repeated measure. Means were separated utilizing the PDIFF option of LSMEANS and differences were considered significant at $\alpha \leq 0.05$.

Results and discussion

Simulated retail display color and myoglobin content

There was not a treatment by day of display interaction for objective color ($P > 0.05$). Furthermore, the main effect of treatment did not affect any of the measures for objective color ($P > 0.05$) including L*, a*, b*, hue angle, chroma, ΔE, or predominate myoglobin state (Table 3.1). However, as day of shelf life progressed, L*, a*, b*, and Chroma values decreased ($P \leq 0.05$) while hue and ΔE values gradually increased ($P \leq 0.05$; Table 2), indicating that samples became darker and less red over time. Further confirming color changes over display life, reflectance ratios showed that oxymyoglobin ratios (OMb; 610 nm / 525 nm) increased ($P \leq 0.05$) while metmyoglobin (MMb; 572 nm

/ 525 nm) ratios decreased ($P \leq 0.05$) as day of display increased. There were no changes ($P > 0.05$) in deoxymyoglobin ratios (DMb; 474 nm / 525 nm) throughout the 7 days of shelf life (Table 3.2). The obtained results are congruent to the findings from McKenna et al. (2005) who reported that as day of display increased, an increase in OMb ratios was indicative of lower levels of OMb, while lower MMb ratios indicated the presence of greater amounts of metmyoglobin in the surface of the meat. Undoubtedly, color and appearance of meat determines how consumers perceive quality and significantly influences consumer purchasing decisions (Carpenter, Cornforth, & Whittier, 2001). Hence, it important that novel antimicrobials do not have any adverse effect on beef color, which is reflected in our findings.

Aerobic plate counts

There was an antimicrobial treatment by day interaction ($P \leq 0.05$; Figure 3.1) for aerobic plate counts. After 7 days in the retail case, the averages of total bacteria recovered from roasts were 5.65, 5.20, 5.78, 3.49, 4.33 log CFU/cm^2 for CON, PUV, EOW, LVA+SDS, and LA, respectively. The prevalence of bacterial population recovered from roasts were significantly ($P \leq 0.05$) affected by the antimicrobial applied. Also, bacterial growth increased ($P \leq 0.05$) as day of display increased, as expected. Markedly, from days 0 to 7, the recovered bacteria populations from roasts treated with LVA+SDS and LA were lower ($P \leq 0.05$) than in the CON group. More noticeably, LVA+SDS was able to suppress bacterial growth and had counts that were less ($P \leq 0.05$) than all other treatment groups for days 1, 2, 3, and 7. For all days, recovered bacteria populations were similar ($P > 0.05$) for CON and EOW, and after day 2, PUV. Overall, there were no differences ($P > 0.05$)

found among the antimicrobials PUV and EOW and the CON group, indicating that these antimicrobials were not very effective in suppressing growth of potential spoilage bacteria.

Keklik, Demirci, & Puri, (2010) investigated the effectiveness of pulse ultra-violet light on the microbial load of boneless chicken breasts and reported that optimal treatment conditions existed at 5 cm from quartz window with 15 s of exposure time (1.27 J/cm^2) for unpackaged samples, resulting in about 2 $\log_{10}$ reduction. While 15 s at 6 cm from the quartz window was not an effective treatment in the current study, a preliminary test showed that exposure over 15 s or at a closer distance to the quartz window resulted in undesirable visual color change. This was confirmed by Keklik et al. (2010), who also noticed that treatments that exceeded 15 s at either 5 or 8 cm resulted in a comprised visual color change caused by the heat generation in the chamber. While pulse ultra-violet light has shown promise as an antimicrobial technology with various foods and surfaces (Gomez-Lopez, Ragaert, Debevere, & Devlieghere, 2007), sample heating is perhaps the most important limiting factor. The effect of acidic electrolyzed oxidizing water on vegetables and food contact surfaces has been extensively studied (Al-Haq, Seo, Oshita, & Kawagoe, 2002; Guentzel, Lam, Callan, Emmons, & Dunham, 2008; Park et al., 2004; Venkitanarayanan, Ezeike, Hung, & Doyle, 1999; Yang, Swem, & Li, 2003). The aforementioned studies have reported effective reductions in populations or observed complete inactivation of *E. coli*, *Salmonella enteritidis*, and *L. monocytogenes.* However, the acidic electrolyzed oxidizing water was not effective at limiting the proliferation of spoilage bacteria in the current study. Reduced aerobic plate counts were achieved when 5% levulinic acid (v/v) was combined with 0.5% sodium dodecyl sulfate (w/v). Zhao et al., (2014) reported that treating beef trim at 8°C with 3% levulinic acid plus 2% sodium

dodecyl sulfate for 1, 2, or 3 min reduced *Salmonella Typhimurium* by 2.1, 2.6, and 5.0 log CFU/cm^2, respectively. Levulinic acid plus sodium dodecyl sulfate was less effective when used at lower concentrations (Zhao et al., 2009; Stelzleni, Ponrajan, & Harrison, 2013). The effectiveness of lactic acid, which is commonly used within the beef industry, on subprimals was confirmed by the current study as well as Gill & Badoni (2004).

Lipid oxidation

There was an antimicrobial treatment by day interaction ($P \leq 0.05$; Figure 3.2) for lipid oxidation. As expected, as day of display increased, so did lipid oxidation for all treatments. Within day of display, all treatments were similar ($P > 0.05$) for days 0 through 5. However, after 7 days of display, roasts treated with LVA+SDS had greater ($P \leq 0.05$) malondialdehyde values compared to all other treatment groups. However, even after 7 days of retail display, lipid oxidation levels were below the threshold of 2 mg MDA/kg that Campo et al. (2006) reported for oxidative rancidity and sensory acceptability in beef.

Cooking and sensory characteristics

Cooking and sensory characteristics are reported in Table 3.3. Antimicrobial interventions did not influence strip loin thaw and cook loss percentages ($P > 0.05$). The results obtained from the trained sensory panelists showed that panelists could not detect any differences ($P > 0.05$) in initial and sustained tenderness, juiciness, beef intensity flavor, or off-flavors among the treatments groups. Similar to sensory tenderness, WBSF values were similar ($P > 0.05$) among all treatment groups.

Conclusion

The antimicrobials PUV and EOW were not effective in controlling psychrotrophic growth in the present study. However, when compared to the other treatments, LVA+SDS was

effective in suppressing psychrotrophic growth on BT roasts. Additionally, none of the antimicrobials applied had an adverse effect on color during retail display or sensory testing. Levulinic acid plus sodium dodecyl sulfate could be used within the meats industry as an antimicrobial on blade tenderized products while maintaining their quality and sensory characteristics.

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Table 3.1. Least squares means and standard errors for objective color and myoglobin content main effects by treatment for roasts from beef strip loins subjected to antimicrobial intervention and blade tenderization.

Variables[2]	Treatment[1]					
	CON	PUV	EOW	LVA+SDS	LA	SEM[3]
L*	39.52	39.57	37.64	36.96	37.79	0.86
a*	26.41	25.82	25.68	26.36	27.44	0.68
b*	21.21	20.96	20.66	21.08	21.98	0.41
Hue	39.01	39.49	39.17	38.96	38.85	0.66
Chroma	33.91	33.30	32.99	33.79	35.18	0.74
ΔE	6.29	7.50	7.54	7.82	6.19	0.84
DMb	0.99	0.92	1.32	1.45	1.28	0.17
OMb	0.21	0.23	0.22	0.21	0.20	0.01
MMb	1.29	1.26	1.27	1.28	1.32	0.02

[1] Antimicrobial intervention: CON = control; blade tenderization only; PUV = pulse ultraviolet light (15 s at 5.754 J/cm^2); EOW = electrolyzed oxidizing water (50 mg/L); LVA+SDS = 5% v/v levulinic acid plus 0.5% w/v sodium dodecyl sulfate; LA = 4.5% v/v lactic acid.

[2] L* = 0 = black to 100 = white; a* = measurement of green to red on color spectrum, high values indicate more red; b* = measurement of yellow to blue on color spectrum, higher values indicate more yellow; Hue = lower values indicate redder color; Chroma = higher value indicates more red saturation; ΔE = total color change over 7 days of simulated retail storage time; DMb = deoxymyoglobin; OMb = oxymyoglobin; MMb = metmyoglobin.

[3] SEM= standard error of means.

Table 3.2. Least squares means and standard errors for objective color and myoglobin content main effects by day for roasts from beef strip loins subjected to antimicrobial intervention and blade tenderization.

Variables[1]	Day of display								
	0	1	2	3	4	5	6	7	SEM[2]
L*	40.11[a]	39.14[b]	39.22[b]	38.45[c]	37.93[d]	37.60[d]	36.95[e]	36.95[e]	0.42
a*	31.77[a]	30.08[b]	28.65[c]	27.59[d]	25.99[e]	24.71[f]	22.14[g]	19.78[h]	0.38
b*	24.65[a]	23.36[b]	22.67[c]	21.69[d]	20.61[e]	20.05[f]	18.65[g]	17.44[h]	0.23
Hue	37.79[a]	37.81[a]	38.37[b]	38.19[ab]	38.47[ab]	39.18[c]	40.43[d]	42.54[e]	0.37
Chroma	40.22[a]	38.09[b]	36.56[c]	35.10[d]	33.19[e]	31.84[f]	28.99[g]	26.67[h]	0.41
ΔE	0.00[a]	2.89[b]	4.53[c]	5.80[d]	7.72[e]	9.21[f]	12.01[g]	14.40[h]	0.46
DMb	1.21	1.24	1.14	1.19	1.18	1.21	1.21	1.21	0.08
OMb	0.17[a]	0.17[b]	0.18[c]	0.20[d]	0.21[e]	0.23[f]	0.26[g]	0.30[h]	0.01
MMb	1.48[a]	1.41[b]	1.37[c]	1.34[d]	1.28[e]	1.23[f]	1.14[g]	1.03[h]	0.02

[a-h] Least squares means within rows with different letters are different ($P \leq 0.05$).
[1] L* = 0 = black to 100 = white; a* = measurement of green to red on color spectrum, high values indicate more red; b* = measurement of yellow to blue on color spectrum, higher values indicate more yellow; Hue = lower values indicate redder color; Chroma = higher value indicates more red saturation; ΔE = total color change over 7 days of simulated retail storage time; DMb = deoxymyoglobin; OMb = oxymyoglobin; MMb = metmyoglobin. [2] SEM= standard error of mean.

Table 3.3. Least squares means and standard errors for the main effect of treatment on cooking and sensory characteristics for steaks from beef strip loins subjected to antimicrobial intervention and blade tenderization

Variables	Treatment[1]					
	CON	PUV	EOW	LVA+SDS	LA	SEM[2]
Thaw Loss[3]	0.49	-	0.41	0.90	0.64	0.18
Cook Loss[3]	14.34	-	15.18	13.20	15.04	1.27
Initial Tenderness[4]	4.55	-	5.22	4.89	5.13	0.31
Sustained Tenderness[4]	4.92	-	5.60	5.47	5.45	0.34
Beef Flavor Intensity[5]	3.95	-	4.37	4.33	4.29	0.28
Juiceness[6]	3.7	-	4.01	4.37	4.14	0.31
Off-flavor[7]	1.33	-	1.38	1.34	1.45	0.16
WBSF[8]	3.08	2.63	2.59	3.20	2.79	0.24

[1] Antimicrobial intervention: CON = control; blade tenderization only; PUV = pulse ultraviolet light (15 s at 5.754 J/cm^2); EOW = electrolyzed oxidizing water (50 mg/L); LVA+SDS = 5% v/v levulinic acid plus 0.5% w/v sodium dodecyl sulfate; LA = 4.5% v/v lactic acid.
[2] SEM = standard error of means.
[3] Values are reported in percentage.
[4] 8 = extremely tender, 7 = very tender, 6 =moderately tender, 5 = slightly tender, 4 = slightly tough, 3 = moderately tough, 2 = very tough, and 1 = extremely tough.

[5] 8 = extremely intense, 7 = very intense, 6 = moderately intense, 5 = slightly intense, 4 = slightly bland, 3 = moderately bland, 2 = very bland and 1 = extremely bland.
[6] 8 = extremely juicy, 7 = very juicy, 6 = moderately juicy, 5 = slightly juicy, 4 = slightly dry, 3 = moderately dry, 2 = very dry, and 1 = extremely dry.
[7] 6 = extreme off-flavor, 5 = very strong off-flavor, 4 = moderate off-flavor, 3 = slight off-flavor, 2 = threshold off-flavor, and 1 = non-detected.
[8] Warner-Bratzler Shear Force, values are reported as kgf.

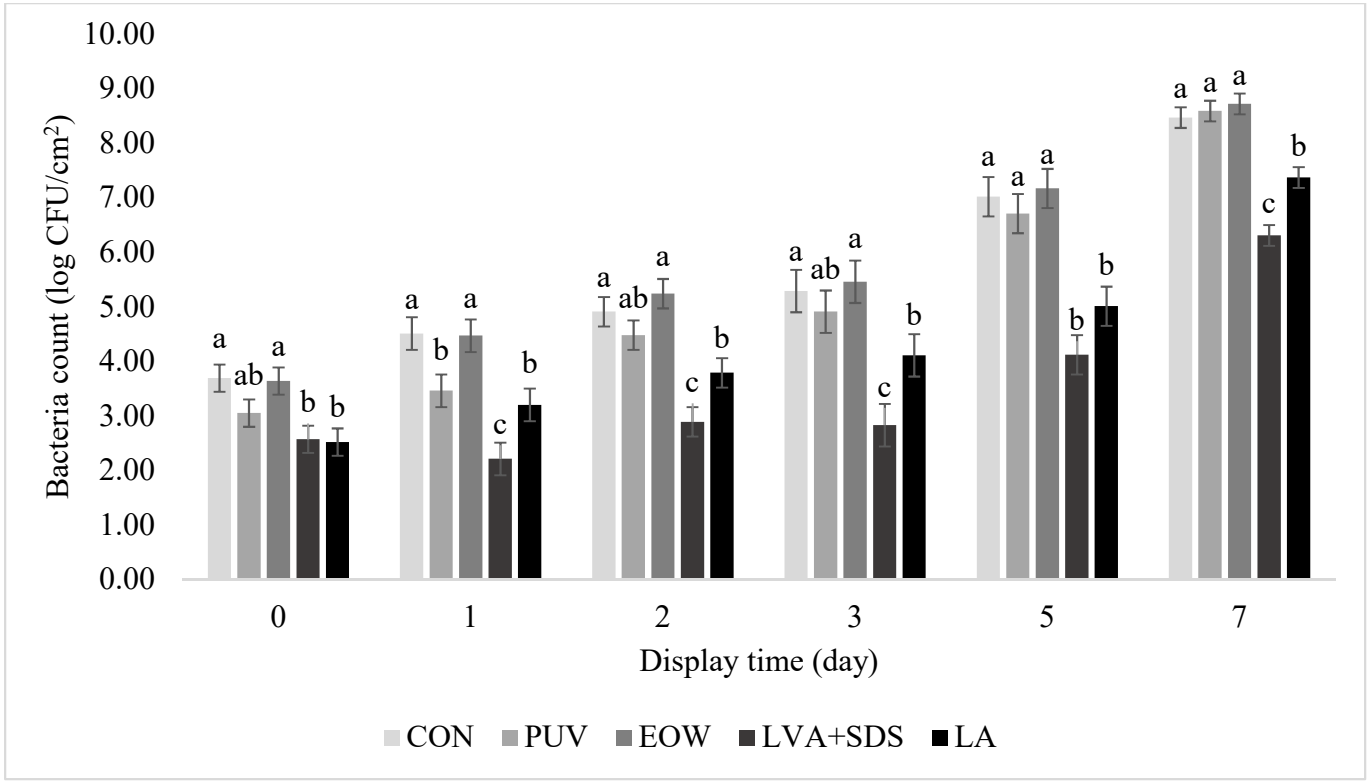

Figure 3.1. Day of display by antimicrobial treatment interaction on psychrotrophic populations (log CFU/cm^2; least squares means ± S.E.) through 7 days of simulated retail storage of roasts from beef strip loins subjected to antimicrobial intervention and blade tenderization. Means within each day of display that do not share a common letter are different ($P \leq 0.05$). Antimicrobial intervention: CON = control; blade tenderization only; PUV = pulse ultraviolet light (15 s at 5.754 J/cm^2); EOW = electrolyzed oxidizing water (50 mg/L); LVA+SDS = 5% v/v levulinic acid plus 0.5% w/v sodium dodecyl sulfate; LA = 4.5% v/v lactic acid.

