Peracetic Acid Effects on Shelf Life and Survival of *Escherichia coli* on Beef Steaks

by

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ABSTRACT

A study was conducted to determine the effectiveness of peracetic acid (PAA) against *Escherichia coli* O157:H7 in beef. In this study, 150 slices of beef ball tips (IMPS: 185B) weighing 100g each were inoculated with either a high (10^6 CFU/mL) or a low (10^2 CFU/mL) inoculation of a cocktail made of five strains of *E. coli* O157:H7. After a 30 minute attachment time at ambient temperature, the inoculated samples were treated with either 0.012% (120ppm) PAA, 0.04% (400ppm) PAA, or were left untreated as the control. The amount of peracetic acid solutions applied to each sample was approximately 3mL in total. After a 5-minute treatment time, samples (including control samples) were sprayed with approximately 1mL of sodium thiosulfate to neutralize the reaction. The neutralized samples were plated onto Cefixime Tellurite Sorbitol MacConkey agar (CT-SMAC) and incubated for 24 hours at 35° C. Three replications were conducted. For the low (10^2 CFU/mL) inoculation level of *E. coli* O157:H7, the 120ppm treatment of PAA reduced bacterial numbers by 0.067 log CFU/g, which was not different ($P>0.05$) from the control. The 400ppm treatment for the low inoculation level of *E. coli* O157:H7 reduced ($P<0.05$) the bacterial numbers by 0.568 log CFU/g. When treatment was applied to the high (10^6 CFU/mL) concentration of *E. coli* O157:H7, both the 120ppm treatment and the 400ppm treatment of PAA reduced ($P<0.05$) the bacterial numbers (reductions were 0.12 log CFU/g and 0.19 log CFU/g, respectively). While both 120ppm PAA and 400ppm PAA reduced the overall

amount of *E. coli* O157:H7 when compared to the positive controls, not all reductions were significant, and treatment with 400ppm PAA was the most effective at reducing *E. coli* O157:H7 on the samples.

Sensory analysis was performed by treating samples with 0.04% (400ppm) or 0.012% (120ppm) peracetic acid, with some samples left untreated as a control. Total volume of peracetic acid solution applied was approximately 3mL per sample. Samples were cooked to an internal temperature of 160° F, then cut (2.54cm x 1cm x 1cm) and placed into plastic cups labeled with random 3-digit codes. Panelists evaluated samples one at a time using an 8-point hedonic scale for initial and sustained tenderness, initial and sustained juiciness, flavor intensity, and off-flavor intensity. Results from the sensory analysis showed no difference between treatments ($P>0.05$) in any of these characteristics.

Data from Warner-Bratzler shear force measurements indicated no difference ($P>0.05$) between treatment with 400ppm peracetic acid and the control, or between treatment with 120ppm or 400ppm peracetic acid, but did indicate an increase ($P<0.05$) in tenderness between treatment with 120ppm peracetic acid and the control. It is of note that the shear force values for treatment with 400ppm peracetic acid were lower than the values for the control, indicating that they were slightly more tender when treated; however, this difference was not significant ($P>0.05$).

In a shelf-life study, 100g slices of beef ball tips (n=108) were inoculated with 100μL of the high (10^6 CFU/mL) inoculum. Following a 30-minute bacterial attachment time, samples were sprayed with 0.012% (120ppm) PAA, 0.04%

(400ppm) PAA, or left untreated as a positive control. Total volume of peracetic acid solution applied was approximately 3mL per sample. After a 5-minute treatment time, samples were sprayed with approximately 1mL of 0.1% sodium thiosulfate to neutralize any remaining reaction from the PAA. Samples were packaged in a Styrofoam tray with overwrap and placed in a walk-in cooler. On days 1, 3, 5, and 7, color was analyzed using a Hunterlab L* a* b* colorimeter, then samples were plated in duplicate onto CT-SMAC, Plate Count Agar, Coliform petrifilms, and Yeast/Mold petrifilms. Plates were incubated 24 hours following the manufacturers' instructions. This process was repeated on different days for a total of three replications. Results showed that, although there were some interactions for color by day and replication of the study, there is no difference ($P>0.05$) in L*, a*, or b* color values between treatment values alone with 120ppm peracetic acid, 400ppm peracetic acid, or the control. Furthermore, results indicate that a treatment of 400ppm peracetic acid is more effective ($P<0.05$) at controlling bacterial growth than 120ppm peracetic acid or the control. In summary, peracetic acid may be viable for use in a multi-hurdle approach to the control of *E. coli* O157:H7 without negative effects to shear force or quality or sensory attributes.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LITERATURE REVIEW

Introduction

According to recent data, roughly 47.8 million people suffer from foodborne illnesses each year in the United States; of that, only 127,839 per year are treated in a hospital (CDC, 2011). According to the CDC (2011), there are 31 foodborne pathogens, which only account for 9.4 million (roughly 19.6%) illnesses, while the others are caused by unknown agents. Out of the estimated 127,839 hospitalizations per year, *E. coli* is responsible for about 2,138 of these cases (CDC, 2011). It is important to note that, while this number may seem small compared to the total hospitalizations, *E. coli* O157:H7 is ranked fifth among foodborne pathogens that result in hospitalization (CDC, 2011). In addition, the economic burden in the United States for *E. coli* O157:H7 related illnesses alone is $271,418,690 per year (Flynn, 2014).

Due to the cost and severity of illness and the ever-present need to protect consumers and develop a safe, pathogen-free product, the meat industry continuously seeks effective means to reduce the bacterial load. Following an outbreak of *E. coli* in 1993, which caused the infection of more than 500 individuals and deaths of 4 people (CDC, 1993), FSIS labeled *E. coli* O157:H7 an adulterant in beef. An adulterant is a poisonous or deleterious substance which may render a product injurious to health (9 CFR 301.2). There is zero tolerance for *E. coli* O157:H7 and the presence of any *E. coli* O157:H7 renders any affected beef

unsellable and unfit for consumption (21 U.S. Code 601 (m)(1), 21 U.S. Code 610). For these reasons, food safety and pathogen reduction is one of the foremost responsibilities of the food industry as a whole.

Escherichia coli O157:H7

Escherichia coli is a bacteria from the family *Enterobacteriaceae* that is normally found in the environment and in the intestines of humans and animals. There are many different types of *E. coli*; some are harmless to humans and some are pathogenic. *E. coli* O157:H7 is a pathogenic strain of *E. coli* that produces a Shiga toxin. There are two types of the toxin that are most commonly found in patients that have ingested *E. coli* O157:H7, namely "Stx1" and "Stx2". These toxins have been shown to act on endothelial cells and cause swelling, resulting in many of the symptoms of *E. coli* O157:H7 poisoning (Acheson et al., 1996). According to Ascheson et al. (1996), Stx1 and Stx2 are able to easily translocate through polarized intestinal cell walls, and can both enter and exit the cell. This author also asserts that even though the Shiga toxin is not always found in the blood and tissue of infected patients, the toxin is still believed to be the cause of illness in those infected with *E. coli* O157:H7. According to Ascheson et al. (1996), the lack of consistent detection of the toxin in the blood or tissue of affected persons is due to the low amount of toxin needed to cause illness. It is worth noting that even though the toxin may not be found in blood or tissue, it will be found in significant quantities in the feces of the infected (Acheson et al., 1996).

Illness is spread through the ingestion of *E. coli*, therefore it is critical to wash your hands, cook food properly, avoid unpasteurized dairy and juices, and to take proper steps to avoid cross contamination. If ingested, the effects of *E. coli* on the body can vary greatly, with the most common side effects being vomiting, stomach cramps, and diarrhea. In a small number of cases, the person infected will develop hemolytic uremic syndrome (HUS), which affects the kidneys and can cause them to shut down completely. People that develop HUS have a considerable chance of permanent damage and, in some cases, this complication results in death. While people of any age are at risk of illness, the very young and the elderly have the highest risk of becoming extremely sick or getting HUS because their immune systems are not as strong. Generally, the incubation period for *E. coli* is 3-4 days, with mild symptoms in the beginning that worsen over time. The symptoms for HUS usually begin about seven days after the first symptoms of illness appear. Even after recovery, it is possible to still shed *E. coli* for several weeks, even months in a few cases (CDC, 2012).

Historically, *E. coli* O157:H7 has been associated with ground beef. From 1982 to 1994, 1127 out of 2334 cases of *E. coli* O157:H7 contamination were from ground beef products (Cassin et al., 1998). Part of the reason that *E. coli* has been found in ground beef is due to the use of grinding, which increases the surface area of meat, providing more favorable conditions for bacterial growth. The grinding process creates more surface area and the entire product can potentially be exposed to contaminants. When meat is ground, it is chopped into smaller pieces and mixed together. In this process, what was previously the outside of the meat is now

chopped and mixed with what was previously the inside of the meat. If there were any bacteria on the outside, it is now spread throughout the whole product and the "inside" can no longer be considered sterile since it has now been exposed. This is why undercooked ground beef has historically been one of the foremost sources of infection from *E. coli* O157:H7 (Luchansky et al., 2008). *Escherichia coli* is not naturally found in the muscles of food animals, but the carcasses of food animals can become contaminated with *E. coli* during harvesting and processing steps such as evisceration or packaging when fecal matter could be transferred onto the muscles (Cassin et al., 1998).

