PACKER APPLIED ANTIMICROBIAL INTERVENTIONS ON BLADE TENDERIZED NON-INTACT BEEF STRIP LOIN AND TOP SIRLOIN

by

REBECCA JO KERSEY

(Under the Direction of Alex Stelzleni)

ABSTRACT

Consumers consider tenderness as one of the most influential factors that contribute to palatability and ultimately the overall perception of quality in beef products. Mechanical tenderization of whole muscle cuts in beef is a commonly used method of tenderization in North America. Mechanical tenderization can introduce pathogen contamination into the interior of the meat. The tenderizing blades may act as a vehicle for surface pathogen translocation carried from the surface of the meat to the inherently sterile interior. The objectives of this study were to use novel antimicrobial interventions, levulinic acid and electrolyzed oxidizing water, on beef strip loin and top sirloin subprimals before blade tenderization and their effects on quality and sensory characteristics compared to industry standard antimicrobial interventions. Results indicated that levulinic acid and electrolyzed oxidizing water were comparable to the industry standard antimicrobial interventions, lactic acid and peroxyacetic acid, in regards to quality and sensory characteristics.

INDEX WORDS: Beef, Antimicrobial, Blade Tenderization, Sensory, Quality

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REBECCA JO KERSEY

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REBECCA JO KERSEY

Major Professor: Alex Stelzleni

Committee: Dean Pringle

Yen-Con Hung

Electronic Version Approved:

Maureen Grasso

Dean of the Graduate School

The University of Georgia

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DEDICATION

I would like to dedicate this work to my friends and family. Especially to my parents who have all been there and supported me thought my endeavors.

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CHAPTER 1

INTRODUCTION

Antemortem factors such as animal maturity, sex class, genetics, diet and nutrition and postmortem factors such as pH, chilling rate, aging time and even muscle location can affect tenderness. The average consumer may not be aware of theses individual antemortem and postmortem factors however, they realize that cuts from different locations vary in acceptable tenderness. Retail markets capitalize on this basic knowledge and place higher monetary value on cuts that are expected to have higher acceptable tenderness. The assurance of acceptable tenderness is important not only to consumers but also to retail, foodservice and restaurant industries. Processors and purveyors have a need for commercial applications to create consistently tender products that will create repeat buyers by satisfying consumer demands. It was in the 1970's when major focus was placed on a commercial application to create marketplace consistency for beef products and thus the use of mechanical tenderization was implemented to help improve tenderness of retail cuts (Davis, Smith, & Carpenter, 1977).

Mechanical tenderization continues to be studied in the meat industry however, the majority of research objectives have shifted from a focus on quality aspects to a microbiology food safety aspect (Gill & McGinnis, 2004; Luchansky, Phebus, Thippareddi, & Call, 2008). In 1993, a major outbreak of *Escherichia coli* O157:H7 due to under cooked ground beef lead to the USDA-FSIS to declare *E.coli* O157:H7 be added to the list of adulterants in ground beef products under the Federal Meat Inspection Act (USDA-FSIS, 1999a). In 1996, U.S. Department of Agriculture (USDA), Food Safety Inspection Services (FSIS) took a proactive, systematic and

preventive approach to food safety and biological hazards in the meat industries production process which led to the Pathogen Reduction Act (HACCP) (USDA-FSIS, 1996a). As a result, the prevalence of *E.coli* O157:H7 in ground beef products was reduced greatly, further reduction of *E.coli* O157:H7 was seen after 1999 when USDA-FSIS added *E.coli* O157:H7 to the list of adulterants in non-intact beef products that were to be further processed (USDA-FSIS, 1999b). In 2003, three major outbreaks of *E.coli* O157:H7 were associated with mechanically tenderized beef products(USDA-FSIS, 2003) . Following these incidents USDA-FSIS responded by requiring establishments producing mechanically tenderized whole muscle, non-intact beef products to reassess their HACCP plans (USDA-FSIS, 2005a). The mandate focused specifically on reassessing the risk of *E.coli* O157:H7 pathogen contamination in mechanically tenderized beef products and evaluation of the establishments' intervention processes to control pathogens during processing.

Mechanical tenderization can introduce pathogen contamination into the interior of the meat (Gill & McGinnis, 2004; Luchansky et al., 2008; Sporing, 1999). The tenderizing blades may act as a vehicle for surface pathogen translocation carried from the surface of the meat to the inherently sterile interior. Whole muscle non-intact beef products can be a health risk due to the cooking method and degree of doneness preferred by consumers, which are often unaware of their procurement of whole muscle non-intact products. Unlike ground beef products usually cooked to an internal temperature of 71°C or medium according to the USDA, some consumers prefer to cook products to an internal temperature corresponding to a rare or medium rare degree of doneness. The internal temperatures of rare and medium rare doneness may not reach temperatures lethal to *E.coli* O157:H7 and other pathogens. A 2003 survey conducted by North American Meat Processors and Food Safety Systems found that 94% of processors surveyed

used mechanical tenderization, and 54% mechanically tenderized their beef products using blade tenderization (NCBA, 2005).

Consumers consider tenderness as one of the most influential factors that contribute to palatability and ultimately the overall perception of quality in beef products (Miller, Carr, Ramsey, Crockett, & Hoover, 2001). Mechanical tenderization of whole muscle cuts in beef is a commonly used method of tenderization in North America. Antimicrobial interventions that are clean label products and have the Food and Drug Administration's Generally Recognized as Safe status (GRAS) are important for packers and processers because of the growing trend is consumer preference for clean-labeled products. However, antimicrobial interventions should not affect quality and consumer perception and therefore effects antimicrobial interventions on quality and sensory characteristics of meat should be studied prior to adoption.

The main objectives of this research are:

1. To investigate the effects of two novel antimicrobial interventions on beef strip loin and top sirloin subprimals applied prior to blade tenderization under two packaging systems.
2. Quantify the effect novel antimicrobials applied to beef strip loin and top sirloin subprimals prior to blade tenderization on the quality and sensory characteristics

CHAPTER 2

LITERATURE REVIEW

Mechanical tenderization background

Blade tenderization works by physically disrupting the connective tissue and myofibrillar contractile system. Closely placed blades penetrate the muscle tissue cutting the muscle fibers into shorter segments. Advantages of mechanical tenderization were justified by Miller (1975) (1) it insures acceptable tenderness of normal table-grade cuts; (2) it equalized tenderness in portioned items containing 2 or more muscles that differed in tenderness; (3) it upgrades cuts not normally used for steaks without enzymatic tenderization and (4) its effects are more uniform and more easily controlled that enzyme treatments.

Mechanical tenderization effects on sensory characteristics

Over the years, numerous reports have evaluated the effects of blade tenderization of beef tenderness and palatability of steaks and roasts. Blade tenderization has shown to significantly improve tenderness. Glover, Forrest, Johnson, Bramblett, and Judge (1975) reported that roasts and loin steaks mechanically tenderized had markedly increased tenderness as measured by Warner-Bratzler shear force. Savell, Carpenter, and Smith (1976) report that blade tenderization (1 pass) decreased shear force for the *gluteus medius* (GM), *semimembranosus*, and *longissimus* (LM) muscles. Similar results were reported by Hayward, Hunt, Kastner, and Kropf (1980)

concluding that blade tenderization significantly reduced meat tenderness significantly reduced Warner-Bratzler shear force values.

Accompanying the reduction in shear force values, many have also reported that mechanical tenderization also improves sensory panel tenderness and tenderness desirability. Blade tenderized steaks had notably improved sensory scores in both initial and residual tenderness and improved tenderness compared to postmortem aging alone while not affecting flavor, juiciness, or overall palatability (Davis et al., 1977; Glover et al., 1975). Additionally, overall palatability ratings were not affected for LM steaks that were blade tenderized and were rated as being lower in connective tissue and more tender than those not blade tenderized (Jeremiah, Gibson, & Cunningham, 1999; Savell et al., 1976). Furthermore, mechanical tenderization improved initial and overall tenderness of the outside round, top sirloin, strip loin, inside round, chuck tender, sirloin tip and overall tenderness in the eye of round while less perceptible connective tissue was detected the in top sirloin, strip loin and eye of round (Jeremiah et al., 1999). Steaks subjected to additional (2 or more) blade tenderization passes showed no significant advantages (Bowling, Smith, Carpenter, Marshall, & Shelton, 1976), however blade tenderized top sirloins steaks had higher myofibrillar, overall tenderness scores and similar ratings for connective tissue amount, flavor, and juiciness compared to non-blade tenderized steaks (George-Evins, Unruh, Waylan, & Marsden, 2004). There are controversial reports about the affect blade tenderization has on other palatability attributes such as cooking time, cooking loss, thaw drip loss, and degree of doneness. Some studies report an increase in cooking loss (Davis, Huffman, & Cordray, 1975; Glover et al., 1975; Hayward et al., 1980) which some suggest the increased cook loss was attributed to moisture loss due to the holes made by the tenderizer (Davis et al., 1975). On the contrary, other studies found cooking losses and

thaw-drip losses from the *semitendinosus* (Seideman, Smith, Carpenter, & Marshall, 1977), LM (Tatum, Smith, & Carpenter, 1978) and *biceps femoris* (BF), *semimembranosus* (SM) (Savell et al., 1976) were not affected blade tenderization. However, some have concluded that mechanical tenderization increased cooking losses only in certain muscles (Jeremiah et al., 1999; Savell et al., 1976). Cooking times of steaks from LM, BF and SM (Jeremiah et al., 1999) muscles were not influenced by blade tenderization (up to three passes) or SM steaks after one pass (Pietrasik & Shand, 2011). On the other hand, shorter cooking times were reported for blade tenderized top sirloin steaks when compared to non-tenderized steaks (Savell et al., 1976).

Reports conclude that mechanical (blade) tenderization increase tenderness. However, mechanical tenderization offers no additional benefits to meat that is of already acceptable tenderness, meaning a cut that would be considered USDA tender couldn't be mechanically tenderized to become USDA very tender. Smith, Seideman, and Carpenter (1979) explained that even though blade tenderization improves tenderness, the amount of improvement is not sufficient to make tough beef equal to tender beef. The effects of tenderization on shear or sensory were insignificant when used with meat of higher quality grades or in addition to other tenderization methods. However, tenderization was favorable for use with lower quality grade, tougher muscles (Davis et al., 1975; Tatum et al., 1978; Wheeler, Savell, Cross, Lunt, & Smith, 1990).