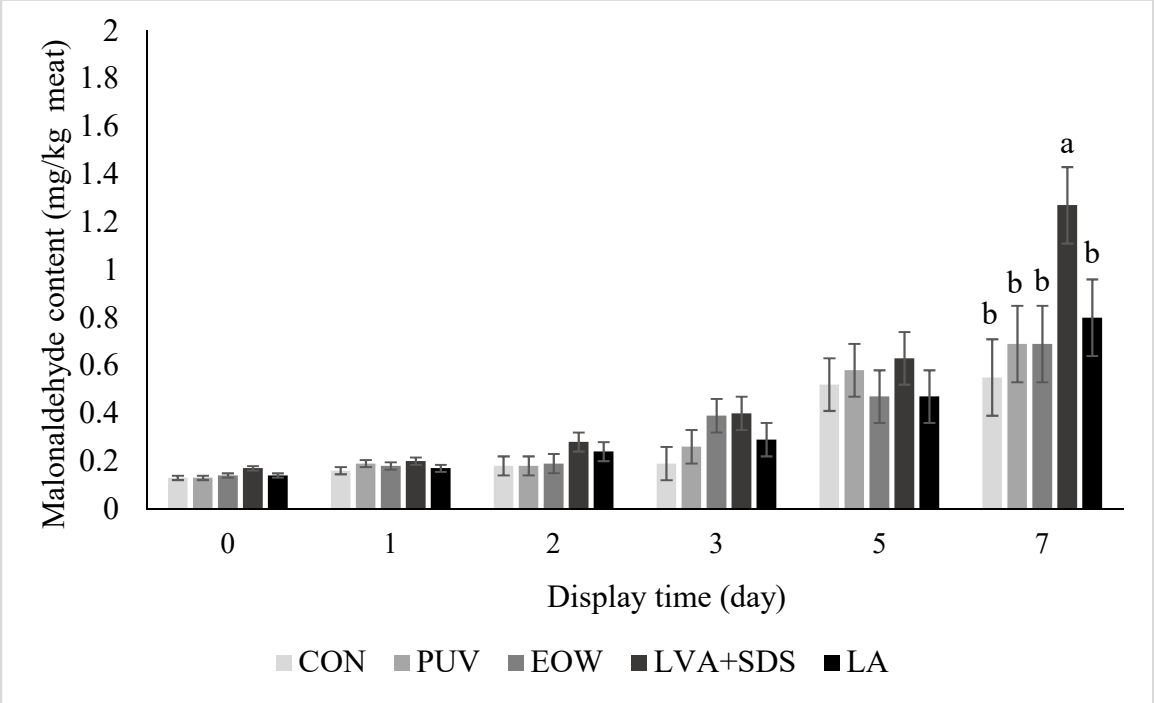

Figure 3.2. Day of display by antimicrobial treatment interaction on thiobarbituric acid reactive substance (mg malonaldehyde (MDA)/kg meat; least squares means ± S.E.) through 7 days of simulated retail storage of roasts from beef strip loins subjected to antimicrobial intervention and blade tenderization. Means within each day of display that do not share a common letter are different ($P \leq 0.05$). Antimicrobial intervention: CON = control; blade tenderization only; PUV = pulse ultraviolet light (15 s at 5.754 J/cm^2); EOW = electrolyzed oxidizing water (50 mg/L); LVA+SDS = 5% v/v levulinic acid plus 0.5% w/v sodium dodecyl sulfate; LA = 4.5% v/v lactic acid.

CHAPTER 4

THE EFFICACY OF ANTIMICROBIAL INTERVENTIONS ON SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* (STEC) SURROGATE POPULATIONS INOCULATED ON BEEF STRIPLOINS PRIOR TO BLADE TENDERIZATION[1]

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Abstract

A common method used to improve meat tenderness is blade tenderization; however, this method is a known vehicle for the transmission of potential surface pathogens into the interior of meat. The US meat industry actively seeks antimicrobials to eliminate adulterants such as Shiga toxin-producing *Escherichia coli* (STEC) on blade tenderized products. To facilitate this, the anterior portion of whole muscle beef striploins (30.48 cm) were inoculated (lean side) across a 10.16 cm band with a ca. 8.00 log CFU/mL cocktail containing non-pathogenic rifampicin resistant surrogate *E. coli* strains. The inoculated striploins were then passed through a spray cabinet and treated with either levulinic acid (5.0%) + sodium dodecyl sulfate (0.50%) (LVA+SDS), peroxyacetic acid (2000 ppm; PAA), acidified sodium chlorite (1200 ppm; ASC), lactic acid (4.5%; LA), or no antimicrobial application (CON) and then blade tenderized. After the inoculated striploins were treated and tenderized, a non-inoculated beef striploin was treated with the same antimicrobial and passed through the same blade tenderizer. For each treatment group, surface and subsurface samples (2.54 cm wide) were collected from the anterior, middle, and posterior end of each striploin. Among the antimicrobial treatments used in this experiment, PAA was the most effective in reducing surrogate *E. coli* ($P \leq 0.05$) on the surface when compared to all other treatment groups, followed by LVA+SDS and ASC, respectively. Likewise, PAA had the lowest ($P \leq 0.05$) amount of recovered *E. coli* subsurface. Sponge samples taken from the blade tenderizer after each treatment showed significant amounts of surrogate STEC remaining on the plastic plate and blades of the blade tenderizer. The recovered bacteria on the plate head and blades of the blade tenderizer

were the same for all treatment groups except for PAA. These results showed that PAA and LVA+SDS could be used to improve the safety of blade tenderized beef.

Keywords: Beef, Antimicrobial intervention, Non-intact, *Escherichia coli*, Surrogate

Introduction

Meat tenderness is one of the most important quality traits that influences consumer acceptability of fresh meat. The US beef industry has placed high priority on producing tender products. Currently, there are several different technologies that are utilized to improve tenderness, namely chemical injection, enzymatic digestion, hydrodynamic shock, and/or blade and needle tenderization (Luchansky et al., 2009). Blade tenderization is commonly used in the beef industry and has been shown to improve the marketability of certain beef cuts (Heller et al., 2007). However, there is an inherent risk of translocating surface pathogens to the interior of the normally sterile intact meat. Research conducted by Thippareddi et al. (2000) identified that blade tenderization could lead to the internalization of 3-4% of the surface bacteria. This demonstrated the potential for pathogens to be translocated (Thippareddi et al., 2000; Gill & McGinnis, 2004; Luchansky et al., 2008; Yoon et al., 2009; Luchansky et al., 2012), and if tenderized products are undercooked, they could present a public health threat and easily lead to foodborne illnesses.

Within the last decade, there have been several recalls and foodborne outbreaks associated with the consumption of nonintact mechanically and/or chemically tenderized steaks (Laine et al., 2005; CDC, 2010; CDC, 2010a; Luchansky et al., 2009). In 1994, the United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) issued a policy stating that raw ground beef is to be considered adulterated when contaminated with *Escherichia coli* (*E. coli*) O157:H7. In 1999, the adulteration policy was further extended to include all raw, nonintact beef contaminated with this pathogen (USDA, 1999). In 2011, the FSIS further determined that a subset of Shiga toxin producing *E. coli* (STEC) serotypes (O26, O45, O103, O111, O121, and O145) would be considered

adulterants in raw nonintact beef. These serotypes are known to cause serious illness when ingested (Kennedy et al., 2006).

According to USDA-FSIS (2019), among the non-intact products, beef presents an increased food safety concern and poses a heightened public health concern. With this heightened food safety concern, beef processors have placed special emphasis on better managing these products. One way to combat the inherent risk of blade tenderization is through the use of effective antimicrobial interventions. Thus, the objectives of the current study were: 1) quantify the efficacy of levulinic acid plus sodium dodecyl sulfate, acidified sodium chlorite, lactic acid, and peroxyacetic acid on *E. coli* surrogates inoculated on beef striploins prior to blade tenderization compared to a non-treated control, and 2) examine the cross-contamination of surrogate STEC from one contaminated striploin to a subsequent uncontaminated striploin.

Methods and Materials

Thirty whole muscle beef striploins (3 replicates; 5 treatment groups; 2 striploins per treatment) were purchased (FPL Food LLC, Augusta, Georgia), transported (161 km; 0±2ºC) to the University of Georgia Meat Science Technology Center (Athens, GA), and subsequently stored frozen (-20ºC) for research purposes. Prior to each trial, 10 striploins were thawed (4±1°C) for 24 h and then cut to 30.48 cm. On the day of the trial, subprimals were randomly assigned to one of the five treatment groups: 1) levulinic acid (5.0%) + sodium dodecyl sulfate (0.50%) (LVA+SDS), 2) peroxyacetic acid (2000 ppm; PAA), 3) acidified sodium chlorite (1200 ppm; ASC), 4) lactic acid (4.5%; LA), and 5) no intervention (CON).

Culture preparation and application

Frozen non-pathogenic surrogate STEC strains (BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431) approved for plant use by the USDA were obtained from the American Type Culture Collection (ATCC; Manassas, VA). Each strain was adapted to rifampicin (Fisher Scientific, Fair Lawn, NJ) in order to obtain rifampicin-resistant strains. Before each replicate, all five strains were grown in separate 10 mL tryptic soy broth (TSB, Becton Dickinson and Company, Sparks, MD) with 0.1 mg/mL of rifampicin and incubated for 24 h at 37ºC in incubation. To prepare the cocktail, 20 mL of each culture was harvested by centrifugation (5488 x *g* for 10 min) and mixed with 450 mL of sterile peptone water (PW; 0.1%) to obtain a final cell count of ca. 8.0 log CFU/mL. From each treatment group, one striploin was selected and inoculated. The anterior portion of each selected striploin was inoculated across (10.16 cm band) the lean side. The inoculation was done using a foam paint brush (Project Source, Scottsdale, AZ). The inoculated striploins were left undisturbed for 15 min at 4ºC to achieve attachment of the inoculum before subsequent antimicrobial treatment application (except for CON) and blade tenderization. For each treatment group, the inoculated striploin was passed lean side up, anterior end first through the spray cabinet, followed by blade tenderization. The second uninoculated striploin immediately followed the first striploin through the spray cabinet and blade tenderizer.

Antimicrobial preparation

All antimicrobial treatments were freshly prepared on the day of trials immediately prior to use. To prepare the levulinic acid plus sodium dodecyl sulfate, 98% levulinic acid (CAS #123-76-2, Acros Organics, Morris, NJ) and 95% sodium dodecyl sulfate (CAS #151-21-3, Sigma Aldrich, St. Louis, MO) were measured to meet the aforementioned

concentration and mixed in a 2000 mL glass media bottle. Immediately before use, the weighted sample of levulinic acid and sodium dodecyl sulfate was added to DI water in the automatic spray tank and brought to volume to create the desired concentration of solution. Concentrated peroxyacetic acid (21%; Microtox ultra, Zee Company, Chattanooga, TN) was prepared to a final concentration of 2000 ppm (PAA; pH 4.7) with DI water (v/v). Acidified sodium chlorite (1200 ppm) was prepared by mixing weighed amounts of sodium chlorite (CAS # 7758-19-2) and citric acid (CAS # 77-92-9, ASC reagent) from Sigma-Aldrich (St. Louis, MO) with DI water immediately before use. A 4.5% lactic acid solution was prepared by diluting a measured amount of 88% concentrate lactic acid (Birko Company, Henderson, CO) in a glass media bottle before being transferring to the automated spray tank and brought to volume with DI water.

Treatment application

All antimicrobial treatments were applied to subprimals within their respective groups using an automated six-nozzle sanitizing cabinet (Chad Co., Olathe, KS). The automatic premixed spray treated all sides of the subprimals with nozzles located above and below the subprimal at a flow rate of 0.42 liters/nozzle per min^{-1} and a pressure of ~ 275.79 kPa. Between each treatment, the spray cabinet tank and the spray cabinet were thoroughly rinsed and flushed with hot potable water (82°C) for 2 min and allowed to cool to room temperature (4±2°C) before proceeding with the next treatment. After each treatment application, the subprimals were transferred to the conveyor belt (1.0 m/min) of the blade tenderizer (model TC700MC, Ross manufacturing, Midland, VA) where they made a single pass, lean side up. The tenderizer blade head consists of seven alternating angled rows containing 32 perpendicular blades (3 mm wide) set 10 mm apart between

rows and columns. The blade tenderizer was also cleaned thoroughly between each treatment groups.

Microbial sampling

Surface and subsurface samples were collected at three locations from each striploin (Figure 4.1) following blade tenderization. A sterile knife was used to remove the entire top surface area of each striploin. Removal of the surface was performed by placing the striploin in a sterilized cutting jig and cutting from the posterior to the anterior end of each striploin. Following removal of the surface, samples were collected every 10.16 cm. This resulted in a 2.54 cm wide cut across the striploin from three different locations: the anterior, middle and posterior ends. All samples were collected beginning from the posterior end going towards the anterior end, and the knife was sterilized between each cut. Following the removal of the surface area, subsurface samples were collected for translocation measurements. The striploins were transferred aseptically to a clean surface and the lean side was placed face down. Afterwards, a sterile knife was used to cut samples (2.54 cm) every 10.16 cm to align with surface samples. Again, all samples were collected starting from the posterior end moving towards the anterior end, and the knife was sterilized between each cut. In addition, sponge samples were collected from the blades and plate of the blade tenderizer after passing the second striploin through. The sponges were hydrated using buffered peptone water with 0.1% rifampicin added.