Because the immune systems of the elderly are not as strong, they are very susceptible to pathogenic bacteria. Children are also extremely susceptible due to the fact that their immune systems are not as well developed as the immune systems of healthy adults. Because of this, many of the major outbreaks of *E. coli* O157:H7 have been manifested in the deaths of children, ultimately leading to a larger public outcry due to the unfortunately young age of the infected. One case in particular was in the Northwest from November 1992 to February 1993. As a result of this outbreak, there were over 500 laboratory confirmed cases of *E. coli* related illness and 4 subsequent deaths. This was a multistate outbreak that included Washington, Idaho, California, and Nevada. A total of five meat U.S. meat packing plants and one Canadian meat packing plant were identified as probable sources of the meat. Multiple slaughter operations could have been possible sites of contamination, but no individual operation was ever identified as the starting point of the contamination. Because of the size of the outbreak and the young age of many

of the infected and deceased, it received increased media coverage (CDC, 1993). This case is an important one to the meat industry because it led to the classification of *E. coli* O157:H7 as and adulterant in ground beef by the USDA Food Safety and Inspection Service. This means that a positive test result for *E. coli* O157:H7 causes the meat to be injurious to human health and it must not be sold (9CFR 301.2, 21 U.S. Code 610).

Historical Events

There have been multiple recalls and outbreaks of *E. coli* O157:H7 in the past that have led to changes and improvements in the way the meat industry seeks to eliminate this bacteria in the food supply. Some of the more impactful outbreaks are listed in the "Timeline of Events Related to *E. coli* O157:H7", published by the USDA Food Safety and Inspection Service (2013). Besides the earlier mentioned outbreak in 1993, this list cites two other major outbreaks that resulted in new publications or studies about controlling *E. coli* O157:H7 and how to properly prevent contamination. The first event happened in Colorado in July 2002. In this outbreak, ConAgra Beef Company was forced to recall 18.6 million pounds of beef products containing ground beef and beef trim due to contamination with *E. coli* O157:H7, making this one of the largest recalls in U.S. history. The outbreak resulted in 43 cases of illness spread over multiple states (CDC, 2002). The second outbreak occurred from September to October 2002 in Wisconsin. In this outbreak, Emmpack Foods, Inc. recalled a total of 2.8 million pounds of products containing ground beef due to contamination with *E. coli* O157:H7 (FSIS, 2002). This was also a multistate

outbreak and 57 total individuals were infected, with 23 of these individuals in Wisconsin (MarlerClark, 2005). The timeline also mentions that in 2003, there were 3 outbreaks of *E. coli* O157:H7 that were associated with mechanically tenderized products, otherwise known as whole muscle, non-intact (WMNI). After all these instances cited in the timeline, the CDC, USDA FSIS, and other relevant groups took firm actions with new studies, meetings, publications, and new handling, processing, and testing methods. These historic outbreaks shaped how the industry and regulatory agencies control *E. coli* O157:H7, and through meticulous regulations and testing, beef is safer today than it ever has been. For example, FSIS requires regular testing of areas including but not limited to domestic and imported ground product, domestic and imported trimmings, machinery, and individual carcasses, as outlined in USDA FSIS directive 10010.1. This directive also states that if a positive test result occurs, FSIS has the authority to decide how much product is considered adulterated, not the company itself. However, to prevent future outbreaks, food safety must continue to evolve over time to ensure the continued safety and quality of its products.

Whole Muscle Non-Intact

Whole muscle non-intact beef is beef in which the inside of the cut of meat can no longer be considered sterile, even though it is a whole muscle cut. There are certain processing techniques that can expose the inside of whole muscle products without cutting or grinding. The process of mechanical tenderization usually involves piercing a less tender cut of meat with blades or needles in order to break

through the connective tissue and disrupt muscle fibers to create a more tender product (Luchansky et al., 2009). Processing steps that yield this type of product are most often mechanical tenderization or injection. Whole muscle non-intact products are favored among consumers because while certain cuts of beef may decrease slightly in juiciness ratings as a result of mechanical tenderization, studies have shown that overall, mechanical tenderization can be used to effectively increase the tenderness of most cuts of beef with no negative impacts to other palatability factors of the meat (Jeremiah et al., 1999). The increase in tenderness is one of the main reasons mechanical tenderization is so widespread, as studies have shown consumers will pay a premium for meat that can be guaranteed to be more tender (Miller et al., 2001). However, there have been concerns about the effect tenderization has on food safety, specifically the prevalence of *E. coli* O157:H7.

Studies have indicated that if a piece of beef is inoculated on the surface with *E. coli*, mechanical tenderization of the beef can result in the surface inoculum being transferred to the previously sterile center of the cut, which raises concerns if the cut is not cooked to the proper internal temperature (Luchansky et al., 2008; Johns et al., 2011; Huang, 2010). In a study by Luchansky et al. (2008), results indicated that 3-4% of the surface inoculum was transferred to the center. In this study, top butt beef subprimals weighing 15-20 pounds each were inoculated with *E. coli* O157:H7 (concentrations up to 3.5 log CFU/g) and passed with the inoculated side up through a mechanical blade tenderizer. Core samples were taken and split into segments that corresponded with different depth measurements of the subprimal. In the inoculated and untenderized control, pathogen concentration in the

uppermost 1cm of top beef butt subprimals were 0.6, 1.46, 2.5, and 3.19 log CFU/g when inoculated with 0.5, 1.5, 2.5, and 3.5 log CFU/g *E. coli* O157:H7, respectively. The pathogen concentration in uppermost 1cm of the tenderized samples was 0.22, 1.06, 2.04, and 2.7 log CFU/g, respectively, when inoculated at the same levels. Therefore, this study determined that there is not a greater risk ($P>0.05$) for *E. coli* related illness in whole muscle non-intact products than there is in intact products (Luchansky et al., 2008). However, the authors stated that this was only true if the meat was cooked to a temperature that addressed the possibility of contamination in the center of the product. This is due to the fact that pathogens were found in the center of the tenderized subprimals, though it is worth noting they were found in levels that were 7- to 34-fold lower in these segments than in the uppermost 1cm segment.

While the study by Luchansky et al. (2008) did not specify an "adequate temperature" that would address the existence of such pathogens in the center of the meat, further studies have confirmed the assertion that cooking tenderized products to appropriate temperatures (60-65° C) is sufficient to kill bacteria that may be transferred to the core of the product. One such study focused on brine-injected steaks inoculated with 8 log CFU/mL *E. coli* and *Listeria innocua* (Gill et al., 2009). The pathogens were mixed into a brine and injected into 3cm thick steaks, which were cooked to an internal temperature of 50, 55, 60, 65, or 70 degrees Celsius. After cooking, core samples were evaluated for pathogen levels. Results indicated that cooking the steaks to an internal temperature between 60-65° C killed all bacteria in the core of the meat. Researchers then injected steaks with a

broth containing *E. coli* and *Listeria monocytogenes* at levels greater than 8 log CFU/mL and cooked the steaks to 60, 63, and 65 degrees Celsius. These results indicated that cooking the steaks to an internal temperature of 65° C reduced all pathogens in the center of the meat by at least 7 log CFU/g (Gill et al., 2009).

Another study on mechanically tenderized beef by Johns et al. (2011) assessed beef striploins to determine the transfer of *E. coli* from one striploin to the other when passed through the same mechanical tenderizer. In this study, one striploin was inoculated with a high ($10^{8.2}$–$10^{10.1}$ CFU/mL) concentration of naladixic acid-resistant *E. coli* and passed through a mechanical tenderizer followed by 5 additional uninoculated striploins immediately following the inoculated striploin. Results of this study indicate that *E. coli* was transferred from Loin 1 (inoculated) to Loin 2 (the first uninoculated), but that for Loins 3, 4, 5, and 6, *E. coli* levels stayed below the limit of detection. Although there was transfer of the pathogen, even Loin 2 showed levels of *E. coli* under 10 CFU/g, therefore this study concluded that while transfer of *E. coli* does occur, levels fall almost immediately below detectable levels with very little product affected.

Sensory Evaluation

While application of antimicrobials is effective for control of pathogens, it is necessary to assure they do not affect meat quality. Sensory evaluation is a vital part of the food industry because it allows companies to assess how consumers may react to their product. In fact, studies have shown that products are less accepted by consumers if they have not undergone sensory testing to confirm consumer

expectations of flavor, texture, and appearance before production (Cardello and Sawyer, 1992). Sensory evaluation also provides feedback on what companies can improve and where they are succeeding with their product. There are many different types of sensory studies available depending on what particular attributes a company wants to test and why. Feedback from these studies can be either qualitative or quantitative (AMSA, 2015). While qualitative data can be useful for gathering a consumer's overall opinion on a product, the current study required quantitative feedback that could be analyzed for product differences.

A trained panel is one example of a sensory panel that is highly effective when evaluating specific attributes of meat, such as tenderness or juiciness. Training is intensive and lengthy and allows companies to teach panelists how to evaluate different attributes. It is precise and provides panelists with references on how to rank the attributes so that each panelist is judging the product in the same way. Trained panels are a technique to standardize consumer responses and ensure accuracy, and the more training a sensory panel receives, the more precise their responses will become (AMSA, 2015).

For beef and other meat products, some of the most important attributes tested during sensory evaluations are tenderness, juiciness, and off-flavor. Tenderness and juiciness are closely related and often fluctuate in relation to each other. Off-flavor is of great importance because it is linked with meat quality. Many different factors can cause off-flavor, which is why it is so heavily tested.

