DEVELOPMENT OF A NOVEL ANTIMICROBIAL ICE APPLICATION FOR MEAT GRINDER SANITATION

By

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DEVELOPMENT OF A NOVEL ANTIMICROBIAL ICE APPLICATION FOR MEAT GRINDER SANITATION

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Abstract: Ground beef is typically produced in a continuous type of production practice. This means that most large-scale ground beef facilities only have a full break down of the grinding equipment at the end of the production day or a partial break down of equipment when mechanical or minor issues occur during production (Gill and McGinnis, 1993; Gill et al., 2003). This presents a situation where large amounts of ground products can be contaminated and unfit for human consumption. The basis of this research is to introduce a novel antimicrobial ice application in order to reduce *Escherichia* coli O157:H7 and *Salmonella* Typhimurium DT104 with a simple process step. In addition to the antimicrobial ice, an additional hurdle in the form of antimicrobial sprays on beef trim were also tested. The antimicrobial ice treatments tested were: peracetic acid (PAA, 350 mg/L; PeroxyChem, PA, USA) and combination PAA with 2% FreshFX® (PAAF; PeroxyChem, PA, USA), 2% Paradigm® (PAAP; PeroxyChem, PA, USA) and 2% lactic acid (PAAL; Brico Co. IN, USA). The spray treatments were: no treatment (NT), de-ionized water spray (DI), 3% Sodium Acid Sulfate (NaHSO4; Jones-Hamilton Co., OH, USA), 5% Lactic Acid (LA; Brico Co. IN, USA), 0.2 % Blitz peracetic acid (Blitz; PeroxyChem, PA, USA), NaHSO4 followed by Blitz (NaHSO4 + Blitz), and LA followed by Blitz (LA + Blitz) The tests were primarily focused on antimicrobial reduction. There was also experiments conducted to determine storage color effects of the antimicrobial spray treatments. The experiments resulted in reduction of over 3 log pathogen transfer from the meat grinder.

Keywords: Ground Beef, *Escherichia* coli O157:H7, *Salmonella* Typhimurium DT104, Lactic Acid, Sodium Acid Sulfate

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CHAPTER I

INTRODUCTION

Ground beef is typically produced in a continuous type of production practice. This means that most large-scale ground beef facilities only have a full break down of the grinding equipment at the end of the production day or a partial break down of equipment when mechanical or minor issues occur during production (Gill and McGinnis, 1993; Gill et al., 2003). With limited separation of ground beef products, foodborne pathogens are not presented an additional antimicrobial intervention to reduce possible cross contamination in current industry practices. There is still much more room left for improvement since "beef is still the third most common" foodborne illness outbreak product (Andrews, 2014). The two pathogens commonly associated in these beef foodborne illness outbreaks are Shige toxin-producing *Escherichia coli* (*E.coli*) and *Salmonella* spp. (Niyonzima et al., 2015). These two pathogens accounted for 58% of the beef outbreaks (Andrews, 2014). Common industry practice is to heavily sanitize on the harvest floor aspect of beef production, but only a few measures are taken directly before grinding of the product (Young et al., 2008). There are many ground products that could potentially result in foodborne illness outbreaks like ground beef patties, sausage, hot dogs, summer sausages, hot links, and numerous other products that experience similar types of grinding processing. These numerous products could benefit from a sanitation intervention step applied directly to the grinding equipment.

Various studies on the effects of antimicrobials on beef trim and the testing of grinder surfaces to determine microbial presence, but these studies there is limited explanation for how these treatments can be applied in an industrial setting or the effects the treatments will have on products when used in a real-world setting. Although this research is extremely significant in the improvement for sanitation, there is a need for research as to how to more effectively apply these antimicrobial treatments (Eisel et al., 1997; Farrell et al., 1998; Gill et al., 2003; Ortiz, 2006; Committee, 2009; Quilo et al., 2009; Ismaïl et al., 2013; Belanger and Stelzleni, 2015; Koohmaraie et al., 2015; Nair et al., 2016; Loukiadis et al., 2017) . The research discussed is looking into the use of an antimicrobial ice made from Peracetic Acid as well as antimicrobial sprays of Lactic Acid, Sodium Acid Sulfate, and Peroxyacetic Acid. The objective of the current research was to present a simple antimicrobial ice application that can significantly reduce pathogens on meat grinder surfaces. This application would also be able to reduce the amount of downtime that would be necessary for partial sanitation of meat grinders for industry use. In order to have a multi-hurdle sanitation approach to this research, other antimicrobial spray applications were tested alongside the antimicrobial ice application.

CHAPTER II

REVIEW OF LITERATURE

Ground Beef History

Ground beef started as a humble meal immigrant made to ease their homesickness. German immigrants, who brought their typical spicy ground products to the Americas, are thought to be responsible for the introduction of ground beef to the United States (Tarshis, 2015). Although it started out being called the "Hamburg Steak" in reference to its German origins (Tarshis, 2015). The reason that the "Hamburg Steak" did not reach popularity very quickly can be attributed to the negatively viewed ground products produced in the United States at the time. Many immigrants, especially in the Chicago, Illinois area worked for large beef packers where there was limited and scarce oversight about how many meat products were made. It was believe by the majority of the general public in that time that ground beef was of extremely low quality and should be avoided at all costs (Sinclair, 1906). The writing of Upton Sinclair's "The Jungle" opened the eyes of the public as to what was going on behind the closed doors of the meat packing plants, even though the public had more open access to them than they do today. This book is the contributed to be the main reason why the 1906 "Federal Meat Inspection Act" was enacted by the government (Marler, 2006). The irony of this is that Sinclair originally wrote the book to discuss the hazardous conditions of workers (Sinclair, 1906).

Various immigrants still used this "low-grade" cut of beef, probably due to cost, and served it both as a raw or cooked meal. Seasoning were added as well as onions and breadcrumbs. This item took on various Italian origin names but was not referred to as ground beef until the meat chopper was invented (Stradley, 2015). An 1845 paten filled by G.A. Coffman closely resembles the modern meat grinders of today. There are many old cookbooks and restaurant that mention either "Hamburger Steak", "Hamburger Beefsteak", "Broiled Meat Cakes", or "Hamburgh Steak" (Stradley, 2015). There is much disputed history between who actually invented the "hamburger" due to the numerous people that have claimed to have served it first (Stradley, 2015; Tarshis, 2015). There are various states that have acknowledged different towns as the "Birth place of the Hamburger", including Tulsa, Oklahoma (Stradley, 2015). One story behind the birth of the hamburger is that during a customer rush, Walt Anderson in Wichita, Kansas, smashed a meatball out of frustration and served it flattened. Years later, Anderson partnered with Billy Ingram to start "White Castle". The goal behind the name was to deem that their hamburgers were fit for nobility (Tarshis, 2015). Regardless of the where the birthplace is, it is safe to say that the hamburger took the American taste palate by storm for the years to follow.

Beef Consumption Trends

Beef consumption in general has seen many highs and lows over the last several decade in the United States. There have been many factors that have affected this like climate, feed, and especially, upcoming consumer trends. In 1995, Putnam and Duewer (1995) publish an article in "FoodReview" that American's were consuming a record high amount of beef, a 64-pound average. One factor that has dramatically changed from this 1995 article to currently industry trends in the increase in vegetarian consumers. Putnam and Duewear (1995) claim that the number of consumers claiming to lead a vegetarian lifestyle was relatively stable over a sixteen-year period (Putnam and Duewer, 1995; Davis and Lin, 2005). Moving forward to 2011, a movement named "Flexitarians" took flight as a consumer trend. Flexitarian meaning that a consumer would reduce and not eliminate the amount of red meat intake for health reasons

(Unknown, 2012). An interesting correlation is that, in 2011, the beef consumption per capita was down 25% compared to consumption in 1980 (Davis, 2011). It was also noted by Zare, Zheng, and Buck (2017) that beef consumption declined more than ten pounds per capita between 2002 and 2015 (Zare et al., 2017). More lean options for meat have pulled consumers away from beef and in order to counteract that movement, the beef industry has offered up new cuts of beef, such as the "Vegas Strip Steak" from Oklahoma State University (NewsOK, 2013). Ground beef has also been re-invented by offering consumers more pre-seasoned hamburgers and other options for consumers (Martin and Brooks, 2012). Introducing these new formulations has given ground beef a chance to "re-invented" for consumers; however, it will remain a staple food item for consumers.

Another negative factor toward beef consumption trends are many health-related recalls that happen with beef. One of the most well-known in recent history was announcement of "Mad Cow Disease" or "Bovine Spongiform Encephalopathy" (BSE). After the release of the concerns with "Mad Cow Disease", beef saw a 20% decrease in consumption (Zare et al., 2017). Similar reactions can be seen with any health-related beef recall, such as *Escherichia coli*, *Salmonella*, and Listeria outbreaks in any beef product. The reason that these pathogens are such a concern, especially E. coli, is because cattle are a natural carrier of certain strains. A secondary factor is that some production practices pose an increased risk of contamination (Juska et al., 2003; Mora Garcia, 2016; Loukiadis et al., 2017).

Despite all this, beef has, once again, seen an increase in consumer spending habits. From 2005 to 2012, ground beef purchases increased 8% (Laudert, 2012). Low-income residents tend to purchase the most ground beef out the income-based household studies. Meanwhile, households with a great income show a steady meat purchasing habit despite economic turns. However, breed specific programs have helped significantly in terms of beef purchasing. In either case, beef is considered to be a food item that is cooked at home rather than when eating out by consumers (Davis and Lin, 2005). The Certified Angus Beef (CAB) program is one of the most

well beef labels on the market today. Since then brand name packages have increased to 36% since CAB first hit retail shelves. Even through the recession of 2007, CAB maintained increasing sales (Davis and Lin, 2005; Henderson, 2014) . This is in direct response to consumer asking for more information about where their grocery purchases come from. Another initiative to give consumers more information about food was the Nutritional Labeling and Education Act and the Food Allergen Labeling and Consumer Protection Act (United States, 1994; United States Committee on Health, 2002). Although these two rulings are separated by 8 years from when it became law, both are attempts to accomplish the goal of helping consumers make an educated and safe decision on their purchases. In a European study conducted over four different countries found that consumers consistently use the nutritional labeling to make health-conscious decisions which results in consuming beef more (Van Wezemael et al., 2010).

Ground Beef Production

Ground beef is made at the very end stages of beef production, which is why there are so many opportunities for ground beef to become contaminated with various biological and physical hazards. Due to the amount of processing points that the entire carcass has to go through in order to produce ground beef, the risk of exposure is increased. A risk assessment determined "several points between slaughter and packaging" that could pose as a point where fecal contamination could come in contact with the exposed meat (Cassin et al., 1998). Ground beef starts out as a whole carcass that is then cut down into primal cut, sub-primal cuts, and eventually retail cuts. The main production practice that poses the most initial risk to beef carcass contamination is the removal of the hide from the actual carcass. The hide is the main external source of fecal contamination to the exterior of the exposed beef carcass, while the lower sections of the internal organs pose a risk to contaminating the internal portion of the carcass (Cassin et al., 1998; Elder et al., 2000; Antic et al., 2010; Arthur et al., 2010). After the carcass is finished with the harvest process, it frozen and stored until it is needed for fabrication. Before it can be fabricated it has to be split between the 12^{th} and 13^{th} rib to expose the *Longdismus dorsi*, otherwise known as the

ribeye, muscle that is used to determine the quality and yield grade factors of the carcass (Tait et al., 2005; USDA-AMS, 2016b). These quality and yield grades do not play a significant factor in determining the final production of ground beef (USDA-FSIS, 2016). The carcass is then broken down into primal cuts (Youssef et al., 2013; USDA-AMS, 2016b). These industry recognized primal cuts are known as the chuck, loin, rib, and round (Tait et al., 2005). The trimmings that are left over making the primal and sub-primal cuts are typically sent to become ground beef (Gill et al., 2003). The United States Department of Agriculture, Food Safety Inspection Service (USDA-FSIS) states that ground beef can also be made from less desirable cuts of beef as well. Less desirable meaning that it is lower quality beef carcasses or cuts of beef that do not have a significant demand within the market. These components are then course ground and finely ground to produce a homogenous beef product to sell to consumers. It can also be called primary and secondary grind. The main point is that trim is processed through a set of grinding plates twice in order to have a through homogenous mixture. While making this homogenous mixture, is when the exposed surface area of the beef trim increases and allows for contamination to be spread. Bacteria that has remained on the outside of the beef trim will be given the opportunity to be thoroughly mixed while making the ground beef. A similar, but different issue can also be seen in mechanical tenderization of meat. While grinding increases exposed surface area of the meat product, tenderization forces the bacterial into the internal tissue of the meat. In both cases properly cleaning the equipment is somewhat difficult in order to prevent the spreading of pathogens (Saha et al., 2016). Although the sanitization practices and procedures between grinders and tenderizers vary, which allows sanitization of a meat grinder to be considered more feasible. This ground beef can come at various different lean points and be label with various different types of ground beef (i.e. chuck, round, angus, etc.). The most amount of fat that can be added to ground beef is 30%, according to the USDA standards and the Federal Labeling standards for ground beef and cannot contain any additives, besides seasonings (USDA-FSIS, 2016).

The task of insuring the safety of meat products is given the USDA Food Safety Inspection Service (USDA-FSIS). This government agency oversees the inspection and labeling of meat products before sold to consumers (Rose et al., 2002). One of the biggest movements in how beef industry inspection was performed occurred after the Jack-in-the-Box outbreak of 1993 and 1994. The result of this nationwide scare was the implementation of a food safety measure known as Hazard Analysis and Critical Control Points, or HACCP (Has-Cip) and the raising of the internal cooking temperature from 140°F to 160°F (Liddle, 2013; USDA-FSIS, 2015). This is considered the first major food safety advancement since the enactment of the 1906 Federal Meat Inspection Act (Tucker, 2014). The HACCP protocol helped categorize the hazards that can be present in meat production into three groups: physical, chemical, biological. While identifying those three main categories of contamination, it also allows for "Critical Control Points" to be determined as well. "Critical Control Points" are those process steps that are considered to be crucial phases of production that cannot be strayed from in order to reduce the risk of a major food safety issues. (Brown, 2000; Lawley, 2012). It formally "defined as a point, step, or procedure at which control can be applied and a food-safety hazard can be prevented, eliminated, or reduced to an acceptable level" (Hulebak and Schlosser, 2002). Since the enactment of this measure, the internal cooking temperature for ground beef is currently set at 160°F (USDA-FSIS, 2016). Despite this increase in cooking temperatures the Centers for Disease Control (CDC) still estimates that between 11% and 28% of consumers still choose to consume undercooked ground beef (Andrews, 2014). This is a high number of consumers that can potentially be at a greater risk for Foodborne pathogenic exposure, which means that it is up to producers to created new ways to effectively reduce that risk in their production, cleaning, and sanitization practices.