Mechanical tenderization and food safety

As processors and purveyors began to value the use of blade tenderization to improve product palatability, studies soon began to focus on the potential to translocate pathogenic bacteria from exposed meat surfaces to the interior portion of the meat. Sporing (1999) found

that inoculated *E.coli* O157:H7 was translocated from the meats surface to an interior depth of 6-cm during blade tenderization. The amount of *E.coli* O157:H7 translocated decreased with increasing depth of penetration, each centimeter of depth penetrated resulted in 0.5 log decrease in *E.coli* O157:H7. Similarly, Luchansky et al. (2012) reported that mechanical tenderization transferred *E.coli* O157:H7 and other Shiga-toxin producing *E.coli* throughout beef subprimals and both types were transferred into the topmost 1-cm than into the deeper tissue. Gill and McGinnis (2004) examined the microbiological conditions of mechanically tenderized beef from four retail stores. Deep muscle tissue and surface tissue were analyzed and results suggested that deep tissue of beef that was mechanically tenderized at retail stores would usually be contaminated with bacteria. Lemmons, Lucia, Hardin, Savell, and Harris (2011) and Chancey et al. (2013) reported similar findings for the translocation of surface bacteria to interior portions of beef muscle tissue. Further investigations continue to determine thermal inactivation or lethality of pathogenic bacteria in non-intact beef products. Gill and McGinnis (2004) reported that cooking steaks from mechanically tenderized subprimals to 70°C destroyed most of the bacteria in deep tissues and aerobes were reduced by approximately 4 log units. Furthermore, intact products that have poor microbiological conditions might cause extensive deep tissue contamination by mechanical tenderization and may require higher cooking temperature. Luchansky et al. (2012) reported that regardless of steak thickness or initial (inoculation) pathogen levels, cooking non-intact steaks from tenderized subprimals to internal temp of 48.9 to 71.1°C resulted in average reduction of 2.0 to 4.1 log of E.coli O157:H7 and 1.5 to 4.5 log of Shiga-toxin producing E.coli.

Mechanical tenderization is confirmed to enhance tenderness in lower quality beef products. However, with improved quality and tenderness comes the apparent risk of

translocating surface pathogenic bacteria to interior portions of the meat. Research efforts have now moved towards a focus of antimicrobial interventions in non-intact beef products to help control and reduce the potentially harmful bacteria in these products.

Antimicrobials in the meat industry

Combinations of preservation factors called hurdles help maintain microbial safety, sensory attributes, and nutritional quality of foods. The most important hurdles used in food preservation are temperature, water activity (aw), acidity (pH), redox potential (ORP), preservatives, and competitive microorganisms (Leistner, 2000). The basic principle of hurdle technology is that reducing the initial microbial load creates fewer microorganism thereby making it easier to further reduce remaining microorganisms in following processing steps (Leistner, 2000).

The internal tissue of a carcass is thought to be sterile and free of bacteria, however carcasses can become contaminated during slaughtering and processing or during the conversion of muscle to meat. Primary sources and routes of bacterial contamination include knives, animal hides, gastrointestinal tract, handling, and equipment (Jay, 2000). Purveyors and processors aim to produce raw meat products with low levels of surface bacteria and no pathogenic bacteria. Unfortunately, because of working conditions the environment is not sterile and pathogenic microorganisms may come into contact with the surface of meat (Huffman, 2002). Strategies have been developed specifically for beef carcass decontamination to help reduce the microbial load. Before the animal reaches the slaughter facility, hurdles are in place to help reduce pathogenic bacteria such as manipulating feed ingredients, dietary modifications, and treated drinking water. However, the majority of carcass decontamination efforts are implemented postmortem. Washing and sanitizing agents have been effective in reducing bacterial populations

and the presence of pathogens on carcasses (Huffman, 2002). The basic and most commonly used carcass decontamination step used in the meat industry is hot water (>74°C), which has bactericidal effects that are thermal and physical. Numerous studies have investigated the effectiveness of hot water washing to reduce bacterial contamination of beef carcasses (Castillo, Lucia, Goodson, Savell, & Acuff, 1998; Dorsa, Cutter, Siragusa, & Koohmaraie, 1996; Ellebracht, Castillo, Lucia, Miller, & Acuff, 1999; Kalchayanand et al., 2012). The effectiveness of hot water sprays in reducing pathogenic contamination in beef depends on factors such as temperature of the water, distance from the nozzle to the carcass surface, volume of the spray, an type of nozzle (Castillo et al., 1998).

Commonly used chemical rinses

Applying organic acids to the exposed surface is a common decontamination practice. There are many advantages to using organic acids such as they are inexpensive, simple and fast, and have an established efficacy (Hinton & Corry, 1999). Additionally, the United States Food and Drug Administration (FDA) has designated most organic acids as generally recognized as safe (GRAS) for meat products. The USDA-FSIS has approved the use of organic acid solutions such as acetic, lactic, and citric acids at concentrations of 1.5-5% (USDA-FSIS, 1996b). Numerous studies have already been conducted on the efficacy of organic acids (Castillo et al., 1998; Castillo, Lucia, Goodson, Savell, & Acuff, 1999; Ellebracht et al., 1999). Organic acids are thought to affect microbial activity by two primary mechanisms: by cytoplasmic acidification with subsequent uncoupling of energy production and regulation and by accumulation of the dissociated acid anion to toxic levels (Taylor et al., 2012). Where the pH of the cellular cytoplasm is higher than that of the surrounding environment the diffusion of an undissociated acid through its microbial membrane creates a transmembrane gradient (Gould, 1989). As

protonated acid diffuses across the membrane, an alkaline environment is encountered, which favors the dissociation of the acid into an acid anion and free proton (Eklund, 1983). The cell reacts by working to efflux the protons, exchanging the proton for some other cation, this is the basis for the chemiosmotic theory (Mitchell, 1961; Mitchell & Moyle, 1969). It's proposed that the microbial membrane is impermeable to protons, and requires active transport to efflux protons and maintain pH homeostasis in the interior of the cell (Brul & Coote, 1999; Hirshfield, Terzulli, & O'Byrne, 2003).

Lactic acid

Lactic acid (LA) has become one of the most common organic acids used in the industry for carcass decontamination (Koohmaraie et al., 2005). Currently, the USDA-FSIS permits the use of organic acids as a wash solutions up to 2.5% as a carcass wash before chilling and 5% hot (55°C) LA rinse on carcasses at pre-or post-chill steps before fabrication (USDA-FSIS, 2005b). Extensive studies have evaluated LA efficacy as an antimicrobial for reduction of pathogenic bacteria on beef carcasses. King et al. (2005) found that 2% LA applied to the beef carcass surface before chilling reduced microbial counts and prevented growth of E. coli O157:H7 and Salmonella Typhimurium during the chilling period. Castillo, Lucia, Mercado, and Acuff (2001) investigated the effects of 4% LA at 55°C applied to chilled beef carcass and reported 3.0 to 3.2 log reductions in aerobic plate counts. Additionally, Castillo et al. (1998) used high pressure water wash (35°C) and carcass trimming alone or in combination with 2% lactic acid spray at 55°C to compare their effectiveness in reducing Salmonella Typhimurium, E.coli O157:H7, aerobic plate counts, Enterobacteriaceae, total coliforms, thermotolerant coliforms and generic E.coli on hot beef carcass surfaces. Although the combined treatments had greater log reductions, the range of log reductions by the 2% lactic acid regardless of washing or trimming

was from 4.6 to >4.9 log CFU/cm^2. Dorsa et al. (1996) used 3% LA spray on beef carcasses and concluded that it was effective as a carcass wash against *Listeria innocua*, *E.coli* O157:H7 and *Clostridium sporogenes* after a 21 d storage period.

Many have reported the effectiveness of LA on beef subprimals and trimmings. Gill and Badoni (2004) applied 2% and 4% LA sprays to beef trimmings from chilled beef carcass to control natural flora. In this study, both 2% and 4% LA showed reductions of aerobes, coliforms and *E.coli* however 4% LA was more effective. Improved microbiological conditions were reported by Prasai et al. (1997) when 1.5% LA spray was applied to beef subprimals before being vacuum packaged and held for up to 126 d after treatment. Despite these studies, others have reported the effect of LA to have little decontamination effect when applied to beef carcasses or cuts in commercial practice (Bacon, Sofos, Belk, & Smith, 2002; Gill & Landers, 2003). The discrepancy in the effectiveness of LA correlates to various factors such as concentration of LA used, temperature of solution, application of treatment, volume of solution applied, and length of time treatment is applied. Others have reported that as temperature of the acid treatment increases the greater the bacterial reductions on beef carcasses (Anderson & Marshall, 1989; Greer & Dilts, 1995). Additional factors that may cause differences in the effectiveness of LA include the application of treatments used before or following the application of LA solution, the temperature of the surface, such as chilled beef carcasses or cuts compared to hot beef carcasses, and in studies where surface inoculation was used, inoculation level. A recent study by Youssef, Yang, Badoni, and Gill (2012) investigated the effects of solution volume, type of surface, and level of inoculation of the survival of *E.coli* O157:H7 on beef. A 5% LA solution was used to spray controlled volumes over cut muscle surfaces with fat (intact fascia) and membrane overlying the muscle tissue. The authors reports the effect of LA

treatments were not consistent. It is suspected that the differences were due to various details of the surfaces of the muscle cuts. For example, difference in surface detail may include the degree of pitting of the surface fat or the extent of separation of the cut end of the muscle fibers. Currently, LA is commonly used in the meat industry for carcass decontamination and antimicrobial intervention.

Peroxyacetic acid

Peroxyacetic acid (PAA), also known as peracetic acid, is a mixture of acetic acid, which is the principle component of vinegar and hydrogen peroxide. With its pH of 2.8 it is a very strong oxidizing agent (1.87 electron Volts or eV), higher than chlorine dioxide (1.57 eV) but less than ozone (2.07 eV) (Gómez-López, 2012). The sanitizing power of peroxyacetic acid is not affected by organic load, solution pH, or temperature, and it can rapidly breakdown into water, oxygen and acetic acid (Wang, Feng, & Luo, 2006). Recently, the use of peroxyacetic acid has been investigated as an antimicrobial for the decontamination of pathogenic bacteria in the meat and poultry industries. When evaluated as a sanitizer for meat contact surfaces it was reported that peroxyacetic acid was effective in reducing the bacterial load but did not completely eliminate *E.coli* O157:H7 (Farrell, Ronner, & Wong, 1998). Kalchayanand et al. (2012) investigated the effectiveness of existing antimicrobial interventions for inactivating non-O157 Shinga toxin producing *E.coli* (STEC) on fresh beef. The use of peroxyacetic acid (200 ppm) as a spray had an immediate effect in reducing levels of non-O157 STEC *E.coli* serogroups O26, O45, O103, O111, O121, O145, O157 and aerobic plate counts compared to untreated controls. Peroxyacetic acid significantly reduced all non-O157 STEC *E.coli* strains except O111. Gill and Badoni (2004) used 0.02% peroxyacetic acid spray to control natural flora on the distal surfaces of pieces of brisket from chilled beef carcasses. However, peroxyacetic acid had little

effect on reducing the number of aerobes, coliforms or *E.coli* on meat surface. Ellebracht et al. (2005) reported that dipping beef trimmings into a peroxyacetic acid solution (200 ppm) reduced *E.coli* O157:H7 and *Salmonella* Typhimurium by 1.01 log CFU/cm^2. Results from King et al. (2005) suggested that spraying the surface of beef carcasses with peroxyacetic acid (1000 ppm) for 15 s reduced *E.coli* O157:H7 by 1.7 log CFU/cm^2 and *Salmonella* Typhimurium by 1.3 log CFU/cm^2. On the other hand, authors also report that peroxyacetic acid was not effective for reducing bacterial counts on chilled carcasses even at increased concentrations. Conversely, Yoder et al. (2012) reported that when used at 200 ppm, peroxyacetic acid appeared to be less effective than water at reducing *Camplyobacter* spp., *E. coli* O157:H7 and aerobic plate count on beef plate pieces.