Off-flavor

Flavor is another characteristic of meat that greatly influences consumer satisfaction. In fact, if tenderness is the same between all cuts of meat, flavor has been found to be the single most important characteristic in consumers' buying decisions (Calkins et al., 2007). There are numerous compounds and reactions that create the flavor in meat, and anything producers and consumers do to the product – from the animals' feed to how consumers cook the product – can influence the final flavor profile (Calkins et al., 2007). Because of the importance of flavor to consumer acceptability, off-flavor in meat is highly undesirable; however, not all off-flavor is the same. There are many different tastes associated with off-flavor, that have been described as metallic, grassy, bloody, vinegar, liver, and many more. One specific type of off-flavor that often occurs is known as "warmed-over" flavor. This taste is a product of lipid oxidation and is noticeable upon reheating of meat that has been precooked and refrigerated for a short time (Brewer, 2006). Warmed over flavor is therefore not an issue with raw product, but can be a concern with pre-cooked or par-cooked products.

The development of any flavor, including off-flavor, is not caused by any one factor in particular, but may be caused by several different factors at a time. Because the production of off-flavors is so unpredictable, it is necessary to test for them when any new ingredient, processing step, or antimicrobial is presented for use in the industry. This testing is extremely important because the existence and intensity of off-flavors is the only characteristic of meat found to have a positive

correlation with the flavor intensity of beef as a whole (Calkins et al., 2007). As the flavor intensity of beef becomes more important to consumers, off-flavor intensity must become more important to producers in order to sell product.

Tenderness and Juiciness

Some of the factors affecting juiciness include the fat content of the meat, the pH of the meat, the temperature the meat is cooked to, the method used to cook the meat, etc. Juiciness is an interesting characteristic of beef due to its effect on the perception of other characteristics, especially tenderness. The degree of juiciness can either positively or negatively impact the degree of tenderness perceived by consumers, and a study by Zimoch and Gullet (1997) confirmed this when results demonstrated a correlation ($P<0.05$) between juiciness and perceived tenderness. In this study, samples having different levels of tenderness and juiciness were evaluated by a sensory panel. Results indicated that samples with a lower juiciness level were given lower tenderness scores while samples with a higher juiciness level were given higher tenderness scores. The reason for this is that increased juiciness lubricates the muscle fibers as well as the consumer's teeth, making mastication easier due to less resistance (Zimoch and Gullet, 1997). Although the shear force may be the same in two different samples of meat, the juicier sample may receive higher tenderness ratings due to this lubrication effect. This lubrication effect is central in the "Lubrication Theory" which states that the lubrication of the muscle fibers and fibrils by intramuscular fat creates a sensation of tenderness for the

consumer, explaining the close correlation between tenderness and juiciness (Savell & Cross, 1988).

Tenderness, like juiciness, is affected by many different factors, not all of which come from the processing of the meat. Some of these factors affecting tenderness include the location of the muscle on the animal's body (whether it is used for locomotion or support), the age of the animal at slaughter, pre-slaughter handling of the animal, pH of the meat, the temperature the meat is cooked to, the method used to cook the meat, and many more (Ferguson et al., 2001). All of these factors affect tenderness by affecting different characteristics of the muscle itself. Four specific characteristics of muscles are considered to be most important: postmortem proteolysis, intramuscular fat or marbling, connective tissue, and the contractile state of the muscle (Belew et al., 2003). Postmortem proteolysis is the degradation of proteins after the death of the animal. This degradation of proteins causes an increase in tenderness of the muscle since less force is needed to shear these proteins as their structural soundness decreases. Marbling can greatly enhance the tenderness of the meat because, as stated by Savell & Cross (1988) and Zimoch & Gullet (1997), the intramuscular fat will melt and lubricate muscle fibers, creating a sensation of tenderness. This can be contrasted with the connective tissue found in the meat, which does not break down as easily and therefore contributes to muscle toughness. Finally, during contraction, cross-links form within the muscle between actin and myosin, contributing to muscle toughness due to the stability of these cross-links. For these reasons, this study concluded that these four characteristics are the most important in the development of muscle

tenderness and can also cause different cuts of meat from the same animal to have differences in tenderness (Belew et al., 2003).

Tenderness can be measured in a variety of ways, including but not limited to, the use of sensory panels and shear force data. A combination of sensory studies and shear force tests are common when assessing the tenderness of meat. Shear force tests can only obtain an objective result about the force used to shear through a sample of meat. Shear force tests cannot determine the acceptability of different levels of tenderness; this can only be learned through consumer sensory panels (AMSA, 1995). Consumer sensory panels are a vital part of assessing tenderness in meat because ultimately the industry revolves around the consumer. Moreover, studies have shown that consumers are willing to pay a higher price if they believe the meat they are purchasing is more tender than other meat available at a lower price (Boleman et al., 1997).

Warner-Bratzler Shear Force

While consumer sensory panels can also evaluate the tenderness of meat, they are subjective; therefore thoroughly trained sensory panels have often been implemented as an objective measurement. However, the need for stronger repeatability led to shear force measurements by machines becoming more common. Using a machine with exact specifications and multiple programs ensures that the measurements can be repeated precisely and allows completely different studies to be compared to each other using common values.

Shear force is a measurement of the force it takes to cut through a sample of meat, thus it is used in research to give an objective value for the tenderness of a sample. One way of measuring shear force is to use the Warner-Bratzler method. This method involves a V-notched blade built to very precise specifications. These specifications are as follows: blade thickness of 1.1684mm (0.046 inches); V-notched (60° angle) cutting blade; cutting edge beveled to a half-round corner of V rounded to a quarter-round of a 2.363mm diameter circle; spacers providing gap for cutting blade to slide through of 2.0828mm thickness (AMSA 2015). This blade is attached to a texture analysis machine and is lowered into a core sample of meat until it shears all the way through the sample. The machine ensures that the blade is lowered at the same speed and to the same position for each sample. A computer gathers the shear force data for each core sample so that the data may be analyzed based on the current research being performed. To obtain the core samples for this method, the meat should be cooked to the appropriate temperature for the individual study (in the current study, steaks were cooked to 71° C) and then refrigerated overnight at 2°-5° C. When the temperature of the meat is between 2°-5° C, a coring device should be used to retrieve uniform core samples, 1.27cm in diameter. These samples should be obtained by running the coring device parallel to the muscle fibers so that the blade cuts perpendicular to the muscle fibers (AMSA, 2015).

It is of note that many studies choose to gather tenderness data from both a shear force measurement and a sensory panel, because only a consumer panel can determine the acceptability of the product. Studies may also combine shear force

measurements and trained sensory panels to obtain a wider range of data. A study by Boleman et al. (1997) found that when beef top loin steaks were separated into categories based on shear force results and given to consumers for evaluation, consumers consistently recognized ($P<0.05$) steaks with lower shear force values as more desirable and were willing to pay a premium for this product due to increased tenderness. However, different studies may result in discrepancies between the two methods. For example, one study in particular found that when Warner-Bratzler shear force measurements were used to divide meat into 3 categories of tenderness, consumers correctly identified the categories only 55.6% of the time when separating tough meat from tender and intermediate, and only 62.3% of the time when separating tender meat from tough and intermediate samples (Destefanis et al., 2008). For this reason, as in the current study, researchers often use both methods of tenderness evaluation in order to obtain a wider range of information.

Current Methods of Control

To ensure the safety of product, the meat industry has adopted a multi-hurdle approach to controlling *E. coli* growth and preventing *E. coli* contamination of beef. This multi-hurdle approach means that there are several different steps at which various treatments are applied to the product to kill bacteria and ensure that no pathogens are found in the plant or on the meat. These treatments can include antimicrobial sprays, washes, and cooking. In addition to treatments like these, testing is done for pathogens at multiple points in the process as well as all over the plant.

In beef slaughter, one of the most important processes to controlling cross-contamination is the full removal of the hide. After being stunned and exsanguinated, a cut is made in the hide and a hide-puller removes the entire hide. This step is important financially due to the monetary value of hides and hide products, but it is even more vital to proper sanitation and meat safety because feces on the hide is considered the original source of contamination due to *E. coli* when cattle come in for harvest (Cassin et al., 1998). *Escherichia coli* is naturally present in the digestive tract of ruminant animals, which is why the feces of these animals is the main source of contamination during the harvest process. Therefore, in addition to feces on the hide, another major source of *E. coli* contamination is the digestive tract. Since the muscles of an animal are not naturally contaminated with *E. coli*, the pathogen could be transferred onto the surface of the muscles during the harvest and processing through cross-contamination (Cassin et al., 1998). The digestive tract contains fecal matter and thus poses a large risk of *E. coli* contamination for the rest of the carcass. Because of the risk of cross-contamination, cautious removal of the hide and digestive tract from the carcass is essential and ensures that the majority of the bacterial load on the animal is removed as well. Furthermore, trimming of the carcass as it progresses through the dressing process allows for removal of any undesirable areas on the carcass that may have come in contact with these sources of contamination and others (Castillo et al., 1998).