Pathogens Associated with Ground Beef

The most well-known pathogenic contamination sources for ground beef are *Escherichia coli (E. coli)*[1]*,Salmonella* ssp., *Listeria monocytogenes (L. monocytogenes),* and *Campylobacter jejuni (C. jejuni)* (Eisel et al., 1997; Mora Garcia, 2016). All of these pathogenic contaminations would be classified under the biological forms of hazards when looking at where these hazards would apply in a HACCP plan for ground beef production.

Significant Ground Beef Foodborne Outbreaks

There have been several significant events that have led to both researchers and consumers to becoming more knowledgeable about food safety and the practices that put food on the grocery store shelves. The first outbreak that happened within ground beef, went unnoticed by most of the general public, and most individuals in the food industry as well. The outbreak started in February of 1982 and was not concluded until May of the same year. This outbreak was isolated to two states, Michigan and Oregon, and is considered by many within the industry to be the first outbreak of *E. coli* O157:H7. The first initial study of HUS after this outbreak was even titled "Hemorrhagic Colitis Associated with a Rare *Escherichia* coli Serotype" (Riley et al., 1983). This first initial outbreak occurred in a fast food chain from contaminated ground beef used in hamburgers; however, some major differences for this outbreak were that the median age was higher than that of the following outbreak and that there no deaths associated with it as well. At the time, the initial cooking temperature was set to 140°F as a part of a national standard, over the years that temperature has increase in small increments as more research was conducted on the pathogen (Riley et al., 1983; Rangel et al., 2005; Benedict, 2013). A decade later, it seemed the whole world would know about *E. coli* O157:H7 and how deadly it could be. Jack in the Box earned their stake in food safety history with one of the most historical foodborne illness outbreaks of 1993 by claiming four lives of small children and infecting hundreds of others

[1] For the purposes of this discussion, the emphasis will be placed on the "Big Seven" of the *E. coli* strains.

(Liddle, 2013; Tarshis, 2015). After much investigation, it was later discovered that the outbreak was caused by a pathogen known as *E. coli* O157: H7 from under cooked ground beef (Tuttle et al., 1998; Juska et al., 2000; Liddle, 2013). One interesting comment on this outbreak is that when it happened, everyone involved had never head of *E. coli* (Benedict, 2013). One fatal mistake that Jack in the Box made was that just before the outbreak the state of Washington health department, where the outbreak was first noted, had increased the required internal cooking temperature of ground beef from 140°F to 155°F. This meant that Jack in the Box was not within regulatory guidelines for the state of Washington, but still within regulation of the federal requirement (Unknown, 2011; Benedict, 2013). Another useful regulation rule that was put into place during this timeframe was called "Hazard Analysis and Critical Control Points" (HACCP). This would allow producers to look at the food production system and identify where points of control could be made against various hazards within their facility (Weingold et al., 1994; Hulebak and Schlosser, 2002). By 2000, 96% of the United States required reporting of *E. coli* O157: H7. Ground beef specifically contributed to 41% of the outbreaks (Rangel et al., 2005). Later on, as hygienic production practices improved, the statistics for beef related Shiga toxin-producing *E.coli* (STEC) outbreaks were reported to be improved by the CDC. From 2010 to 2014, there were a total of 120 outbreaks reported and only 24% percent of those were beef related STEC foodborne diseases (Crowe et al., 2015).

From 1998 to 2008, Laufer et al. (2015) estimated that beef was the fourth most common source of salmonellosis. Ground beef was particularly attributed to MDR strains of *Salmonella* (Laufer et al., 2015). That is why in 1998 *Salmonella* testing was additionally required for beef carcasses, along with *E. coli* O157:H7. Although it is not considered an adulterant in the same way that *E. coli* O157:H7 is in testing results. From 1973 to 2011, there were five deaths associated with salmonellosis outbreaks in beef. Serotype Typhimurium was isolated in 17% of the beef outbreaks reported during this timeline as well. In total salmonellosis is causes 400 deaths each year. In 2004, New Mexico reported a *Salmonella* Typhimurium outbreaks caused by

ground beef source. Although this outbreak was reported to have only lasted 12 days, it still effected three patients within this single state. The same strains were identified in thirty-one total patients across several different states over much longer period of time. The median age over the entire outbreak was 35 years-old (Cronquist et al., 2006). In the same report by Crowe et al. (2015) *Salmonella* was the leading cause of foodborne illness outbreaks, with beef contributing to five of those outbreaks (Crowe et al., 2015). With beef products specifically *Salmonella* accounts of 32.9% of the outbreaks documented, according to a 2015 document (Niyonzima et al., 2015). Though, thanks to better production practices and documentation, the prevalence of this pathogen in food production systems has been steadily decreasing over the years. *Salmonella* presence was shown to have decreased to 2.4% reported United States prevalence in 2011 (Cabrera-Diaz et al., 2013). The first case of *S.* Typhimurium DT 104 was not reported until 2003 (Dechet et al., 2006). This outbreak was originated from ground beef that re-ground at grocery store facilities and then sold to customers. The supplier was similar for all grocery stores involved in this outbreak. During the entirety of this 2003 outbreak the Center for Disease Control claimed a total of thirty infected people across six states, but no deaths occurred from this outbreak. One interesting point about this outbreak is that it was determined that this strain of *Salmonella* was resistant to no greater that five antibiotics; however, majority of patients involved in this outbreak did requires antibiotic treatments (Dechet et al., 2006). This outbreak is what lead researchers to attribute *S.* Typhimurium DT104 as a "multi-drug resistant" (MDR) pathogen (Sahu et al., 2013).

Escherichia coli O157:H7

It is a common misconception that all forms of *E. coli* are pathogenic, or harmful, to humans; however, this bacterium is a part of normal gastrointestinal biome for most warm-blooded creatures. It is when strains are passed between animals and humans or from human to human that concerns for gastrointestinal issues arise. Other sources that can become contaminated with *E. coli* strains are animal feed, soil/pasture/field, water, and even other wildlife species (Nazareth, 2017). Cattle are not known for being "clean" animals in terms of where fecal material

is distributed in herds. In an 1998 article by Farrell, Ronner, and Lee Wong, fecal material contained 0.8% to 5% *E.coli* O157:H7 when comparing various studies (Farrell et al., 1998). This attribute in cattle is why the hide is significant source of contamination; although transmission can occur from food sources, water sources, human, and animals in a variety of pathways (Cassin et al., 1998; Rangel et al., 2005; Soon et al., 2011; Jadeja and Hung, 2014). However, the amount of bacterial contamination seen on a specific carcass is proportional to the amount contamination initially present on the hide (Cassin et al., 1998). The reason that *E. coli* O157:H7 is specifically pointed out in meat production is because of its ability to produce Shiga toxins and the fact that specific strain is isolated from outbreaks time and time again. *Escherichia coli* belongs to the *Enterobacteriaceae* family. *Escherichia coli* O157:H7, a species, presents morphology as a gram-negative rod that has facultative respiration with an growth temperature range between 30°C and 45°C . It does not produce spores under stress to ensure survival, which helps contribute to the 160°F lethality temperature (Mora Garcia, 2016). These rod-shaped pathogenic bacteria can result in serious, sometimes even fatal, concerns within the gastrointestinal tract of humans, especially those individuals that are immunocompromised and cannot combat the pathogenic infection (Cassin et al., 1998; Law, 2000; Rangel et al., 2005). These specific strains of the pathogen are known as "Shiga Toxin Producing *Escherichia coli"* or STEC (S-Tec). These Shiga toxins are what cause the gastrointestinal issues in human if digested. This pathogen is able to inflict such issues due to its ability to make attaching and effacing lesions, along with its resistance to an acidic environment (Cassin et al., 1998; Law, 2000; Rangel et al., 2005). These lesions then cause abdominal cramps, diarrhea, bloody diarrhea. These can lead to more severe conditions like hemorrhagic colitis, hemolytic-uremic syndrome (HUS), or, sometimes, death. Hemorrhagic colitis and HUS can result in permanent damage to the patient's gastrointestinal system by requiring transfusions and dialysis of the blood (Friedrich et al., 2002; Smith et al., 2013). According to Soon, Chad, and Baines (2011) Beef accounted for 44% of the *E. coli* O157:H7 outbreaks in the United States from 1988 to 2007. In terms of specifically considering STEC

species, beef contributes 75% of foodborne illness outbreaks (Hauge et al., 2015). In terms of controlling the spread of this bacteria from farm to fork, there are numerous interventions and practices being researched for reduction by feeding practices before harvest and the use of steam cabinets on fresh carcasses after harvest (Smith et al., 2013).

Salmonella Typhimurium

Another member of the *Enterobacteriaceae* family is *Salmonella species. Salmonella enterica* is a gram-negative, facultative anaerobe that does not form spores, similar to *E.coli* O157:H7 (Alvarez-Ordonez et al., 2015; Azriel et al., 2016). Its optimum growth temperature is 37°C with a neutral pH; however it can survive in a range of environments for its short life cycle (Lianou and Koutsoumanis, 2012; Sahu et al., 2013). Despite not being able to form spores, *Salmonella* still has a quick adaptive skill, compared to most other organisms, in order to survive through stressful environments. One of these adaptations is the formation of biofilms, while research about this particular characteristic is still ongoing (Chiu et al., 2004; Lianou and Koutsoumanis, 2012; Azriel et al., 2016). The skill is the ability to change certain characteristics within strains in order to survive in the unfavorable environment (Azriel et al., 2016). The virulence factors of *Salmonella* can be complex in nature since there are so many serotypes that express different pathogenicity. This pathogen is able to occupy the human gastrointestinal biome by using what are reference to as "*Salmonella* pathogenicity islands" (SPIs) (Bugarel et al., 2011). These islands can be thought of as the command center or base for the pathogen to establish itself within the host. These islands are where initial proteins are allowed to start the attack on the host healthy cells, primarily within the gastrointestinal tract. Then what is called a "secretion system". There are three types of secretion systems that can be used individually or in combination with each other. This of course depends on the specific strain of *Salmonella* as well (Bugarel et al., 2011). Common symptoms are similar *E. coli*, since it presents itself as stomach pain or cramps, nausea, and diarrhea, which can increase to vomiting, bloody diarrhea if the infection is severe enough. These symptoms can last for seven days or longer. The longer the

symptoms present themselves, the increasing likelihood that hospitalization will occur (Kivi et al., 2007). Bugarel et al. estimated that this pathogen caused 1.4 million illness in the United States alone (Bugarel et al., 2011). The knowledge of *Salmonella* has grown tremendously in previous years and the names of the various species and serotypes has changed as well. For instance, the O antigens present in the pathogen have been defined and broken into serotypes (Chiu et al., 2004). Typhimurium and Enteritidis are the two main serotypes of concerns when it comes to the production of animal products for human consumption (Bugarel et al., 2011). Identifying specific genes and their expression has given researchers a great knowledge of each serotype's virulence factors. Enteritidis is the most prominently associated with foodborne illness, while Typhimurium is second (Sahu et al., 2013). There has been more recent attention to the Typhimurium strain DT104. The first key in understanding *Salmonella* Typhimurium DT104 is by breaking down the name. The full name can also be referred to as *Salmonella enterica* Typhimurium DT104 (Cloeckaert and Schwarz, 2001). *Salmonella* the species, while Typhimurium is the serotype. The final part of the name "DT104" is known as "Definitive phage Type 104". This specific DNA sequence within the *Salmonella* Typhimurium group is identified through phage libraries (Bugarel et al., 2011). *Salmonella* Typhimurium DT104 can be seen with and without multidrug resistant (MDR) attributes. This means that certain antimicrobials have little to no effect on this strain, which includes streptomycin, tetracycline, and ampicillin. Although the MDR characteristics do not affect the initial potency of bacteria (Sahu et al., 2013). It should also be noted that this group MDR *Salmonella* are small in number; however, there is concern for this number to increase over time (Bosilevac et al., 2009). MDR does seem to increase the fatality incidences among infected people (Poppe et al., 2002).

Antimicrobials

Since the first outbreaks concerning the pathogen previously noted, antimicrobials have been introduced in the many ways to food processing systems. Directive 7120.1 is the complete listings of antimicrobial and natural substances that are approved for the use on meat, poultry, and

food contact surfaces (FSIS, 2018). This directive is extensive in providing how various acids and combinations of acids can be added and/or used on meat and meat products. When considering which acids to focus on for research purposes, looking at current research is the best way to approach reaching novel antimicrobials that can be tested against previous research. When looking into current research three antimicrobials repeatedly appear in the literature. Those acids would be lactic acid, citric-based acids, and peroxyacetic acid (Edwards and Fung, 2006; Ortiz, 2006; Laury et al., 2009; Quilo et al., 2009; Zhao et al., 2014; Belanger and Stelzleni, 2015; Nair et al., 2016; Zhang et al., 2016; Eastwood et al., 2018; van Asselt et al., 2018). The reason that this antimicrobial is discussed or even considered for beef antimicrobial research is because of the demands that consumers have to see natural ingredients and additives being used. Sodium acid sulfate provides this agenda to the consumer. Using this natural antimicrobial could provide a gateway to a novel antimicrobial application to beef and meat products that was once only considered for fruits and vegetables.

Lactic Acid

The first product that most individuals will discuss heavily when it comes to the production of beef in the United States is Lactic Acid (LA). LA is used to target bacteria and is used primarily in meat and fermented meat products in order to reduced total bacterial load. It can be primarily used to target both *Salmonella* and *E. coli* species. It is use on whole poultry and beef carcass has been researched thoroughly by many scientists in the field, which has led government agencies (USDA and FDA) to approve its use in food production across many products. For the purposes of this discussion, emphasis will be placed on the beef, beef trimmings, and ground beef production. Lactic acid is produced through either respiration or fermentation by bacteria and is produced in one of two forms. One is called the "*L* isomer" while the other is called "Lactate". Lactate is primarily used for increasing the flavor and color profile of a food, while the "*L* isomer" is used as an antimicrobials (Mani-López et al., 2012). Lactic acid is typically applied in less than or equal to 5% solution for carcass washes (Bosilevac et al., 2006;

Mani-López et al., 2012). LA is effective as an antimicrobial because it produces bacteria that are called “Bacteriocins”, which are proteins that attack other bacteria like *Salmonella*, *E. coli,* and several other bacteria species. These bacteriocins are typically isolated from meat products as well. Which helps them be “Generally Regarded as Safe” (GRAS) (Lewus et al., 1991; Mani-López et al., 2012). When applied to trimmings or cuts of beef the percentage use typically decreases to around 2%. According to the 1973 FAO table, there was no limitations on the amount of lactic acid or any of its other forms for human consumption (Mani-López et al., 2012) . Although there has been much debate between lactic acid and hot water/steam usage on carcass interventions. Lactic acid seems to remain the choice of many producers (Bosilevac et al., 2006). During Bosilevac et al. study (2006) with a 2% lactic acid spray was able to reduce *E.coli* O157:H7 by 35%. In Bosilevac’s discussion of the results it is noted that laboratory strains of both *E.coli* O157:H7 and *Salmonella* Typhimurium were “reduced by up to 4 log CFU”, while natural microflora were only reduced by about 1 log CFU in two different sets of studies (Bosilevac et al., 2006). In a study discusses by Laury et al. (2009) it is noted that lactic acid can reduce *E. coli* O157:H7 by around 1.0 log CFU/100cm^2. When discussing the effects of lactic acid against *S.* Typhimurium, a 1% to 3% lactic acid spray was able replicate the same amount of reduction (Laury et al., 2009).