All collected samples were stored on ice (0±1°C) in a styrofoam cooler until taken to the microbiology lab. The samples were stored overnight at 0±1°C. Following overnight storage, individual samples were minced and blended (Cuisinart, East Windsor, NJ). After blending, 25 g of meat was weighed and placed in a stomacher bag with 90 mL of 0.1% peptone water supplemented with rifampicin (PW^R), placed in a stomacher, and mixed for

2 min. Serial dilutions (1:10) were made for all samples (including sponge samples) using 9 mL of 0.1% PW^{R} and plated in duplicate onto APC Petrifilm (3M, Saint Paul, MN) following the manufacturer's instructions. Petrifilm plates were then incubated at 37ºC for 48h, followed by enumeration.

Statistical analysis

Data was analyzed using Proc Mixed (SAS Inst., v.9.4; Cary, NC) as a completely randomized split-plot design were subprimal was the whole-plot and the sectional cuts within the subprimal was the sub-plot. Subprimal identification within replication by treatment was considered as the random variable. Subprimal was the experimental unit and the three sectional cuts were considered the observational units. The microbial counts for surface and translocation were log transformed and then analyzed for the main effects of antimicrobial treatment, location, and their interactions. Differences were considered significant at $\alpha \leq 0.05$.

Results and Discussion

There was not an antimicrobial treatment by sample location interaction ($P > 0.05$). Therefore, only the main effects of antimicrobial treatment and sample location are presented. The Least squares means of the surrogate *E. coli* recovered (log CFU/g) after antimicrobial intervention and blade tenderization for the surface and subsurface samples are presented in Table 4.1. For surface and subsurface samples, the recovered populations of surrogate *E. coli* were different ($P \leq 0.05$) among the antimicrobial treatments groups. Among the antimicrobial treatments used in this experiment, PAA was the most effective in reducing surrogate *E. coli* ($P \leq 0.05$) for both surface (1.79 log CFU/g) and subsurface (1.56 log CFU/g) when compared to the other chemical antimicrobial treatments and the CON group. Levulinic acid and ASC caused reductions that were lower ($P \leq 0.05$) than

CON on the surface (1.00 and 0.58 log/CFU/g, respectively). Lactic acid resulted in reductions that were similar ($P > 0.05$) to the CON. For the subsurface samples, again, compared to the other treatment groups, PAA had the lowest ($P \leq 0.05$; 1.80 log CFU/g) amount of recovered surrogate *E. coli* that was translocated to the interior of the muscle. Both LA and LVA+SDS had counts (3.04 and 2.94 log CFU/g, respectively) that were similar ($P > 0.05$) to CON (3.36 log CFU/g). Acidified sodium chlorite had counts (2.78 log CFU/g) that were similar ($P > 0.05$) to LA and LVA+SDS but lower than ($P \leq 0.05$) CON.

The main focus of the present study was to evaluate the efficacy of the aforementioned antimicrobials in reducing surrogate *E. coli* populations on inoculated beef striploins prior to blade tenderization. As stated previously, the use of peroxyacetic acid at the concentration of 2000 ppm was the most effective in reducing STEC surrogates on beef striploins. Several experiments conducted by King et al. (2005) showed that applying peroxyacetic acid at low concentrations, such as 200-600 ppm, on chilled beef surfaces had minimal effect on inoculated *E. coli* O157:H7 when applied at varying temperatures. However, when 1000 ppm was applied at 55°C, reductions were similar to the current study at and 1.70 log CFU/cm^2. Liao et al. (2015), using 200 ppm of peroxyacetic acid (25°C), observed only a 0.31 and 0.37 log CFU/cm^2 reduction of non-O157:H7 and O157:H7 STEC, respectively, that was inoculated on blade tenderized beef subprimals. The low reductions reported by Liao et al. (2015) could be explained by the concentration of peroxyacetic acid used in the study. The use of levulinic acid plus sodium dodecyl is currently being tested for use in the US meat industry. The US Food and Drug Administration (FDA) has designated levulinic acid (21 CFR, 172.515; US FDA, 2018)

and SDS (21 CFR 172.822; US FDA, 2018a) as being generally recognized as safe for specific uses in food. Zhao et al., (2014), treated cold (4°C) beef trim with of 0.5% levulinic acid and 0.05% sodium dodecyl sulfate at 21°C for 30 s and achieved reductions of 1.0 log CFU/cm^2 for *E. coli* O157:H7. These reductions are similar to the reductions achieved in the current study. However, Zhao et al. (2014) reported that LVA+SDS is temperature dependent. Therefore, at cooler temperatures it took a greater concentration to achieve similar results. In addition to its effectiveness with surrogate *E. coli*, previous research has shown that LVA+SDS was effective in reducing and suppressing the growth of spoilage bacteria on blade tenderized striploins without causing negative impacts on quality or sensory characteristics (Chapter 3). Acidified sodium chlorite is approved for use in the meat industry at concentration between 500-1200 ppm (USDA-FSIS, 2017). The current study utilized 1200 ppm, and at this concentration minimal reductions (0.60 log CFU/g) was achieved that were similar to the non-treated group. Muriana et al. (2019) reported similar reductions (~0.50 - 0.75 log CFU/cm^2) on surface cold beef surfaces that were inoculated with *E. coli* O157:H7. Of all the chemical antimicrobial interventions used in the current study, lactic acid was the least effective in reducing surrogate *E. coli*. Only a 0.40 log CFU/g reduction was observed, which is similar to the surface reductions of 0.50 and 0.48 log CFU/cm^2 for non-O157:H7 STEC and *E. coli* O157:H7, respectively, reported by Liao et al. (2015) after using a 5% lactic acid. Heller et al. (2007) reported a slightly greater (1.1 log CFU/cm^2) reductions on the surface of blade tenderized beef subprimals. However, Heller et al. (2007) applied 5% lactic acid at 55°C, which was higher than the 20°C used in the current study. The difference in temperature account for the differences in reductions observed between their study and the current one.

Differences ($P \leq 0.05$) were found among the sample locations (Figure 4.2) for both the surface and subsurface samples. With locations (L), samples taken from the surface of inoculated striploin were labelled L1, L2, and L3, representing the anterior, middle, posterior (illustrated in Figure 4.1), while samples taken from the surface of the noninoculated striploin was designated L4, L5, and L6 (anterior to posterior). For the surface, L1 (the first 2.54 cm) recovered populations of surrogate *E. coli* were greater ($P \leq 0.05$) than the other samples collected thereafter. All the L1 surface samples were collected from the inoculated area, and it was expected that these samples would have greater recovery of *E. coli* than the other surface locations. The samples collected from the inoculated striploin decreased ($P \leq 0.05$) from location 1 to 3 for the surface samples. The cross-contamination from the first striploin to the second showed that similar ($P > 0.05$) amounts of surrogate *E. coli* were transferred from the end of the first striploin (L3) to the second striploin (L4). Lower counts were observed for L5 and L6 surface samples than in L4. However, L5 and L6 were similar ($P > 0.05$) to each other. The subsurface samples followed a similar pattern as the surface samples going from the anterior to posterior ends, showing recovered populations of *E. coli* that decreased ($P \leq 0.05$) with each location except for L6, where the counts were greater ($P \leq 0.05$) than L5. A previous study conducted by Johns et al. (2011) examining the translocation of surface *E. coli* from 1 whole inoculated striploin to 5 other non-inoculated striploins showed that the population of *E. coli* for striploin 1 (the inoculated striploin) was more than 1 log CFU/mL higher than that of the other striploins. Loin 2 did not differ from loin 3 but had a higher population than did loins 4, 5, and 6. The populations on loins 3, 4, 5, and 6 did not differ from each other in their study. In the current study, only one non-inoculated striploin was passed

through the blade tenderizer after the inoculated striploins. As there was only a 10.16 cm inoculated area, we did observe similar results were L1 was more than 1 log CFU/g higher than all other locations for both the surface and subsurface samples.

The results from the swab samples taken from the blade tenderizer after each treatment (Table 4.2) showed significant amounts of surrogate STEC remaining on the plastic plate and blades of the blade tenderizer (Figure 3). On the plastic plate, the recovered populations of surrogate *E. coli* were 4.01, 3.31, 3.08, 3.42 and 1.40 log CFU/cm^2 for CON, LA, ASC, LVA+SDS, and PAA, respectively. As for the blades, the recovered populations of STEC surrogates were 3.31, 2.76, 2.50, 2.91, and 0.97 log CFU/cm^2 for CON, LA, ASC, LVA+SDS, and PAA, respectively. The recovered bacteria on the plate head and blades of the blade tenderizer were the same for all treatment groups, except for PAA. This reiterates findings from previous studies (Thippareddi et al., 2000; Gill & McGinnis, 2004; Luchansky et al., 2008; Yoon et al., 2009; Luchansky et al., 2012) that showed that the blade tenderized is a potential vehicle for the translocation of bacteria in blade tenderizer products. As such, processors should pay significant attention to making sure that equipment is properly cleaned and sanitized in order to prevent the translocation and internalization of potential pathogens.

Conclusion

The benefits of blade tenderization in improving meat tenderness is well documented. However, when blade tenderizing there is an inherent risk of transferring surface pathogens into the interior of the meat. This requires processors who commonly blade tenderize to follow good manufacturing practices and apply antimicrobial interventions to reduce surface pathogens prior to blade tenderization. Results from this

study show that due to the reduced efficacy of antimicrobials on cold meat surfaces, higher levels of peroxyacetic acid than what are commonly reported might be necessary to ensure the lethality of surface pathogens. In addition, results from this study show that LVA+SDS could be a suitable substitute to lactic acid, which is commonly used in the beef industry, today.

Acknowledgements

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Table 4.1. Least squares means (± SE) of surrogate *E. coli* recovered from surface and sub-surface of inoculated beef striploin.

Treatment Group[1]	Recovered population of STEC (log CFU/g)	
	Surface	Sub-surface
CON	4.54 ± 0.18^{a}	3.36 ± 0.15^{a}
LA	4.14 ± 0.18^{ab}	3.04 ± 0.15^{ab}
ASC	3.96 ± 0.18^{bc}	2.77 ± 0.15^{b}
LVA+SDS	3.56 ± 0.18^{c}	2.90 ± 0.15^{ab}
PAA	2.75 ± 0.18^{d}	1.80 ± 0.15^{c}

[a-d] Least squares means within columns that do not share a common letter are different ($P \leq 0.05$).

[1] Antimicrobial intervention: CON = control, blade tenderization only; LA = 4.5% lactic acid; ASC = acidified sodium chlorite (1200 ppm); LVA+SDS = 5% levulinic acid plus 0.5% sodium dodecyl sulfate; PAA= peroxyacetic acid (2000 ppm).

Table 4.2. Least squares means (± SE) of recovered rifampicin resistant surrogate *E. coli* recovered from the blade tenderizer after each treatment.

Treatment[1]	Location on the Blade Tenderizer (log CFU/cm^2)	
	Plastic plate	Blades
CON	4.01 ± 0.34[a]	3.32 ± 0.35[a]
LA	3.31 ± 0.41[a]	2.76 ± 0.42[a]
ASC	3.08 ± 0.34[a]	2.50 ± 0.35[a]
LVA+SDS	3.42 ± 0.34[a]	2.90 ± 0.35[a]
PAA	1.40 ± 0.34[b]	0.97 ± 0.35[b]

[1] Antimicrobial intervention: CON = control, blade tenderization only; LA = 4.5% lactic acid; ASC = acidified sodium chlorite (1200 ppm); LVA+SDS = 5% levulinic acid plus 0.5% sodium dodecyl sulfate; PAA= peroxyacetic acid (2000 ppm).
[a-b] Least squares means within columns that do not share a common letter are different ($P \leq 0.05$).

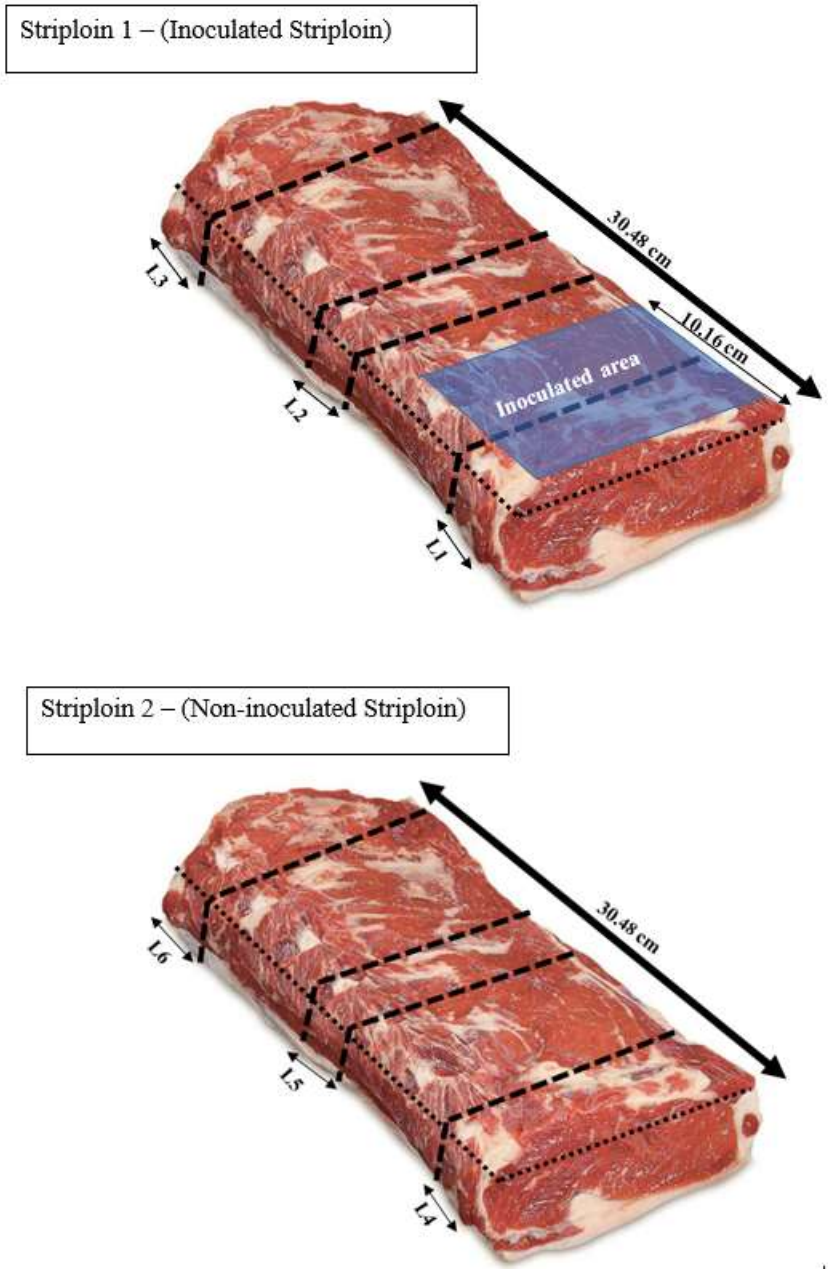

Figure 4.1. Diagram illustrating the; inoculated region (10.16 cm), the 2.54 cm sections that was removed from the anterior (L1), middle (L2), and posterior (L3) ends of striploin 1, and the anterior (L4), middle (L5), and posterior (L6) ends of striploin 2. Beef striploin (NAMP #180) used with permission from Canada beef (Available at: http://elearn.canadabeef.ca/carcass/loin).