At multiple points during the harvest process, the carcasses are sprayed with both water and an antimicrobial. The antimicrobial used may vary depending on

the plant, but it is most often an organic acid. While the exact mechanism of organic acids is not confirmed, research indicates that the dissociated molecules within the organic acid permeate the cell membrane and alter the pH within the cell, lysing the cell wall (Brul & Coote, 1999). Furthermore, organic acids have proven to be highly capable of both bacteriostatic and bactericidal effects and can be used as highly effective antimicrobials (Ricke, 2003). A study conducted by Castillo et al. (1998) compared the effectiveness of hot water washes, lactic acid treatment, and trimming of the carcass against bacteria, including *E. coli* O157:H7. Specifically, the treatments were hot (95° C) water wash, 2% lactic acid spray (applied at a temperature of 55° C), hot water before lactic acid spray, and lactic acid spray before hot water. All treatments were used in combination with trimming, because they found that log reductions were greater ($P<0.05$) with trimming than without. Results of this study indicate that washing the carcass with water and then trimming it can spread the bacterial load over a greater area of the carcass; however, this method reduced the overall contamination despite this spreading effect. Researchers stated that the most effective method of killing fecal bacteria such as *E. coli* O157:H7 is to follow the wash and trim steps with a hot water wash followed by a lactic acid spray; a method that is very common in the meat industry today (Castillo et al., 1998).

Peracetic Acid

Peracetic acid is an antimicrobial that exists as an aqueous solution with peracetic acid in equilibrium with acetic acid, water, and hydrogen peroxide

(USNLM, 2011). It is often used in the healthcare field as both a disinfectant and a sanitizer and is highly biocidal (CDC, 2009). The exact mechanism of action for peracetic acid is not known; however, it is an oxidizing agent. It is assumed that peracetic acid works by disrupting and penetrating the cell wall, denaturing proteins. Moreover, peracetic acid has proven itself to be extremely effective against both gram-positive and gram-negative bacteria at concentrations below 100ppm (CDC, 2009). Different industries use different concentrations of peracetic acid, depending on the material being disinfected. Concentrations used in the current study were established at 120ppm and 400ppm peracetic acid, 120ppm being reflective of more common levels in food industries, and 400ppm being an example of an extremely high usage.

Besides being effective against a wide range of bacteria, peracetic acid is also a desirable antimicrobial to use because of how fast it degrades. In tests done with the blood from rats, the half-life of peracetic acid was found to be under five minutes in blood diluted 1000 times, which leads researchers to expect a half life of mere seconds in undiluted rats blood (UNSLM, 2011). This quick degradation of peracetic acid is most likely due to its instability. Because the solution is unstable, it constantly strives to re-equilibrate itself by breaking down into acetic acid and hydrogen peroxide. When tested at low levels, peracetic acid showed no harm to animal or human subjects through touch or ingestion. There was also no impact found on reproductive organs. Due to the data confirming that low levels of peracetic acid are not harmful to humans or animals, peracetic acid can only be used at low levels in the food industry, ensuring the safety of treated products. Further

testing showed that not only is peracetic acid highly biodegradable, but after being treated by a water treatment facility, there would be no peracetic acid residue entering into any aquatic environment (USNLM, 2011).

There have been many studies done showing the benefits of peracetic acid. Gonzalez et al. (2004) reported that peracetic acid is not affected by organic load, as estimated by chemical oxygen demand (COD) levels, which is desirable for use in the food industry. Rodgers et al. (2004) confirmed the effectiveness of peracetic acid in the presence of high organic loads and went on to state that peracetic acid remains unaffected by temperature and is also non-corrosive. In research by Nagel et al. (2012), peracetic acid was compared with conventional antimicrobial methods when used to reduce the amount of *Campylobacter jejuni* and *Salmonella* spp. in poultry. In this study, chicken breasts were inoculated with *Campylobacter jejuni* and *Salmonella* spp., and then 0.04% (400ppm) and 0.1% (1000ppm) concentrations of peracetic acid were tested against the widely accepted methods of 0.004% (40ppm) chlorine and 0.5% (5000ppm) lysozyme, and all were applied to chicken breasts using a post-chill immersion tank, a typical method widely used in poultry processing. Results indicated that, when compared to the positive control, the 0.04% peracetic acid treatment caused a 2.02-log cfu/mL reduction in *Salmonella* spp. and the 0.1% peracetic acid treatment caused a 2.14-log cfu/ mL reduction. The 0.004% chlorine treatment and both the lysozyme treatments had less than a 1-log cfu/mL reduction in *Salmonella* spp. when compared to the positive control (Figure 2). Results also showed that the 0.04% peracetic acid treatment resulted in a 1.93-log cfu/mL reduction in *Campylobacter jejuni* and the 0.1%

peracetic acid treatment resulted in a 2.03-log cfu/mL reduction in *Campylobacter jejuni* compared to the positive control (Figure 1). This is compared with the 0.004% chlorine as well as 0.1% and 0.5% lysozyme treatments, which had less than a 1-log cfu/mL reduction in *Campylobacter jejuni* when compared to the positive control. These results clearly show that peracetic acid reduces ($P<0.05$) the amount of bacteria on chicken carcasses even when compared to standard industry treatments already in place. This study also indicates that according to sensory analysis, product quality (appearance, flavor, and juiciness) was not negatively affected, and even improved in some cases (Nagel et al., 2012).

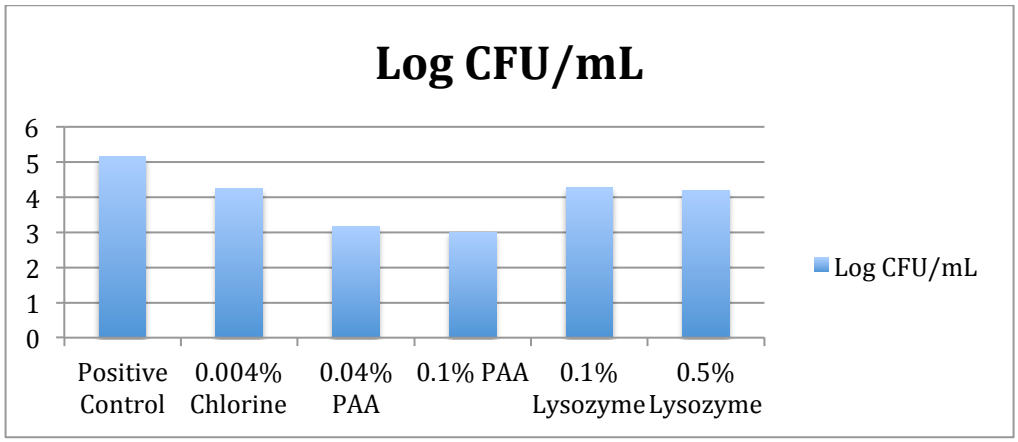

Figure 1: *Campylobacter jejuni* recovered from inoculated carcasses treated with various antimicrobials (Nagel et al., 2012)

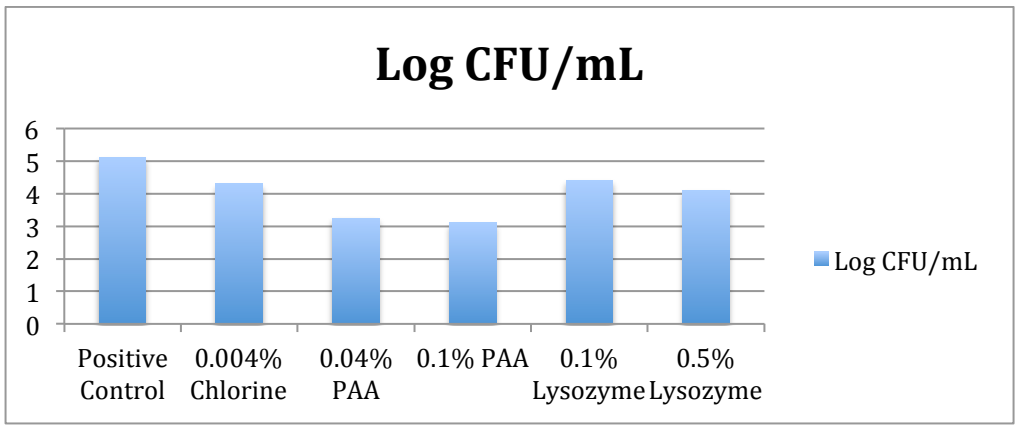

Figure 2: *Salmonella* spp. Recovered from inoculated carcasses treated with various antimicrobials (Nagel et al., 2012)

The results from that particular research are an important factor driving the current study with peracetic acid in beef because they clearly show that peracetic acid can be used in the poultry industry safely and effectively and at no detriment to the final product. The objectives of the current study are to test the effectiveness of peracetic acid in controlling growth of *E. coli* O157:H7 in fresh beef products and to determine the impact, if any, of peracetic acid on product quality and shelf life. While there are many similarities between the two studies, there are many differences that require an independent beef study be done. First, the composition of beef is very different from that of chicken. When compared, beef has much more saturated fat than chicken has. It is not yet clear if or how this will change the effectiveness of peracetic acid as an antimicrobial, but it is worth noting. Also, the application methods are different: the chicken was treated through a post-chill dip tank where the carcasses were completely immersed in the solution while the beef in the current research was treated through a spray application. Both are true to industry applications, but the differences in exposure to oxygen could affect the

rapid degradation and possibly the effectiveness of peracetic acid. Immersion treatment of beef samples by Ransom et al. (2003) resulted in a 1.4 log CFU/cm^2 reduction on inoculated (10^5–10^6 CFU/mL) beef carcass tissue when applying peracetic acid compared to the control. On inoculated (10^5-10^6 CFU/mL) lean tissue pieces, this reduction was reported to be 1.0 log CFU/g when using peracetic acid as compared to the control. In this study, inoculated samples were submersed in treatments for 30 seconds instead of using a spray application as in the current experiment. It will be interesting to compare the results of different application methods in beef. Finally, the most obvious and most important difference in the two experiments though is that the studies use different bacteria. The resistance could differ for *E. coli*, though it is not expected to differ much due to the wide range of bacteria that peracetic acid is effective against. Furthermore, the study by Ransom et al. (2003) utilized *E. coli* O157:H7 for their inoculum, indicating that peracetic acid can be effective against this pathogen.