Peroxyacetic Acid

There is very limited, but promising research concerning peroxyacetic acid (PAA) and its use as a biological intervention in beef production concerning grinding products. Quilo et al.(2009) performed a study on the use of peroxyacetic acid and compared it to the results of potassium lactate, sodium metasilicate, and acidified sodium chlorite. This document briefly described some of the other studies that were focused on beef trim interventions (Quilo et al., 2009). According to the 47th revision of directive 7120.1, peroxyacetic acid was approved for use on meat and poultry carcasses and trimming with a PAA solution of no more than 400 parts per million (*ppm*) when applied to whole carcasses. When applied as a spray to trim, the *ppm* is

limited to 2000 (FSIS, 2018). The typical use of PAA is for direct contact washes with meat or poultry products and sanitation of direct contact surfaces. PAA can also be called peracetic acid or per acid. PAA can simply be broken down as a combination of hydrogen peroxide and acetic acid (USDA-AMS, 2000). The beneficial aspects of PAA is that it will maintain more consumer appeal in ground products, it effectively reduces microbial load, and appears to be the most cost effective antimicrobial available for producers to use in such high volumes (Quilo et al., 2009; Mohan et al., 2012). Another beneficial factor to peroxyacetic acid is the fact that it can be applied to both warm and cold temperature meat surfaces. This makes PAA what can be called "temperature blind", meaning that it can be applied at consistent ratios and achieve the same amount microbial reduction. Lactic acid can be increased to create the same amount of reduction, but this increases the overall cost of the product as well. Increasing the percent of LA will not guarantee the same effects on the beef products as the lower contraction, which presents a dilemma for producers (Gill and Badoni, 2004). Despite these presented issues, lactic acid still remains a primary choice for microbial reduction in beef production.

Sodium Acid Sulfate

The use of sodium acid sulfate (SAS) has been used in fruit and vegetable production for its properties as anti-browning component and as an additive to reduce emissions in livestock production. The chemical formula for this agent is $NaHSO_4$ and is also known as sodium bisulfate. The FDA does consider this compound as GRAS, but does not guarantee that the USDA will consider it in the same fashion for its use on meat products, including ground beef (Fan et al., 2009). This product can also be referred to as "bisulfate of soda, sodium hydrogen sulfate, and sodium bisulfate" (Kim et al., 2018). One study done by Fan et al. (2009) looked into the antibrowning and antimicrobial properties of a 3% SAS solution on apple slices. The antimicrobial portion of this study looked at "total plate count" or TPC. The SAS treatments sample started at "1.5 log CFU/g" and maintained the least amount of microflora throughout the shelf-life study (Fan et al., 2009). In a recently published study using SAS, the reduction of

Listeria innocua was tested in whole apples (Kim et al., 2018). 1% SAS with 60*ppm* PAA, 3% SAS with PAA at 60*ppm*, 3% SAS with 60*ppm* PAA and a surfactant sticker. The solutions were used to wash whole apples that were tested for *L. innocua* growth over a 14-day storage period. Over the storage period both 3% SAS treatments showed the most reduction. The SAS treatments showed a significant reduction when compared to the reduction displayed by the control and chlorine treatments. By day 14 the 3% SAS solution was able to complete a 5.56 log CFU/g (Kim et al., 2018). The upcoming research on this compound is still new to the field of food microbiology, although limited research on its use for meat applications, it is definitely coming on as a compound for continued investigation.

Summary of Proposed Objectives

Keeping in mind the previously discussed background information, it is clear to see that there is still room for improvement in terms of ground beef food safety. It is a goal to hopefully try to eliminate the possibility of pathogens being exposed to food sources, but realistically that goal will not become reality. This leaves the area of continuously improving the types of cleaning and sanitation practices available to producers. It is an obvious sign that this new sanitation and cleaning options need to be highly effective against both *E. coli*, more importantly *E. coli* O157 strains, and *Salmonella* strains. Another highly important factor is the how well these new techniques will be able to be implemented into an industry setting for continued use. That is why the main goal of this research is to provide a quick sanitation step for meat grinders that does not require a full break of the equipment. “Breaking down equipment” is slang phrase that industry personnel to use to reference to process of taking equipment a part in order to effectively and thoroughly clean it. This process usually costs time at the end of production time frame and at the startup of a production shift in order to put the equipment back together. Of course, the research discussed in this paper is not intended to replace that entire process; however, it is not feasible, nor economical, to say that this process should occur more often to decrease the likelihood of pathogens cross-contaminating large amounts of product. The reason for choosing to focus on

meat grinders is that most meat grinders are similar in their design and function, although there is a wide range of grinder sizes being used in the industry. In order for this research to applicable to these wide range of grinder sizes, this novel sanitation is that it must provide enough surface contact with the meat grinder casing in order to closely mimic the sanitation results when compared to a full scale break down. This is the reason why a liquid solution would not work and why ice is the state of choice for this research. Ice is an extremely easy resource to make and since all meat production facilities typically contain a freezer of some size, access to make it is already established. By adding a chemical to water, an antimicrobial solution can be turned into an antimicrobial ice product. This antimicrobial ice can then be processed through the meat grinder in the same way that beef trim is, meaning that production would not have to be stopped for a significant amount of time in order to apply the ice. The antimicrobials chosen to be tested for this research have already been discussed, but two of these antimicrobials are already heavily used in the meat industry. Sodium Acid Sulfate is used primarily in fruit and vegetable production, but there is no research to date that shows its applications on ground beef products. Another common practice in the meat industry is to spray beef trimmings with an antimicrobial. Research discussed in this document will show how this ice can be used in addition to that spray process, and what effects were seen in ground beef patties over a shelf life study.

REFERENCES

Alvarez-Ordonez, A., V. Broussolle, P. Colin, C. Nguyen-The, and M. Prieto. 2015. The adaptive response of bacterial food-borne pathogens in the environment, host and food: Implications for food safety. International Journal of Food Microbiology 213:99-109. doi: 10.1016/j.ijfoodmicro.2015.06.004

Andrews, J. 2014. CDC Shares Data on E. Coli and *Salmonella* in Beef. Food Safety News

Antic, D., B. Blagojevic, M. Ducic, I. Nastasijevic, R. Mitrovic, and S. Buncic. 2010. Distribution of microflora on cattle hides and its transmission to meat via direct contact. Food Control 21(7):1025-1029. doi: https://doi.org/10.1016/j.foodcont.2009.12.022

Arthur, T. M., D. M. Brichta-Harhay, J. M. Bosilevac, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2010. Super shedding of *Escherichia coli* O157:H7 by cattle and the impact on beef carcass contamination. Meat Science 86(1):32-37. doi: https://doi.org/10.1016/j.meatsci.2010.04.019

Azriel, S., A. Goren, G. Rahav, and O. Gal-Mor. 2016. The Stringent Response Regulator DksA Is Required for *Salmonella* enterica Serovar Typhimurium Growth in Minimal Medium, Motility, Biofilm Formation, and Intestinal Colonization. Infect Immun 84(1):375-384. doi: 10.1128/IAI.01135-15

Belanger, S., and A. Stelzleni. 2015. Effects of antimicrobials on shelf life characteristics of ground beef. Meat Science 101:134-134. doi: 10.1016/j.meatsci.2014.09.082

Benedict, J. 2013. Poisoned : the true story of the deadly E. coli outbreak that changed the way Americans eat. New York, NY : February Books.

Bosilevac, J. M., M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2009. Prevalence and characterization of *Salmonell*ae in commercial ground beef in the United States. Appl Environ Microbiol 75(7):1892-1900. doi: 10.1128/AEM.02530-08

Bosilevac, J. M., X. Nou, G. A. Barkocy-Gallagher, T. M. Arthur, and M. Koohmaraie. 2006. Treatments Using Hot Water Instead of Lactic Acid Reduce Levels of Aerobic Bacteria and Enterobacteriaceae and Reduce the Prevalence of *Escherichia coli* O157:H7 on Preevisceration Beef Carcasses. Journal of Food Protection 69(8):1808-1813. doi: 10.4315/0362-028x-69.8.1808

Brown, M. 2000. HACCP in the Meat Industry. Boca Raton : CRC Press ; Cambridge, England : Woodhead Pub., Boca Raton : Cambridge, England Boca Raton, Fla. : Cambridge, England.

Bugarel, M., S. A. Granier, F. X. Weill, P. Fach, and A. Brisabois. 2011. A multiplex real-time PCR assay targeting virulence and resistance genes in *Salmonella* enterica serotype Typhimurium. BMC Microbiology 11:151. doi: 10.1186/1471-2180-11-151

Cabrera-Diaz, E., C. M. Barbosa-Cardenas, J. A. Perez-Montano, D. Gonzalez-Aguilar, C. Pacheco-Gallardo, and J. Barba. 2013. Occurrence, serotype diversity, and antimicrobial resistance of *Salmonella* in ground beef at retail stores in Jalisco state, Mexico. Journal of Food Protection 76(12):2004-2010. doi: 10.4315/0362-028X.JFP-13-109

Alvarez-Ordonez, A., V. Broussolle, P. Colin, C. Nguyen-The, and M. Prieto. 2015. The adaptive response of bacterial food-borne pathogens in the environment, host and food: Implications for food safety. International Journal of Food Microbiology 213:99-109. doi: 10.1016/j.ijfoodmicro.2015.06.004

Andrews, J. 2014. CDC Shares Data on E. Coli and Salmonella in Beef. Food Safety News

Antic, D., B. Blagojevic, M. Ducic, I. Nastasijevic, R. Mitrovic, and S. Buncic. 2010. Distribution of microflora on cattle hides and its transmission to meat via direct contact. Food Control 21(7):1025-1029. doi: https://doi.org/10.1016/j.foodcont.2009.12.022

Arthur, T. M., D. M. Brichta-Harhay, J. M. Bosilevac, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2010. Super shedding of Escherichia coli O157:H7 by cattle and the impact on beef carcass contamination. Meat Science 86(1):32-37. doi: https://doi.org/10.1016/j.meatsci.2010.04.019

Azriel, S., A. Goren, G. Rahav, and O. Gal-Mor. 2016. The Stringent Response Regulator DksA Is Required for Salmonella enterica Serovar Typhimurium Growth in Minimal Medium, Motility, Biofilm Formation, and Intestinal Colonization. Infect Immun 84(1):375-384. doi: 10.1128/IAI.01135-15

Belanger, S., and A. Stelzleni. 2015. Effects of antimicrobials on shelf life characteristics of ground beef. Meat Science 101:134-134. doi: 10.1016/j.meatsci.2014.09.082

Benedict, J. 2013. Poisoned : the true story of the deadly E. coli outbreak that changed the way Americans eat. New York, NY : February Books.

Bosilevac, J. M., M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2009. Prevalence and characterization of salmonellae in commercial ground beef in the United States. Appl Environ Microbiol 75(7):1892-1900. doi: 10.1128/AEM.02530-08

Bosilevac, J. M., X. Nou, G. A. Barkocy-Gallagher, T. M. Arthur, and M. Koohmaraie. 2006. Treatments Using Hot Water Instead of Lactic Acid Reduce Levels of Aerobic Bacteria and Enterobacteriaceae and Reduce the Prevalence of Escherichia coli O157:H7 on Preevisceration Beef Carcasses. Journal of Food Protection 69(8):1808-1813. doi: 10.4315/0362-028x-69.8.1808

Brown, M. 2000. HACCP in the Meat Industry. Boca Raton : CRC Press ; Cambridge, England : Woodhead Pub., Boca Raton : Cambridge, England

Boca Raton, Fla. : Cambridge, England.

Bugarel, M., S. A. Granier, F. X. Weill, P. Fach, and A. Brisabois. 2011. A multiplex real-time PCR assay targeting virulence and resistance genes in Salmonella enterica serotype Typhimurium. BMC Microbiol 11:151. doi: 10.1186/1471-2180-11-151

Cabrera-Diaz, E., C. M. Barbosa-Cardenas, J. A. Perez-Montano, D. Gonzalez-Aguilar, C. Pacheco-Gallardo, and J. Barba. 2013. Occurrence, serotype diversity, and antimicrobial resistance of salmonella in ground beef at retail stores in Jalisco state, Mexico. Journal of Food Protection 76(12):2004-2010. doi: 10.4315/0362-028X.JFP-13-109

Cassin, M. H., A. M. Lammerding, E. C. D. Todd, W. Ross, and R. S. McColl. 1998. Quantitative risk assessment for Escherichia coli O157:H7 in ground beef hamburgers. Int J Food Microbiol 41(1):21-44. doi: https://doi.org/10.1016/S0168-1605(98)00028-2

Chiu, C. H., L. H. Su, and C. Chu. 2004. Salmonella enterica Serotype Choleraesuis: Epidemiology, Pathogenesis, Clinical Disease, and Treatment. Clinical Microbiology Reviews 17(2):311-322. doi: 10.1128/cmr.17.2.311-322.2004

Cloeckaert, A., and S. Schwarz. 2001. Molecular characterization, spread and evolution of multidrug resistance in Salmonella enterica typhimurium DT104. Vet Res 32(3-4):301-310. doi: 10.1051/vetres:2001126

Committee, B. I. F. S. C. E. 2009. Best Practices for Raw Ground Products.52.

Cronquist, A., S. Wedel, B. Albanese, C. M. Sewell, D. Hoang-Johnson, T. Ihry, M. Lynch, J. Lockett, N. Kazerouni, C. O'Reilly, and D. Ferguson. 2006. Multistate outbreak of Salmonella Typhimurium infections associated with eating ground beef--United States, 2004. MMWR. 55(7):180-182.

Crowe, S., B. Mahon, A. Vieira, and L. Gould. 2015. Vital Signs: Multistate Foodborne Outbreaks - United States, 2010-2014. MMWR. 64(43):1221-1225. doi: 10.15585/mmwr.mm6443a4

Davis, C. G., and B.-H. Lin. 2005. Factors Affecting U.S. Beef Consumption.

Davis, M. 2011. Where's the Beef: U.S. beef consumption in decline.