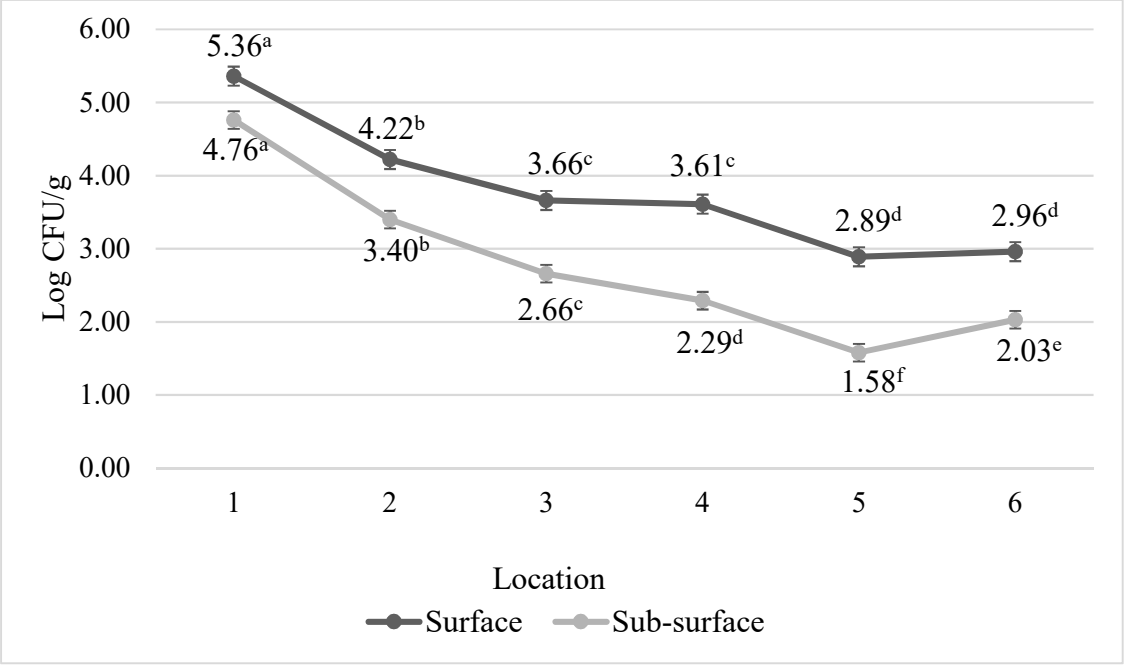

Figure 4.2. Least squares means (± SE) of the surrogate *E. coli* populations recovered from surface and sub-surface samples from beef striploins that were subjected to antimicrobial treatment and blade tenderization. Means among surface and subsurface sample that do not share a common letter are different ($P \leq 0.05$). Antimicrobial intervention: CON = control; blade tenderization only; LA = 4.5% lactic acid; ASC = acidified sodium chlorite (1200 ppm); LVA+SDS = 5% levulinic acid plus 0.5% sodium dodecyl sulfate; PAA= peroxyacetic acid (2000 ppm).

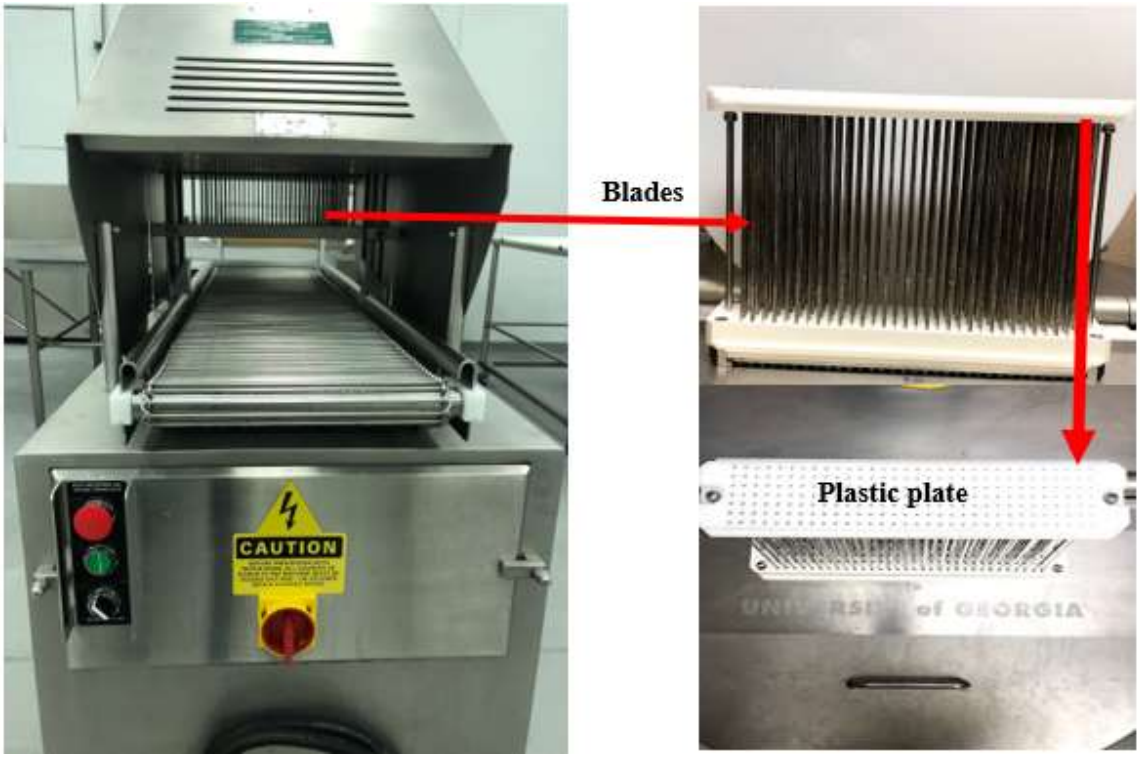

Figure 4.3. Pictures of the plastic plate and blades that were sampled after running the treated beef striploins through the blade tenderizer.

CHAPTER 5

VALIDATION OF ANTIMICROBIAL INTERVENTIONS FOR REDUCING SHIGA TOXIN–PRODUCING *ESCHERICHIA COLI* SURROGATE POPULATIONS DURING GOAT SLAUGHTER AND CARCASS CHILLING[1]

[1] Thomas, C.L., A.M. Stelzleni, A.G. Rincon, S. Kumar, M. Rigdon, R.W. McKee, and H. Thippareddi. 2019. *Journal of Food Protection*. 82:364-370. doi: 10.4315/0362-028X.JFP-18-298. Reprinted here with permission of the publisher.

Abstract

Demand and consumption of goat meat is increasing in the United States due to an increase in ethnic populations that prefer goat meat. As ruminant animals, goats are known reservoirs for Shiga toxin producing *Escherichia coli* (STEC) and proper handling, especially during slaughter, is imperative to reduce the likelihood of carcass and meat contamination. However, the majority of antimicrobial intervention studies during the slaughter of ruminant species have focused on beef, highlighting the need for validation studies targeting small ruminants, such as goats, during slaughter and chilling procedures. The objective of this research was to evaluate 4.5% lactic acid (LA; pH 2.1), peroxyacetic acid (PAA; 400 ppm; pH 4.7), a hydrochloric and citric acid blend (Citrilow [CL]; pH 1.2), 5% levulinic acid plus 0.5% sodium dodecyl sulfate (LVA+SDS; pH 2.60), and a nontreated control (CON) for their efficacy in reducing STEC surrogates and their effect on carcass color from slaughter through 24-h chill. Fifteen goat carcasses across three replicates were inoculated with a five-strain cocktail (ca. 5 log CFU/cm2 attachment), containing rifampin-resistant surrogate *E. coli* (BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431) and were randomly assigned to an antimicrobial treatment. Antimicrobials were applied prechill and 24 h post-chill. Mean log reductions achieved after prechill treatment with LA, PAA, CL, and LVA+SDS were 2.00, 1.86, 2.26, and 1.90 log CFU/cm2, respectively. Antimicrobial treatment after the 24-h chilling, resulted in additional reductions of surrogate *E. coli* by 0.99, 1.03, 1.94, and 0.47 log CFU/cm2 for LA, PAA, CL, and LVA+SDS, respectively. Antimicrobial treatments did not impact goat carcass objective color (L* and a*), except for b*. The antimicrobials tested in this study

were able to effectively reduce surrogate STEC populations during slaughter and subsequent chilling without compromising carcass color.

Key words: Antimicrobial intervention; Color*; Escherichia coli*; Goat; Surrogate

Introduction

Within the past two decades, goat meat consumption in the United States has increased as the number of consumers including goat meat in their diet has grown by over 100% (Solaiman, 2007). The increase in goat meat consumption has led the United States to become a net importer of goat meat. In the United States, goats slaughtered in federally inspected plants, as well as goat meat imported from other parts of the world (primarily Australia and New Zealand), have increased since 1999 (Solaiman, 2007). Although goats are rarely the primary animal production enterprise in the United States, they are important contributors to the income of many producers (Glimp, 1995) and present new marketing opportunities for the small farmer or rancher (USDA, 2013).

The number of foodborne illness outbreaks associated with meat from small ruminants is low; however, all meat and meat products, especially those from ruminants, are possible vectors for zoonotic pathogens. Therefore, as goat meat production and consumption increases, the need to address microbial safety of the product should be considered. Recent Shiga toxin–producing *Escherichia coli* (STEC) illnesses associated with small ruminant contact at livestock exhibitions serve as an important reminder that these animals are a source of STEC (CDC, 2012). Additionally, consumption of goat products has recently been associated with human STEC infections (Jacob et al., 2013). Although small ruminants are known reservoirs for STEC, the epidemiology and ecology of the organisms are not extensively studied in these species (Jacob et al., 2013), highlighting the need for validated antimicrobial intervention strategies during small ruminant slaughter.

Within the meat industry, focus has been placed on finding various decontamination techniques to reduce or eliminate bacteria that are human pathogens, as well as those that may cause meat spoilage (Huffman, 2002; Sofos, 2005). Most studies have focused on the postharvest and prechill phase to prevent bacteria from firmly attaching to the meat surface. However, even after postharvest application of antimicrobials, surviving bacterial populations may continue to grow if the product is temperature abused or because of slow chilling of the carcass surfaces (Pitman et al., 2012). Therefore, there is a need to identify post-chilling interventions that will further reduce microbial numbers on meat products prior to fabrication (King et al., 2005).

Organic acids, such as acetic, citric, and lactic acids (LA), are some of the more widely studied antimicrobial agents (Hamby et al., 1987; Dorsa, Cutter, & Siragusa, 1997; Belk, 2001). Numerous studies have validated the efficacy of LA as an antimicrobial for reduction of pathogenic bacteria on beef carcasses (Hamby et al., 1987; Hardin et al., 1995; Dorsa, Cutter, & Siragusa, 1997; Castillo et al., 1998b; Castillo et al., 2001; King et al., 2005; Koohmaraie et al., 2005). Peroxyacetic acid (PAA) has also been proven to be an effective antimicrobial for the decontamination of spoilage and pathogenic bacteria in the meat and poultry industries (Farrell, Ronner, & Wong, 1998; Gill & Badoni, 2004; King et al., 2005; Koohmaraie et al., 2005), and a combination of citric and hydrochloric acids in water has been validated by Beers, Cook, & Coleman (2011) and Cook, Beer, & Coleman (2011). Additionally, levulinic acid (LVA), in combination with the surfactant sodium dodecyl sulfate (SDS) has shown promise as a potential antimicrobial agent for various foods (Zhao, Zhao, & Doyle, 2009; Zhao et al., 2014). Both LVA and SDS have been

recognized by the U.S. Food and Drug Administration as safe for direct addition to food as a flavoring substance or adjunct (US FDA, 2016; US FDA, 2016b).

Currently, there is a lack of research on validated antimicrobial treatments effective in reducing STEC on goat carcasses pre- and post-chill. Therefore, the objectives of this study were to characterize the reduction of surrogate STECs and the effect on carcass color when LA, PAA, Citrilow (CL), and LVA plus SDS (LVA+SDS) were used both pre- and post-carcass chilling on goat carcasses.

Material and Methods

Animal handling and slaughter

All experimental procedures were reviewed and approved by the University of Georgia Institutional Animal Care and Use Committee. Fifteen goats of similar age (approximately 1 year, 28 ± 6 kg) across three replicates (5 goats per replicate) were purchased and transported (ca. 31 km) to the University of Georgia Meat Science and Technology Center, 12 to 16 h prior to slaughter. Goats were housed in holding pens, withheld from feed, but had *ad libitum* access to fresh water. On the day of slaughter, animals were randomly assigned to an order and slaughtered following federal guidelines.

Bacterial strains

Frozen rifampicin-resistant, non-pathogenic surrogate *E. coli* strains (BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431; American Type Culture Collection, Manassas, VA) approved for in-plant use by the U.S. Department of Agriculture (USDA) were used. Each surrogate strain was thawed and revived by placing 10 µL into 10 mL of sterile tryptic soy broth (TSB; Remel, Lenexa, KS) containing 0.1 mg/mL rifampin (Fisher Scientific, Fair Lawn, NJ) and incubated at 37°C for 24 h. All five strains were grown in

separate 10 mL of TSB (four tubes per strain) with 0.1 mg/mL rifampicin. Rifampicin resistance was confirmed by streaking cultures onto Trypticase soy agar rifampin plates (BD, Sparks, MD) and incubated for 24 h at 37°C. Rifampicin-resistant colonies were then used for further propagation and inoculated into TSB and incubated for 24 h at 37°C. Cultures were maintained weekly by transferring colonies from each strain into fresh TSB and incubating at 37°C for 24 h. Fresh STEC surrogate inoculum was prepared prior to each slaughter day. To prepare the inoculum for trials, 40 mL of each culture was harvested by centrifugation (2,410 x *g* for 15 min; CR 312, Jouan Inc., Winchester, VA), washed twice with peptone water (PW), and suspended in sterile PW (0.1%) to obtain a final cell concentration of ca. 8.0 log CFU/mL. Bacterial concentrations were confirmed by plating 1-mL portions of appropriately diluted culture on aerobic plate count Petrifilm (3M, St. Paul, MN) and incubating at 37°C for 48 h.