Conclusion

Due to the severe health risks it poses, *E. coli* O157:H7 is an adulterant in ground beef. It costs the U.S. millions of dollars annually and numerous hospitalizations. With a constantly growing meat industry, food safety must continue to evolve in order to protect both producers and consumers. Because of this, research is continuously being done to find the best way to control and eliminate pathogens such as *E. coli* O157:H7. One of the ways the industry has been so successful in this area is through the use of new antimicrobials, like peracetic

acid. The effectiveness of peracetic acid in reducing pathogens in poultry processing makes it an ideal candidate for research in controlling *E. coli* O157:H7 in beef. However, in order to be successful, it must not only kill *E. coli* O157:H7, it must do so while not negatively impacting the important characteristics of beef that drive consumer satisfaction, including tenderness, juiciness, and flavor. The results of both laboratory and sensory testing will provide an answer as to whether this is a viable option for the meat industry moving forward.

MATERIALS AND METHODS

Antimicrobial Study

A total of 450 beef samples (25 slices X 3 replications X 6 treatments) were used to validate the effectiveness of peracetic acid (PAA) as an antimicrobial agent against the growth of *Escherichia coli* O157:H7. Beef ball tips (IMPS: 185B) were sliced into individual samples weighing 100g each. Samples were placed onto individual Styrofoam trays (size 1, Genpak, Glens Falls, NY) and inoculated with 1mL of *E. coli* O157:H7. Half of the samples (75 samples per replication) were inoculated with a low *E. coli* concentration (10^2 CFU/mL) while the other half were inoculated with a high *E. coli* concentration (10^6 CFU/mL), followed by a 30 minute bacterial attachment time. After the thirty minute attachment period, the samples were treated with approximately 3mL of either 0.012% (120ppm) PAA, 0.04% (400ppm) PAA, or left untreated as a positive control. Each treatment group contained 25 high inoculum samples (10^6 CFU/mL) and 25 low inoculum samples (10^2 CFU/mL), for a total of 50 samples receiving each treatment. All samples were sprayed with approximately 1mL of 0.1% sodium thiosulfate to neutralize any remaining reaction after a 5-minute contact time. Peracetic acid breaks down rapidly, and it was assumed that any reaction would have stopped before the neutralization by sodium thiosulfate. However, the use of sodium thiosulfate ensured that if there were any remaining reaction, it would be stopped at exactly 5 minutes to create the same environment for every sample. Samples were stomached (Seward Stomacher 400,

260rpm, Davie, FL) for two minutes in individual WhirlPak bags (6x9", Nasco, Atkinson, WI) containing 100mL of 0.1% Peptone solution. 100μL from each sample was serially diluted onto Sorbitol MacConkey agar supplemented with Cefixime Tellurite to select for *E. coli* O157:H7 growth and placed in an incubator at 37° C for 24 hours.

Escherichia coli Inoculum Preparation

Each inoculum contained five strains of *E. coli* O157:H7. These strains are listed in Table 1. One milliliter of each strain was transferred to individual test tubes containing 9mL of tryptic soy broth and incubated at 37° C for 24 hours. 250μL of each strain was then plated onto an individual CT-SMAC plate and incubated at 37° C for 24 hours to achieve individual colonies. One colony per strain was transferred to individual test tubes containing 9mL of tryptic soy broth which were then incubated at 37° C for another 24 hours. The contents of each test tube were poured into individual 50mL centrifuge tubes and centrifuged at 3650rpm for 20 minutes at 37° C which resulted in ~8 log CFU/mL. The supernatant was discarded from the tubes, then each pellet was re-suspended in 9mL of 0.1% Peptone solution. For the high inoculum, 200μL from each tube was transferred into a single vessel containing 99mL of 0.1% Peptone solution which resulted in ~6 log CFU/mL. For the low inoculum, each tube was serially diluted 4 times, then 200μL from each tube was transferred into a single vessel containing 99mL of 0.1% Peptone solution in order to generate ~2 log CFU/mL. Both inoculum levels were verified through direct plating.

Shelf-Life

Samples were inoculated with 100μL of the high (10^6 CFU/mL) inoculum (the low (10^2 CFU/mL) inoculum was not used for this part of the study because at the time of inoculation, only the high inoculum was showing favorable results). Following a 30 minute bacterial attachment time, 12 of the samples were sprayed with approximately 3mL of 0.012% (120ppm) PAA, 12 of the samples were sprayed with approximately 3mL of 0.04% (400ppm) PAA, and 12 of the samples were left untreated as a positive control (amount of samples here is per replication). All samples were sprayed with approximately 1mL of 0.1% sodium thiosulfate to neutralize any remaining reaction from the PAA following a 5-minute contact time. Samples were packaged in a Styrofoam tray (size 1, Genpak, Glens Falls, NY) with overwrap and placed in a walk-in cooler (4 ± 2° C). On days 1, 3, 5, and 7, color was analyzed for lightness (L*), redness (a*), and yellowness (b*) using a Hunter Miniscan XE Plus (model MSXP-4500C; Hunter Laboratories, Reston, VA). This study utilized illuminant D65 at 10° observance angle and a 3.5cm aperture. Color was taken from 3 samples per treatment on each of these days, then samples were placed into WhirlPak (6x9", Nasco, Atkinson, WI) bags and stomached (Seward Stomacher 400, 260rpm, Davie, FL) for 2 minutes. Proper dilutions were made for each sample, and then 100μL from each dilution was plated in duplicate onto CT-SMAC agar, Plate Count Agar, Coliform petrifilm, and Yeast/Mold petrifilm. Plates were incubated at 37° C for 24 hours anaerobically or aerobically according to the manufacturers' directions.

Sensory Evaluation

In a sensory analysis, a total of six 2.54cm thick slices of beef ball tips were treated with approximately 3mL of either 0.012% (120ppm) peracetic acid, 0.04% (400ppm) peracetic acid, or left untreated as a control. Slices were cooked to an internal temperature of 71° C. Upon reaching this temperature, slices were cut into cubes (2.54cm x 1cm x 1cm) and placed into individual 1oz. plastic containers with lids. Each container was labeled with a random 3-digit code. Nine sensory panelists evaluated samples one at a time using an 8-point hedonic scale. Each panelist was placed in a partitioned booth with 250Lx of red incandescent light and received two cubes from each treatment, in random order. Prior to evaluation, panelists were given a sample to discuss and score to standardize results. The panelists analyzed initial and sustained juiciness (8= extremely juicy, 1=extremely dry), initial and sustained tenderness (8=extremely tender, 1=extremely tough), flavor intensity (8=extremely intense, 1=extremely bland), and off-flavor intensity (8=extreme off-flavor, 1=none). If panelists recognized an off-flavor, they were asked to provide an off-flavor descriptor. Options for this descriptor were metallic, salty, livery, sour, sweet, vinegar, bloody, or other (panelists were asked to explain a choice of "other"). Between each sample, panelists were required to cleanse the palate by ingesting an un-salted saltine cracker and a drink of plain water.

Warner-Bratzler shear force was evaluated according to the AMSA (2015) guidelines. Samples were cooked on a clamshell grill to an internal temperature of

71 ± 2° C. Temperature was monitored with copper-constantan K-type thermocouples attached to a Data Logger Thermometer (Omega, HH309A, Stamford, CT). The thermocouples were placed in the approximate geometric center of each sample and used to record internal temperature. After cooking, samples were refrigerated overnight at 2° C before coring. Six core samples (1.27cm in diameter) were taken parallel to the muscle fibers of each sample using a handheld coring device. Each core sample was sheared once in the middle of the sample using a V-notched blade manufactured to AMSA (2015) standards. These standards included a "blade thickness of 1.1684mm (0.046 inches); V-notched (60° angle) cutting blade; cutting edge beveled to a half-round corner of V rounded to a quarter-round of a 2.363mm diameter circle; spacers providing gap for cutting blade to slide through of 2.0828mm thickness" (AMSA 2015). The crosshead speed of the blade was 200mm per minute.

Statistical Analysis

Bacterial counts were converted to log colony-forming units per gram. Data was analyzed using both PROC GLM and PROC MIXED models of SAS version 9.4 and least squares means were separated using the PDIFF procedure. The dependent variables in this study were bacterial growth, color, shear force, and sensory attributes while the independent variables were day, replication, and treatment. The interaction between day and treatment as well as replication and treatment were present in the model. Statistical significance was reported as *P* values being ≤ 0.05.

RESULTS AND DISCUSSION

Evaluation of the Antimicrobial Efficacy of Peracetic Acid

For the low (10^2 CFU/mL) inoculation level of *E. coli* O157:H7 (Table 2), the 120ppm treatment of PAA reduced bacterial growth by 0.067 log CFU/g, which was not different ($P>0.05$) than the control. The 400ppm treatment for the low inoculation level of *E. coli* O157:H7 reduced the bacterial growth by 0.568 log CFU/g ($P<0.05$) from the control (0.762 log CFU/g). For the high (10^6 CFU/mL) inoculation level of *E. coli* O157:H7 (Table 2), the 120ppm treatment of PAA reduced bacterial growth by 0.12 log CFU/g, and the 400ppm treatment reduced bacterial growth by 0.19 log CFU/g from the control (4.96 log CFU/g). Both of these treatments were different from the control ($P<0.05$). These results indicate treatment with 400ppm PAA was the most effective at reducing *E. coli* O157:H7 on the samples.