Dechet, A. M., E. Scallan, K. Gensheimer, R. Hoekstra, J. Gunderman-King, J. Lockett, D. Wrigley, W. Chege, J. Sobel, and G. Multistate Working. 2006. Outbreak of multidrug-resistant Salmonella enterica serotype Typhimurium Definitive Type 104 infection linked to commercial ground beef, northeastern United States, 2003-2004. Clinical Infectious Diseases 42(6):747-752. doi: 10.1086/500320

Eastwood, L. C., A. N. Arnold, R. K. Miller, K. B. Gehring, and J. W. Savell. 2018. Impact of Multiple Antimicrobial Interventions on Ground Beef Quality. Meat and Muscle Biology 2(1)doi: 10.22175/mmb2017.07.0039

Edwards, J. R., and D. Y. C. Fung. 2006. Prevention and decontamination of Escherichia coli O157 : H7 on raw beef carcasses in commercial beef abattoirs. J Rapid Meth Aut Mic 14(1):1-95. doi: DOI 10.1111/j.1745-4581.2006.00037.x

Eisel, W. G., R. H. Linton, and P. M. Muriana. 1997. A survey of microbial levels for incoming raw beef, environmental sources, and ground beef in a red meat processing plant. Food Microbiol 14(3):273-282. doi: http://dx.doi.org/10.1006/fmic.1996.0094

Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic Escherichia coli O157 prevalence in feces, hides, and carcasses of beef cattle during processing. doi: 10.1073/pnas.97.7.2999

Fan, X., K. J. Sokorai, C. H. Liao, P. Cooke, and H. Q. Zhang. 2009. Antibrowning and antimicrobial properties of sodium acid sulfate in apple slices. Journal of Food Protection 74(9):M485-492. doi: 10.1111/j.1750-3841.2009.01362.x

Farrell, B. L., A. B. Ronner, and A. C. Lee Wong. 1998. Attachment of Escherichia coli O157:H7 in Ground Beef to Meat Grinders and Survival after Sanitation with Chlorine and Peroxyacetic Acid. Journal of Food Protection 61(7):817-822. doi: 10.4315/0362-028x-61.7.817

Friedrich, A. W., M. Bielaszewska, W. L. Zhang, M. Pulz, T. Kuczius, A. Ammon, and H. Karch. 2002. Escherichia coli harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. J Infect Dis 185(1):74-84. doi: 10.1086/338115

FSIS, U. 2018. FSIS Directive 7120.1. Safe and suitable ingredients used in the production of meat, poultry, and egg products. In: USDA-FSIS (ed.).

Gill, C. O., and M. Badoni. 2004. Effects of peroxyacetic acid, acidified sodium chlorite or lactic acid solutions on the microflora of chilled beef carcasses. Int J Food Microbiol 91(1):43-50. doi: 10.1016/s0168-1605(03)00329-5

Gill, C. O., J. Bryant, and C. Landers. 2003. Identification of critical control points for control of microbiological contamination in processes leading to the production of ground beef at a packing plant. Food Microbiol 20(6):641-650. doi: 10.1016/s0740-0020(03)00024-8

Gill, C. O., and C. McGinnis. 1993. Changes in the microflora on commercial beef trimmings during their collection, distribution and preparation for retail sale as ground beef. Int J Food Microbiol 18(4):321-332. doi: 10.1016/0168-1605(93)90154-9

Hauge, S. J., T. Nesbakken, B. Moen, O. J. Rotterud, S. Dommersnes, O. Nesteng, O. Ostensvik, and O. Alvseike. 2015. The significance of clean and dirty animals for bacterial dynamics along the beef chain. Int J Food Microbiol 214:70-76. doi: 10.1016/j.ijfoodmicro.2015.07.026

Henderson, M. K. 2014. Comparison of the Effect of the Economic Recession on Certified Angus Beef to All Beef Products on Restaurant Menus. In: D. L. VanOverbeke, G. G. Hilton and F. B. Norwood (eds.). Oklahoma State University.

Hoyle Parks, A. R., M. M. Brashears, J. N. Martin, W. D. Woerner, L. D. Thompson, and J. C. Brooks. 2012. Shelf life and stability traits of traditionally and modified atmosphere packaged ground beef patties treated with lactic acid bacteria, rosemary oleoresin, or both prior to retail display. Meat Science 90(1):20-27. doi: 10.1016/j.meatsci.2011.05.020

Hulebak, K. L., and W. Schlosser. 2002. Hazard Analysis and Critical Control Point (HACCP) History and Conceptual Overview. Risk Anal. 22(3):547-552. doi: 10.1111/0272-4332.00038

Ismaïl, R., F. Aviat, V. Michel, I. Le Bayon, P. Gay-Perret, M. Kutnik, and M. Fédérighi. 2013. Methods for Recovering Microorganisms from Solid Surfaces Used in the Food Industry: A Review of the Literature No. 10. p 6169-6183. MDPI AG, Basel.

Jadeja, R., and Y.-C. Hung. 2014. Efficacy of near neutral and alkaline pH electrolyzed oxidizing waters to controlEscherichia coliO157:H7 andSalmonellaTyphimurium DT 104 from beef hides. Food Control 41:17-20. doi: 10.1016/j.foodcont.2013.12.030

Juska, A., L. Gouveia, J. Gabriel, and S. Koneck. 2000. Negotiating Bacteriological Meat Contamination Standards in the US: The Case of E.coli O157:H7. Sociologia Ruralis 40(2):249-271. doi: 10.1111/1467-9523.00146

Juska, A., L. Gouveia, J. Gabriel, and K. Stanley. 2003. Manufacturing bacteriological contamination outbreaks in industrialized meat production systems: The case of E. coli O157:H7. Journal of the Agriculture, Food, and Human Values Society 20(1):3-19. doi: 10.1023/A:1022416727626

Kiermeier, A., I. Jenson, and J. Sumner. 2015. Risk Assessment of Escherichia coli O157 Illness from Consumption of Hamburgers in the United States Made from Australian Manufacturing Beef. Risk Anal. 35(1):77-89. doi: 10.1111/risa.12248

Kim, S. A., S. H. Park, C. Knueven, R. Basel, and S. C. Ricke. 2018. A decontamination approach using a combination of bisulfate of soda and peracetic acid against Listeria innocua inoculated on whole apples. Food Control 84:106-110. doi: 10.1016/j.foodcont.2017.07.036

Kivi, M., A. Hofhuis, D. W. Notermans, W. J. Wannet, M. E. Heck, A. W. Van De Giessen, Y. T. Van Duynhoven, O. F. Stenvers, A. Bosman, and W. Van Pelt. 2007. A beef-associated outbreak of Salmonella Typhimurium DT104 in The Netherlands with implications for national and international policy. Epidemiology and Infection 135(6):890-899. doi: 10.1017/S0950268807007972

Koohmaraie, M., J. Bosilevac, M. De La Zerda, A. Motlagh, and M. Samadpour. 2015. Distribution of Escherichia coli Passaged through Processing Equipment during Ground Beef Production Using Inoculated Trimmings. Journal of Food Protection 78(2):273-280. doi: 10.4315/0362-028X.JFP-14-369

Laudert, S. 2012. Consumption trends No. 48. p 36, Minneapolis.

Laufer, A. S., J. Grass, K. Holt, J. M. Whichard, P. M. Griffin, and L. H. Gould. 2015. Outbreaks of Salmonella infections attributed to beef – United States, 1973–2011. 143(9):2003-2013. doi: 10.1017/S0950268814003112

Laury, A. M., M. V. Alvarado, G. Nace, C. Z. Alvarado, J. C. Brooks, A. Echeverry, and M. M. Brashears. 2009. Validation of a Lactic Acid– and Citric Acid–Based Antimicrobial Product for the Reduction of Escherichia coli O157:H7 and Salmonella on Beef Tips and Whole Chicken Carcasses. Journal of Food Protection 72(10):2208-2211. doi: 10.4315/0362-028x-72.10.2208

Law, D. 2000. Virulence factors of Escherichia coli O157 and other Shiga toxin-producing E. coli. Journal of Applied Microbiology 88(5):729-745. doi: 10.1046/j.1365-2672.2000.01031.x

Lawley, R. 2012. The food safety hazard guidebook. 2nd ed.. ed. Cambridge, U.K. : RSC Publishing, Cambridge, U.K.

Lewus, C. B., A. Kaiser, and T. J. Montville. 1991. Inhibition of food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl Environ Microbiol 57(6):1683-1688.

Lianou, A., and K. P. Koutsoumanis. 2012. Strain variability of the biofilm-forming ability of Salmonella enterica under various environmental conditions. Int J Food Microbiol 160(2):171-178. doi: 10.1016/j.ijfoodmicro.2012.10.002

Liddle, A. J. 2013. 1993 outbreak still drives food safety advances. Nation's Restaurant News 47(3):1-12. (Article)

Loukiadis, E., C. Bièche-Terrier, C. Malayrat, F. Ferré, P. Cartier, and J.-C. Augustin. 2017. Distribution of Escherichia coli O157:H7 in ground beef: Assessing the clustering intensity for an industrial-scale grinder and a low and localized initial contamination. Int J Food Microbiol 250:75-81. doi: 10.1016/j.ijfoodmicro.2017.03.009

Mani-López, E., H. S. García, and A. López-Malo. 2012. Organic acids as antimicrobials to control Salmonella in meat and poultry products. Food Research International 45(2):713-721. doi: 10.1016/j.foodres.2011.04.043

Marler, W. 2006. It is still a jungle out there. National Provisioner 220(8):8.

Martin, J., and C. Brooks. 2012. Ground beef shelf life National Provisioner No. 226. p 14, Northbrook.

Mitacek, R. 2017. The Effects of Aging on Beef Biochemical Properties. In: R. Ramanathan, G. Mafi and D. VanOverbeke (eds.). ProQuest Dissertations Publishing.

Mohan, A., F. W. Pohlman, J. A. McDaniel, and M. C. Hunt. 2012. Role of peroxyacetic acid, octanoic acid, malic acid, and potassium lactate on the microbiological and instrumental color characteristics of ground beef. Journal of Food Protection 77(4):M188-193. doi: 10.1111/j.1750-3841.2011.02600.x

Mora Garcia, M. 2016. Presence of possible pathogens in ground beef during basic food-handling practices. In: L. A. Kinman, R. Harp and J. Speshock (eds.). ProQuest Dissertations Publishing.

Nair, M., P. Lau, K. Belskie, S. Fancher, C. H. Chen, D. Karumathil, H. B. Yin, F. Ma, Y. Liu, I. Upadhyaya, A. Upadhyay, R. Mancini, and K. Venkitanarayanan. 2016. Enhancing the thermal destruction of Escherichia coli O157:H7 in ground beef patties by natural antimicrobials No. 112. p 163-163.

Nazareth, J. 2017. Prevalence of Salmonella Species and Escherichia coli O157:H7 in Organically Managed Cattle and Food Safety Status of Selected Meat Products, ProQuest Dissertations Publishing.

NewsOK. 2013. Oklahoma State University's Vegas Strip Steak Patent Application Moves Forward, Minneapolis.

Niyonzima, E., M. P. Ongol, A. Kimonyo, and M. Sindic. 2015. Risk Factors and Control Measures for Bacterial Contamination in the Bovine Meat Chain: A Review on Salmonella and Pathogenic E.coli. doi: 10.5539/jfr.v4n5p98

Ortiz, S. A. Q. 2006. Impact of Antimicrobial Treatment on Meat Microbial and Quality Characteristics.

Poppe, C., K. Ziebell, L. Martin, and K. Allen. 2002. Diversity in Antimicrobial Resistance and Other Characteristics Among Salmonella Typhimurium DT104 Isolates. Microbial Drug Resistance 8(2):107-122. doi: 10.1089/107662902760190653

Putnam, J., and L. Duewer. 1995. U.S. per capita food consumption: Record-high meat and sugars in 1994. Food Review 18(2):2.

Quilo, S. A., F. W. Pohlman, A. H. Brown, P. G. Crandall, P. N. Dias-Morse, R. T. Baublits, and J. L. Aparicio. 2009. Effects of potassium lactate, sodium metasilicate, peroxyacetic acid, and acidified sodium chlorite on physical, chemical, and sensory properties of ground beef patties. Meat Science 82(1):44-52. doi: 10.1016/j.meatsci.2008.12.002

Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin, and D. L. Swerdlow. 2005. Epidemiology of Escherichia coli O157:H7 outbreaks, United States, 1982-2002. Emerging Infectious Diseases 11(4):603-609. doi: 10.3201/eid1104.040739

Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare Escherichia coli serotype. The New England Journal of Medicine 308(12):681-685. doi: 10.1056/NEJM198303243081203

Rose, B. E., W. E. Hill, R. Umholtz, G. M. Ransom, and W. O. James. 2002. Testing for Salmonella in Raw Meat and Poultry Products Collected at Federally Inspected Establishments in the United States, 1998 through 2000. Journal of Food Protection 65(6):937-947. doi: 10.4315/0362-028x-65.6.937

Saha, J., P. Kaur Litt, D. Jaroni, and R. Jadeja. 2016. Labeling of Mechanically Tenderized Beef Products: A Mini Review. Labeling of Mechanically Tenderized Beef Products: A Mini Review 3(2)doi: 10.15406/mojfpt.2016.03.00067

Sahu, S. N., Y. Anriany, C. J. Grim, S. Kim, Z. Chang, S. W. Joseph, and H. N. Cinar. 2013. Identification of virulence properties in Salmonella Typhimurium DT104 using Caenorhabditis elegans. PLoS One 8(10):e76673. doi: 10.1371/journal.pone.0076673

Shang, X., and G. T. Tonsor. 2017. Food safety recall effects across meat products and regions. Food Policy 69:145-153. doi: 10.1016/j.foodpol.2017.04.002

Sinclair, U. 1906. The Jungle. p. 134-136.

Smith, B. A., A. Fazil, and A. M. Lammerding. 2013. A risk assessment model for Escherichia coli O157:H7 in ground beef and beef cuts in Canada: Evaluating the effects of interventions. Food Control 29(2):364-381. doi: 10.1016/j.foodcont.2012.03.003

Soon, J. M., S. A. Chadd, and R. N. Baines. 2011. Escherichia coli O157:H7 in beef cattle: on farm contamination and pre-slaughter control methods. Animal Health Research Reviews 12(2):197-211. doi: 10.1017/S1466252311000132

Tait, R. G., Jr., D. E. Wilson, and G. H. Rouse. 2005. Prediction of retail product and trimmable fat yields from the four primal cuts in beef cattle using ultrasound or carcass data. Journal of Animal Science 83(6):1353-1360. doi: 10.2527/2005.8361353x

Tarshis, L. 2015. Hamburger History. Storyworks 22(4):10-11. (Article)

Tucker, J. 2014. When Government Spreads Disease: The 1906 Meat Inspection Act. Freeman 64(10):14-16.