Antimicrobial preparation.

All antimicrobial treatments were prepared immediately prior to use. Levulinic acid (5%, v/v) plus SDS (0.5%, w/v; pH 2.6) was prepared by dissolving the appropriate amount of SDS (CAS 151-21-3, Sigma Aldrich, St. Louis, MO) into LVA (CAS 123-76-2, Acros Organics, Morris, NJ) in a 50-mL beaker, and the solution brought to volume in a 2,000-mL glass media bottle. The LA solution (4.5%, pH 2.1) was prepared by diluting 88% concentrated LA (v/v; Birko Company, Henderson, CO) with water in a 2,000-mL glass media bottle and bringing it to volume. Concentrated PAA acid (16%; Peragonn, Safe Foods Corporation, North Little Rock, AR) was prepared with a final concentration of 400 ppm (pH 4.7) with water (v/v) following the LaMotte hydrogen peroxide and peracetic acid kit (CAS 7191-02, LaMotte Company, Chestertown, MD). A commercial blend of

hydrochloric and citric acid (20%; CL, Safe Foods Corporation) was prepared by diluting a measured amount with water (v/v) according to manufacturer's instructions to obtain a final pH of 1.2.

Carcass treatment

After hide removal and evisceration, the exterior of each carcass was evenly inoculated to achieve ca. 5 log CFU/cm^2 attachment with the five-strain rifampicin-resistant cocktail of surrogate *E. coli* by using a foam paint brush (Project Source, Scottsdale, AZ). After inoculation, the carcasses were held on the slaughter line for 30 min (25ºC) for attachment before subsequent water washing and antimicrobial treatment application. The following antimicrobial treatments were randomly assigned to each carcass on trial day: (i) LVA+SDS, (ii) LA, (iii) PAA, (iv) CL, and (v) nontreated control (CON; no antimicrobial treatment). After the 30-min inoculum attachment, each carcass was washed with warm water (55°C; 551.58 kPa; 1 L/min) for 1 min. After water washing, each carcass was allowed to drip for 5 min before the assigned antimicrobial was applied. For all treatments, except CON, the assigned antimicrobial spray (25°C; 172.37 kPa ; 380 mL/min) was applied evenly over the entire carcass using a low pressure hand-held fan spray nozzle (0.635 cm; DeWalt Manufacturing Company, Baltimore, MD) and allowed to drip for 5 min at ambient temperature (25°C) before being placed in the carcass cooler (0 ± 1°C) for 24 h. Following the 24-h chilling, a second antimicrobial spray was applied to each carcass, congruent with the previous treatment application.

Microbial sampling

Each carcass (except CON) was sampled at five different points during processing: (i) after inoculation and a 30-min attachment, (ii) 5 min after the standard water wash, (iii)

5 min after the prechill carcass antimicrobial spray application (not for CON), (iv) after 24-h chilling, and (v) 5 min after the 24-h chill carcass antimicrobial spray application (not for CON) to determine surrogate *E. coli* bacteria population counts. For each sampling time point, three surface samples were excised from one of five randomly assigned anatomical locations from each side of the carcass (Figure 1). An anatomical location was only sampled at one time point for each carcass side. A sterilized, stainless steel metal coring device (2.54 cm in diameter) was used to create an impression on the matched anatomical surface locations on each carcass. The marked surface area was then aseptically excised to a depth of approximately 0.2 cm using a sterile scalpel. Within sampling time point, the cores from both carcass sides were pooled, placed in sterile bags, and kept on ice until further analysis.

The samples were transferred to a stomacher bag containing 90 mL PW supplemented with 0.1 mg/mL rifampicin (PW^R) and stomached (Neutec Group Inc., Barcelona, Spain) for 1 min. The suspension was serially diluted (1:10) in PW^R, and 1.0 mL of each dilution was plated in duplicate onto aerobic plate count Petrifilm following manufacturer's instructions. Petrifilm plates were incubated at 37ºC for 48 h and enumerated.

Color evaluation

Objective CIE L*, a*, and b* color of each carcass was measured (Hunter-Lab MiniScan XE, Hunter Associates Laboratory, Reston, WV) using illuminant A with a 10º viewing angle and standardized using white and black tile standards before each use to determine the effects of antimicrobial treatment on carcass color. Three objective color readings were recorded and averaged. Hue angle [$\tan^{-1}$ (b^*/a^*)], chroma [$(a^{*2} + b^{*2})^{0.5}$],

and ΔE $\{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}\}$ were subsequently calculated according to American Meat Science Association Color measurement guidelines (AMSA, 2012). Color readings were taken from both sides of each carcass posterior to the hipbone on a smooth surface that was not sampled for microbial populations (Figure 5.1). Carcass color was measured prior to antimicrobial treatment, after antimicrobial treatment, 1 h post-chill, 24 h postchill, and after 24 h antimicrobial treatment.

Statistical analysis

Microbial counts and color values were analyzed by using general linear models procedure (v.9.4, SAS Institute Inc., Cary, NC), with sample time point as a repeated measure. Microbial counts were transformed into log CFU per square centimeter and analyzed for the main effects of antimicrobial treatment, sampling points, and their interaction. Color values were also analyzed for the main effects of antimicrobial treatment, sampling points, and their interaction. If an interaction was detected, the means were separated and presented within time point and antimicrobial treatment. Means were separated using least-squares means and the PDIFF option. Mean differences were considered significant at $\alpha \leq 0.05$.

Results and Discussion

Antimicrobial effectiveness. The least-squares means for log CFU per square centimeter counts of surrogate *E. coli* recovered after inoculation, water wash, antimicrobial application, 24-h chilling, and a post 24-h chill antimicrobial application are presented in Table 5.1. After inoculation, the average population of surrogate *E. coli* recovered was 5.15 ± 0.24 CFU/cm^2 and was similar ($P > 0.05$) among all treatments. After washing the carcasses with water (55°C; 551.58 kPa; for 1 min) the average reduction for

all treatment groups was 0.62 log CFU/cm^2. The reduction of surrogate *E. coli* population after washing with warm water was similar ($P > 0.05$) for all treatments. The results from the current study indicate that warm water washing followed by carcass chilling procedures, was not effective in reducing surrogate *E. coli* populations, while others have indicated that the use of hot water has been effective (Smith, 1992; Gorman et al., 1997; Castillo et al., 1998a). Reductions from the current study were less than those reported by Sevart et al. (2016) despite similar water temperature conditions (50°C) and application volume (0.48 versus 0.55 MPa) applied to veal carcasses. Differences in microbial reduction between the two studies could be owing to different application methods, such as the distance from the carcass surface, and differences in carcass tissue composition (veal versus goat). Greater *E. coli* O157:H7 reductions were also reported by Castillo et al. (1998) on beef primal cuts from hot carcasses, using lower temperature water (35ºC) in a two-step procedure, including a low pressure manual wash for 90 s, followed by a high pressure automated spray cabinet wash for 9 s. King et al. (2005), following methods similar to Castillo et al. (1998), also reported a greater reduction of *E. coli* Type I and *E. coli* O157:H7 on inoculated beef primals than what was observed in the current study. In the latter study, the high-pressure wash was likely to have a greater impact on microbial reductions observed than the temperature of the water.

The prechill carcass antimicrobial applications resulted in surrogate *E. coli* reductions ($P \leq 0.05$) of 1.90, 2.00, 1.86, and 2.26 log CFU/cm^2 for LVA+SDS, LA, PAA, and CL, respectively, and resulted in all treated carcasses having lower counts ($P \leq 0.05$) than CON carcasses. Carcasses treated with LA and LVA+SDS showed additional reduction (1.75 and 0.88 log CFU/cm^2, respectively) subsequent to 24-h of chilling ($P \leq$

0.05). Additional reductions achieved during the chilling were probably due to the residual acid effect, especially for the LA-treated group. After the 24-h chilling, carcasses treated with PAA and CL prior to chilling had a minimal increase (0.47 and 0.79 log CFU/cm^2, respectively) in surrogate *E. coli* counts ($P > 0.05$). Also, the CON carcasses had minimal reduction (0.25 log CFU/cm^2) after 24-h chilling and surrogate *E. coli* population was similar ($P > 0.05$) to that of the prechill, postwash carcasses. A second antimicrobial application to the chilled carcasses resulted in further reductions ($P \leq 0.05$) for LA (0.99 log CFU/cm^2), PAA (1.03 log CFU/cm^2), and CL (1.94 log CFU/cm^2). Chilled carcasses treated with a second application of LVA+SDS had an additional reduction of 0.47 log CFU/cm^2 but were similar ($P > 0.05$) to that of the 24-h postchill. Considering the entire process (warm water wash through postchill antimicrobial treatment), the surrogate *E. coli* population was reduced ($P \leq 0.05$) on goat carcasses by 5.15, 3.90, 3.74, and 3.15 log CFU/cm^2 for LA, CL, LVA+SDS, and PAA, respectively, compared to the 1.21-log CFU/cm^2 reduction from the CON carcass that was only washed with warm water and chilled for 24 h.

As stated previously, all antimicrobials utilized in this study effectively reduced surrogate STEC population on goat carcasses via application at prechill or postchill or both stages. Although the antimicrobial LVA+SDS was able to result in a 1.90 log CFU/cm^2 reduction prechill, there was only a 0.47-log CFU/cm^2 reduction after a postchill application. Zhao et al. (2014) reported that the reduction of STEC on beef trim was directly related to the surface temperature of the treated beef trim, which may explain the decreased efficacy of LVA+SDS on surrogate *E. coli* post-chill versus pre-chill. Compared to the other antimicrobials used in the current study, application of LA resulted in the

greater reductions (5.15 log CFU/cm^2; $P \leq 0.05$) in surrogate *E. coli* populations. In a similar study, carried out on veal carcasses, Sevart et al. (2016) reported only a 0.60- and 0.05-log CFU/cm^2 reduction after applying a 4.5% lactic acid (20°C) spray prechill, and 24-h chill, respectively. Notably, Sevart et al (2016) reported an increase (1.20 to 1.60 log CFU/cm^2) in the surrogate *E. coli* population after a postchill antimicrobial application. Because the concentration, temperature, and the volume of the antimicrobial applied were similar in both studies, the differences in microbial reduction could be owing to the application method of the antimicrobial, such as distance from carcasses or differences in carcass tissue composition. On the basis of results obtained in the current study, LA was able to further reduce surrogate *E. coli* population during chilling and was also effective as a postchill intervention.

Until recently, the maximum allowed concentration of peroxyacetic acid for use as an antimicrobial for surface application in the meat industry was 200 ppm but has since been increased to 400 ppm for washing, rinsing, cooling, or otherwise processing of fresh beef carcasses (Wheeler, Kalchayanand, & Bosilevac, 2014). Results from this study suggest that PAA, although able to reduce STEC population on carcasses prior to chilling, did not show continued efficacy during chilling. However, a second application of PAA elicited further reductions (1.03 log CFU/cm^2; $P \leq 0.05$) of surrogate *E. coli*, such that final counts were similar to that of LVA+SDS and CL. Penney et al. (2007) evaluated the efficacy of a peroxyacetic acid formulation (180 ppm; 20ºC) at reducing *E. coli* O157:H7 contamination on external carcass surfaces of hot-boned beef and veal flaps. Peroxyacetic acid treatment resulted in a substantial reduction of 3.56 log CFU/cm^2 on veal and 3.59 log CFU/cm^2 on beef. These reductions were greater than the 1.86-log CFU/cm^2 reduction

achieved in this study and may be because of the method of application. Penney et al. (2007) applied peroxyacetic acid formulations to hot-boned bobby calf and beef flaps (30 cm from the spray nozzle) using a spray cabinet. Greater reductions achieved by Penney et al. (2007) might be owing to the distance between the beef and veal flaps compared with the distance between the handheld spray nozzle and carcass, size difference, along with the carcass tissue composition. Citrilow, a hydrochloric acid and citric acid blend, can sustain a good pH range that is effective in controlling pathogenic bacterial growth (Pohlman et al., 2010). Contrary to the results observed in the current study, Sevart et al. (2016) reported only a 0.4-log CFU/cm^2 reduction on veal carcasses sprayed with CL (pH 1.2; 20ºC) on prerigor carcasses and no additional STEC surrogate population reductions after 24 h of chilling. Differences in findings may be attributed to the difference in experimental method. Beers et al. (2011) reported a 1.80 log CFU/cm^2 reduction of aerobic bacteria on sections of chilled beef brisket subprimals treated with CL at pH 1.5, which corresponds to the results found in the current study. Microbial counts for CL increased by 0.79 log CFU/cm^2 log after 24-h chill. On the basis of these results, it appears that CL is not effective in further reducing or suppressing surrogate *E. coli* growth during chilling. However, after a second antimicrobial application, CL was effective in eliciting further reductions of surrogate *E. coli* ($P \leq 0.05$; 1.94 log CFU/cm^2). Additionally, final counts were similar ($P > 0.05$) to LVA+SDS- and PAA-treated carcasses.

Effect of antimicrobials on color of goat carcass

There was no treatment by sample time interactions ($P > 0.05$) for objective color; thus, only main effects of treatment and sampling time are reported in Table 5.2 and 5.3, respectively. There were no significant differences ($P > 0.05$) observed among treatment

groups for L*, a*, hue, chroma, and ΔE values. However, differences ($P \leq 0.05$) were noticed for b* values among treatment groups. The b* values among the CON, LA, and PAA treatment groups were similar ($P > 0.05$); however, b* values of LVA+SDS and CL were lower ($P \leq 0.05$) than the CON group. Also, goat carcasses that were treated with CL had b* values that were lower ($P \leq 0.05$) compared with carcasses that were treated with LA. As expected, color recordings differed ($P \leq 0.05$) between sampling times (Table 5.3). Goat carcasses became darker ($P \leq 0.05$) during chilling. Similarly, as time progressed, there was a reduction ($P \leq 0.05$) in redness after prechill antimicrobial application, and it continued to decrease ($P \leq 0.05$) through chilling. Low b* values were recorded before chilling, but there was an increase ($P \leq 0.05$) in b* values 1 h and 24 h postchill. Hue values were similar ($P > 0.05$) for pretreatment and after prechill antimicrobial application, but increased ($P \leq 0.05$) hue values were observed after a post 1-h chilling. Chroma values decreased after the first antimicrobial application, but these values increased ($P \leq 0.05$) during chilling, indicating an increase in vividness. As expected, ΔE values, the combined changes in L*, a*, and b* values over a selected period of time (AMSA, 2012), increased ($P \leq 0.05$) over time.

Conclusion

Overall, the application of LA was more effective ($P \leq 0.05$) in reducing surrogate *E. coli* populations on goat carcasses compared to all other treatment groups. However, final *E. coli* populations were similar among carcasses sprayed with LVA+SDS, PAA, and CL solutions, resulting in a ≥ 3.15 log CFU/cm^2 reduction, and were significantly different from the CON carcasses. Lactic acid and LVA+SDS were able to suppress and further reduce surrogate *E. coli* populations during the chilling process unlike CL and PAA.