In a previous study (Nagel et al., 2012), the efficacy of peracetic acid was tested against *Salmonella* Typhimurium and *Campylobacter jejuni*. While the Nagel et al. (2012) study showed a greater reduction of bacterial numbers (a 2-log reduction for both bacterial species), results were similar to the present study in that treatment with peracetic acid at both a low and high concentration yielded a significant overall reduction at the inoculation level of 10^6 CFU/mL. Differences in the amount of reduction seen in the two studies may be due to the use of different

bacteria or to the application methods of the peracetic acid. In the current study, peracetic acid was applied by a spray application, as is the industry standard for beef production. In comparison, the Nagel et al. (2012) study utilized a FinalKill® Finishing Chiller® (model FC-8WHS-S, Morris & Associates, Garner, NC.) in which samples were fully submersed for approximately 20 seconds. This application is consistent to industry standards for poultry processing.

A study by Ransom et al. (2003) compared methods of *E. coli* O157:H7 reduction in beef, which included the application of peracetic acid at 200ppm. Results from this study demonstrate a 1.4 log CFU/cm^2 reduction on inoculated (10^5–10^6 CFU/mL) beef carcass tissue when applying peracetic acid compared to the control. On inoculated (10^5-10^6 CFU/mL) lean tissue pieces, this reduction was reported to be 1.0 log CFU/g when using peracetic acid as compared to the control. There is a greater bacterial reduction in this study as well as in the study by Nagel et al. (2012). In this case, the bacteria and species used are the same, but in the study by Ransom et al. (2003) inoculated samples were submersed in treatments for 30 seconds instead of using a spray application as in the current experiment.

Another study by King et al. (2005) applied 200ppm peracetic acid to beef samples inoculated with *Escherichia coli* O157:H7 and *Salmonella* Typhimurium using a spray application as used in the current study. Results from the King et al. (2005) study showed that 200ppm peracetic acid had no effect ($P>0.05$) on bacterial load of either pathogen on chilled carcass surfaces. However, when applied to hot carcasses, spray application of peracetic acid resulted in a 0.7 log CFU/cm^2

reduction in both pathogens. This strengthens the theory that application methods could impact the amount of reduction. However, both studies show a significant reduction in the bacterial load on the samples after treatment with peracetic acid.

Quality Determination

Sensory analysis results showed no difference ($P>0.05$) for any attributes for 120ppm peracetic acid, 400ppm peracetic acid, or control samples (Table 3). Therefore, the use of peracetic acid at either 120ppm or 400ppm in beef will not negatively impact the sensory characteristics of juiciness, tenderness, flavor, or off-flavor. These results were similar to studies by Nagel et al. (2012) and Bauermeister et al. (2008), which showed no quality defects in poultry treated with peracetic acid and no difference ($P<0.05$) in consumer acceptance of poultry treated with peracetic acid when compared to poultry treated with traditional treatments, such as chlorine.

Values associated with colorimeter measurements (L*=lightness/darkness, a*=red/green, b*=yellow/blue) are shown in Table 4. There were no differences ($P>0.05$) in color due to treatment alone. These results are important because color is one of the foremost attributes on which consumers base quality. As shown by Carpenter et al. (2001), color impacted consumer perception of meat quality at purchase, even though sensory testing showed that the consumers did not report differences in these products after cooking.

There were slight differences in color when analyzed by treatment by replication and treatment by day. As shown in Table 5a, lightness (L*) of the

samples treated with 120ppm peracetic acid showed no difference (P>0.05) between days; however, control samples were different (P<0.05) between Day 5 and Day 7, but the same (P>0.05) for all other comparisons (Table 5a). Furthermore, for samples treated with 400ppm peracetic acid, there was a difference (P<0.05) in lightness when comparing Day 1 and Day 5, but not with any other comparisons (Table 5a). Redness (a*) decreased (P<0.05) over time during the study for all treatments, but deterioration was seen more quickly (Day 3) for samples treated with 120ppm or 400ppm peracetic acid (Table 5b). It is important to note that a decrease in redness was expected since fresh beef products naturally decrease in redness over time due to a change in the metmyoglobin concentration of the meat. Yellowness (b*) decreased with time as well, showing a difference (P<0.05) between Day 7 and all other days across all treatments (Table 5c). As stated previously, the b* value is a measure of the yellow-blue color spectrum. A decrease in yellow pigment corresponds with an increase in blue pigment. Due to an increase in metmyoglobin over time, brown or gray color development occurs in fresh beef products naturally over time (MacDougall, 1981). For this reason, the results showing a decrease in yellowness were also expected, since graying of the product would be associated with an increase in blue pigmentation.

While lightness (L*) did not differ (P>0.05) between replications (Table 6a), redness (a*) was different (P<0.05) in Replication 3 for the control samples and samples treated with 400ppm peracetic acid and was different in Replication 1 for samples treated with 120ppm peracetic acid (Table 6b). Yellowness (b*) was different (P<0.05) in Replication 1 for samples treated with 120ppm peracetic acid

and in Replication 3 for control samples and those treated with 400ppm peracetic acid (Table 6c). Both a* and b* values increased in Replication 3 for all treatments (Table 6b, Table 6c). Color could vary for many reasons, such as pH, temperature, age, and exposure to oxygen. As meat is stored, color change over days should be expected in fresh meat products based on factors such as light and oxygen exposure (MacDougall, 1981). Metmyoglobin is the pigment that develops in meat over storage time, causing a brown color that is undesirable to consumers. Studies have revealed the central factors in this formation of metyoglobin to be exposure to oxygen (oxygen forms metmyoglobin through the oxidation on oxymyoglobin) and light, which hastens the process (MacDougall, 1981). Even in the most controlled experiment, samples will at some point in the research be exposed to both light and oxygen, which will cause changes to color over time. However, even with some differences in replication or day, results for treatment still show no difference ($P>0.05$) in color change for any color attribute. This study showed more desirable color results than a previous study in poultry (Bauermeister et al., 2008), which reported a "bleached" color on carcasses treated with peracetic acid when compared to the control. The study reported no differences ($P>0.05$) in color when treatment with peracetic acid was compared to treatment with the industry standard, chlorine; however, the study did indicate a difference ($P<0.05$) from the control, which the current study does not show in beef.

The results for yeast and mold growth showed zero growth for any petrifilm used, those results were expected and will not be further discussed in this study. The results for bacterial growth on Plate Count Agar (PCA), Sorbitol MacConkey

agar supplemented with Cefixime Tellurite (CT-SMAC), and Coliform petrifilm are shown in Table 7. Results for every type of agar and petrifilm indicate that treatment with 400ppm peracetic acid is more effective ($P<0.05$) than treatment with 120ppm peracetic acid or the control at controlling bacterial growth. Treatment with 400ppm peracetic acid does reduce ($P<0.05$) growth of *E. coli* O157:H7 in raw beef products.

Growth on PCA plates represents total bacterial load on the sample. Data for PCA plates indicated no difference ($P>0.05$) between days of the study for treatment with either 120ppm or 400ppm peracetic acid, but did show an increase ($P<0.05$) in growth in the control samples after Day 1 (Table 9a). This suggests that treatment with peracetic acid in either concentration controls growth of total bacteria over time, due to the increase of bacteria in the control that was not seen in the treated samples. However, while PCA plates were representative of total aerobic bacterial growth, this bacterial growth likely consisted of mostly *E. coli* O157:H7, as that was the bacteria used in the inoculum.

Results for treatment with 120ppm peracetic acid did not differ ($P>0.05$) between replications for PCA plates; however, there was a difference ($P<0.05$) in Replication 3 for the control samples and a difference between Replication 1 and Replication 2 for the samples treated with 400ppm peracetic acid. These differences in replication are all under 1 log CFU/g (Table 8a).

Sorbital MacConkey plates were supplemented with Cefixime Tellurite so that growth on this agar showed only the growth of *E. coli* O157:H7. Data for these plates showed that Replication 3 is different ($P<0.05$) than replications 1 and 2 in

the control samples and those treated with 120ppm peracetic acid, and Replication 1 is different ($P<0.05$) than replications 2 and 3 for treatment with 400ppm peracetic acid; however, the difference is still less than 1 log CFU/g (Table 8b).

Results for CT-SMAC plates also showed a difference ($P<0.05$) between Day 1 and days 3, 5, and 7 for the control and treatment with 120ppm peracetic acid and a difference ($P<0.05$) between Day 5 and Day 7 for treatment with 400ppm peracetic acid (Table 9b). These differences, while also under 1 log CFU/g, show a decrease in *E. coli* O157:H7 prevalence from Day 1 to Day 7.

Finally, growth on Coliform petrifilm represented the total coliform count for *Escherichia coli*. Results showed a difference ($P<0.05$) between Replication 1 and replications 2 and 3 for the control and treatment with 120ppm peracetic acid, but no difference ($P>0.05$) in replications for treatment with 400ppm peracetic acid; however, the differences were less than 1 log CFU/g (Table 8c).