Tuttle, J., T. Gomes, M. P. Doyle, J. G. Wells, T. Zhao, R. V. Tauxe, and P. M. Griffin. 1998. Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties. Epidemiology and Infection 1999(122):185-192.

United States Committee on Health, E. L. a. P. 2002. Food Allergen Labeling and Consumer Protection Act : report (to accompany S. 2499), Washington, D.C. : U.S. G.P.O., Washington, D.C.

Washington, D.C.].

United States, F. 1994. Guide to Nutritional Labeling and Education Act (NLEA) requirements, Washington, D.C.? : Division of Field Investigations, Office of Regional Operations, Office of Regulatory Affairs, U.S. Food & Drug Administration, Washington, D.C.?].

Unknown. 2011. How Burgers Went To, And Came Back From, The Brink. 95(11):24. (Trade Journal)

Unknown. 2012. National Consumer Research Institute: Top Health Trends for 2012. Close-Up Media, Inc., Jacksonville.

USDA-AMS. 2000. Peracetic Acid Technical Report Handling.

USDA-AMS. 2016a. Federal Purchase Program Specification (FPPS) For Ground Beef Items, Frozen. In: USDA-AMS (ed.) 10,010.1, Revision 4. p 28.

USDA-AMS. 2016b. United States Standards for Grades of Carcass Beef. In: USDA-AMS (ed.). p 17.

USDA-FSIS. 2015. Safe minimum internal temperature chart. (Accessed October 14,2018.

USDA-FSIS. 2016. Ground Beef and Food Safety. https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/meat-preparation/ground-beef-and-food-safety/ct_index (Accessed 06/26/2018 2018).

van Asselt, E. D., M. Y. Noordam, M. G. Pikkemaat, and F. O. Dorgelo. 2018. Risk-based monitoring of chemical substances in food: Prioritization by decision trees. Food Control 93:112-120. doi: 10.1016/j.foodcont.2018.06.001

Van Wezemael, L., W. Verbeke, M. D. de Barcellos, J. Scholderer, and F. Perez-Cueto. 2010. Consumer perceptions of beef healthiness: results from a qualitative study in four European countries. BMC Public Health 10(1):342-342. doi: 10.1186/1471-2458-10-342

Weingold, S. E., J. J. Guzewich, and J. K. Fudala. 1994. Use of Foodborne Disease Data for HACCP Risk Assessment. Journal of Food Protection 57(9):820-830. doi: 10.4315/0362-028x-57.9.820

Young, B., L. White, and T. Biela. 2008. GROUND BEEF TECHNOLOGY 101: PART TWO*. National Provisioner 222(4):50-59.

Youssef, M. K., M. Badoni, X. Yang, and C. O. Gill. 2013. Sources of Escherichia coli deposited on beef during breaking of carcasses carrying few E. coli at two packing plants. Food Control 31(1):166-171. doi: 10.1016/j.foodcont.2012.09.045

Zare, S., Y. Zheng, and S. Buck. 2017. Examining th Effect of Food Recalls on Demand: The Case the of Ground Beef in the U.S. p 27. University of Kentucky.

Zhang, C., B. Li, R. Jadeja, J. Fang, and Y.-C. Hung. 2016. Effects of bacterial concentrations and centrifugations on susceptibility of Bacillus subtilis vegetative cells and Escherichia coli O157:H7 to various electrolyzed oxidizing water treatments. Food Control 60:440-446. doi: 10.1016/j.foodcont.2015.08.018

Zhao, T., P. Zhao, D. Chen, R. Jadeja, Y.-C. Hung, and M. P. Doyle. 2014. Reductions of Shiga Toxin-Producing Escherichia coli and Salmonella Typhimurium on Beef Trim by Lactic Acid, Levulinic Acid, and Sodium Dodecyl Sulfate Treatments - ProQuest. Journal of Food Protection 77(4):528.

CHAPTER III

NOVEL ANTIMICROBIAL ICE BASED CLEANING – IN – PLACE MEAT GRINDER SANITATION PROCESS DEVELOPMENT AND OPTIMIZATION

Introduction

Shiga toxin- producing *E. coli* (STEC), and *Salmonella enterica* are two major groups of foodborne pathogens in the United States. Scallen et al., (2011) estimated that STEC infections are responsible for approximately 2409 hospitalizations yearly and among these cases, over 2100 cases are caused by *E. coli* O157: H7. In the same study, Scallen et al., (2011) reported that various *Salmonella* spp. are responsible for 1,027,561 illnesses and 19,336 hospitalizations each year in the United States.

Cattle are a well-known source of *E. coli* O157: H7 and *S. enterica,* and because of that, beef products carry a significant risk of contamination with these foodborne pathogens (Koohmaraie, Bosilevac, Zerda, Motlagh and Samadpour, 2015). The muscles of a healthy animal are free of pathogens, but during slaughtering and especially during hide removal process, pathogens may get transferred to the surface of the carcass and may cause consumer illness if not handled properly. Ground beef is usually prepared from beef trimmings that are generally obtained from the surfaces of whole muscle cuts during fabrication and hence, has higher possibilities to contain pathogens (Loukiadis, Bièche-Terrier, Ferre, Cartier and Augustin, 2017).

Ground beef is typically produced in a continuous type of production practice. This means that most large-scale ground beef facilities only have a full break of the grinding equipment at the end of the production day or a partial break down of equipment when mechanical or minor issues occur during production (Gill, and McGinnis 1993; Gill, Bryant, and Landers, 2003). There are existing protocols and control points in the meat processing or retail operations, which specify the frequency and proper procedures of grinder sanitization. However, if contamination occurs between two cleaning operations, the grinder will potentially cross-contaminate large amount of products. An increase in the frequency of dissembling the grinder for cleaning will lead to increase in operating cost and reduced productivity. Therefore, there is a need for new rapid interventions which could be employed during ground beef processing to improve the overall safety of ground beef.

Peracetic acid (PAA) and is known for its antimicrobial properties. PAA has been proven effective in reducing the variety of foodborne pathogens from food matrices and food contact surfaces (Farrell, Ronner and Wong, 1998). In the same study, authors have also noticed that the number of positive samples from the metal chips those were glued to the augur housing surface decreased significantly after PAA treatment.

These research studies indicate that PAA could be an antimicrobial of choice for meat grinder sanitation. Therefore, the objective of this study was to develop and optimize a rapid meat grinder sanitization process using antimicrobial ice and solutions prepared from PAA and combinations of PAA with commercial products FreshFx®, Paradigm® and lactic acid.

Materials and Methods

Bacterial Cultures

In this study, a total of ten strains of *E. coli* O157: H7 and *S.* Typhimurium DT 104 were used. The five strains of *E. coli* O157: H7 were 1 (Beef isolate), 5 (human isolate), 932 (human isolate), E009 (Beef isolate), and E0122 (cattle isolate); and five strains of *Salmonella*

Typhimurium DT104 were H2662 (cattle isolate), 11942A (cattle isolate), 13068A (cattle isolate), 152N17-1 (dairy isolate) and H3279 (human isolate). All *E. coli* O157: H7 strains were adapted to 50 mg/L nalidixic acid for ease of isolation. Before each experiment, *E. coli* O157:H7 strains were grown individually in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) supplemented with 50 mg/L nalidixic acid and *S.* Typhimurium DT 104 strains were grown in TSB supplemented with 32 mg/l ampicillin, 16 mg/l tetracycline, and 64 mg/l streptomycin. Each overnight grown strain was then washed by centrifugation (3,000Xg for 15 min), and the pellet was resuspended in phosphate buffered saline (PBS). Pathogen mixtures were made by creating five strain mixtures prepared by mixing 2 mL of individual strains of respective the pathogens. Appropriate dilutions were made using PBS to achieve final concentrations of approximately 9 logs CFU/ml, and (high inoculum) and 6 logs CFU/ml (low inoculum).

Antimicrobial ice and solution preparation

For this study, antimicrobial efficacy of four types of ice; 350 mg/L peracetic ice (PAA), PAA+ 2% FreshFX (PAAF), PAA+2% Paradigm (PAAP), PAA+ 2% lactic acid (PAAL) and deionized water ice (DI) were investigated. PAA ice was prepared from a 15% peracetic acid solution (PeroxyChem, PA, USA). FreshFX and Paradigm were also acquired from PeroxyChem, USA. 2% lactic acid was prepared from 85% lactic acid solution (ThermoFisher Scientific, NJ, USA). For each experiment, a fresh treatment solution was prepared by mixing appropriate volumes of chemicals into the deionized water. Antimicrobial ice was prepared by freezing above mentioned solutions in plastic ice trays at -20^{O}C overnight.

Inoculation procedures

The meat grinders were inoculated with target pathogens by processing artificially contaminated beef. The beef samples were obtained from the Robert M. Kerr Food and Agricultural Products Center (Oklahoma State University, OK, USA). In the first step, two approximately 200 g uninoculated beef pieces (4”L X 4”W, prepared from beef shoulder clods,

temperature of beef- 2.0 ± 2 ºC) were processed through a bench top grinder (Model # 781, LEM products, OK, USA) to create food matrix inside the grinder to simulate commercial grinding operation. The meat grinders were then contaminated by double grinding two beef pieces (200 g each, 4"L X 4"W), prepared by inoculating 200 µl bacterial suspension/piece at high or low levels. Pathogens were allowed to attach for 15 min at room temperature. The attachment time was found to be optimal for these experiments (data not presented).

Bacterial transfer and decontamination experiments

After inoculation, meat grinders were treated by processing 1000 g antimicrobial ice and 500 ml of corresponding antimicrobial solution simultaneously (e.g., 1000 *g* PAA ice+ 500 ml PAA solution). Processed ice samples were collected in sterilized stomacher bags and analyzed for the presence of pathogens. After each treatment, two uninoculated beef pieces (approximately 200 *g* each) were processed through the grinder to determine bacterial transfer from contaminated meat grinder to beef prices. The resulting ground beef portion was collected in sterile stomacher bags (Seward, Worthing, UK). For all experiments, efficacies of antimicrobial ice were compared with DI treatment (1000 *g* DI ice+500 mL DI water) and no treatment controls.

Microbiological analysis

Ground beef corresponding to each treatment was mixed with PBS followed by mixing for 1 min using a stomacher (Seward, Ltd., London, UK). Further appropriate dilutions were made and 0.1 mL portions were plated on sorbitol MacConkey agar (SMAC; Oxoid, Basingstoke, UK) supplemented with 50 mg/L nalidixic acid for *E. coli* O157:H7 or xylose lysine deoxycholate agar (XLD; Becton Dickinson, Sparks, MD) supplemented with 32 mg/L ampicillin, 16 mg/L tetracycline, and 64 mg/L streptomycin for *S.* Typhimurium DT 104. All strains of *E.coli* O157:H7 were adapted to 50mg/L nalidixic aid (Jadeja *et al.*, 2013) Plates were stored at 37ºC for 24 h before counting. At the end of the incubation period, plates were observed for typical *E. coli* O157: H7 (colorless) and *Salmonella* (black) colonies. Selection and

confirmation of *E. coli* O157: H7 and *S.* Typhimurium DT 104 isolates were carried out using the procedure described in a previously published study. The presence of pathogens was also determined from processed ice samples. Briefly, after each treatment, 10 ml of processed ice and treatment solutions (the processed ice had the texture of runny slush) were collected and neutralized by mixing with 10 ml of 10% sodium thiosulfate solution. The resulting solutions were then enriched using previously published method to detect the presence of target pathogens.

Statistical analysis

All results presented are outcomes of at least three independent experimental replicates. Statistical analysis was performed using JMP PRO 13 (SAS Institute, Inc., Cary, NC). Tukey-Kramer test at the probability level of $P \leq 0.05$ was used for pairwise comparisons of means.

Results and Discussion

In order to mimic the contamination conditions, normally found in the processing environment, meat grinders were inoculated with target pathogens by processing artificially spiked meat pieces.

Efficacy of antimicrobial ice treatments to reduce cross-contamination from the meat grinders inoculated with low levels of pathogens

For low levels of inoculation, all ice treatments except DI, were able to to reduce *Escherichia* coli O157:H7 and *S.* Typhimurium DT 104 to non-detectable levels by direct plating. In comparison to the no treatment controls, after DI treatment, bacterial transfer was reduced from 2.93 to 1.62 and 2.75 to 1.48 log CFU/g for *E. coli* O157:H7 and *S.* Typhimurium DT 104, respectively. Enrichment of the all samples showed positive results for target pathogens, indicating that either surviving cell numbers were lower than the method's detection limit (1.3 log CFU/*g*) or potentially injured microorganisms were present in the ground beef which needed additional time to recover.

Efficacy of antimicrobial ice treatments to reduce cross-contamination from the meat grinders inoculated with high levels of pathogens

The effectiveness of various antimicrobial ice to reduce pathogens were also determined on meat grinders inoculated with high levels of *E. coli* O157: H7 and *S.* Typhimurium DT 104. All antimicrobial ice treatments were able to significantly ($P \leq 0.05$) reduced the cross-contamination of *E. coli* O157: H7 and *S.* Typhimurium DT 104 in ground beef in comparison to no treatment control and DI treatments(Figure 1 and 2). For grinders inoculated with *E. coli* O157:H7, NT, DI, PAAP, PAAL, PAAF and PAA yielded bacterial recoveries of 5.95,4.26, 3.79, 3.58, 3.54 and 3.50 log CFU/g, respectively. In case of *S.* Typhimurium DT 104 recoveries were 5.86, 4.18, 3.63, 3.23, 3.35 and 3.46 log CFU/g for treatments NT, DI, PAAP, PAAL, PAAF, and PAA, respectively. The PAAL treatment for *S.* Typhimurium DT 104 and PAA for *E. coli* O157: H7 resulted in a slightly higher reduction in cross-contamination, but cross-contamination reductions after all the antimicrobial treatments were not significantly ($P \leq 0.05$) different from each other.

In order to understand why all of the antimicrobial ice treatments yielded almost similar reduction in cross-contamination, meat grinder head was carefully disassembled and inspected after each treatment. It was observed that ice treatments were very effective in cleaning grinder augur and housing (visual observation), but for all the cases, beef accumulation between grinder plate and retainer ring were observed. We hypothesize that the antimicrobial ice was able to push-out meat debris and sanitize most parts of the augur and grinder head but, the meat that accumulated between grinder plate and retainer ring protected pathogens from the antimicrobial ice.

Survival of pathogens in processed ice samples

In order to determine the survival of pathogens in the ice after meat grinder treatments, all processed ice samples were analyzed for the presence of the pathogen by enrichment. All but

one DI samples were tested negative for the presence of target pathogens. As DI sample did not have antimicrobials, it was expected to have surviving pathogens. It is very important to ensure that the processed ice after the sanitation treatment does not become the source of contamination for the meat processing facilities. Therefore, complete destruction of pathogens in ice is essential.