Nonetheless, CL and PAA were able to achieve ≥ 1-log reductions when applied to chilled carcasses, which implies these treatments would be effective if applied before fabrication. While LA caused greater reductions compared with the other antimicrobials, the results showed that all antimicrobials used in this study were able to effectively reduce surrogate STEC populations during slaughter and chilling without compromising carcass color.

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Table 5.1. Least squares means (± SD) for populations of rifampicin-resistant surrogate *E. coli* recovered from goat carcasses sprayed with various antimicrobial solutions[1].

Treatment[2]	Inoculation	Water wash[3]	Prechill treatment	24-h chill	Postchill treatment
CON[4]	5.23 ± 0.35^{ax}	4.27 ± 0.38^{bx}	4.27 ± 0.38^{bx}	4.02 ± 0.36^{bx}	4.02 ± 0.36^{bx}
LVA+SDS	5.05 ± 0.27^{ax}	4.56 ± 0.31^{ax}	2.66 ± 0.72^{by}	1.78 ± 0.52^{cy}	1.31 ± 0.45^{cy}
LA	5.21 ± 0.32^{ax}	4.80 ± 0.36^{ax}	2.80 ± 0.93^{by}	1.05 ± 1.21^{cy}	$< 0.47^{dz5}$
PAA	5.03 ± 0.17^{ax}	4.30 ± 0.19^{ax}	2.44 ± 0.40^{bcy}	2.91 ± 78^{bz}	1.88 ± 0.70^{cy}
CL	5.21 ± 0.14^{ax}	4.72 ± 0.42^{ax}	2.46 ± 0.96^{by}	3.25 ± 0.85^{bxz}	1.31 ± 0.63^{cy}

[1] Values are means ± standard deviations in log CFU per square centimeter.
[2] Treatment groups: CON, control (no antimicrobial application); LVA+SDS, 5.0% levulinic acid plus 0.5% sodium dodecyl sulfate (pH 2.6); LA, 4.5% lactic acid (pH 2.0); PAA, peroxyacetic acid (400 ppm; pH 4.7); CL, citrilow (pH 1.2).
[3] Water wash (55°C for 1 min) was applied by using a handheld hose with spray nozzle.
[4] The control carcass did not receive any antimicrobial spray treatment; therefore, least-squares means of surrogate recoveries were reported to be the same as the previous sampling point for the prechill and postchill antimicrobial sampling points.
[5] Recovered population of surrogate *E. coli* was below the detection limit (< 0.47 log CFU/cm^2).
[a-d] Least square means within treatments (within rows) that do not share a common letter are different ($P \leq 0.05$); [x-z] Least square means within sampling points (within columns) that do not share a common letter are different ($P \leq 0.05$).

Table 5.2. Least squares means (± SD) for objective color main effects by treatment of goat carcasses subjected to antimicrobial interventions[1].

Variables[2]	CON	LVA+SDS	LA	PAA	CL
*L**	48.59 ± 6.03	51.4 ± 6.47	49.27 ± 6.03	48.94 ± 6.63	49.38 ± 7.43
*a**	14.77 ± 2.14	13.68 ± 4.07	13.38 ± 3.05	14.18 ± 3.48	11.96 ± 3.10
*b**	8.25 ± 2.55[a]	6.46 ± 3.54[bc]	7.4 ± 3.12[ab]	7.14 ± 2.31[abc]	5.57 ± 3.35[c]
Hue	28.51 ± 4.86	23.88 ± 8.31	27.97 ± 7.14	26.71 ± 5.73	23.04 ± 9.09
Chroma	16.97 ± 3.00	15.26 ± 4.96	15.38 ± 3.98	15.94 ± 3.90	13.33 ± 4.11
Delta *E*	6.35 ± 3.76	7.18 ± 4.43	6.72 ± 6.44	6.49 ± 6.57	7.60 ± 7.46

[1] Least squares means ± standard deviations. All carcasses were subjected to a standard water wash (55°C for 1 min), followed by one of four spray treatments: LVA+SDS, 5.0% levulinic acid plus 0.5% sodium dodecyl sulfate (pH 2.6); LA, 4.5% lactic acid (pH 2.0); PAA, peroxyacetic acid (400 ppm; pH 4.7); CL, citrilow (pH 1.2).
[2] L*, 0 = black to 100 = white; a*, measurement of green to red on color spectrum (high values indicate more red); b*, measurement of yellow to blue on color spectrum (higher values indicate more yellow); hue, lower values indicate redder color; chroma, higher values indicate more red saturation; ΔE, the combined changes in L*, a*, and b* values over time.
[a-c] Least-squares means within the row with different letters are different ($P \leq 0.05$).

Table 5.3. Least squares means (± SD) for objective color main effects by sample time of goat carcasses subjected to antimicrobial intervention[1].

Variables[2]	Pretreatment	Prechill treatment	Post 1-h chill	Post 24-h chill	Postchill treatment
*L**	53.33 ± 2.86^{ab}	55.47 ± 4.06^{a}	51.47 ± 5.62^{b}	43.53 ± 4.13^{c}	43.77 ± 3.74^{c}
*a**	12.42 ± 1.88^{a}	10.30 ± 1.92^{b}	12.45 ± 2.80^{a}	16.11 ± 2.45^{c}	16.67 ± 2.16^{c}
*b**	4.91 ± 1.66^{a}	4.04 ± 1.81^{a}	6.46 ± 2.08^{b}	9.61 ± 2.30^{c}	9.80 ± 2.00^{c}
Hue	21.11 ± 6.59^{a}	20.65 ± 7.32^{a}	27.35 ± 6.43^{b}	30.77 ± 5.64^{b}	30.22 ± 3.57^{b}
Chroma	13.42 ± 2.11^{a}	11.14 ± 2.27^{b}	14.10 ± 3.14^{a}	18.84 ±2.77^{c}	19.37 ± 2.62^{c}
Delta *E*	0.00 ± 0.00^{a}	4.87 ± 4.92^{b}	5.81 ± 4.47^{b}	11.81 ± 4.31^{c}	12.83 ± 2.39^{c}

[1] Least squares means ± standard deviations. All carcasses were subjected to a standard water wash (55°C for 1 min), followed by one of four spray treatments: LVA+SDS, 5.0% levulinic acid plus 0.5% sodium dodecyl sulfate (pH 2.6); LA, 4.5% lactic acid (pH 2.0); PAA, peroxyacetic acid (400 ppm; pH 4.7); CL, citrilow (pH 1.2).

[2] L*, 0 = black to 100 = white; a*, measurement of green to red on color spectrum (high values indicate more red); b*, measurement of yellow to blue on color spectrum (higher values indicate more yellow); hue, lower values indicate redder color; chroma, higher values indicate more red saturation; ΔE, the combined changes in L*, a*, and b* values over time.

[a-c] Least-squares means within the row with different letters are different ($P \leq 0.05$)

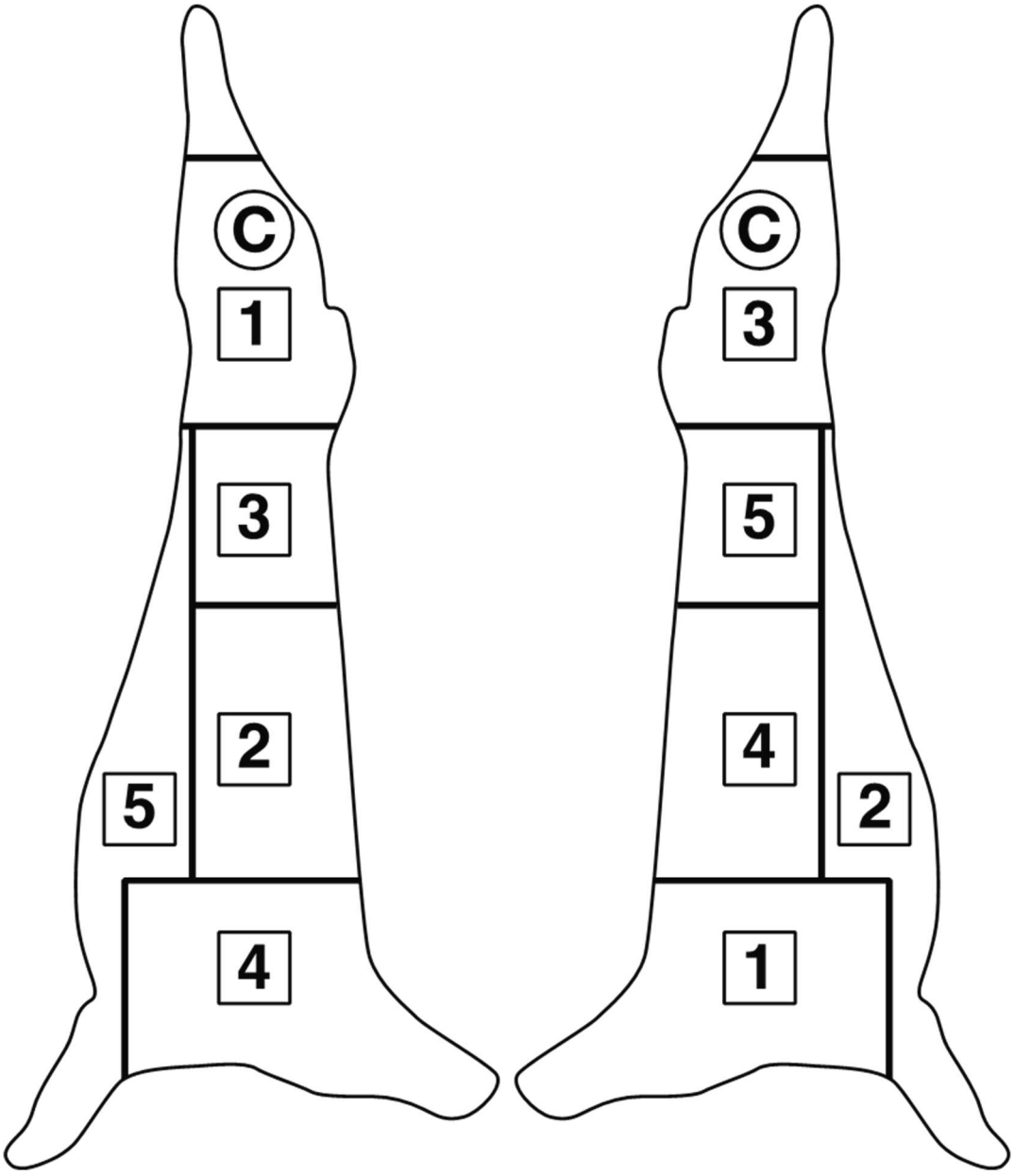

Figure 5.1. Anatomical locations where matched random samples for microbiological enumeration were taken from each goat carcass side for pooling of the samples. Carcass color was measured at the anatomical location labeled C.

CHAPTER 6

VALIDATION OF COMMONLY USED ANTIMICROBIALS ON VEAL CARCASSES INOCULATED WITH SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* SURROGATES[1]

[1] Thomas, C.L., H. Thippareddi, S. Kumar, M. Rigdon, R.W. McKee, and A.M. Stelzleni. To be submitted to *Journal of Food Protection*.

Abstract

Ruminants are natural reservoirs of Shiga toxin producing *Escherichia coli* (STEC) and these STEC can be easily transferred to carcasses during the conversion of animals to meat. The literature on the efficacy of antimicrobial interventions for reducing STEC populations on veal carcasses is limited. Three experiments were conducted to validate lactic acid (4%; LA), peroxyacetic acid (300 ppm; PAA), and hot water (80˚C; HW) for their individual or combined abilities to reduce STEC surrogates on bob veal carcasses pre- and post-chill and through fabrication. In experiment 1, hot carcasses (n=9) were inoculated with a 5-strain cocktail (ca. 8 log CFU/mL) containing rifampicin-resistant surrogate *Escherichia coli* (*E. coli*; BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431) and then treated with HW, LA, or PAA. Carcasses were then chilled (0±1°C; 24 h), split in half, and each side was treated with LA or PAA. In experiment 2, hot carcasses (n=3) were inoculated and chilled (24 h). After 24 h, the carcasses were split, and each side was treated with either LA or PAA. For experiment 3, carcasses (n=3) were chilled for 24 h, split, inoculated, and treated with either LA or PAA. After chilling, all carcasses were fabricated to subprimals and cut surfaces were sampled for translocation. Experiment 1 showed that LA+LA was the most effective ($P \leq 0.05$) treatment for reducing surrogate *E. coli* on veal. In experiments 2 and 3, LA and PAA were similar ($P > 0.05$) in their ability to reduce *E. coli* on chilled veal carcasses. Samples collected from cut surfaces after fabrication showed that all antimicrobial treatments resulted in undetectable levels (< 0.47 log CFU/cm^2) of surrogate *E. coli* in experiments 1 and 2 and low levels (1.66 and 0.97 log CFU/cm^2 for LA and PAA, respectively) in experiment 3. Of the antimicrobial

interventions utilized, lactic acid was overall more effective in reducing STEC surrogate populations on veal carcasses, pre- and/or post-chill.

Keywords: Bob veal, Antimicrobial intervention, *Escherichia coli*, Surrogate

Introduction

Meat processors aim to produce raw products that have low levels of bacteria and, most importantly, no pathogenic bacteria (Huffman, 2002). However, ruminants are natural reservoir of Shiga toxin producing *Escherichia coli* (STEC; Gyles, 2007) that can easily be transferred to carcasses during the conversion of animals to meat, especially in veal. Meat carcasses are exposed to potential sources of contamination during multiple stages at harvest. Meat carcasses can become contaminated from fecal matter, paunch contents, and during hide removal. In addition, processing tools, equipment, and human contact are known sources for carcass contamination (Huffman, 2002).

Shiga toxin-producing *Escherichia coli* has been implicated as the causative agent in multiple human diseases outbreaks. According to Scallan et al. (2011) and the CDC (2012), STEC are estimated to cause more than 265,000 illnesses each year in the United States, with more than 3,600 hospitalizations and 30 deaths. These diseases range from mild diarrhea to very severe and life-threatening conditions, such as hemolytic-uremic syndrome. The multiple cases of reported outbreaks and sporadic cases of human infections associated with STEC has led to increased concerns regarding the safety of beef and veal (Hussein and Bollinger, 2005). There have not been any reported cases of foodborne illness outbreak due to the consumption of veal product. However, veal carcasses are more likely to become contaminated with STEC compared to beef. This has been further substantiated by several recalls of veal products over the last few years due to contamination with *E. coli* serotypes O157:H7, O145 and O45 (Horton, 2009; USDA-FSIS, 2013; Kulas et al., 2015).