For the control group on Coliform petrifilm, there was a difference ($P<0.05$) between Day 1 and all other days. For treatment with 120ppm peracetic acid, Days 1 and 3 were different ($P<0.05$) than Days 5 and 7. Finally, treatment with 400ppm peracetic acid showed a difference ($P<0.05$) between Day 3 and all other days. These differences (all under 1 log CFU/g) showing an overall decrease ($P<0.05$) over time in the prevalence of Escherichia coli (Table 9c).

Data from Warner-Bratzler shear force measurements indicated no difference ($P>0.05$) between treatment with 400ppm peracetic acid and the control, or between treatment with 120ppm or 400ppm peracetic acid, but did indicate an increase ($P<0.05$) in tenderness between treatment with 120ppm peracetic acid and

the control, with shear values decreasing from 3.09g to 2.36g of force needed to shear samples (Table 10). It is of note that while there was not a difference in P values, the shear force values for treatment with 400ppm peracetic acid were lower than the values for the control (shear decreased from 3.09g to 2.78g of force), indicating that samples were slightly more tender when treated with peracetic acid than when untreated (Table 10). Due to treatment with 400ppm peracetic acid failing to show a significant decrease ($P>0.05$) in shear force values, it is possible that peracetic acid does not increase tenderness as indicated by treatment with 120ppm peracetic acid. Instead, the higher shear force value seen in the control may have been due to less liquid being present at cooking, since the treatment samples had liquid added in the form of the peracetic acid solution while the control samples had no liquid added. Further studies would need to be performed to evaluate this effect on tenderness; however, the application of peracetic acid has not been shown in the current study to decrease tenderness in any way. When shear force results are compared to the quality results for tenderness from the trained sensory panel, data indicates that, while shear force decreases ($P<0.05$) for samples treated with 120ppm peracetic acid, the panel was not able to detect a difference ($P>0.05$) in sustained or initial tenderness between any samples, treated or control. This could be due to the differences in shear force being less than 1kg, which is the threshold at which consumers are able to detect a difference in tenderness (Shackelford et al., 2007). As studied by Destefanis et al. (2008), consumers are able to correctly identify differences in tenderness only 62.3% of the time, at best. The importance of the tenderness and shear force results in the current study is the

indication that using peracetic acid does not increase toughness of the product or create any discernable negative difference in eating experience for the consumer.

Results from both the antimicrobial and shelf life portions of this study show that peracetic acid does reduce ($P<0.05$) growth of *E. coli* O157:H7 and other bacteria at levels of 400ppm. In addition, quality results show that peracetic acid can be used with no negative effects on the sensory attributes of color, juiciness, tenderness, flavor, or off-flavor ($P<0.05$). A study by Bauermeister et al. (2008) showed that the use of peracetic acid could extend ($P<0.05$) shelf life in fresh poultry products due to reduced growth of *Salmonella* Typhimurium and *Campylobacter jejuni* in treated samples when compared to traditional methods. The current study utilized a different pathogen as well as a different species; however results for the current study are in agreement that the use of peracetic acid could extend shelf-life, due to the resulting decrease in bacterial counts overall. Also in agreement with the current study, Bauermeister et al. (2008) indicated that peracetic acid did not have a negative impact on sensory or quality characteristics of the meat. However, it is important to note that while peracetic acid has been shown to reduce bacteria in poultry products to acceptable levels, *E. coli* O157:H7 is considered an adulterant in beef. While peracetic acid reduces the amount of *E. coli* O157:H7, it has not yet been shown to eradicate it fully, or to reduce levels as much as current industry standards, such as lactic acid. It is for this reason that, even though results between these studies are similar, the use of peracetic acid in beef products specifically must be incorporated as a multi-hurdle approach to pathogen reduction and should not be used as the sole method of control. If producers

desired to incorporate peracetic acid as one of several means of control, it is advised that these producers evaluate the effectiveness of peracetic acid using the specific mode of application used in their individual plant, since application method does appear to affect efficacy.

Table 1. Strains of *Escherichia coli*

Microorganism	ATCC number or ID Code	Source
Escherichia coli O157:H7	ATCC 35150	Human – HC
Escherichia coli O157:H7	ATCC 43894	Human – HC
Escherichia coli O157:H7	AU – 1	Laboratory strain (301)
Escherichia coli O157:H7	AU – 2	Laboratory strain (505B)
Escherichia coli O157:H7	AU - 3	Laboratory strain

Table 2. Standard error and mean CFU/g of beef inoculated with low inoculum (10^2 CFU/mL) and high inoculum (10^6 CFU/mL) *E. coli* O157:H7 and treated with different concentrations of peracetic acid

	10^2		10^6	
Treatment	**Mean CFU/g**	**SEM**	**Mean CFU/g**	**SEM**
Control	0.76[a]	0.085	4.96[a]	0.029
120ppm	0.70[a]	0.084	4.84[b]	0.029
400ppm	0.19[b]	0.087	4.77[b]	0.029

[a,b]within a column, means without a common superscript differ (P<0.05)

Table 3. Means and standard error of initial and sustained juiciness, initial and sustained tenderness, flavor intensity, and off-flavor intensity for beef treated with different concentrations of peracetic acid (no difference (P>0.05) was found between treatments for any attribute)

Treatment	Initial Juiciness	Sustained Juiciness	Initial Tenderness	Sustained Tenderness	Flavor intensity	Off-flavor Intensity
Control	6.56±0.55	6.17±0.42	6.73±0.39	6.62±0.36	5.34±0.23	2.45±0.15
120ppm	4.45±0.55	4.06±0.42	4.89±0.39	4.89±0.36	4.50±0.23	1.92±0.15
400ppm	5.22±0.55	4.61±0.42	6.00±0.39	5.95±0.36	5.17±0.23	2.22±0.15

[1]Scale: 1 = extremely dry (tough, bland) and no off-flavor, 2 = very dry (tough, bland) and slight off-flavor, 3 = moderately dry (tough, bland) and small off-flavor, 4 = slightly dry (tough, bland) and modest off-flavor, 5 = slightly juicy (tender, intense) and moderate off-flavor, 6 = moderately juicy (tender, intense) and very off-flavor, 7 = juicy (tender, intense) and intense off-flavor, 8 = extremely juicy (tender, intense) and extreme off-flavor

Table 4. Means ± standard error of L*, a*, and b* color values for different treatments with peracetic acid (no difference (P>0.05) was found between treatments for any attribute)

Treatment	L*	a*	b*
0	46.28 ± 0.96	17.22 ± 0.60	17.63 ± 0.62
120ppm	46.60 ± 0.96	18.06 ± 0.60	18.81 ± 0.62
400ppm	46.86 ± 0.96	17.21 ± 0.60	18.67 ± 0.62

Table 5a. Means ± standard error of Treatment x Day for L* color values for beef inoculated with 10^6 CFU/mL *E. coli* O157:H7 and treated with different concentrations of peracetic acid

Day	Treatment		
	0	**120ppm***	**400ppm**
1	47.25 ± 1.96^{ac}	49.09 ± 1.96	50.39 ± 1.96^{bc}
3	46.35 ± 1.96^{ac}	45.54 ± 1.96	$46.18 \pm 1{,}96^{ac}$
5	42.36 ± 1.96^{a}	45.53 ± 1.96	43.95 ± 1.96^{a}
7	49.18 ± 1.96^{bc}	46.23 ± 1.96	46.98 ± 1.96^{ac}

^{a,b,c}within a column, means without a common superscript differ (P<0.05)
*there was no difference between Days (P>0.05)

Table 5b. Means ± standard error of Treatment x Day for a* color values for beef inoculated with 10^6 CFU/mL *E. coli* O157:H7 and treated with different concentrations of peracetic acid

Day	Treatment		
	0	**120ppm**	**400ppm**
1	23.70 ± 1.13^{a}	25.02 ± 1.13^{a}	21.07 ± 1.13^{ae}
3	23.21 ± 1.13^{a}	21.79 ± 1.13^{b}	21.52 ± 1.13^{be}
5	13.08 ± 1.13^{b}	16.47 ± 1.13^{c}	17.41 ± 1.13^{c}
7	8.89 ± 1.13^{c}	8.96 ± 1.13^{d}	8.83 ± 1.13^{d}

^{a,b,c,d,e} within a column, means without a common superscript differ (P<0.05)

Table 5c. Means ± standard error of Treatment x Day for b* color values for beef inoculated with 10^6 CFU/mL *E. coli* O157:H7 and treated with different concentrations of peracetic acid

Day	Treatment		
	0	**120ppm**	**400ppm**
1	20.24 ± 1.26^{a}	22.84 ± 1.26^{a}	21.05 ± 1.26^{a}
3	19.19 ± 1.26^{a}	19.80 ± 1.26^{a}	20.23 ± 1.26^{a}
5	17.56 ± 1.26^{a}	19.51 ± 1.26^{a}	19.91 ± 1.26^{a}
7	13.55 ± 1.26^{b}	13.10 ± 1.26^{b}	13.48 ± 1.26^{b}

^{a,b}within a column, means without a common superscript differ (P<0.05)

Table 6a. Means ± standard error of Treatment x Replication for L* color values for beef inoculated with 10^6 CFU/mL *E. coli* O157:H7 and treated with different concentrations of peracetic acid (no difference (P>0.05) was found between replications for any treatment)

Replication	Treatment		
	0	**120ppm**	**400ppm**
1	45.58 ± 1.70	47.86 ± 1.70	47.01 ± 1.70
2	47.39 ± 1.70	46.61 ± 1.70	47.36 ± 1.70
3	45.88 ± 1.70	45.32 ± 1.70	46.20 ± 1.70