Levulinic acid and sodium dodecyl sulfate

Levulinic acid (LVA) has increased interest from the food industry as a potential antimicrobial agent and been investigated as a novel antimicrobial for food sanitation. Recently, LVA has been approved by the United FDA as GRAS for a direct additive to food as a flavoring substance or adjunct (21 CRF 172.515). Its chemical properties, such as high boiling point, pKa 4.59 and its ability to be readily soluble in water, ethanol, acetone and many organic solvents make it useful in the food industry. Vasavada, Carpenter, Cornforth, and Ghorpade (2003) found that 1.4% sodium levulinate was just as inhibitory to spoilage bacteria as 2.7% sodium lactate in fresh pork and turkey sausage and concluded that sodium levulinate may have potential as an antimicrobial agent in fresh sausage. Thompson, Carpenter, Martini, and Broadbent (2008) investigated sodium levulinate and found that it was at least as effective at preventing the growth of *Listeria monocytogenes* in ready-to-eat meats (bologna and turkey rolls) as 2% sodium lactate and 2% combination of sodium lactate and sodium diacetate. Additionally, 2% or greater sodium

levulinate suppressed the growth of *Listeria monocytogenes* to less than 1 log during refrigerated storage.

Sodium dodecyl sulfate (SDS) is also used in household products such as toothpastes, shaving foams, bubble baths, and shampoos (Zhao, Zhao, & Doyle, 2009). However, in the food industry SDS is approved for foods including egg whites, fruit juices, vegetable oils, and gelatin (FDA 2007) since it holds GRAS status with the FDA for multipurpose additives (21 CRF 172.822). The SDS molecule contains a 12 carbon atom tail that attaches to a sulfate group, creating the amphiphilic properties that are needed for a detergent (Zhao et al., 2009). Sodium dodecyl sulfate can denature protein surfaces and damage cell membranes, and its bactericidal effect can be increased when the pH is reduced to between 1.5 and 3.0. Sodium dodecyl sulfate has also been found to facilitate the detachment of viruses from produce surfaces, thereby making them more available to the liquid disinfectant (Liu, Duan, & Su, 2006). Similar conclusions were reported by Cannon et al. (2012) who investigated LVA, SDS and the combination of both as a sanitizer on the inactivation of Human Norovirus and feline calicivirus on stainless steel surfaces. The author suggests that the detergent properties of SDS in LVA plus SDS solutions may have played a role in virus detachment, increasing the likelihood of virus particles to become inactivated.

Combining LVA with SDS dramatically increased the bactericidal activity of the two compounds (Ortega, Torres, & Tatum, 2011). Zhao et al. (2009) validated the antimicrobial efficacy of LVA and SDS for *E. coli O157:H7* and *Salmonella* on fresh produce, chicken wings and skin, and water contaminated with chicken feces or feathers. Zhao et al. (2009) reported that a combination of 3% LVA plus 1% SDS reduced *Salmonella* and *E. coli* O157:H7 populations on lettuce and reductions of *Salmonella* and aerobic bacteria on chicken wings. Furthermore,

reductions were seen when using a combination of 3% LVA plus 2% SDS in water that was heavily contaminated with chicken feces. In another study, Zhao, Zhao, and Doyle (2010) reported that soaking chicken carcasses in 3% LVA plus 2% SDS at room temperature for 5 min can substantially reduce *Salmonella* populations on skin and feathers. Furthermore, the study found that concentrations 3% LVA plus 2% SDS used as either a foam or liquid for 10 min was needed for substantial and rapid reduction of *Salmonella* in biofilms on equipment or processing surfaces SDS. Stelzleni, Ponrajan, and Harrison (2013) included a combination of 1.0% LVA plus 0.1% SDS added at 10% w/v in ground beef patties as an intervention against *Salmonella* Typhimurium and found that LVA plus SDS treated ground beef patties had the largest reduction (0.70 log CFU/g) of *Salmonella*. Additionally, ground beef patties treated with LVA plus SDS had increased growth of psychrotrophic organisms and reduced color scores after 3 d of aerobic retail display. Chen, Zhao, and Doyle (2014) investigated cross contamination between deli foods and slicers by *Listeria monocytogenes, Salmonella,* and *E. coli* O157:H7 and the subsequent inactivation by LVA plus SDS on slicers. The antimicrobial efficacy of LVA +SDS sanitizers in three concentrations (0.5% LVA + 0.05% SDS, 1% LVA + 0.1% SDS, and 2% LVA + 0.5% SDS) as either liquids or foams was determined for decontamination of the pathogens on the slicer at 21°C. The authors report that inactivation was greater when concentrations of LVA and SDS was increased. *Listeria monocytogenes* populations were reduced to undetectable levels within one minuet in all three foam concentrations. Within one minute, *Salmonella* was reduced by 3.5 logs when treated with 0.5% LVA + 0.05% SDS as a liquid or was reduced 6.0 logs when treated with any of the concentrations as a foam. One minute exposure of 0.5% LVA + 0.05% SDS and 1% LVA + 0.1% SDS as a liquid did not significantly decrease *E. coli* O157:H7. However, when applied as a foam *E. coli* O157:H7 cell

numbers were reduced to undetectable levels within 1 to 2 min by 1% LVA + 0.1% SDS and 0.5% LVA + 0.05% SDS respectively. The authors postulate that the differences in antimicrobial efficacy of the liquids versus the foam treatments may be related to the penetration rate, with the foam having greater penetration into the hard to reach places on the slicer and blades. The authors conclude that combining LVA and SDS at appropriate concentration and especially when applied as a foam is highly effective in killing *Listeria monocytogenes*, *Salmonella* Typhimurium and *E. coli* O157:H7 on slicers. Most recently, Zhao et al. (2014) investigated the efficacy of LVA plus SDS treatments for inactivation of STEC in pure culture and for reducing STEC and *Salmonella* on beef trim held under various temperature conditions. Study results showed that LVA plus SDS based intervention were effective in reducing STEC contamination on beef, however many factors can interfere with their efficacy. The authors explain that the reductions of STEC on beef were directly related to the surface temperature of the treated beef trim and the greatest reductions occurred at temperatures $\geq 8°C$. Zhao et al. (2014) discusses when the surface temperature of the beef trim was 4°C the surface was solid and the LVA plus SDS treatments were not able to penetrate therefore decreasing treatment effectiveness. Higher surface temperatures softened the beef trim allowing LVA plus SDS treatments to be more effective in killing pathogens. Additionally noted, immersion of beef samples into treatment solution was more effective at reducing STEC on beef than spraying surfaces however, hand massaging after immersion had the greatest effect for reducing STECs, especially at higher surface temperatures. Lastly, the author concludes that STEC does not survive in LVA plus SDS solution alleviating the possibility of contamination by fluid retention after application. In agreement, Zhao et al. (2010) reported when applied individually at equivalent concentrations and under equivalent conditions LVA and SDS antimicrobial activities are substantially reduced or insignificant.

These findings are parallel to those found by Ortega et al. (2011) stating that when applied individually LVA and SDS had very limited effect on *E. coli* O157:H7 viability but in combination they were highly effective at 30 and 60 min of incubation.

Electrolyzed water

Electrolyzed oxidizing (EO) water has been gaining considerable attention as an antimicrobial agent. Japan has already implemented EO water as a disinfectant in various fields such as agriculture, dentistry, medicine and food industries (Huang, Hung, Hsu, Huang, & Hwang, 2008). Electrolyzed oxidizing water is a general term used to describe electrolyzed water, however because of the various properties many different types of EO water can be produced that have different pH, oxidation-reduction potential (ORP) and free available chlorine concentrations (FCC). Electrolyzed water is generated by electrolysis of NaCl solution through an electrolyzic cell. A salt solution passes through the electrolyzic cell, which contains an anodic and cathodic side separated by a membrane. Electrolyzing the electrodes with direct current voltage creates negatively charged ions from the anode electrode, such as chloride and hydroxide, and positively charged ions from the cathode electrode such as dilute sodium hydroxide. Two types of water with very different properties are produced simultaneously. From the anodic side, acidic electrolyzed water is produced and has an approximate pH < 2.7, ORP >1,100 mV, and chlorine base reactants 10-90 ppm. Produced from cathode side electrolyzed is reduced water also called alkaline electrolyzed water, or basic electrolyzed water and has an approximate pH >11 and ORP <-800 mV. Neutral electrolyzed water can be produced by mixing acidic and alkaline electrolyzed waters or by not using a separating membrane to divide the product of the electrolytic cell (Al-Haq, Sugiyama, & Isobe, 2005; Gómez-López, 2012).

There are many advantages when using EO water in the food industry. Among the top advantages is its cost effectiveness and environmental friendly properties. After the initial purchase and setup of the generation device, EO water can be produced on site with only NaCl and water. Thereby EO water can readily revert to normal water when encountering organic matter making it environmental friendly. Only containing water and NaCl the cost and potential hazards of buying, handling, storing and transporting chemicals is greatly reduced. On the other hand, a disadvantage or concern of EO water is that when producing or using acidic electrolyzed water chlorine gas is emitted, metal corrosion may occur, and synthetic resin degradation can take place, due to its strong acidity and free chlorine concentration (Huang et al., 2008)

The main property contributing to EO antimicrobial activity remains debatable. The ORP of a solution is an indicator of its oxidizing or reducing strength, with higher positive ORP values indicating a greater oxidizing strength (Park, Hung, & Chung, 2004). The ORP value has been thought to be of greater importance than the concentration of residual (free) or total chlorine. Reasons supporting this include as the pH of the water changes the equilibrium between the two forms of chlorine shifts, as pH increases $HOCl^-$ is converted to OCl^- or hypochlorite ion a weaker and slower acting oxidizer. Additionally, when chlorine combines with amines or other stabilizers it forms combined chlorine, which is known to be less effective as a disinfectant (McPherson, 1993). Oxidation of microbes can damage cell membranes by interruption in the cells metabolic processes eventually lysing the cell. One theory for inactivation of bacteria is the high ORP of EO water causing damage to the cell membranes. Liao, Chen, and Xiao (2007) concluded through fluorescent and spectroscopic analysis that ORP could damage the outer and inner membranes of *E. coli* O157:H7 thereby killing the cell. It was suggested that ORP could affect and damage the redox state of GSSG/2GHS and then penetrate

the outer and inner membranes of E. coli, which would result in necrosis. The pH of EO water will also affect its bactericidal activity, because it can change the relative fractions of chlorine species in the solution (Len, Hung, Erickson, & Kim, 2000). As the pH of a chlorine-containing solution increases, the oxidizing capability decreases which is reflected directly in the ORP reading (McPherson, 1993). Generally, bacteria grow in a pH range of 4-9; aerobic bacteria grow mostly at ORP range +200 to 800 mV, while anaerobic bacteria grow well at -700 to +200 mV. The high ORP in the EO water could cause the modification of metabolic fluxes and ATP, probably due to the change in the electron flow in cells (Huang et al., 2008). Oomori, Oka, Inuta, and Arata (2000) state that the bactericidal activity of acidic electrolyze water is correlated with the concentration of hypochlorous acid. Hypochlorous acid, the most effective form of chlorine compounds, kills microbial cells by inhibiting glucose oxidation by chlorine-oxidizing sulfhydryl groups of certain enzymes important in carbohydrate metabolism (Rahman, Park, Song, Al-Harbi, & Oh, 2012). Available chlorine is the term used to describe the three chlorine species (Cl_2, HClO, & ClO^-) in an aqueous medium that are available for disinfection. The concentration of theses chlorine species which are unreacted are called free available chlorine. Free available chlorine becomes depleted through oxidation-reduction reactions with a variety of inorganic and organic materials. Nonetheless, the combination of hydrogen ion concentration, oxidation-reduction potential and free chlorine gives EO its potent antimicrobial effect.