The Food Safety and Inspection Service has placed renewed emphasis on the justification of interventions included in HACCP programs during processing (King et al.,

2005). Therefore, veal production and processing needs validation for actual process and product and should be targeted as areas in which interventions can be applied to reduce contamination and the consumption of contaminated veal products. Literature on the efficacy of antimicrobial interventions in reducing STEC populations on veal carcasses is limited. Lack of literature on veal shows a need to help veal and small ruminant processors. Therefore, three experiments were conducted to validate the efficacy of hot water (80˚C; HW), lactic acid (4%; LA), and peroxyacetic acid (300 ppm; PAA) for their individual or combined abilities to reduce STEC surrogates on bob veal carcasses pre- and post-chill and through fabrication.

Materials and Methods

Animal handling and slaughter

All experimental procedures were reviewed and approved by the University of Georgia Institutional Animal Care and Use Committee. Fifteen bob veal (1-2 weeks, 23±2 kg) were purchased and transferred to the University of Georgia Meat Science and Technology Center for research purposes. The animals were held overnight in holding pens with no feed but *ad libitum* access to fresh water. Over 3 replications (5 animals per rep), animals were randomly assigned to a treatment group and humanely harvested following federal guidelines.

Bacterial strains

The non-pathogenic surrogate *E. coli* strains BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431, which are approved for in-plant use by USDA, were obtained from American Type Culture Collection (Manassas, VA). For each replication, the frozen surrogate strains were thawed and revived by placing 10 µL into 10 mL of sterile tryptic

soy broth (TSB; Remel, Lenexa, KS) supplemented with 0.1 mg/mL rifampicin (Fisher Scientific, Fair Lawn, NJ). The mixture was incubated at 37ºC for 24 h. Each strain was grown in separate 10 ml TSB supplemented with 0.1 mg/mL of rifampicin. Rifampicin-resistance was then confirmed by streaking cultures onto trypticase soy agar rifampicin plates (TSA; Becton Dickinson and Company, Sparks, MD) and incubating for 24 h at 37ºC. Rifampicin-resistant colonies were taken from the TSA plates and used for further propagation. Cultures were maintained weekly by transferring colonies from each strain into fresh TSB and incubating at 37ºC for 24 h. Fresh STEC surrogate inoculum was prepared prior to each slaughter day. To prepare the inoculum for trials, 20 mL of each culture was harvested by centrifugation (2,410 x *g* for 15 min; CR 312, Jouan Inc., Winchester, VA), washed twice with sterile peptone water (PW), and suspended in sterile PW (0.1%) to obtain a final cell concentration of ca. 8.0 log CFU/mL. Bacterial concentrations were confirmed by plating 1 mL portions of appropriately diluted culture (in duplicates) on Aerobic Plate Count (APC) Petrifilm (3M, Saint Paul, MN) and incubating at 37ºC for 48 h.

Antimicrobial preparation

Three antimicrobial agents were evaluated: hot water (HW; 80 ºC), lactic acid (LA; 4%; pH 2.1; 68ºC), and peroxyacetic acid (PAA; 300 ppm; pH 3.3; 24ºC). Prior to conducting each experiment, fresh antimicrobial treatments were prepared. The lactic acid solution was prepared by diluting 88% concentrated lactic acid (Birko Company, Henderson, CO) with water. Concentrated peroxyacetic acid (26%; Microtox ultra (Zee Company; Chattanooga, TN) was prepared to a final concentration of 300 ppm (PAA; pH 3.3; 24ºC) with water.

Carcass treatment and microbial sampling

Experiment 1

Hot carcasses (n=9; 3 per replication) were evenly inoculated with a 5-strain cocktail (ca. 8 log CFU/mL; 5 log CFU/mL attachment) containing rifampicin-resistant surrogate *E. coli* (BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431) to simulate carcass contamination during slaughter. Each carcass was inoculated using a foam paint brush (Project Source, Scottsdale, AZ). After inoculation, the carcasses were held undisturbed on the slaughter line for 30 min (25ºC), to allow for attachment before a standard water wash (25ºC; 551.58 kPa) was performed. After the water wash, antimicrobial treatments HW, LA, or PAA were randomly assigned and subsequently applied to each carcass using a low-pressure hand-held fan spray nozzle (0.635 cm; Dewalt Manufacturing Company, Baltimore, MD) for 1 min. Carcasses were allowed to drip for 5 min and then placed in the carcass cooler (0 ± 1°C) for 24 h. After 24 h, each carcass was split in half (down the vertebral column), and each side was treated with LA or PAA. Each carcass side was then fabricated in to primals including: shoulder, shank/breast, rack, loin and leg. Each carcass was sampled at six different points during processing: 1) after inoculation and a 30 min attachment period, 2) 5 min after the standard water wash, 3) 5 min after the pre-chill carcass antimicrobial spray application, 4) post-24-h chilling, 5) 5 min after the 24 h post-chill carcass antimicrobial spray application, and 6) after fabrication to determine surrogate *E. coli* bacteria population counts. Fabrication samples were collected from cut surfaces to measure the translocation of surface bacteria unto cut surfaces.

Experiment 2

Hot carcasses (n=3; 1 per replication) were inoculated with the same 5-strain cocktail as experiment 1 and allowed a 30 min attachment period. Following attachment, carcasses received the standard water wash, and were then chilled for 24 h. After chilling, each carcass was split in half (down the vertebral column), and each half treated was with LA or PAA. After antimicrobial treatment, each side was fabricated. For this experiment, each carcass was sampled at five different points during processing: 1) after inoculation and a 30 min attachment period, 2) 5 min after the standard water wash, 3) post-24-h chilling, 4) 5 min after the 24 h post-chill carcass antimicrobial spray application, and 5) after fabrication to determine surrogate *E. coli* bacteria population counts.

Experiment 3

After hide removal and evisceration, hot carcasses (n=3) were placed directly in the carcass cooler and chilled for 24 h. After 24 h, each carcass was removed from the carcass cooler and split into sides down the vertebral column, inoculated, and treated with either LA or PAA. For experiment 3, each carcass was sampled at three points during processing: 1) after inoculation, 2) 5 min after the 24 h post-chill carcass antimicrobial spray application, and 3) after fabrication.

For each sampling time point in all experiments, three surface samples were excised from 1 of 3 randomly assigned anatomical locations from each side of the carcasses. A sterilized, stainless steel metal coring device (2.54 cm diameter) was used to create an impression on the matched anatomical surface location on each carcass. The marked surface area was then aseptically excised to a depth of approximately 0.2 cm using a sterile scalpel. Within sampling time point, the cores collected were placed in sterile bags and

kept on ice until further analysis. For analysis, core samples were transferred to a stomacher bag containing 90 mL PW supplemented with 0.1 mg/mL rifampicin (PW^R) and stomached (Neutec Group Inc., Barcelona, Spain) for 90 s. The suspension was serially diluted (1:10) in PW^R, and 1.0 mL of each dilution was plated in duplicate onto APC Petrifilm following the manufacturer's instructions. Petrifilm plates were incubated at 37ºC for 48 h and enumerated.

Statistical Analysis

Experiment 1 was designed as a randomized split-plot with carcass as the whole plot and side as the sub-plot. Experiments 2 and 3 were completely randomized designs with side as the experimental unit. For each experiment, *E. coli* population (log CFU/cm^2) was analyzed using PROC Mixed (SAS V.9.4; Cary, NC) for the main effects of antimicrobial treatment, sampling time point, and their interaction, when applicable. If an interaction was detected, the means were separated and presented within time point and antimicrobial treatment. In experiment 2 and 3, the data was reanalyzed and separated by sampling time points to confirm no difference existed between inoculated carcass sides designated for LA or PAA, prior to antimicrobial application. Means were considered different at $\alpha \leq 0.05$.

Results and Discussion

Experiment 1

The first experiment utilized sequential chemical washes, with the aim of reducing and further preventing the growth of pathogenic bacteria. As described by Leistner (2002) and Huffman (2002), the principle of hurdle technology is to substantially reduce the initial microbial load by the first decontamination method, leading to fewer microorganisms

present and making it easier to inhibit/remove theses microorganisms in subsequent processing steps. The results from experiment 1, showed that there was an antimicrobial treatment by sampling time point interaction ($P \leq 0.05$; Table 6.1). After inoculation, the recovered populations of surrogate *E. coli* were similar ($P > 0.05$) for all treatment groups. Similarly, after a standard water wash (24ºC), the recovered surrogate *E. coli* populations were similar ($P > 0.05$) for all treatment groups, with less than 0.50 log CFU/cm^2 reduction achieved. A pre-chill antimicrobial application of HW (80ºC), showed similar ($P > 0.05$) recovered populations of *E. coli* (4.10 log CFU/cm^2) to the *E. coli* populations (4.90 log CFU/cm^2) that was recovered after a standard water wash (24ºC). Pre-chilled application of LA, and PAA resulted in 2.73, and 1.65 log CFU/cm^2 reduction ($P \leq 0.05$), respectively. After chilling carcasses for 24 h (0±1ºC), the carcasses that were treated with LA and PAA showed additional reductions ($P \leq 0.05$; 1.11 and 1.30 log CFU/cm^2, respectively).

In the current study, the standard water wash (25ºC) was not able to significantly lower surrogate *E. coli* populations on veal carcasses. The average reduction achieved was lower than the 0.9 log CFU/cm^2 reduction reported by Sevart et al. (2016) using water at 50ºC. The higher reduction reported by Sevart et al. (2016) may be due to the differences in the temperature of water used to wash the veal carcasses. Greater temperatures are known to have a more robust sanitizing effect. Phebus et al. (1997), using water (35ºC) slightly higher in temperature than in the current study, was able to achieve similar reductions as Sevart el al. (2016). However, Phebus et al. (1997) applied water to individual beef carcass surfaces that were hanging in a cabinet, while the current study sprayed the entire veal carcass. Application temperatures and methods used by Phebus et al. (1997) might have led to differences in the observed reductions.

Various studies have validated the effects of hot water (> 74ºC), and it has been accepted as an appropriate sanitizer by USDA-FSIS. According to Huffman (2002), hot water wash application to carcasses normally reduce bacterial counts by 1 to 3 logs. However, the reductions in the current study were lower than the reductions, reported by Huffman (2002). The differences observed in the current study and the reported effectiveness of hot water may be affected by various parameters such as meat bacterial attachment time, carcass composition, and most importantly, the microorganism being assayed. In the current study, the application of LA (4%) on pre-rigor veal carcasses was the most effective in reducing surrogate *E. coli*. The reductions achieved in the current study, was similar to the 2.00 log CFU/cm^2 reductions by reported by Thomas et al., (2019) after applying a 4.5% lactic acid spray to goat hot carcasses. Contrary to the aforementioned studies, Sevart et al. (2016) reported only a 0.60 log CFU/cm^2 reduction of surrogate *E. coli* on pre-rigor veal carcasses. Sevart el al. (2016) applied 4.5% lactic acid at 20ºC compared to 68ºC used in the current study, which likely contributes to some of the difference between the two studies. When PAA (300 ppm) was applied to pre-rigor veal carcasses, a 1.65 log reduction was achieved. This was similar to the1.86 log CFU/cm^2 reductions achieved by Thomas et al., (2019) on goat carcasses. Penny et al. (2007) reported greater reductions (2.29 and 2.67) of *E. coli* O157:H7 on beef and veal carcasses after being treated with 180 ppm of peroxyacetic acid (20ºC). The reductions achieved by Penny et al. (2007) might be due to applications method, where the antimicrobial was applied to individual pieces of meat using a spray cabinet.

Following 24 h chilling, each carcass was split in half, and each side was treated with either LA or PAA. For the carcasses that were treated initially with HW and

subsequently with LA, the mean reduction achieved was 2.08 log CFU/cm^2 (HW+LA; $P \leq 0.05$). For the carcasses that were treated initially with HW and subsequently treated PAA, the mean reduction achieved was 1.27 log CFU/cm^2 (HW+PAA; $P \leq 0.05$). For the carcasses that were initially treated with LA, the sides treated with LA (LA+LA) showed a reduction of 1.67 log CFU/cm^2 ($P \leq 0.05$), while the sides that were treated with LA+PAA showed no further reduction ($P > 0.05$). When PAA was the first antimicrobial administered and was followed by any of the two antimicrobials (LA or PAA), no further reductions ($P > 0.05$) were achieved on the chilled veal carcasses. Considering the entire process (warm water wash through post-chill antimicrobial treatment), the greatest reduction of surrogate *E. coli* populations on veal carcasses was achieved by the LA+LA treated group (5.00 log CFU/cm^2) followed by HW+LA (4.05 log CFU/cm^2), LA+PAA (3.87 log CFU/cm^2), HW+PAA (3.24 log CFU/cm^2), PAA+LA (2.96 log CFU/cm^2), and PAA+PAA (2.63 log CFU/cm^2).

Based on these results, PAA had less bactericidal activity than LA on chilled veal carcass. The reductions achieved after applying LA to post-chilled carcasses were similar to the reductions observed by Thomas et al. (2019), who observed a 0.99 log CFU/cm^2 reduction on chilled goat carcasses. Contrary to the current study, Sevart et al. (2016) reported that no additional reduction was achieved when 4.5% lactic acid was applied to chilled veal carcasses. The use of PAA was not as effective as LA when applied as a secondary antimicrobial post-chilled. At 300 ppm PAA was less effective on the chilled carcass, these results confirmed findings by King et al. (2005). King et al. (2005) applied peroxyacetic acid (200 ppm; 43 ºC) for 15 s on chilled beef carcass surface, and found that

peroxyacetic acid had no effect on microbial counts of *E. coli* O157:H7 and *Salmonella Typhimurium*.

After fabrication, the recovered populations of surrogate *E. coli* on the cut surfaces were below the detection limit (≤ 0.17 log CFU/cm^2) for all combinations. This data indicates that at low levels of contamination, only a small amount of microorganisms is transferred to the cut lean surfaces.

Experiment 2

Experiment 2 was designed to simulate the possibility of a deviation, where the carcass was contaminated during slaughter but there was a failure to apply an antimicrobial or the antimicrobial was not applied properly. As such, carcasses were inoculated, given a standard water wash, and then chilled for 24 h before an antimicrobial was applied. There was not a treatment by sampling time point interaction ($P > 0.05$), or a main effect of antimicrobial treatment ($P > 0.05$). However, the main effect of sampling time point affected the recovered *E. coli* population ($P \leq 0.05$) and is presented in Figure 6.1. The recovered population of *E. coli* gradually reduced after each sampling time point. To confirm that there was no difference between inoculated sides designated for LA or PAA prior to antimicrobial application, the data was reanalyzed and separated by main effect of sampling time points. No difference ($P > 0.05$) was found with any time points, including post chill antimicrobial application.