Use of PAA and other antimicrobials to improve the microbiological quality of food products is well documented but, to the best of our knowledge, this is the first attempt to develop the clean-in-place type of process for meat grinders using antimicrobial ice, and hence, direct comparison of data is not possible with previously published literature. Current industry practices involve cleaning and sanitization of meat grinders at the end of the shift, and there is no intervention currently available to control cross-contamination during processing. One of the benefits of using antimicrobial ice based method is that it does not increase the temperature of the grinder unit which is very important to maintain the quality and safe temperature of the ground products. Cleaning/sanitizing effect of PAA based ice could be the functions of its antimicrobial capabilities and physical abrasion process. Use of antimicrobial ice does not require disassembling of the grinder, therefore; this intervention could be easily applied during a shift to reduce microbial cross-contamination in ground beef products. The sanitation process discussed in this study could not only improve food safety by reducing the chances of cross-contamination but also dramatically reduce the size (quantity of product) of product recalls.

Acknowledgements

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Tables and Figures

Table 1: Survival of *E. coli* O157:H7 and *S.* Typhimurium DT 104 in processed ice samples

Treatment	*E. coli* O157:H7		*S.* Typhimurium DT 104	
	High inoculation	Low inoculation	High inoculation	Low inoculation
DI	+*	+	+	+
PAA	-#	-	-	-
PAAF	-	-	-	-
PAAP	-	-	-	-
PAAL	-	-	-	-

* Indicates detection of target pathogen after enrichment

# Indicates no-detection of target pathogen after enrichment

Figure 1

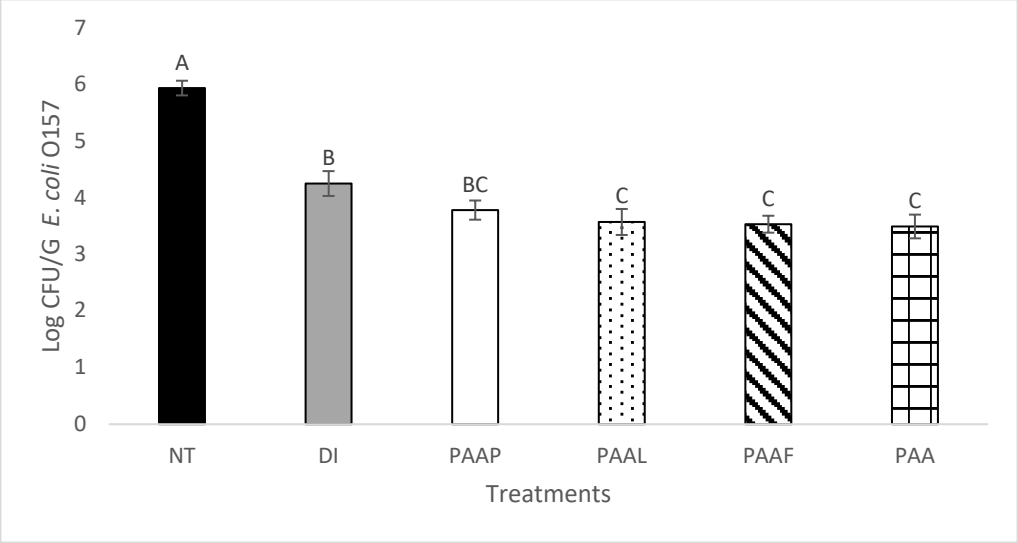

Figure: 1 Efficacy of antimicrobial ice to reduce *E. coli* O157: H7 from the meat grinder inoculated at high levels

NT: No treatment, DI: Deionized ice and water treatments, PAA: 350 mg/L peracetic ice+ liquid, PAAF: PAA+ 2% FreshFX ice +liquid, PAAP: PAA+2% Paradigm ice+ liquid, PAAL: PAA+ 2% lactic acid ice + liquid. For all the treatments 1000 g ice and 500 ml of liquid were used.

A-C, means bearing with no common letter are significantly different ($P \leq 0.05$)

Figure 2

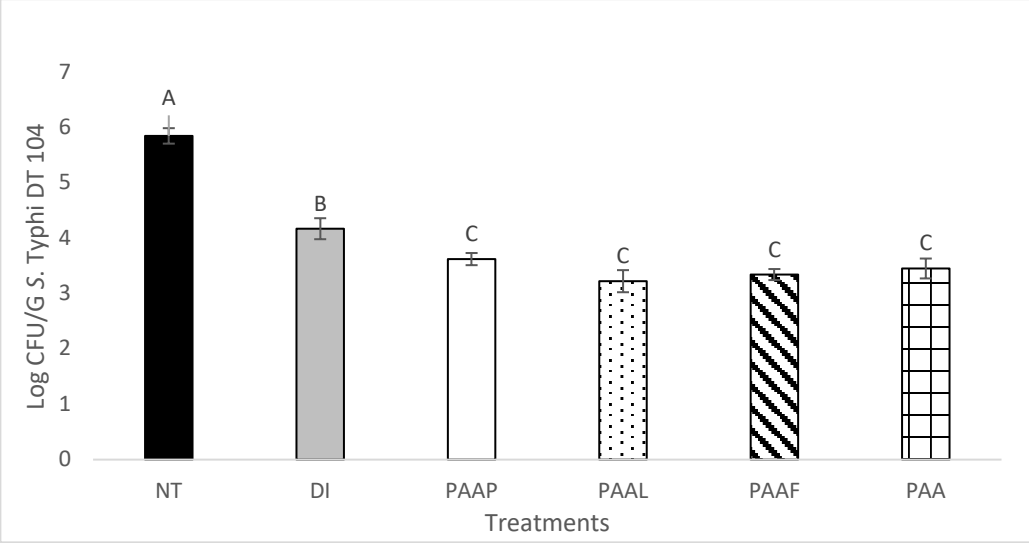

Figure: 2 Efficacy of antimicrobial ice to reduce *S.* Typhimurium DT 104 from the meat grinder inoculated at high levels

NT: no treatment, DI: Deionized ice and water treatments, PAA: 350 mg/L peracetic ice+ liquid, PAAF: PAA+ 2% FreshFX ice +liquid, PAAP: PAA+2% Paradigm ice+ liquid, PAAL: PAA+ 2% lactic acid ice + liquid. For all the treatments 1000 g ice and 500 ml of liquid were used.

A-C, means bearing with no common letter are significantly different ($P \leq 0.05$)

Figure 3

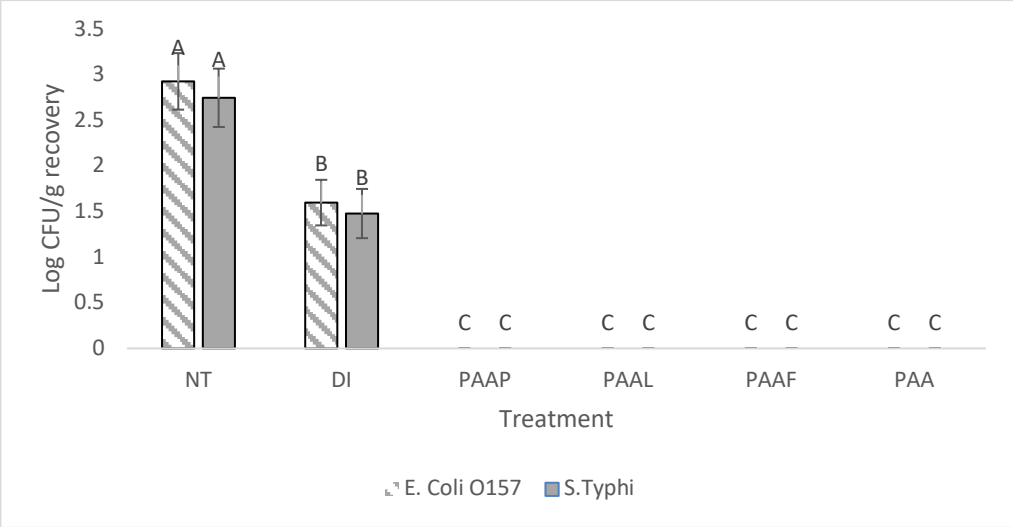

Figure: 3 Efficacy of antimicrobial ice to reduce *E. coli* O157:H7 and *S.* Typhimurium DT 104 from the meat grinder inoculated at low levels

NT: no treatment, DI: Deionized ice and water treatments, PAA: 350 mg/L peracetic ice+ liquid, PAAF: PAA+ 2% FreshFX ice +liquid, PAAP: PAA+2% Paradigm ice+ liquid, PAAL: PAA+ 2% lactic acid ice + liquid. For all the treatments 1000 g ice and 500 ml of liquid were used.

A-C, means bearing with no common letter are significantly different ($P \leq 0.05$)

REFERENCES

Farrell, B. L., A. B. Ronner, and A. C. Lee Wong. 1998. Attachment of *Escherichia coli* O157:H7 in Ground Beef to Meat Grinders and Survival after Sanitation with Chlorine and Peroxyacetic Acid. Journal of Food Protection. 61:817-822.

Gill, C. O., and C. McGinnis. 1993. Changes in the microflora on commercial beef trimmings during their collection, distribution and preparation for retail sale as ground beef. International Journal of Food Microbiology. 18:321-332.

Gill, C. O., J. Bryant, and C. Landers. 2003. Identification of critical control points for control of microbiological contamination in processes leading to the production of ground beef at a packing plant. Food Microbiology. 20:641-650.

Jadeja, R., Y. Hung, and J. M. Bosilevac. 2013. Resistance of Various Shiga Toxin-Producing *Escherichia coli* to Electrolyzed Oxidizing Water. Food Control. 30:580-584.

Koohmaraie, M., J. Bosilevac, M. De La Zerda, A. Motlagh, and M. Samadpour. 2015. Distribution of *Escherichia coli* Passaged through Processing Equipment during Ground Beef Production Using Inoculated Trimmings. Journal of Food Protection. 78:273-280.

Loukiadis, E., C. Bièche-Terrier, C. Malayrat, F. Ferré, P. Cartier, and J.-C. Augustin. 2017. Distribution of *Escherichia coli* O157:H7 in ground beef: Assessing the clustering intensity for an industrial-scale grinder and a low and localized initial contamination. International Journal of Food Microbiology. 250:75-81.

Scallan, E. R Hoekstra, F. Angulo, R. Taue, M. Widdowson, S. Roy, J. Jones and P. Griffin. 2011. Foodborne illness acquired in the United States- major pathogens. Emerging Infecious Diseases. 17(1) 7-15

CHAPTER IV

THE DEVELOPMENT OF MULTIPLE HURDLE APPROACH TO IMPROVE MICROBIAL SAFETY OF GROUND BEEF

Introduction

According to Section 331.1.1 of the "Federal Purchase Program Specification (FPPS) for Ground Beef Items, Frozen" (April 2016), the process of making ground beef is simply put as trimmings that will be ground twice (USDA-AMS, 2016a). With such a simple process, how is there still such a large, continuing issue with ground beef foodborne illness outbreaks? In 2010, the average cost of an *Escherichia coli* O157: H7 outbreak was $9,606, while *Salmonella spp.* costs $4312 per case (Shang and Tonsor, 2017). In addition to the economic cost, consumers can experience adverse health conditions that have the possibility to be fatal, especially for immunocompromised consumers (Farrell et al., 1998; Loukiadis et al., 2017). These pathogenic microorganisms become contaminates in beef, primarily from fecal contact with the carcass during the hide removal process (Ortiz, 2006; Mora Garcia, 2016). This contamination is spread further during processing (Ortiz, 2006; Niyonzima et al., 2015), which is why the pathogens are commonly associated with ground beef since exposed raw meat surface area is increased during fabrication and double grinding (Loukiadis et al., 2017).

In 1982, the first case of this pathogenic strain emerged in Oregon and Michigan (Kiermeier et al., 2015). However, the effects of this type of beef contamination were noted nationwide during the 1993 Jack-in-the-Box outbreak that infected hundreds including children and claimed four lives (Liddle, 2013; Tarshis, 2015). After much investigation and thousands of pounds in recalled product, it was later discovered that the outbreak was caused undercooked ground beef contaminated with *E. coli* O157:H7 (Tuttle et al., 1998; Juska et al., 2003; Liddle, 2013). The result of this was changed implementations that included Hazard Analysis and Critical Control Points (HACCP) systems in beef production facilities, along with supporting programs like Good Manufacturing Practices (GMPs) and Sanitation Standard Operating Procedures (SSOP's), into beef production food safety systems, and raising the beef internal cooking temperature from 140 degrees Fahrenheit to 160 degrees Fahrenheit (Eisel et al., 1997; Liddle, 2013; USDA-AMS, 2016a; USDA-FSIS, 2016).

While *E. coli* O157: H7 was the main focus of the Jack-in-the-Box outbreak, *Salmonella* spp. continues to play a role in beef-related foodborne illness outbreaks. Although it is not considered an adulterant, testing for this pathogen is still required in ground beef in many cases. The increasing concern with *Salmonella spp*. strains is antimicrobial resistance with the bacterial species (Andrews, 2014). There have been numerous studies on the use of various antimicrobials and organic acids in the meat industry. In an extensive research study published by Sergio Ortiz, the effects of potassium lactate, sodium metasilicate, peroxyacetic acid, and acidified sodium chlorite were tested on beef trimmings to determine the effects on color, sensory, lipid oxidation, pH, shear force, and cook loss percentage. While the results discussed the positive and negative aspects of each treatment, it was concluded that each treatment could offer the benefit of a neutral aspect to the industry desired characteristics (Ortiz, 2006). Quilo et al (2009) used a similar study in ground beef patties and came to the same conclusions with the same treatments as the previously discussed study (Quilo et al., 2009). Both of these experiments proved that the antimicrobials could be applied to the beef trim and not affect the end product characteristics.

These studies primarily focus on the application of antimicrobials to the beef trim only, still using the single intervention mindset. However, there is a growing interest in researching potential applications of antimicrobial solutions on meat grinding equipment in order to increase cleaning and sanitation options during production. In our previous study [unpublished data], the effectiveness of peroxyacetic acid (PAA) ice cubes were tested against both *E. coli* O157: H7 and *Salmonella spp.* In order to continue optimizing and developing that research, the objective of this second study is to introduce multiple interventions to the meat grinding process that will reduce the risk of cross-contamination from *E. coli* O157: H7 and *Salmonella spp.* in ground beef.

Materials and Methods

Beef Sample Preparation

Whole beef clod cuts were obtained from the Robert M. Kerr Food and Agricultural Product Center (Oklahoma State University, OK, USA) under USDA inspection. Clods were cut into 4-inch-long by 4-inch-wide squares weighing 200 grams per piece approximately. All beef sample pieces were prepared using this method for the microbial study. For the color analysis shelf life study, fresh, never frozen clods were cut into identical sample size and pieces. A total of 400g (2 sample pieces) were ground in for each section of the experiment process that required beef set.