Numerous research efforts have focused on acidic electrolyzed water that is characterized by a low pH and high ORP. Venkitanarayanan, Ezeike, Hung, and Doyle (1999) reported EO water was effective in inactivating *Listeria mono* and *E. coli* O157:H7 on kitchen cutting boards. Others conveyed EO water was successful at reducing bacterial populations of foodborne pathogens *Listeria mono.*, *E. coli* O157:H7 and *B .cereus.* (Park et al., 2004). Fabrizio, Sharma,

Demirci, and Cutter (2002) reported EO water can reduce *Salmonella* Typhimurium on poultry surfaces following extended refrigerated storage. Additionally, Park, Hung, and Brackett (2002) demonstrated that EO water was effective in reducing populations of *C. jejuni* on chicken while also preventing cross contamination during processing.

Recently, the use of EO water that is only slightly acidic or near neutral pH has been a focus of investigation in the study of EO water. There are numerous advantages to using low concentration electrolyzed water such as being non-corrosive to equipment, low current and minimum time required for production, the absence of residues left on food because of the low content of available chlorine concentration, and the reduced potential of health hazards to workers due to the lack of Cl_2 gas production (Ding, Rahman, Purev, & Oh, 2010). Issa-Zacharia et al (2010) indicated that slightly acidic EO water with low available chlorine concentration and near neutral pH could potentially reduce *E. coli* and *S. aureus.* A study done by Park et al., (2004) showed that EO water was very effective at inhibiting *E. coli* O157:H7 and *Listeria mono* in a wide pH range (2.6-7.0) if sufficient residual (free) chlorine is present (>2 mg/L).

In summary applying EO water in the meat industry allows packers and processors an antimicrobial that will make food safer and more appealing to consumers by answering their demands for a chemical free product. Electrolyzed oxidizing water has gain significant interest in other aspects of the food industry for its use as an antimicrobial agent. It has been shown to be an effective antimicrobial agent against pathogens on food processing equipment and food surfaces and against food spoilage in vegetables. The exact mechanism in which EO water works as an antimicrobial agent is still in debate. Many suggest that it is the chlorine species that is present while others suggest that it is due the pH but some also support that the antimicrobial mechanisms is due the oxidation reduction potential. However, most will agree that it is the

trifecta of pH, ORP and chlorine content that contribute to the success of the disinfectant properties of EO water.

Effects of antimicrobials on sensory and quality attributes in meat

Shelf-life is defined as the length of time that the quality of food products remain acceptable under certain conditions of distribution, storage and display (Gyesley, 1991). In contrast when foods deteriorate to the point of undesirable or unsuitable for sale for consumption or considered non-edible for human consumption it has undergone a process known as spoilage. Because of its high water activity and available nutrients, meat is the perfect medium for microbial growth that can lead to off-odors, off-tastes, texture changes, and formation of slime. Additionally, lipid oxidation can contribute to discoloration and meat rancidity. Meat spoilage is characterized by bacterial growth and metabolism, which causes formation of objectionable compounds, including those causing off-odors, gas, slime, and oxidation of lipids and pigments to cause undesirable flavors and discoloration (Sun & Holley, 2012).

Specific characteristics of meat tissue such as residual glucose and pH, storage conditions, and packaging material can influence the extent of spoilage in meat (Mani-Lopez, Garcia, & Lopez-Malo, 2012). The use of antimicrobials on beef carcasses, subprimals and cuts is a common practice. Although these antimicrobials can reduce harmful or detrimental pathogens on the meat surface and my extend shelf-life from a bacteriologic point of view, they could also have properties that have adverse effects on color, organoliptic, and sensory aspects such as juiciness, tenderness, and flavor.

Sensory

Sensory changes in meat products are usually noticeable at a count of 10^6 to 10^9 CFU spoilage microorganism/g meat (Bruhn et al., 2004; Gill & Gill, 2005). Gram-negative bacteria such as genera *Pseudomonas Acinetobacter, psychrobacter* and *Moraxella* have the greatest potential for meat spoilage. Members of these genera have a rapid growth rate and greater potential for spoilage in aerobically chilled fresh meat products (Mani-Lopez et al., 2012). Under normal aerobic storage conditions, the dominant spoilage microorganisms such as *pseudomonas* can deplete available glucose and amino acids metabolites, producing ammonia, amines and organic sulfides resulting in offensive spoilage usually around 10^8 bacteria/cm^2. When *pseudomonas* spoilage microorganisms are suppressed, in the absence of O_2, LA bacteria (*lactobacilli, leuconostocs, carnobacteria*) will predominate but will eventually produce offensive off flavors (Sun & Holley, 2012). Numerous studies have reported the use of antimicrobial interventions to extend shelf-life of fresh meat. However, there have been few studies reporting the effects of novel antimicrobial interventions have on sensory characteristics of meat, especially beef.

Woolthuis and Smulders (1985) reported flavor scores of meat treated with 2% LA were not statistically different from controls but a treatment with 4% v/v LA could be identified. Another study by Semler et al. (2013) showed consumer evaluations reported when 4.17% LA was sprayed directly on the surface of *psoas major* beef steaks they were more desirable for juiciness and flavor compared to control, bromine (560 ppm), and 2.48% commercial blend samples and there were no significant differences for off-flavors among treatments. Sawyer, Apple, and Johnson (2008) enhanced dark cutter beef strip loins with various concentrations of LA. Their findings were that concentrations exceeding 1.0% was extremely detrimental to fresh

and cook meat characteristics such as pH, water holding capacity and sensory taste. However, LA enhancement at 0.5%-1.0 percentage may add value to dark cutter beef. A further study by Sawyer, Apple, Johnson, Baublits, and Yancey (2009) reported that dark cutter steaks enhanced with 0.25% LA can improve fresh and cooked beef color similar to that of normal pH beef. Although not many sensory and quality investigations of new antimicrobial technologies such as LVA and electrolyzed have been reported, Stelzleni et al. (2013) incorporated 1.0% LVA plus 0.1% SDS into beef trimmings prior to grinding and reported an increase in thaw loss, juiciness and purge in ground beef patties. The author suggested that the lower pH of LVA plus SDS treated patties was the likely contributor to the greater moisture loss, purge loss, and cooking loss that was observed.

Color

Myoglobin is the main protein responsible for meat color. This globular heme protein is found in skeletal and cardiac muscles and its main function is to bind and store oxygen. There are three common form of myoglobin; deoxymyoglobin, where meat is purple or purplish-red in color and is typically associated with vacuum packaged products. Oxymyoglobin forms when oxygenation of deoxymyoglobin occurs and is associated with a bright red color in beef and metmyoglobin that is associated with brown color in meat formed by oxidation of oxymyoglobin and is often describe as surface discoloration (Bekhit & Faustman, 2005; Mancini & Hunt, 2005). It is common to see an increase in surface discoloration of meat as day of display increases. This is the result of the oxidation process that shifts oxymyoglobin and deoxymyoglobin to metmyoglobin and will normally cause the reflection rather than the absorption of light at the surface of the meat. This results in the brown-discoloration that is associated with spoilage (Quilo et al., 2009).

Objective color or instrumental color is often used to evaluate meat color. Color measurement guidelines produced by AMSA suggest for *Commission Internationale de l'Eclairage* (CIE) *L*a**, and *b** color values using illuminant A with a 10˚ viewing for whole muscle beef color evaluation. Subjective color panelist can more easily evaluate colors representing *L** and *a** when assessing meat color. However, panelists often relate *b** to browning rather than blue to yellow and usually require more training in order to evaluate colors represent by *b** values (O'Sullivan, Byrne, & Martens, 2003). Nonetheless, subjective color panels can provide benefit to meat quality research as the can offer insight into consumer perception (Mancini & Hunt, 2005)

At the point of purchase, two major factors greatly influenced consumers they are color or discoloration of fresh meat and packaging. Consumers visual scores for appearance and the likelihood to purchase were correlated (r =0.9) and scores decreased in order of red > purple > brown. This is a conformation of the close link between color preference an purchasing decisions where consumers prefer the bright red color of fresh meat over purple and brown color (Carpenter, Cornforth, & Whittler, 2001). Packaging type can influence meat color and thereby influence consumer purchasing. Carpenter et al. (2001) evaluated CIE *L* a* b** (D-65, Illuminate A, 10° observer) on beef loin steaks and reported *L*, a** and *b** values of 31.1 ± 0.1, 13.7 ± 0.2, 8.5 ± 0.5 for vacuum packaging and 31.1 ± 1.3, 13.1 ± 1.1, 14.1 ± 1.0 for polyvinyl chloride (PVC) overwrapped packaged steaks. The author postulates that the greater *b** values seen in PVC steaks may be attributed to metmyoglobin formation as oxygen diffused through the PVC overwrap. The study also reported that visual scores of steaks were greater for PVC packaged steaks followed by vacuum packaged and then modified atmosphere packaging steaks.