Table 6b. Means ± standard error of Treatment x Replication for a* color values for beef inoculated with 10^6 CFU/mL *E. coli* O157:H7 and treated with different concentrations of peracetic acid

Replication	Treatment		
	0	**120ppm**	**400ppm**
1	14.24 ± 0.98^{a}	15.29 ± 0.98^{a}	14.56 ± 0.98^{a}
2	14.06 ± 0.98^{a}	18.87 ± 0.98^{b}	15.62 ± 0.98^{a}
3	23.36 ± 0.98^{b}	20.02 ± 0.98^{b}	21.44 ± 0.98^{b}

^{a,b}within a column, means without a common superscript differ (P<0.05)

Table 6c. Means ± standard error of Treatment x Replication for b* color values for beef inoculated with 10^6 CFU/mL *E. coli* O157:H7 and treated with different concentrations of peracetic acid

Replication	Treatment		
	0	**120ppm**	**400ppm**
1	14.87 ± 1.09^{a}	16.05 ± 1.09^{a}	16.07 ± 1.09^{a}
2	17.17 ± 1.09^{a}	19.63 ± 1.09^{b}	18.42 ± 1.09^{a}
3	20.86 ± 1.09^{b}	20.76 ± 1.09^{b}	21.51 ± 1.09^{b}

^{a,b}within a column, means without a common superscript differ (P<0.05)

Table 7. Means (throughout storage) ± standard error of PCA, CT-SMAC, and Coliform bacterial growth values for *E. coli* O157:H7 on beef samples inoculated with 10^6 CFU/mL of *E. coli* O157:H7 and receiving different treatments with peracetic acid (log CFU/g)

Treatment	PCA	CT-SMAC	Coliform
0	6.43 ± 0.10^a	5.64 ± 0.08^a	4.65 ± 0.09^a
120ppm	6.44 ± 0.10^a	5.68 ± 0.07^a	4.53 ± 0.07^a
400ppm	6.11 ± 0.10^b	5.40 ± 0.07^b	4.26 ± 0.07^b

^{a,b}within a column, means without a common superscript differ ($P<0.05$)

Table 8a. Means ± standard error of Treatment x Replication for PCA bacterial growth values for *E. coli* O157:H7 on beef samples inoculated with 10^6 CFU/mL of *E. coli* O157:H7 and treated with different concentrations of peracetic acid (log CFU/g)

Replication	Treatment		
	0	**120ppm**	**400ppm**
1	5.88 ± 0.19[a]	6.67 ± 0.15	6.43 ± 0.15[a]
2	6.28 ± 0.15[a]	6.35 ± 0.15	5.89 ± 0.15[bc]
3	6.88 ±0.15[b]	6.30 ± 0.15	6.01 ± 0.15[ac]

[a,b,c]within a column, means without a common superscript differ (P<0.05)

Table 8b. Means ± standard error of Treatment x Replication for CT-SMAC bacterial growth values for *E. coli* O157:H7 on beef samples inoculated with 10^6 CFU/mL of *E. coli* O157:H7 and treated with different concentrations of peracetic acid (log CFU/g)

Replication	Treatment		
	0	**120ppm**	**400ppm**
1	5.84 ± 0.17[a]	6.00 ± 0.14[a]	5.60 ± 0.13[a]
2	5.84 ± 0.13[a]	5.78 ± 0.12[a]	5.46 ± 0.12[ac]
3	5.24 ± 0.12[b]	5.28 ± 0.12[b]	5.13 ± 0.12[bc]

[a,b,c]within a column, means without a common superscript differ (P<0.05)

Table 8c. Means ± standard error of Treatment x Replication for Coliform bacterial growth values for *E. coli* O157:H7 on beef samples inoculated with 10^6 CFU/mL of *E. coli* O157:H7 and treated with different concentrations of peracetic acid (log CFU/g)

Replication	Treatment		
	0	**120ppm**	**400ppm**
1	5.10 ± 0.18[a]	4.99 ± 0.11[a]	4.30 ± 0.11
2	4.45 ± 0.14[b]	4.31 ± 0.11[b]	4.16 ± 0.12
3	4.50 ± 0.11[b]	4.29 ± 0.11[b]	4.34 ± 0.11

[a,b]within a column, means without a common superscript differ (P<0.05)

Table 9a. Means ± standard error of Treatment x Day for PCA bacterial growth values for *E. coli* O157:H7 on beef samples inoculated with 10^6 CFU/mL of *E. coli* O157:H7 and treated with different concentrations of peracetic acid (log CFU/g)

Day	Treatment		
	0	**120ppm***	**400ppm**
1	5.70 ± 0.22[a]	6.58 ± 0.17	5.98 ± 0.17
3	6.57 ± 0.18[b]	6.54 ± 0.17	5.96 ± 0.17
5	6.63 ± 0.17[b]	6.24 ± 0.17	6.25 ± 0.17
7	6.48 ± 0.17[b]	6.40 ± 0.17	6.26 ± 0.17

[a,b]within a column, means without a common superscript differ (P<0.05)
*there was no difference between Days (P>0.05)

Table 9b. Means ± standard error of Treatment x Day for CT-SMAC bacterial growth values for *E. coli* O157:H7 on beef samples inoculated with 10^6 CFU/mL of *E. coli* O157:H7 and treated with different concentrations of peracetic acid (log CFU/g)

Day	Treatment		
	0	**120ppm**	**400ppm**
1	6.23 ± 0.20[a]	6.13 ± 0.14[a]	5.47 ± 0.14[ab]
3	5.60 ±0.17[b]	5.64 ± 0.16[b]	5.34 ± 0.15[ab]
5	5.35 ± 0.14[b]	5.52 ± 0.14[b]	5.61 ± 0.14[b]
7	5.37 ± 0.14[b]	5.46 ± 0.14[b]	5.18 ± 0.14[a]

[a,b]within a column, means without a common superscript differ (P<0.05)

Table 9c. Means ± standard error of Treatment x Day for Coliform bacterial growth values for *E. coli* O157:H7 on beef samples inoculated with 10^6 CFU/mL of *E. coli* O157:H7 and treated with different concentrations of peracetic acid (log CFU/g)

Day	Treatment		
	0	**120ppm**	**400ppm**
1	5.40 ± 0.24[a]	4.98 ± 0.13[a]	4.02 ± 0.13[a]
3	4.65 ± 0.17[b]	4.81 ± 0.13[a]	4.66 ± 0.13[b]
5	4.35 ± 0.13[b]	4.16 ± 0.13[b]	4.18 ± 0.13[a]
7	4.33 ± 0.13[b]	4.18 ± 0.13[b]	4.21 ± 0.13[a]

[a,b]within a column, means without a common superscript differ (P<0.05)

Table 10. Means ± standard error of Warner-Bratzler shear force values of beef treated with different concentrations of peracetic acid (g)

Treatment	LSMEAN	Standard Error
0	3.09[a]	2.07
120ppm	2.36[bc]	2.07
400ppm	2.78[ac]	2.07

[a,b,c]within a column, means without a common superscript differ (P<0.05)

IMPLICATIONS

Results from this study suggest that at low levels of contamination, applying 400ppm peracetic acid as an antimicrobial in beef could be effective in reducing *E. coli* O157:H7. However, with zero tolerance of *E. coli* O157:H7 in beef, these results do not show enough reduction in bacterial growth to use peracetic acid as the sole method of controlling *E. coli* O157:H7. Processors may consider peracetic acid as a hurdle strategy in combination with other hurdles tailored to individual processes. Overall, shelf-life and sensory results show that if processors choose to incorporate peracetic acid into their multi-hurdle strategies, they can do so with little effect on the quality attributes of their products.

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APPENDICES

Appendix A. Sensory Evaluation Form (Part A)

Beef Sensory Evaluation Form

Sample Number	Initial Juiciness	Sustained Juiciness	Initial Tenderness	Sustained Tenderness	Flavor Intensity	Aroma Intensity	Off Flavor Intensity	Off Flavor Descriptor

Appendix B. Sensory Evaluation Form (Part B)

Juiciness	Tenderness	Flavor Intensity	Aroma Intensity	Off Flavor Intensity	Off Flavor Descriptor
8 = Extremely Juicy	8 = Extremely tender	8 = Extremely intense	8 = Extremely intense	8 = Extreme off flavor	8 = Metallic
7 = Very juicy	7 = Very tender	7 = Very intense	7 = Very intense	7 = Intense off flavor	7 = Salty
6 = Moderately juicy	6 = Moderately tender	6 = Moderately intense	6 = Moderately intense	6 = Very off flavor	6 = Livery
5 = Slightly juicy	5 = Slightly tender	5 = Slightly intense	5 = Slightly intense	5 = Moderate off flavor	5 = Sour
4 = Slightly dry	4 = Slightly tough	4 = Slightly bland	4 = Slightly bland	4 = Modest off flavor	4 = Sweet
3 = Moderately dry	3 = Moderately tough	3 = Moderately bland	3 = Moderately bland	3 = Small off flavor	3 = Vinegar
2 = Very dry	2 = Very tough	2 = Very bland	2 = Very bland	2 = Slight off flavor	2 = Bloody
1 = Extremely dry	1 = Extremely tough	1 = Extremely bland	1 = Extremely bland	1 = None	1 = Other, explain