Bacterial Culture Preparation

There were 5 strains of *Escherichia coli* O157:H7 used in this experiment. The five strains were as follows: 1 (Beef Isolate), 5 (Human Isolate), 932 (Human Isolate), E009 (Beef Isolate), and E0122 (Cattle Isolate). All strains were prepared and cultured in the same manner. Each strain was grown individually in tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD) that was supplemented with 50 mg/L nalidixic acid. Each strain was grown over night, approximately 12 hours, and then centrifuged to form a bacterial pellet (3,000 × *g* for 15 minutes). The pellet was re-suspended in phosphate buffered saline (PBS). In addition to the *E.*

coli O157:H7 strains, there were five strains of *S.* Typhimurium DT104. Each strain was as follows: H2662 (Cattle Isolate), 11942A (Cattle isolate), 13068A (Cattle Isolate), 152N17-1 (Dairy Isolate), and H3279 (Human Isolate). Each strain was grown individually on TSB supplemented with 32mg/L ampicillin, 16 mg/L tetracycline, and 64 mg/L streptomycin. *S.* Typhimurium DT104 strains were prepared in the same manner previously mentioned. In the case of both pathogens, all five strains were combined by taking 2mL of each. This created a five-strain mixture that was then used to make appropriate dilutions using PBS to achieve a 7 log CFU/mL.

Preparation of Antimicrobial Spray

A total of five antimicrobials were tested for their efficacy as a spray application: de-ionized water spray (DI), 3% Sodium Acid Sulfate (SAS; Jones-Hamilton Co., OH USA), 5% Lactic Acid (LA; PeroxyChem, PA, USA), 0.2 % Blitz® (PAA; PeroxyChem, PA, USA), SAS followed by PAA, and LA followed by PAA. This resulted in a total of seven treatments. All were prepared according to the manufacturer recommendations. These were prepared fresh for each experiment trial in individual 500mL volumes. Each type of spray (PAA, LA and SAS) was transferred into individual spray bottles that were calibrated to spray approximately 30mL of liquid on to the beef set. During the combination treatments, 15mL of each was sprayed on to the beef sample, making the total amount of solution sprayed 30mL. For de-ionized water spray (DI), 30mL was sprayed as well in the same manner. There was also a no treatment (NT) phase of the experiment that was used as control, in addition to the de-ionized water treatment. In terms of no treatment, no spray or ice was applied throughout the experiment process.

Preparation of Antimicrobial Ice

In addition to the antimicrobial spray treatments, one type of antimicrobial ice was tested. This ice was tested in previous experiments and proved to be the most effective (data for this experiment is not yet published). Ice was prepared fresh and frozen for 12 hours directly before

experimentation. Commercial PAA was used to created 1000g of ice for each treatment at 325 ppm. In addition to the PAA ice, 1000g of sterilized water ice was prepared. For each set of ice, there was matching pair of 500 mL of liquid solution to be processed with the ice through the bench top grinder (Model #781, LEM products, OK, USA). The ice and liquid were only ground once and were directly collected for microbial analysis.

Microbial Experiment Procedures

As previously mentioned, for each part requiring a beef set, there were 3 sample pieces (approximately 400g total) double ground to simulate industry practices. To create an environment a clean beef set was processed through a bench top grinder twice. The next step was to inoculate the second beef set. Each sample piece was spot inoculated with 200µL of the target bacteria, (400 µL total) across entire beef set, and allowed a fifteen-minute attachment time. After the attachment, 30mL of the determined spray treatment was applied with the calibrated spray bottles. Then contaminated pieces were processed through the benchtop meat grinder in the same manner. There was a second fifteen-minute attachment time immediately following this. Both attachment times occurred at room temperature. Following the fifteen minutes, the ice and matching liquid solution was processed. For each treatment, ground ice samples were collected and analyzed for microbial presence. The final step was double grinding a beef set that had been sprayed with an antimicrobial treatment. This last beef set was then analyzed for microbial presence due to cross-contamination.

Microbial Analysis

In order to determine microbial presence, the final ground beef set was stomached with an equal amount of PBS for thirty seconds at 230 rpm. Dilutions were made by transferring 1mL of the sample into 9mL of PBS accordingly, and plating was completed by transferring 0.1mL portions on to either sorbitol MacConkey agar (SMA; Oxoid, Basingstoke, UK) for *E.coli* O157:H7 or xylose lysine deoxycholate agar (XLD; Becton Dickinson, Sparks, MD) for *S.*

Typhimurium DT104. These plates were supplemented in the same manner as the TSB tubes mentioned earlier. After a 24-hour incubation period, plates were observed and counted for typical microbial presence (colorless *E. coli* O157:H7 colonies and black *S.* Typhimurium DT104 colonies). The microbial presence for the ice was determined by stomaching the ice sample alone and dilutions made from the stomached sample. Confirmation for the presence of *E. coli* O157:H7 and *S.* Typhimurium DT104 was carried out using a previously described method by Zhao et al. (2014).

Color analysis

In order to determine the effect of the antimicrobial spray on beef color, the same experiment procedure was followed as mentioned above, but no inoculated beef was introduced since the experiment was conducted in a non-BSL-2 space. The final beef sample was collected and formed into a ¼" beef patty using the adjust-a-burger patty press, place on a 3.75" by 6.75" soaker pad (Walton's, Item: 4610029, Wichita, KS, USA) in a 17S white foam tray (Walton's, Item: 4610100, Wichita, KS, USA) and overwrapped with food grade hand wrapping 18-inch plastic (Walton's, Item: 46092100, Wichita, KS, USA). Storage occurred in a coffin case cooler maintained at an average of 34°F and not exceeding 39°F for the duration of the study. Three random color readings of each beef patty sample were taken with a HunterLab MiniScan XE Plus spectrophotometer (HunterLab Associates, Reston, VA, USA) for each sample. Readings were taken on days 0 and 4. The HunterLab MiniScan XE Plus spectrophotometer was standardized according to manufacturing recommendations before each daily reading.

Statistical Analysis

All experiments were completed in triplicate to allow for independent replication analysis. Analysis was performed using JMP PRO 13 (SAS Institute, Inc., Cary, NC) and the Tukey-Kramer test was used for a pairwise comparisons of means. A probability level of $P \leq 0.05$ was set.

Results and Discussion

Figures 1 and 2 show the results for microbial reduction. All antimicrobial treatments showed a significant reduction from beef samples compared to no treatment control ($P \leq 0.05$). SAS treatment was found to be most effective and reduce the cross-contamination from meat grinder to uninoculated beef to non-detectable levels. But, after enrichment presence of target pathogens were found in the beef samples. Except for SAS, all other spray treatments were not significantly different from reducing bacterial cross-contamination from inoculated meat grinder. The hurdle approach resulted in *E. coli* O157:H7 recoveries of 3.58, 1.95, 1.53, 1.44, 1.5 and 1.81 for treatments NT, DI, LA, PAA, SAS+PAA and LA+PAA, respectively. The similar pattern of recoveries was observed with *S.* Typhimurium DT 104 except PAA reduced more (no significant difference) target microorganisms in comparison of LA treatment. It was also observed that deionized water spray was also effective in removing *E. coli* O157:H7 and *S. Typhimurium* DT 104. The treatment with LA was the second most effective treatment for *E. coli* O157:H7 and *S.* Typhimurium DT 104 from inoculated trim. It is believed that the effect of deionized water spray is a function of simple washing off effect. As there is no antimicrobial present in the deionized water, runoff resulted due to washing may carry live pathogens and could present increased cross-contamination risk. As this is first of its kind study, direct comparison of results is not possible with previous research. But, there are several studied which investigated the effect of antimicrobial treatments on beef trim.

In one such study, Zhao at el, (2014) investigated the antimicrobial efficacy of lactic acid as beef trim treatment. In the same study, authors have reported that at 3% application, lactic acid reduced *E. coli* O157:H7 to 0.9 log CFU/cm^2 , while at 5% it was reduced to 1.35 log CFU/cm^2 (Zhao at el, 2014) . Though, direct comparison is not possible with this study and our results due to different application methods and reporting units, our multiple hurdle approach resulted to greater target pathogen reduction in comparison of single intervention treatment.

Figure 3 depicts a*, chroma values, and hue for the shelf life color analysis, respectively. The larger the chroma value the more intense the red color. Hue determine how much discoloration was seen in the sample over time, the larger the number the more discoloration or less red was seen in the sample. a* values reference the change of color from red(+a*) to green (-a*). There was no significant difference between the treatments so that is why the graph does not show the timeline across each treatment. When looking at a* values SAS + Blitz® reported the highest values at 21.75, just behind NT and DI (controls). LA was the lowest overall value at 19.36. Across chroma values, the results were similar with SAS + Blitz® reporting the highest value (30.21) and LA recording the lowest (28.91). This means that SAS + Blitz® display a brighter, more cherry red color for a longer period of time, while LA did not. Finally, in looking at hue, LA + Blitz® recorded the highest number at 45.42, which means that is displayed the most discoloration over the shelf life period.

Discoloration of meat is the leading reason why consumers do purchase meat off of the grocery store shelf (Mitacek, 2017). This is why understanding how a change in the pH of meat will affect the shelf life color display (Mitacek, 2017). There are numerous studies determining how different packaging, such as Modified Atmosphere Packaging (MAP), and the changing of pH in meat (Hoyle Parks et al., 2012). An experiment was completed comparing “lactic acid bacteria (LAB)” and “rosemary extracts” in both traditional and MAP packaging in ground beef patty storage (Hoyle Parks, et al., 2012). LAB was noted to have displayed a more intense red color after initial application, but then display more discoloration as the storage time continued. However, the overall display of the ground beef patties in the traditional type of packaging, there were no significant differences in the color (Hoyle Parks, et al., 2012). These results by Hoyle Parks et al. (2012) were also in agreement with the study done by Quilo et al. (2009). Once again there was no observable color change from the application of antimicrobials. Hoyle Parks et al. (2012) observed that the reason no significant change in color from LAB was that it reduced the “accumulation of lipid oxidation by-products” (Hoyle Parks et al., 2012).

In conclusion, our multi-hurdle approach was effective in reducing cross-contamination from inoculated meat grinder to beef. SAS showed the most significant reduction of both *E. coli* O157:H7 and *S.* Typhimurium DT104 compared to both NT and DI control treatments ($P \leq 0.05$). This significant reduction has the potential to be an additional antimicrobial treatment that the industry can use to decrease bacterial presence ($P \leq 0.05$). SAS + PAA and PAA displayed the most benefits in maintaining a bright cherry red color which will increase consumer appeal on the shelf. With keeping both color and microbial reduction in mind, SAS would be the recommendation for further use based on current data. This study gives a future direction in order to obtain more useful and reliable data. Further studies will be needed to confirm color analysis, if there is a possibility for microbial reduction over the time of shelf life display, and industrial specifications needed to apply these interventions logically.

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Tables and Figures

Figure 1

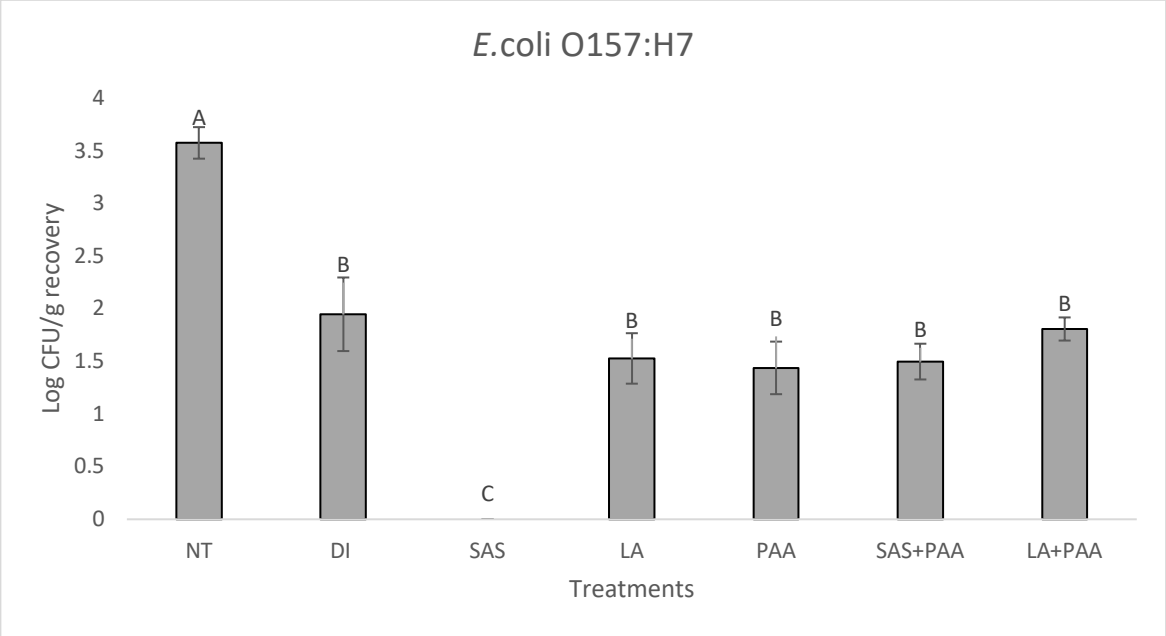

*Figure 1-Recovery of *E. coli* O157:H7. No Treatment (NT), de-ionized water spray (DI), 3% Sodium Acid Sulfate (SAS), 5% Lactic Acid (LA), 0.2 % Blitz® (PAA;), SAS followed by Blitz® (SAS + PAA), and LA followed by Blitz® (LA + PAA). A-C, means bearing no common letters are significantly different ($P \leq 0.05$).