Investigations of antimicrobial treatments on beef carcasses and cuts predominantly focus on controlling bacteria and extending shelf-life and little attention on the effects antimicrobials have on color. Studies continue to find the ideal antimicrobial treatment that minimizes microbial growth while either not affecting or improving product color (Mancini & Hunt, 2005). There have been reports of LA having a bleaching effect and discoloration of carcass lean. Lactic acid concentrations exceeding 1.25% v/v will produce undesirable discoloration of the subcutaneous fat cover on the carcass (Ockerman, Borton, Cahill, Parrett, & Hoffman, 1974). On the other hand, after this fat has been trimmed off primal cuts may be treated with concentrations as high as 2% v/v without causing discoloration (Snijders, Schoenmakers, Gerats, & Depijper, 1979). When surfaces of beef steaks were sprayed with 1% LA, scores for lean color, surface discoloration, fat color, overall appearance and, off-odor showed no consistent differences than non-treated steaks (Dixon, Vanderzant, Acuff, Savell, & Jones, 1987). PAA treat ground beef patties were noted by sensory panelist to have a brighter red color, were redder (greater *a** values) than untreated ground beef patties across 7 days of display (Quilo et al., 2009). Mohan, Pohlman, McDaniel, and Hunt (2012) investigated the use of PAA on beef trimmings before grinding and reported that PAA did not affect instrumental color during simulated retail display and concluded that PAA can reduce bacterial loads with having adverse effects on overall quality attributes of ground beef. However, ground beef treated with 200-ppm peroxyacetic acid was reported to have extensive discoloration by d 7 of display (Jimenez-Villarreal, Pohlman, Johnson, & Brown, 2003). Few studies have focused on the effects of novel antimicrobial interventions on beef subprimals and cuts. Stelzleni et al. (2013) reported 1.0% LVA plus 0.1% SDS treated ground beef patties had a reduced color scores after 3 days of aerobic retail display. Recently, Zhao et al. (2014) used 3% LVA plus 2% SDS at 21°C

on beef trim and noted there was no color change of treated meat cuts was observed with the naked eye. Additionally, *L** values were higher in 2.7% sodium levulinate at day 0 of display than untreated turkey sausage, although generally speaking sausage color not affected by treatment (Vasavada et al., 2003). Novel antimicrobial findings were similar to those were reported by Fabrizio and Cutter (2005) where the use of acid and basic electrolyzed water on ready-to-eat meat was expected to have a "bleaching effect" due to the high ORP characteristic of electrolyzed water, however it was reported that there was no significant differences on Hunter L* a* b* values on d 0 and d 7.

Lipid oxidation

Lipid oxidation is one of the main components to meat deterioration. Lipid oxidation leads to discoloration, off odor and off taste and can produce toxic compounds (Morrissee 1998).

Interestingly, there is an apparent relationship between lipid oxidation and discoloration (Faustman, Sun, Mancini, & Suman, 2010). In meat, the rate of discoloration and lipid oxidation are muscle specific (O'Keeffe & Hood, 1982). Various factors influence lipid and myoglobin oxidation. Muscles with a greater proportion of red fibers versus white fibers contain more iron and phospholipids. Additionally an increased concentration of unsaturated fatty acids will undergo lipid and myoglobin oxidation more rapidly (Faustman et al., 2010) as carbonyl compounds accumulated during oxidation of unsaturated fatty acid and phospholipids and which have been correlated with myoglobin oxidation in meat (Rodas-Gonzalez et al., 2011).

Numerous studies have reported as day of display increased lipid oxidation also increased, which would be expected as air and oxygen catalyzes lipid oxidation in steaks (Park et al., 2008; Pietrasik, Dhanda, Shand, & Pegg, 2006). However, use of antimicrobial interventions

may affect the rate of lipid oxidation in meat products. Although PAA is known to have a high oxidizing potential, PAA treated ground beef had less lipid oxidation that untreated ground beef across days of display (Quilo et al., 2009). Similarly, LVA was reported to have prooxidant properties (Yi & Kim, 1982) however its use as an antimicrobial agent in meat products have not reported such effects on lipid oxidation. In uncooked pork and turkey sausage sodium levulinate did not affect lipid oxidation (Vasavada et al., 2003). The use of slightly acidic low concentration electrolyzed water (10 ppm and 50 ppm available chlorine concentration) was more preventative of lipid oxidation in fresh chicken breast than untreated chicken breast after 6 days of aerobic retail display (Rahman et al., 2012).

Conclusion

Translocation of pathogenic pathogens by blade tenderization has an inherently low risk. However, due do to consumer safety efforts antimicrobial interventions continue to be investigated to measure the efficacy of commonly used antimicrobials and to develop new technologies that can both reduce microbial load and improve shelf-life and quality characteristics in beef carcasses and cuts.

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CHAPTER 3

ANTIMICROBIAL INTERVENTIONS FOR BLADE TENDERIZED WHOLE MUSCLE, NON-INTACT BEEF BONELESS STRIP LOINS: EVALUATION OF SHELF-LIFE AND SENSORY CHARACTERISTICS[1]

[1] Kersey, R.J., Hung, Y.C., and A.M.Stelzleni. To be submitted to *Meat Science*

Abstract

Our objective was to determine the quality and shelf-life implications of two novel antimicrobials on blade tenderized (BT) beef strip loins (SL) for two packaging conditions. Strip loins (n=60) were assigned to one of five treatments prior to BT including; BT only (CON), electrolyzed oxidizing water (50 ppm Cl; EOW), peroxyacetic acid (200 ppm; PAA), 4.5% lactic acid (LA), or 0.2% levulinic acid + 0.02% sodium dodecyl sulfate (LVA+SDS). After BT, SL were halved into posterior and anterior ends and equally assigned to oxygen permeable (PVC) or vacuum packaging (VP) for retail display. Two medial steaks were selected for Warner-Bratzler shear force (WBSF) and sensory. Steak color and lipid oxidation were not affect by treatment ($P<0.05$). Spoilage bacteria were lower ($P<0.05$) for LA and PAA compared to CON, EOW, and LVA+SDS. No differences were detected in sensory characteristics. These results suggest that all treatments would be acceptable for use on BT SL without impacting quality and shelf-life.

Keywords: Beef, antimicrobial, blade tenderization

Introduction

Consumers consider tenderness one of the most influential factors that contribute to palatability and ultimately the overall perception of quality in beef products (Miller, Carr, Ramsey, Crockett, & Hoover, 2001; Savell et al., 1987). The assurance of acceptable tenderness is important not only to the consumer but also to retail, foodservice and restaurant industries. Processors commonly use commercial applications, such as blade tenderization (BT), to ensure a consistently tender product and therefore overall consumer satisfaction.

Blade tenderization works by physically disrupting the connective tissue and myofibrillar contractile system (Pietrasik & Shand, 2004). Closely placed blades penetrate the muscle tissue cutting the muscle fibers into shorter segments. Advantages of mechanical tenderization include (1) insuring acceptable tenderness of normal table-grade cuts, (2) equalizing tenderness in portioned items containing two or more muscles that differ in tenderness and (3) its effects are more uniform and more easily controlled that enzyme treatments (Miller, 1975). Blade tenderization has shown to improve mechanical tenderness (Hayward, Hunt, Kastner, & Kropf, 1980; Savell, Carpenter, & Smith, 1976) while also improving sensory panel tenderness and tenderness desirability (Davis, Smith, & Carpenter, 1977; Glover, Forrest, Johnson, Bramblett, & Judge, 1975; Jeremiah, Gibson, & Cunningham, 1999; Savell et al., 1976).

In 2003, three major outbreaks of *Escherichia coli* O157:H7 were associated with mechanically tenderized beef products (USDA-FSIS, 2003). Following these incidents USDA-FSIS responded by requiring establishments producing mechanically tenderized whole muscle, non-intact beef products to reassess their HACCP plans (USDA-FSIS, 2005). The mandate focused specifically on reassessing for the risk of *E.coli* O157:H7 contamination in mechanically

tenderized beef products and evaluation of the establishment's intervention processes to control this pathogen during processing.

The primary issue with mechanical tenderization is the potential of introducing pathogens into the interior of the meat. The tenderizing blades may act as a vehicle for surface pathogen translocation carried from the surface of the meat to the inherently sterile interior (Sporing, 1999). Whole muscle non-intact beef may increase risk due to the cooking method and degree of doneness preferred by consumers, which are often unaware of their procurement of whole muscle, non-intact products. It is well understood that ground beef, which is easily identifiable should be cooked to an internal temperature of 71.1°C according to USDA-FSIS (2013). However, consumers may prefer to cook whole muscle products to an internal temperature corresponding to rare (57°C) or medium rare (62°C) degree of doneness. The internal temperatures associated with rare and medium rare doneness may not reach the time, temperature combination that is lethal to *E.coli* O157:H7 and other pathogens (Gill, Moza, & Barbut, 2009; Luchansky, Phebus, Thippareddi, & Call, 2008; Luchansky et al., 2012).

Clean label antimicrobials that have the U.S. Food and Drug Administration's Generally Recognized as Safe status (GRAS) are of increased interest for meat processers due to the growing trend in consumer preference for clean-labeled products. It is important that antimicrobial interventions do not affect quality or consumer perception. Therefore, objective of this study was to evaluate the effect of two novel and two industry standard antimicrobial interventions on the shelf-life and quality characteristics of steaks from blade-tenderized beef strip loins.

Method and materials

Meat procurement and enhancement

Twenty boneless beef strip loins (SL), Institutional Meat Purchase Specifications (IMPS) 180 (*longissimus lumborum*) were purchased (Arko Veal company, Forest Park GA) 10 ± 1 d postmortem and transported (0 ± 2˚C) to the University of Georgia Meat Science Technology Center (Athens, GA) for each of the replications (n = 60). Upon arrival, subprimals were stored (2 ± 1˚C) for 4 d. On the fifth day after receiving, subprimals were unpackaged, trimmed of external fat (to 0.60-cm), weighed, and randomly assigned to one of five treatments including: BT only (CON), electrolyzed oxidizing water (50 ppm Cl; EOW), peroxyacetic acid (200 ppm; PAA), 4.5% lactic acid (LA), or 0.2% levulinic acid + 0.02% sodium dodecyl sulfate (LVA+SDS). Treatments were applied to the subprimals using an automated six-nozzle sanitizing cabinet (Chad Co., Olathe, KS). The automatic premixed spray treated all sides of the subprimal with nozzles located above and below the subprimal at a flow rate of 0.42 liters/nozzle•min^{-1} with a pressure of ~ 275.79 kPa. Following treatment application, subprimals continued on the conveyor belt (1.0 m/min) and made a single pass, lean side up, through a mechanical tenderizer (model TC700MC, Ross manufacturing, Midland, VA). The tenderizer blade head consisted of seven alternating angle rows containing 32 perpendicular blades (3-mm wide) and set 10-mm apart between rows and column. After tenderization, subprimals were halved perpendicular to the muscle orientation. Within each treatment, each loin anterior and posterior halves were alternated and assigned to aerobic or vacuum packaged (VP) retail display to simulate retail or processor fabrication and packaging, respectively. The first two steaks from the medial portion end of the VP halves were designated for Warner-Bratzler shear force (WBSF) and sensory analysis. Halves designated for PVC were vacuum packaged (30 to 50 mL

of O_2/m^2/24 h; 101,325 Pa; 23°C; B-620 series, Cryovac Sealed Air Corporation, Duncan, SC), boxed and held in cooler an (2 ± 1°C) for additional 4 d. Strip loin halves designated for VP had three additional steaks (2.54-cm thick) cut and which were randomly assigned to 0, 7, or 15 d of display. Steaks were individually vacuum packaged, boxed and held for 4 d (2 ± 1°C). The additional holding time was to simulate transportation and storage time before further processing by the end users such as restaurants, retailers, or supermarkets (Guelker et al., 2013). Between treatments the spray cabinet tank, spray cabinet, tenderizer cabinet, and tenderizer head were rinsed and flushed with hot potable water (50°C) for 2 min and allowed to cool to room temperature (3 ± 1°C) before proceeding with the next treatment.