The results obtained in experiment 2, confirmed the finding of experiment 1, where post chill applications of LA led to greater numerically reductions compared to PAA when applied to chilled veal carcasses. As stated earlier, Thomas et al. (2019) observed similar results on chilled goat carcasses. Higher reductions were reported by Pittman et al. (2007)

and Castillo et al. (2001) with notable reductions ~2.4 log CFU/cm^2 of *E. coli* O157:H7 on chilled beef subprimal treated with 5% and 4% lactic acid solutions, respectively. Again, the greater reductions reported in the aforementioned studies may be due to the methods used to apply antimicrobial. As for peroxyacetic acid, King et al. (2005) found that at lower concentration (200-600 ppm; 43ºC), peroxyacetic acid was not effective in reducing rifampicin-resistant *E. coli* on chilled beef carcass surfaces. However, only when a higher concentration and temperature were applied (1000 ppm; 55ºC) did King et al. (2005) achieved a 1.7 log CFU/cm^2 reduction. Therefore, both the temperature and concentration used in this current study may explain the reductions obtained from PAA compared to LA.

As expected, as sampling progress recovered population of *E. coli* would eventually reduce. The reductions at each time point resulted in an additive reduction throughout the process. Similar to experiment 1, after fabrication, the recovered populations of surrogate *E. coli* on the cut surfaces were below the detection limit (< 0.17 log CFU/cm^2). Indication again, that at low levels of contaminations, little is transferred to the cut lean surface.

Experiment 3

Meat processors strive to produce raw products that have low levels of bacteria on the surface and no pathogens; however, slaughter and fabrication processes are not conducted in a sterile environment and contamination is unavoidable (Huffman 2002). As such, meat may become contaminated prior to fabrication. Therefore, a third challenge study was performed to test which common antimicrobial is more suitable to be used on veal carcasses that might have been contaminated close or just prior to fabrication. For veal carcasses that were inoculated and treated 24 h post chill, there was not a treatment by sampling time point interaction ($P > 0.05$). Furthermore, the main effect of treatment did

not affect the recovered *E. coli* populations ($P > 0.05$). Treatment with both LA and PAA reduced ($P \leq 0.05$) surrogate loads by 1.67 and 2.42 log CFU/cm^2, respectively, however, the reductions were not different from each other ($P > 0.05$). However, the main effect of sampling time point, affected recovered population of *E. coli* on chilled veal carcasses (Figure 6.2). The recovered populations of surrogate *E. coli* were lower ($P \leq 0.05$) after the antimicrobial sprays, compared with the recovered population after inoculation. Similar to experiment 2, to confirm that there was no difference between inoculated sides designated for LA or PAA prior to antimicrobial application, the data was reanalyzed and separated by main effect of sampling time points, and no difference was found ($P > 0.05$). After fabrication, the recovered populations of surrogate *E. coli* were lower ($P \leq 0.05$) compared to after inoculation and antimicrobial application, as expected. Despite no difference found between the two antimicrobial treatments applied, the results show that if contamination should occur just before fabrication, the application LA or PAA could reduce carcass contamination, before fabricating.

Conclusion

As stated earlier, even though meat processors strive to produce raw products that have low levels of surface pathogens, and ideally free of all pathogenic bacteria, this can be difficult to achieve. Of the antimicrobial interventions utilized, lactic acid was overall more effective in reducing STEC surrogate populations on veal carcasses pre- and/or post-chill. As expected, greater reductions in microbial populations were achieved when decontaminating treatments were used on meat in combination than when they were applied individually. The results from the current study support the concept of using sequential decontamination processes in veal processing as a means of improving the

microbiological quality of veal carcasses. These results showed that for the tested concentrations and temperatures, lactic acid was a more favorable antimicrobial for veal carcasses when applied both pre- and post-chill. However, further studies should test the efficacy of different concentration or temperature of peroxyacetic acid on veal carcasses.

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Table 6.1. Least squares means and (± SD) of rifampicin resistant surrogate *E. coli* (log CFU/cm^2) found on bob veal carcasses treated with different combinations of antimicrobial interventions.

Antimicrobial Combinations[1]	Inoculation	Water wash[2]	Prechill antimicrobial	Post 24 h chill	Postchill antimicrobial	Fabrication[3]
HW+LA	5.28 ± 0.29^{a}	4.90 ± 0.31^{ab}	4.10 ± 0.44^{bcw}	3.31 ± 0.99^{cw}	1.23 ± 0.18^{dy}	$< 0.17^{ex}$
HW+PAA	5.28 ± 0.29^{a}	4.90 ± 0.31^{ab}	4.10 ± 0.44^{bcw}	3.31 ± 0.99^{cw}	2.04 ± 0.15^{dwxy}	$< 0.17^{ex}$
LA+LA	5.26 ± 0.18^{a}	4.70 ± 0.06^{a}	1.97 ± 0.60^{by}	0.86 ± 0.99^{cy}	$< 0.17^{cz}$	$< 0.17^{cx}$
LA+PAA	5.26 ± 0.18^{a}	4.70 ± 0.06^{a}	1.97 ± 0.60^{by}	0.86 ± 0.99^{cy}	1.39 ± 1.39^{cxy}	$< 0.17^{dx}$
PAA+LA	5.12 ± 0.02^{a}	4.78 ± 0.10^{a}	3.13 ± 0.81^{bx}	1.83 ± 1.31^{cx}	2.16 ± 0.63^{bcwx}	$< 0.17^{dx}$
PAA+PAA	5.12 ± 0.02^{a}	4.78 ± 0.10^{a}	3.13 ± 0.81^{bx}	1.83 ± 1.31^{cx}	2.49 ± 0.16^{bcw}	$< 0.17^{dx}$

[a-e] Least square means (± SD) within treatment combinations (within rows) that do not share a common letter are different ($P \leq 0.05$); [w-z] Means within sampling point (within column) that do not share a common letter are different ($P \leq 0.05$).

[1] Antimicrobial interventions: hot water (80˚C; HW), lactic acid (4%; LA), peroxyacetic acid (300 ppm; PAA).

[2] All carcasses were subjected to a standard water wash (24ºC) prior to antimicrobial application.

[3] Fabrication samples collected from all antimicrobial treatment combinations were below the detection limit (< 0.17 log CFU/cm^2).

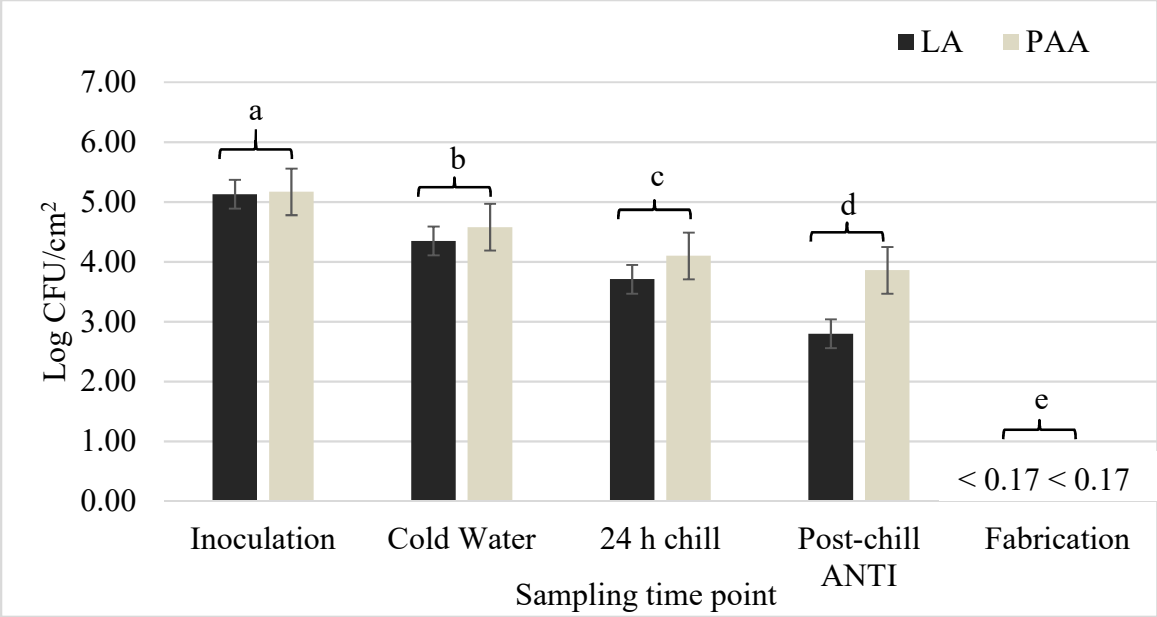

Figure 6.1. Rifampicin resistant surrogate *E. coli* populations recovered (± SD) from bob veal carcasses that were inoculated and subsequently chilled for 24 h before antimicrobial interventions of lactic acid (4%; LA) or peroxyacetic acid (300 ppm; PAA) were applied. Means among sampling time points that do not share a common letter are statistically different ($P \leq 0.05$). Fabrication samples were below the detection limit (< 0.17 log CFU/cm^2) for both treatment groups.

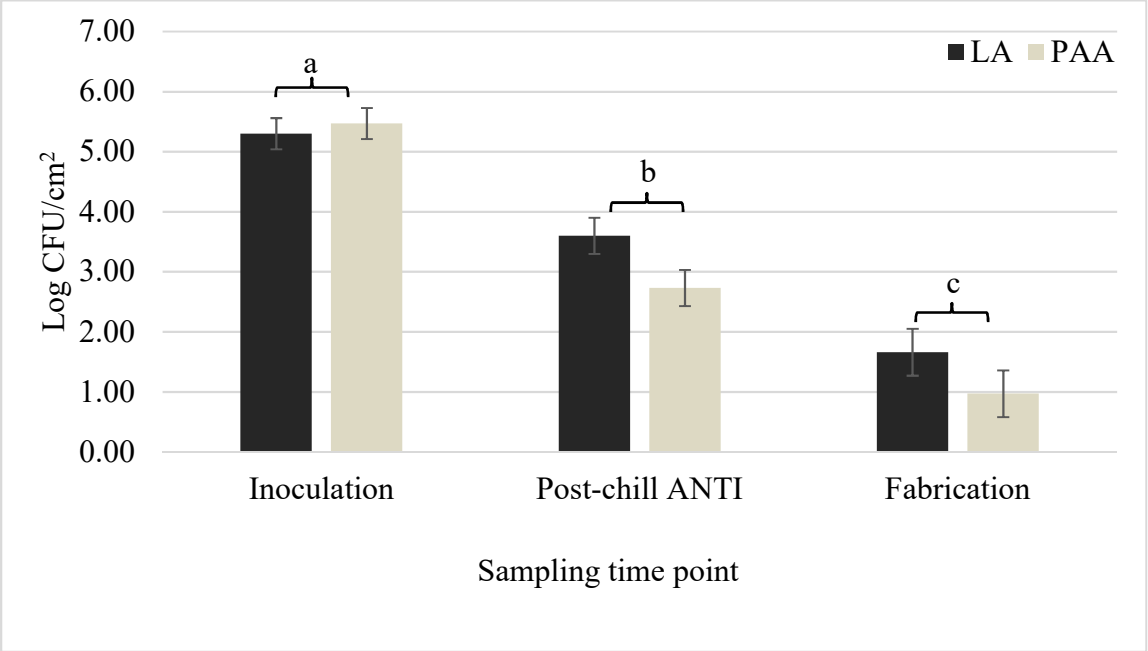

Figure 6.2. Rifampicin resistant surrogate *E. coli* populations (± SD) recovered from bob veal carcasses that were inoculated and treated with antimicrobial interventions lactic acid (4%; LA) or peroxyacetic acid (300 ppm; PAA) after 24 h chill. Means among sampling time points that do not share a common letter are statistically different ($P \leq 0.05$).

CHAPTER 7

CONCLUSION

It is predicted that by 2020, the world's population will surpass 7.5 billion people. As the population increases, so does the consumption of meat and meat products. The increase in population will cause a greater strain on the food industry, especially the meat industry. Like it has been in the past and currently, there is no doubt that in the future, food safety will continue to be a challenge for the industry. *Escherichia coli* O157:H7 along with the other non-O157 Shiga toxin producing *Escherichia coli* (STEC) continues to be the main of concern in the meat industry. Shiga toxin producing *Escherichia coli* generate toxins that can cause severe hemolytic uremic syndrome and other foodborne illnesses in humans. Shiga toxin producing *Escherichia coli* contamination has historically been linked to the meat from ruminant animals, especially non-intact beef products. Although meat processors strive to produce raw products that have low levels of surface contamination, and products free of pathogenic bacteria, this can be difficult to achieve. As such, FSIS recommends that processing establishments incorporate additional procedures into their HACCP systems to support that STEC is not a hazard in consumer ready products. In response to safety concerns and government regulations processors have employed various antimicrobials.

Several new and already established antimicrobials were tested on non-intact beef, as well as veal and goat carcasses. For non-intact beef, use of pulse ultra violet light and electrolyzed oxidizing water were not effective in controlling spoilage bacteria. However,

an emerging antimicrobial, levulinic acid plus sodium dodecyl sulfate (LVA+SDS), could be used as an antimicrobial on blade tenderized products to prevent the growth of both spoilage and pathogenic bacteria, while maintaining their quality and sensory characteristics.

For goat carcasses, the application of lactic acid (LA) was the most effective in reducing surrogate STEC populations, when compared to all other antimicrobial treatment examined. However, other antimicrobials tested including LVA+SDS, peroxyacetic acid (PAA), and citrilow (CL) solutions elicited reductions ≥ 3.15 log CFU/cm^2, and were significantly different from the CON treated carcasses. In addition, LA and LVA+SDS were able to suppress and further reduce surrogate STEC populations during chilling. Citrilow and peroxyacetic acid were able to achieve ≥ 1 log reductions when applied to chilled carcasses, which implies these treatments would also be effective on goat carcasses, if applied before fabrication. The validation study done on goat carcasses, showed that LA, LVA+SDS, CL, and PAA were all able to effectively reduce surrogate STEC populations during slaughter and chilling without compromising goat carcass color.

The validation study conducted for veal carcasses, showed that LA was more effective in reducing STEC surrogate populations, pre- and/or post-chill when compared with hot water and PAA. Greater reductions in microbial populations were achieved when treatments were used on meat sequentially than when they were applied individually. The results from the current study supports the concept of using sequential decontamination processes in veal processing as a means of improving the microbiological safety. Results showed that for the tested concentrations, lactic acid was a more favorable antimicrobial for veal carcasses when applied both pre- and post-chill. However, further studies should

be conducted testing the efficacy of higher concentrations and temperatures of peroxyacetic acid when applied to veal carcasses decontamination.

Shiga toxin producing *Escherichia coli* contamination is a food safety hazard during the slaughter and processing of raw meat products, for which lactic acid and peroxyacetic acid is commonly used to control. However, there are many factors that may affect their efficacy. The experiments conducted have highlighted and emphasized the importance of each establishment conducting their own hazard analysis (as required by USDA-FSIS) to determine the hazards that are reasonably likely to occur, and the effect antimicrobials might have on their product. More importantly, these series of experiment have shown that levulinic acid plus sodium dodecyl sulfate could be substituted for industry standard antimicrobials such as lactic acid and peroxyacetic acid.