Figure 2

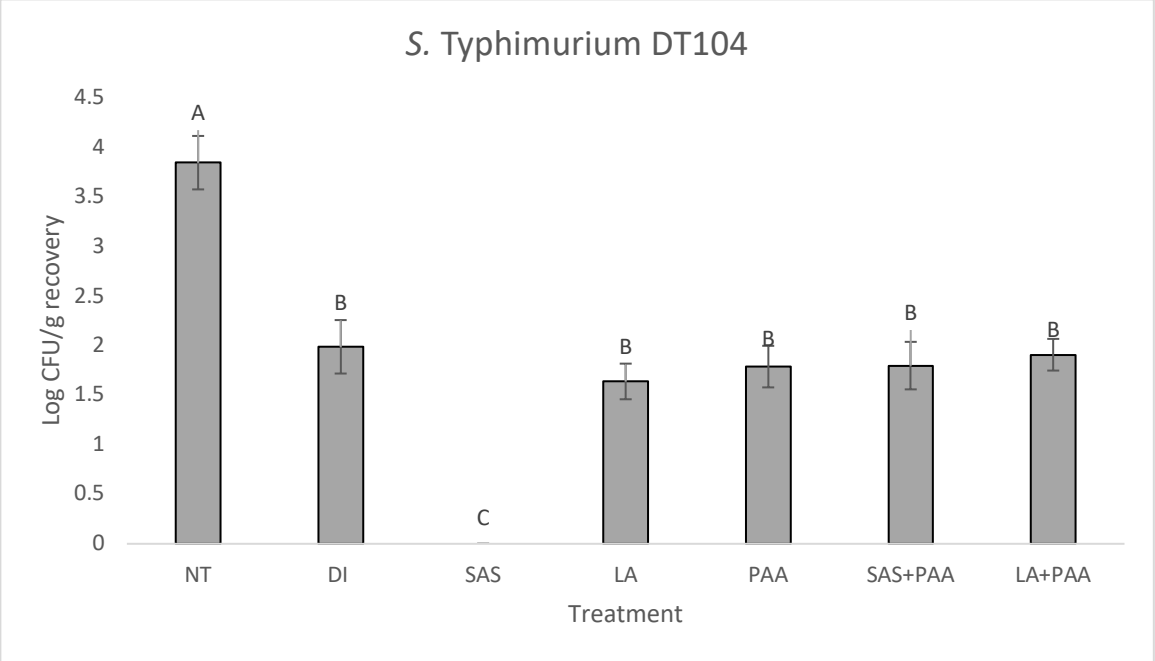

*Figure 2-Recovery of *S.* Typhimurium DT104. No Treatment (NT), de-ionized water spray (DI), 3% Sodium Acid Sulfate (SAS), 5% Lactic Acid (LA), 0.2 % Blitz® (PAA), SAS followed by Blitz® (SAS + PAA), and LA followed by Blitz® (LA + PAA). A-C, means bearing no common letters are significantly different ($P \leq 0.05$).

Figure 3

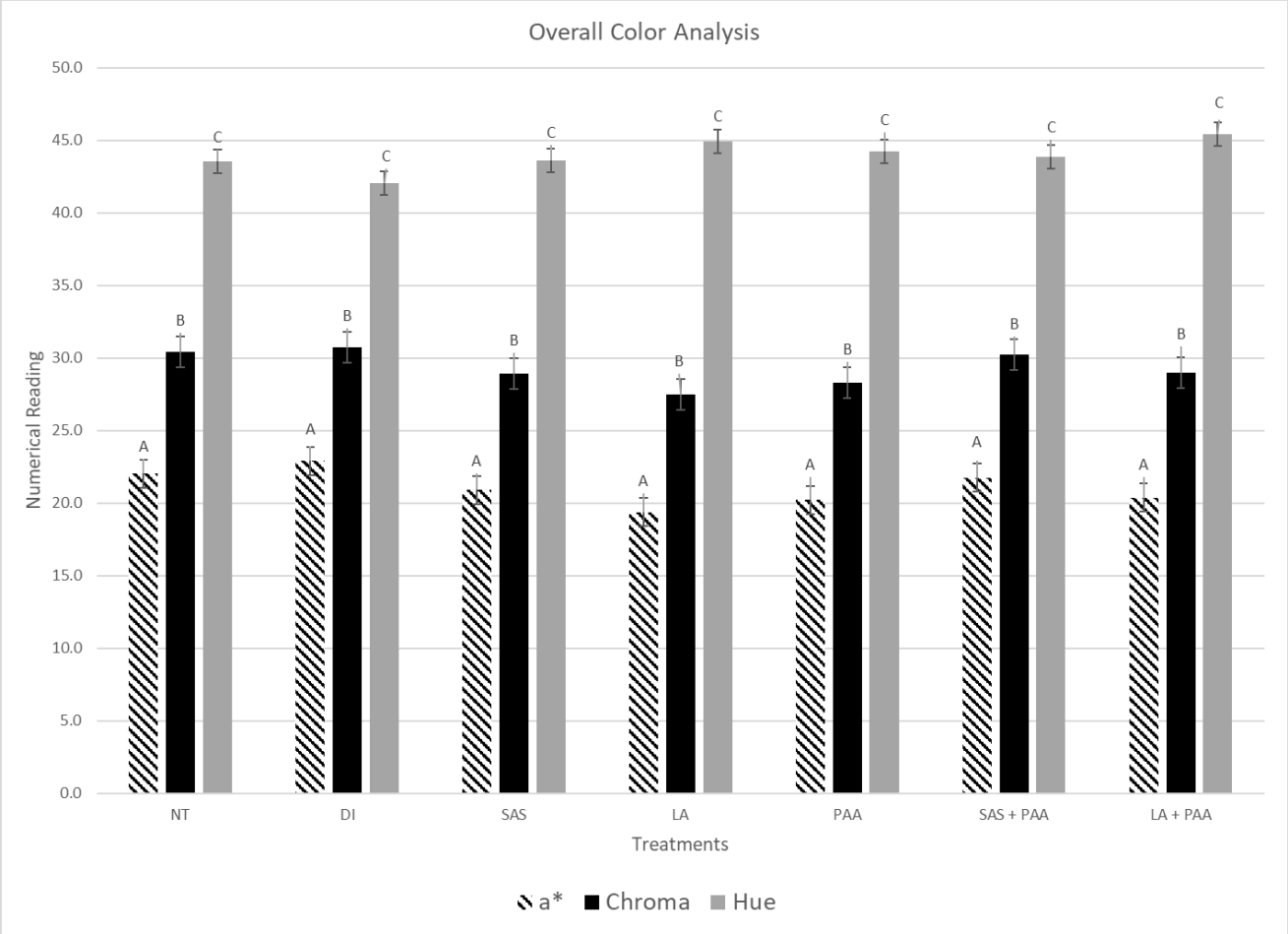

Figure 3- Comparison of a, Hue, and Chroma. No Treatment (NT), de-ionized water spray (DI), 3% Sodium Acid Sulfate (SAS), 5% Lactic Acid (LA), 0.2 % Blitz® (PAA), SAS followed by Blitz® (SAS + PAA), and LA followed by PAA (LA + PAA). A-C, means bearing no common letters are significantly different ($P \leq 0.05$).

Figure 4

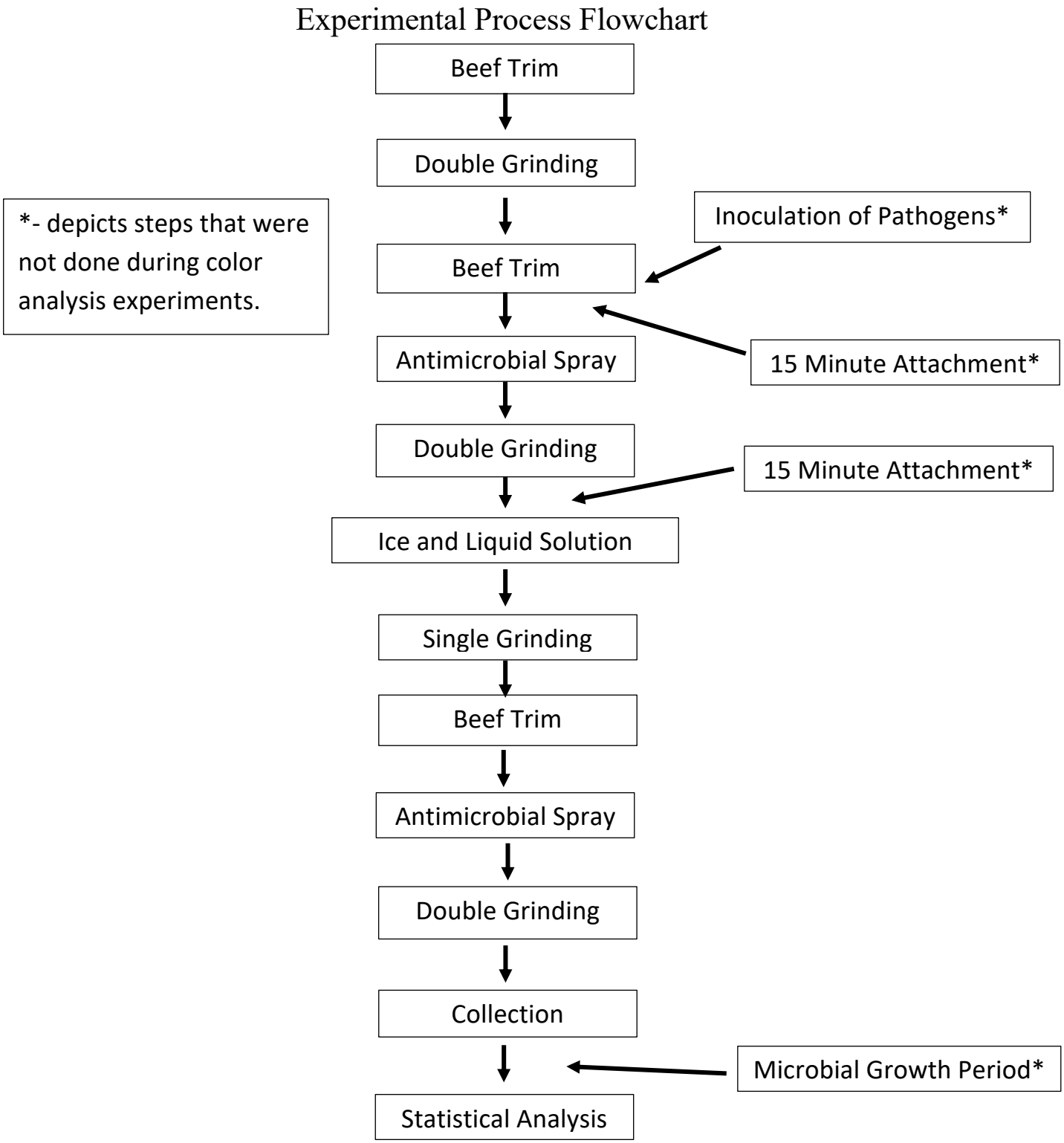

Figure 4: Experimental process flowchart used for the experiments within this chapter.

REFERENCES

Andrews, J. 2014. CDC Shares Data on E. Coli and *Salmonella* in Beef. Food Safety News

Eisel, W. G., R. H. Linton, and P. M. Muriana. 1997. A survey of microbial levels for incoming raw beef, environmental sources, and ground beef in a red meat processing plant. Food Microbiology 14(3):273-282. doi: http://dx.doi.org/10.1006/fmic.1996.0094

Farrell, B. L., A. B. Ronner, and A. C. Lee Wong. 1998. Attachment of *Escherichia coli* O157:H7 in Ground Beef to Meat Grinders and Survival after Sanitation with Chlorine and Peroxyacetic Acid. Journal of Food Protection 61(7):817-822. doi: 10.4315/0362-028x-61.7.817

Hoyle Parks, A. R., M. M. Brashears, J. N. Martin, W. D. Woerner, L. D. Thompson, and J. C. Brooks. 2012. Shelf life and stability traits of traditionally and modified atmosphere packaged ground beef patties treated with lactic acid bacteria, rosemary oleoresin, or both prior to retail display. Meat Sci 90(1):20-27. doi: 10.1016/j.meatsci.2011.05.020

Juska, A., L. Gouveia, J. Gabriel, and K. Stanley. 2003. Manufacturing bacteriological contamination outbreaks in industrialized meat production systems: The case of E. coli O157:H7. Journal of the Agriculture, Food, and Human Values Society 20(1):3-19. doi: 10.1023/A:1022416727626

Kiermeier, A., I. Jenson, and J. Sumner. 2015. Risk Assessment of *Escherichia coli* O157 Illness from Consumption of Hamburgers in the United States Made from Australian Manufacturing Beef. Risk Anal. 35(1):77-89. doi: 10.1111/risa.12248

Liddle, A. J. 2013. 1993 outbreak still drives food safety advances. Nation's Restaurant News 47(3):1-12. (Article)

Loukiadis, E., C. Bièche-Terrier, C. Malayrat, F. Ferré, P. Cartier, and J.-C. Augustin. 2017. Distribution of *Escherichia coli* O157:H7 in ground beef: Assessing the clustering intensity for an industrial-scale grinder and a low and localized initial contamination. International Journal of Food Microbiology 250:75-81. doi: 10.1016/j.ijfoodmicro.2017.03.009

Mitacek, R. 2017. The Effects of Aging on Beef Biochemical Properties. In: R. Ramanathan, G. Mafi and D. VanOverbeke (eds.). ProQuest Dissertations Publishing.

Mora Garcia, M. 2016. Presence of possible pathogens in ground beef during basic food-handling practices. In: L. A. Kinman, R. Harp and J. Speshock (eds.). ProQuest Dissertations Publishing.

Niyonzima, E., M. P. Ongol, A. Kimonyo, and M. Sindic. 2015. Risk Factors and Control Measures for Bacterial Contamination in the Bovine Meat Chain: A Review on *Salmonella* and Pathogenic E.coli. doi: 10.5539/jfr.v4n5p98

Ortiz, S. A. Q. 2006. Impact of Antimicrobial Treatment on Meat Microbial and Quality Characteristics.

Quilo, S. A., F. W. Pohlman, A. H. Brown, P. G. Crandall, P. N. Dias-Morse, R. T. Baublits, and J. L. Aparicio. 2009. Effects of potassium lactate, sodium metasilicate, peroxyacetic acid, and acidified sodium chlorite on physical, chemical, and sensory properties of ground beef patties. Meat Science 82(1):44-52. doi: 10.1016/j.meatsci.2008.12.002

Shang, X., and G. T. Tonsor. 2017. Food safety recall effects across meat products and regions. Food Policy 69:145-153. doi: 10.1016/j.foodpol.2017.04.002

Tarshis, L. 2015. Hamburger History. Storyworks 22(4):10-11. (Article)

Tuttle, J., T. Gomes, M. P. Doyle, J. G. Wells, T. Zhao, R. V. Tauxe, and P. M. Griffin. 1998. Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties. Epidemiol. Infect. 1999(122):185-192.

USDA-AMS. 2016. Federal Purchase Program Specification (FPPS) For Ground Beef Items, Frozen. In: USDA-AMS (ed.) 10,010.1, Revision 4. p 28.

USDA-FSIS. 2016. Ground Beef and Food Safety. https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/meat-preparation/ground-beef-and-food-safety/ct_index (Accessed 06/26/2018 2018).

Zhao, T., P. Zhao, D. Chen, R. Jadeja, Y.-C. Hung, and M. P. Doyle. 2014. Reductions of Shiga Toxin-Producing *Escherichia coli* and *Salmonella* Typhimurium on Beef Trim by Lactic Acid, Levulinic Acid, and Sodium Dodecyl Sulfate Treatments - ProQuest. Journal of food protection 77(4):528.

VITA

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