Following the 4 d storage time, PVC designated shelf-life were cut into steaks (2.54-cm thick) and randomly assigned 0, 1, 3, 5, or 7 d of display. Steaks were placed on absorbent pads (Dri-Loc AC-40, Cryovac Sealed Air, Duncan, SC) in Styrofoam trays and wrapped with an oxygen permeable polyvinylchloride (PVC) overwrap (O_2 transmission=23,250 mL/m^2/24 h, 72 gage; Pro Pack Group, Oakland, NJ, USA). Previously VP steaks were removed from cold storage and set out for retail display. Steaks for simulated retail display were placed in open-topped coffin style display cases (3 ± 2°C, with one defrost cycle every 24-h; M1X-E, Hussmann, Bridgeton, MO) with 24-h continuous lighting between 1600- 2100 lux (Octron/ECO; 30000K; F032/830/ECO; Sylvania Company, Versailles, KY). Steaks were rotated within the case daily. Retail case temperature was monitored and recorded by continuous data loggers (TR-50U2, T&D Corp., Japan) placed at package height. Steaks designated for WBSF and sensory were frozen (-20˚C) until further analysis. This study was conducted over three replicates with samples and treatments equally represented in each replicate.

Preparation of treatments

Electrolyzed oxidizing water was produced using an electrolyzed oxidizing water generator (ROX-20TA-U, Hoshizaki Electric, Japan) by electrolysis of a NaCl solution according to Park, Hung, and Brackett (2002). Briefly, a 9.4 % salt solution (CAS # 7647-14-5, J.T Baker, Center Valley, PA) and deionized water were simultaneously pumped through the generator at approximately 10 volts and 18 amps producing acidic and alkaline water. Electrolyzed oxidizing alkaline water (pH >11, ORP +1100 mV) from the cathodic side, and acidic water (pH= 2.3- 2.7, ORP ~ -800 mV) from the anodic side and, which contained approximately 50-90 ppm free chlorine concentration were collected in separate sealable containers the morning of experiment. Immediately before use, alkaline and acidic water portions were mixed to produce approximately 57 liters with a pH of 6.2-6.5 and have an ORP between +800 to + 850 mV. Free chlorine concentration and ORP were determined using a pH meter and an ORP single junction ion electrode (model WD-35649-50, Oakion Instruments, Vernon Hills, IL). Free chlorine content was determined using the Hach DPD-FEAS (diethyl-*p*-phenylenediamine - 0.00564 *N*-ferrous ethylenediammonium sulfate) titration method (Hach CO., Loveland, CO). The sample was diluted by two 10-fold dilutions before 25-mL was transferred into a 50-mL beaker. Then, a DPD free chlorine powder pillow was added to the sample and was swirled to mix. The sample was titrated using a digital titrator (product # 1690001, Hach CO., Loveland CO,) using FEAS to a colorless endpoint. Free chlorine concentration was calculated from the number obtained following titration, inclusive of the dilution factor (1:100).

A 4.5% lactic acid solution was prepared by diluting 88% concentrate lactic acid (Birko Company, Henderson, CO) with water in the automated spray tank by bringing it to volume (56.78-L) with tap water.

A concentrated peroxyacetic acid solution was prepared by mixing PAA (Envirotec, Perason MP-2, Modesto, CA) with 1-L deionized water in a glass amber screw top bottle the morning of the experiment. Immediately before use, the PAA concentrate was added to approximately 32.85-L of tap water in spray tank, and then brought to volume (56.78-L).

The morning of experiment 113-mL of levulinic acid (LVA) (CAS #123-76-2, Acros Organics, New Jersey, U.S.) and 11.3-g of sodium dodecyl sulfate (SDS) (CAS #151-21-3, Sigma Aldrich, St. Louis, MO) mixed with approximately 4-L of tap water.. Immediately before use, LVA +SDS was added to approximately 26.5-L of tap water in the automatic spray tank and brought to volume (56.78-L) with of tap water to create 0.2% LVA + 0.02% SDS solution.

Retail display color

Objective color was measured on d 0, 1, 3, 5 and 7 for PVC and d 0, 7 and 15 for VP steaks as a repeated measure of d 7 and 15 unopened packaged steaks, respectively, with a Hunter-Lab MiniScan XE (Hunter Associates Laboratory, Reston, West Virginia) using illuminant A with a 10˚ viewing angle and standardized using a white tile, black tile and a saturated red tile working standard before each use. Three objective color readings were taken on each day and the average was recorded for *Commission Internationale de l'Eclairage* (CIE) L^*, a^*, and b^* color values. Hue angle ($\tan^{-1}(b^*/a^*)$), chroma ($((a^{*2} + b^{*2})^{0.5}$), and. ΔE ($[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$) were calculated. In addition, the visible spectrum from 630:580nm was

measured to estimate the redness due to either the oxymyoglobin or deoxymyoglobin fraction of the myoglobin pigment (AMSA, 2012).

For subjective color, a six member trained panel evaluated steak color on d 0, 1, 3, 5 and 7 for PVC and 0, 7 and 15 for VP. Regardless of day, panelists reported the color of d 7 or d 15 steaks for PVC and VP, respectively. Panelists evaluated PVC overwrapped steaks for overall color (8 = Extremely dark red, 7 = Dark red, 6 = Moderately dark red, 5 = Slightly dark cherry-red, 4 = Slightly bright cherry-red, 3 = Moderately bright cherry-red, 2 = Bright cherry-red, and 1 = Extremely bright cherry-red), worst point color **(**8 = Extremely dark red, 7 = Dark red, 6 = Moderately dark red, 5 = Slight dark cherry-red, 4 = Slight bright cherry-red, 3 = Moderately bright cherry-red, 2 = Bright cherry-red and 1 = Extremely bright cherry-red), and surface discoloration (8 = No discoloration, 7 = 1-5%, 6 = 6-10%, 5 = 11-25%, 4 = 26-50%, 3 = 51-75%, 2 = 76-90% and 1 = 91-100%) and VP steaks for overall color (5 = Bright purple-red, 4 = Dull purple-red, 3 = Slightly brownish-red, 2 = Moderately brownish-red, and 1 = Brown), amount of browning (6 = Dark brown, 5 = Brown, 4 = Brownish-grey, 3 = Grayish, 2 = Dull, and 1 = No evidence of browning), and surface discoloration (5 = Extreme, 4 = Moderate, 3 = Small, 2 = Slight, and 1 = None) according to AMSA (2012).

Psychrotrophic growth

Following the USDA-FSIS Microbiology Laboratory Guidebook 3.01 method (USDA-FSIS, 1998), total aerobic bacteria plate count (APC) was conducted on d 0,1,3,5 and 7 of PVC steaks. To sample each steak, a sterile 5 x 5 cm^2 metal template was used to create an impression on the steak before cutting. The 5 x 5 cm^2 surface area of the steak was aseptically excised to a depth of approximately 0.2-cm using a sterile scalpel handle and blade. Samples were placed in a

sterile stomacher bags and stomached for 2.5 min at 230 rpm. All utensils were sterilized by flame, rinsed with diluted chlorine and wiped free of residual chlorine. Serial dilutions were made for all samples using 9 ml of 0.1% peptone. One milliliter of each sample dilution was plated 3M Petrifilm (3M Manufacture, St. Paul, MN) following the manufacturer's instructions. Petrifilm plates were incubated at 35 ± 1˚C for 48 ± 2 h. Plates were counted and reported in CFU/g of meat.

Vacuum packaged steaks were evaluated for lactic acid producing bacteria on d 0, 7, and 15 (USDA-FSIS, 1998). To avoid cross contamination, the same aseptic procedures followed for APC were followed for enumerating lactic acid producing bacteria. Serial dilutions were made for all samples using 9 ml of 0.1% peptone. A combination of 0.5-mL from the sample dilution and 0.5-mL lactic acid bacteria MRS broth was plated on the Petrifilm following the manufacturer's instructions (3M Manufacture, ST. Paul, MN). Petrifilm plates were incubated anaerobically at 35˚ ± 1˚C for 48 ± 2h. Plates were counted and reported in CFU/g of meat.

Lipid oxidation

Thiobarbituric acid reactive substance analysis (TBARS) was adopted from Ahn et al. (1998). Briefly, steak samples were thawed (4 ± 1˚C) overnight, homogenized and a 5 g sample was placed in a 50 mL centrifuge tube with 15 mL deionized water and homogenized for 30 sec then centrifuged at 3077 x *g* (CR 312, Jouan INC., Winchester, VA) for 10 min. One milliliter of supernatant was transferred to a disposable glass test tube (13 x 100 mm). Fifty microliters of butylated hydroxyanisole (7.2%) and 2 mL of thiobarbituric acid/trichloroacetic acid were added to the homogenate. Samples were vortexed and incubated in a hot water bath (90˚C) for 15 min. After color development, the sample was placed in a cool water (20°C) bath for 10 min. The

samples were then centrifuged at 3077 x *g* (CR 312, Jouan INC., Winchester, VA USA) for 15 min and the supernatant was separated for spectrophotometric analysis (model DU 640; Beckman Coulter, Indianapolis, IN, USA;). The absorbance of the supernatant was measured at 531 nm and fitted against a standard curve. Lipid oxidation values were expressed as milligrams of malonaldehyde (MDA) per kilogram of meat.

Sensory analysis

Steaks for sensory analysis were thawed (4 ± 1˚C) for 18 h. Steaks were cooked to an internal temperature of 71˚C on preheated clamshell grills (George Formen, Saltotn Inc., Miramar, FL) and were monitored by a Digi-Sense 12-Channel Scanning thermometer with copper-constantan thermocouples inserted into the geometric center of each steak. After the steaks are cooked they were served in warmed yogurt makers (Euro Cuisine, Inc., Los Angeles, CA) to an eight member trained sensory panel according to AMSA (1995). Panelists evaluated two cubes per steak (1.27-cm^3) and evaluated seven steaks per session, with two sessions per day. The panelists received samples from each treatment randomly at each session. The loaded yogurt makers were passed through a breadbasket door from the sensory kitchen to the sensory analysis room, equipped with negative pressure ventilation and eight individual booths with red lighting to minimize panelist influence and mask differences in cook steak color. Panelists evaluated each sample for initial tenderness (8 = Extremely tender, 7 = Very tender, 6 = Moderately tender, 5 = Slightly tender, 4 = Slightly tough, 3 = Moderately tough, 2 = Very tough, and 1 = Extremely tough), sustained tenderness (8 = Extremely juicy, 7 = Very juicy, 6 = Moderately juicy, 5 = Slightly juicy, 4 = Slightly dry, 3 = Moderately dry, 2 = Very dry, and 1 = Extremely dry), beef intensity flavor (8 = Extremely intense, 7 = Very intense, 6 = Moderately intense, 5 = Slightly intense, 4 = Slightly bland, 3 = Moderately bland, 2 = Very bland, and 1 =

Extremely bland), overall juiciness (8 = Extremely juicy, 7 = Very juicy, 6 = Moderately juicy, 5 = Slightly juicy, 4 = Slightly dry, 3 = Moderately dry, 2 = Very dry, and 1 = Extremely dry), and off-flavor (6 = Extreme off-flavor, 5 = Very strong off-flavor, 4 = Moderate off-flavor, 3 = Slight off-flavor, 2 = Threshold off-flavor, and 1 = None detected).

Warner-Bratzler shear force

Steaks that were previously designated and frozen for WBSF were thawed and cooked following procedures used for sensory. Cooked were cooled to room temperature, covered in plastic wrap, and chilled overnight (4 ± 1˚C). Six 1.27-cm diameter cores were removed parallel to the longitudinal orientation of the muscle fibers of each steak using a hand held coring device. Cores were sheared perpendicular to the longitudinal orientation of the muscle fibers using a Universal Testing Machine (Instron Dual Column Model 3365, Instron corp., Norwood, MA) equipped with a Warner-Bratzler shear head with a 51 kgf load cell with a cross head speed of 25 cm/min. The peak shear forces (kgf) for each core was recorded (Bluehill software, Instron Corp.) and averaged for the shear force value of each steak.

Statistical analysis

Data were analyzed using Proc Mixed of SAS (version 9.3) as a completely randomized split-plot were subprimal was the whole-plot and steak within subprimal as the sub-plot. Subprimal identification within replication by treatment was included as the random variable. Subprimal was considered the experimental unit and steak was considered the observational unit. Main effects and all treatment by day interactions were tested when applicable. Differences were considered significant at $\alpha \leq 0.05$.

Results and discussion

Simulated retail display color

For PVC steaks, there was not treatment by day interaction ($P \geq 0.66$) thus main effects of treatment and day are discussed. As day of display increased L^* and b^*, and chroma values decreased ($P < 0.05$) indicating steaks became darker and less vivid (Table 3.1). Additionally, a^*, and 630:580 decreased and hue angle increased ($P < 0.05$) showing that steaks became less red as in retail display proceeded. Delta E increased ($P < 0.05$) as time in retail display increased which is related to a greater change in color as time progressed. Although d 1 showed a numerically greater difference from d 0 than did d 3 or 5, this difference was not significant and may be related to the greater bloom that occurred after 1 d of display. When samples are initially placed in aerobic packaging and the conversion from deoxymyoglobin to oxymyoglobin starts the initial oxymyoglobin layer is shallow with subsequent layers of metmyoglobin and deoxymyoglobin (Mancini & Hunt, 2005). Therefore, when objective color is recorded on d 0 samples the values may indicate a darker (as noted by the numerically lower L^* in the present study) color. As the samples continued to bloom, the oxymyoglobin layer would potentially become thicker (Mancini & Hunt, 2005) after a full day of exposure allowing for the greater numeric color difference noted between d 0 and 1. After further exposure and continued oxidation of myoglobin the formation of metmyoglobin could increase, creating a color that is objectively more similar to d 0 after 3 d of display than what was observed on d 1. Furthermore, by d 3 surface oxymyoglobin begins to fad while the subsequent layer of metmyoglobin become thicker and become closer to the surface of the meat. This contrast of layers contribute to the lighter or increased L^* values seen on d 3. Changes over day of display were similar to trends found by King, Shackelford, Kalchayanand, and Wheeler (2012) where similar trends of a^* and

hue angle had slow changes early in display but increased as time increased. Additionally, small changes in *L** occurred more rapidly early in display compared to end of display period while *b** and chroma show delay at the beginning of display followed by a noticeable decline.

After 15 d of simulated display VP steaks were similar ($P > 0.05$) in lightness (*L**) compared to d 0 and 7 (Table 3.2), Steaks were also similar ($P > 0.05$) between d 0 and 15 for *a** and hue, however on d 7 steaks were less red (as indicated by *a** and hue angle) than on d 0 or 15. The cause of this change is not readily understood as these steaks had been in VP for a total of 11 d by this point. After 11 d it would be expected that changes in color would have stabilized. Nanke, Sebranek, and Olson (1998) reported that after a 12 wk study investigating objective color for vacuum packaged steaks that received different irradiation dose levels *L**, *a**, *b** trends were similar to those found in the current study. Additionally, Nanke et al. (1998) reported *a** decreased similarly for all irradiation doses across days of display. Furthermore, the hue angle reported by Nanke et al. (1998) were unchanged by irradiation doses and was comparable to the current study between 39.1 and 36.04.

For PVC (Table 3.3) and VP (Table 3.4) steaks there was no difference in objective color between treatments ($P > 0.05$). Novel antimicrobial findings were similar to those reported by Fabrizio and Cutter (2005) where the use of acidic and basic electrolyzed water on ready-to-eat meat was expected to have a "bleaching effect" due to the high ORP characteristic of electrolyzed water, however, it was reported that there was no significant differences for Hunter *L**, *a**, or *b** values after 7 d. On the contrary, Stelzleni, Ponrajan, and Harrison (2013) reported that ground beef patties treated with 1% levulinic acid plus 0.1% sodium dodecyl sulfate were less red after d 1 and less red and less vivid on d 3 when compared to untreated beef patties.

Subjective color scores for PVC steaks (Figure 3.1) had a treatment by day interaction for worst point color ($P < 0.01$) and surface discoloration ($P < 0.01$). However, only day of display was different for overall color, with all samples becoming darker ($P < 0.01$) in their red color as time on display progressed. The changes in overall color that were observed by the panelists correspond to the same trends noted for a^* and chroma. Worst point color became darker red ($P < 0.01$) for all treatments as days of display increased. All treatments were similar to each other between d 0 and 1, however, by d 3 worst point color became darker ($P < 0.05$) and was different for all respective treatments than on the previous days. Furthermore, after 5 d LVA+SDS had a darker ($P < 0.05$) worst point color score than LA and CON and 7 d LA had the lowest ($P < 0.05$) worst point color score and was brighter red than all other treatments. As expected, percent surface discoloration increased as days of display increased ($P < 0.01$). Percentage surface discoloration differences became noticeable after 3 d as EOW had less ($P < 0.05$) surface discoloration than PAA but was similar ($P > 0.05$) to all other treatments. After 5 d in display, all treatments were similar ($P > 0.05$) to each other and remained similar after 7 d with 76-90 % of the steak surface being discolored. Similar findings were reported by Jimenez-Villarreal, Pohlman, Johnson, Brown, and Baublits (2003) where peroxyacetic acid treated ground beef had extensive discoloration by 7 d.

Subjective color scores for VP steaks are shown in Figure 3.2. There was not treatment by day interaction ($P = 0.63$), treatment ($P = 0.36$), or day of display ($P = 0.06$) effect for overall color. Overall color was similar across days of display. There was a treatment by day interaction ($P < 0.01$) for amount of browning with LA and CON increasing for amount of browning as days on display increased, whereas EOW and LVA+SDS remained the same from d 0 to 7 ($P > 0.05$) but increased by d 15 ($P < 0.05$). Peroxyacetic acid treated steaks were not different ($P < 0.05$) in

amount of browning across days of display. There was not a treatment by day of retail display interaction for discoloration ($P = 0.06$) but total amount of surface discoloration increased as days of display increased for all treatments. Although statistically different, mean scores from panelist for discoloration after 15 d were still rated between no discoloration and slight discoloration.

Aerobic plate counts and lactic acid producing bacteria

There was not a treatment by day interaction for psychrotrophic growth in PVC steaks ($P = 0.16$) or VP steak ($P = 0.93$), thus main effects of treatment and day are presented. Psychrotrophic growth increased as days of display increased for PVC ($P < 0.05$; Table 3.5) and VP ($P < 0.05$; Table 3.7) steaks. An increased number of psychrotrophic bacteria can cause a decrease in retail shelf-life (Lambert, Smith, & Dodds, 1991). However, by the end of simulated retail display, psychrotrophic growth for all treatments was still below the commonly held spoilage level of 10^6 CFU/g of meat, which is the normal indicator of sensory changes indicative of spoilage (Bruhn et al., 2004; Gill & Gill, 2005). King et al. (2012) found that APC increased during display, however, LAB did not change over an 11 d display period. In both PVC and VP steaks, LA and PAA ($P < 0.05$) hindered psychrotrophic organism growth more ($P < 0.05$) than EOW and LVA +SDS, which were similar ($P > 0.05$) to CON. The lack of effectiveness for LVA+SDS and EOW were surprising. Chen, Zhao, and Doyle (2014) reported LVA+SDS being and effective antimicrobial against *Listeria monocytogenes, Salmonella* and *E. coli* O157:H7 at concentrations of 1% levulinic acid plus 0.1% sodium dodecyl sulfate, and Park, Hung, and Chung (2004) reported EOW being an effective antimicrobial against *Campylobacter jejuni*. However, Stelzleni, Ponrajan, and Harrison (2013) reported similar results where 1% Levulinic acid plus 0.1% sodium dodecyl sulfate treated beef patties were found to have similar

psychrotrophic organism growth when compared to untreated beef patties. The lower level of levulinic acid and sodium dodecyl sulfate were selected for the current project because Zhao et al. (2014) found that it was effective against *E. coli* O157:H7, O26:H11, and O11:NM at 0.5% levulinic acid plus 0.05% sodium dodecyl sulfate and because Stelzleni et al. (2013) found that at the concentration of 1.0% levulinic acid plus 0.1% sodium dodecyl sulfate there were negative color consequences. Additionally, 4% lactic acid sprayed on cold beef carcasses was found to significantly reduce overall microbial growth (Castillo et al., 2001; Gill & Badoni, 2004). However, the use of peroxyacetic acid on chilled beef carcasses had little effect on aerobes, coliforms, or *E. coli* (Gill & Badoni, 2004; King et al., 2005).

Lipid oxidation

There was not a treatment by day interaction for lipid oxidation for PVC steaks (P = 0.76) or VP steaks (P = 0.06). Lipid oxidation increased as days of display increased (Table 3.5) in PVC steaks ($P < 0.0001$); however, there were no differences among treatments (P = 0.81; Table 3.6). For VP steaks there were no differences in lipid oxidation among days of display (P = 0.27; Table 3.7) or between treatments (P = 0.39; Table 3.8). Although, hydroxide and hypochlorous acid are found in EOW and are considered to have antioxidant effects (Rahman, Park, Song, Al-Harbi, & Oh, 2012), the use of EOW in this study did not have significant impact on PVC or VP treated steaks. Regardless, by day 7 of PVC steaks and d 15 of VP steaks lipid oxidation levels of MDA were below the threshold of sensory acceptability of 2 mg/kg of MDA (Campo et al., 2006; Jayasingh, Cornforth, Brennand, Carpenter, & Whittier, 2002).

Sensory characteristics

Panelists were unable to detect differences in initial and sustained tenderness, juiciness, beef intensity flavor, or off-flavors between treatments (Table 3.9). However, PAA had a greater percent of cook loss ($P < 0.05$) than CON, LA and LVA+SDS but was similar ($P > 0.05$) to that of EOW. Warner-Bratzler shear force values were similar ($P > 0.05$) among CON, EOW, PAA, and LVA+SDS. However, LA had the greatest WBSF value (3.93 kgf) but was similar to EOW (3.28 kgf). Even though there was a statistical difference found, it is not understood what would cause this difference. Furthermore, all treatments were well under the USDA Tender Claim of 4.4 kgf (USDA-AMS, 2012). This is expected as steaks were subjected to blade tenderization. Savell et al. (1976) reported that blade tenderization (1 pass) decreased shear force of lower quality grade *gluteus medius*, *semimembranosus*, and *longissimus* muscles by approximately 1 kgf. However, blade tenderization offers no additional benefits to meat that is of already acceptable tenderness, meaning a cut that would be considered USDA Tender cannot be mechanically tenderized to become USDA Very Tender. Smith, Seideman, and Carpenter (1979) found that the effects of tenderization on shear or sensory were insignificant when used with meat of higher quality, while others have reported that tenderization was favorable for use with lower quality grade and tougher muscles (Davis, Huffman, & Cordray, 1975; Tatum, Smith, & Carpenter, 1978; Wheeler, Savell, Cross, Lunt, & Smith, 1990).

Conclusion

Commonly used antimicrobials, LA and PAA hindered psychrotrophic growth more than the novel antimicrobials or the non-treated subprimals in the present study. Antimicrobial treatment prior to blade tenderization did not influence objective color or have a significant impact on quality traits, as measured by shear force and sensory analysis. These results suggest

that along with LA and PAA the two novel antimicrobial interventions, EOW and LVA+SDS, would be acceptable for use on beef strip loin subprimals subjected to blade tenderization without detrimental effects to quality and shelf-life at the present concentrations. Further investigation regarding increased levels of levulinic acid plus sodium dodecyl sulfate are warranted based on previous reports about its antimicrobial properties.

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Table 3.1
Least squares means and standard errors for objective color main effects by day for PVC overwrapped steaks from beef strip loins subjected to antimicrobial intervention and blade tenderization

	Day of Display[1]					
Trait	0	1	3	5	7	SEM[2]
*L**[3]	38.30[b]	40.28[b]	49.90[a]	36.93[b]	35.29[b]	2.13
*a**[3]	29.07[a]	27.99[a]	25.72[b]	14.21[d]	19.67[c]	0.67
*b**[3]	23.35[a]	23.39[a]	24.03[a]	17.94[b]	15.59[c]	0.44
Hue[3]	38.82[c]	40.25[c]	43.85[b]	42.89[b]	48.89[a]	0.73
Chroma[3]	37.34[ab]	35.55[a]	35.32[b]	26.76[c]	21.26[d]	0.72
630/580 nm[3]	7.99[a]	6.73[b]	5.44[c]	3.84[d]	2.05[e]	0.20
Δ*E* [3]	-	19.72[b]	17.35[b]	18.79[b]	25.37[a]	1.45

[abcde] Least squares means within a row with different superscripts are different ($P < 0.05$).
[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]*L** = 0 = black to 100 = white; *a** = measurement of green to red on color spectrum, high values indicate more red; *b** = measurement of yellow to blue on color spectrum, higher values indicate more yellow; Hue = lower values indicate redder color; Chroma = higher value indicates more red saturation.

Table 3.2
Least squares means and standard errors for objective color main effects by day for vacuum packaged steaks from beef strip loins subjected to antimicrobial intervention and blade tenderization

	Day of Display[1]			
Trait	0	7	15	SEM[2]
*L**[3]	36.48	37.13	37.31	0.76
*a**[3]	25.79[a]	24.47[b]	25.25[a]	0.24
*b**[3]	18.74[a]	19.45[a]	17.43[b]	0.29
Hue[3]	36.04[b]	38.50[a]	34.54[b]	0.61
Chroma[3]	32.26[a]	31.28[b]	30.82[c]	0.18

[abc] Least squares means within row with different superscripts are different ($P < 0.05$).
[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]*L** = 0 = black to 100 = white; *a** = measurement of green to red on color spectrum, high values indicate more red; *b** = measurement of yellow to blue on color spectrum, higher values indicate more yellow; Hue = lower values indicate redder color; Chroma = higher value indicates more red saturation.

Table 3.3
Least squares means and standard errors for objective color main effects by treatment for PVC overwrapped steaks from beef strip loins subjected to antimicrobial intervention and blade tenderization

	Treatment[1]					
Trait	CON	EOW	PAA	LA	LVA+SDS	SEM[2]
L^*[3]	40.92	40.55	40.29	40.76	38.21	2.64
a^*[3]	24.13	23.17	22.96	23.16	23.23	0.75
b^*[3]	21.29	20.91	20.83	20.72	20.55	0.56
Hue[3]	38.82	40.25	43.85	42.89	48.24	0.73
Chroma[3]	32.34	31.33	31.20	31.21	31.15	0.86
630/580 nm[3]	5.57	4.99	4.99	5.36	5.12	0.34
ΔE [3]	18.91	20.33	20.74	22.37	19.18	1.90

[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]L^* = 0 = black to 100 = white; a^* = measurement of green to red on color spectrum, high values indicate more red; b^* = measurement of yellow to blue on color spectrum, higher values indicate more yellow; Hue = lower values indicate redder color; Chroma = higher value indicates more red saturation.

Table 3.4
Least squares means and standard errors for objective color main effects by treatment for vacuum packaged steaks from beef strip loins subjected to antimicrobial intervention and blade tenderization

	Treatment[1]					
Trait	CON	EOW	PAA	LA	LVA+SDS	SEM[2]
*L**[3]	37.90	36.31	37.64	36.81	36.20	1.14
*a**[3]	24.98	25.16	25.19	25.12	25.38	0.31
*b**[3]	18.58	18.31	18.63	18.58	18.60	0.38
Hue[3]	36.61	36.04	36.47	36.45	36.22	0.78
Chroma[3]	31.29	31.34	31.54	31.42	31.66	0.30

[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]*L** = 0 = black to 100 = white; *a** = measurement of green to red on color spectrum, high values indicate more red; *b** = measurement of yellow to blue on color spectrum, higher values indicate more yellow; Hue = lower values indicate redder color; Chroma = higher value indicates more red saturation.

Table 3.5
Least squares means and standard errors for aerobic plate count and thiobarbituric acid reactive substance analysis (TBARS) main effects by day for PVC overwrapped steaks from beef strip loins subjected to antimicrobial intervention and blade tenderization

	Day of Display[1]					
Trait	0	1	3	5	7	SEM[2]
Aerobic Plate Count[3]	2.58[e]	2.87[d]	4.00[c]	4.45[b]	5.84[a]	0.09
TBARS[4]	0.19[e]	0.24[d]	0.41[c]	0.68[b]	0.92[a]	0.05

[abcde] Least squares means within row with different superscripts are different ($P < 0.05$).
[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3] Values are reported as CFU/g.
[4] Values are reported in mg MDA/kg meat.

Table 3.6
Least squares means for lactic acid bacteria and thiobarbituric acid reactive substance analysis (TBARS) main effects by day for vacuum packaged steaks from beef strip loins subjected to antimicrobial intervention and blade tenderization

	Day of Display[1]			
Trait	0	7	15	SEM[2]
Lactic Acid Bacteria[3]	2.39[c]	4.97[b]	5.75[a]	0.11
TBARS[4]	0.35	0.35	0.36	0.03

[abc] Least squares means within row with different superscripts are different ($P < 0.05$).
[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]Values are reported as CFU/g.
[4]Values are reported in mg MDA/kg meat.

Table 3.7
Least squares means and standard errors for aerobic plate count and thiobarbituric acid reactive substance analysis (TBARS) main effects by treatment for PVC overwrapped steaks from beef strip loins subjected to antimicrobial intervention and blade tenderization

	Treatment[1]					
Trait	CON	EOW	PAA	LA	LVA+SDS	SEM[4]
Aerobic Plate Count[3]	4.19[a]	4.20[a]	3.65[b]	3.64[b]	4.07[a]	0.11
TBARS[4]	0.43	0.47	0.52	0.47	0.54	0.07

[ab] Least squares means within row with different superscripts are different ($P < 0.05$).
[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]Values are reported as CFU/g.
[4]Values are reported in mg MDA/kg meat.

Table 3.8
Least squares means and standard errors for lactic acid bacteria and thiobarbituric acid reactive substance analysis (TBARS) main effects by treatment for vacuum package steaks from beef strip loins subjected to antimicrobial intervention and blade tenderization

	Treatment[1]					
Trait	CON	EOW	PAA	LA	LVA+SDS	SEM[2]
Lactic Acid Bacteria[3]	4.72[a]	4.64[a]	4.12[b]	4.01[b]	4.32[a]	0.18
TBARS[4]	0.36	0.27	0.33	0.33	0.42	0.52

[ab] Least squares means within row with different superscripts are different ($P < 0.05$).
[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]Values are reported as CFU/g.
[4]Values are reported in mg MDA/kg meat.

Table 3.9
Least squares means and standard error main effects for sensory and cooking characteristics for steaks from beef top sirloin subjected to antimicrobial intervention and blade tenderization

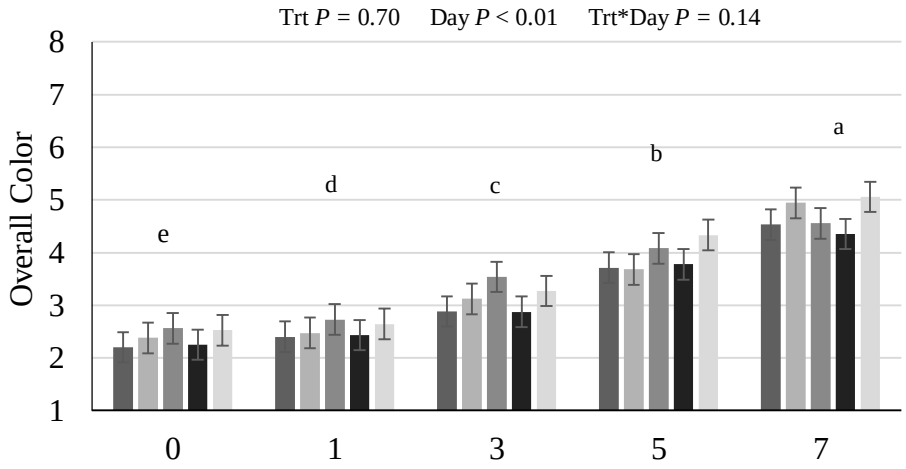
	Treatments[1]					
Trait	CON	EOW	PAA	LA	LVA+SDS	SEM[2]
Sensory Characteristics						
Initial Tenderness[3]	5.82	5.64	5.04	5.17	5.96	0.39
Sustained Tenderness[3]	5.62	5.47	4.84	5.02	5.64	0.40
Beef Flavor Intensity[4]	5.12	5.00	5.26	5.19	5.29	0.25
Juiciness[5]	4.77	4.67	4.35	4.62	4.77	0.37
Off-Flavor[6]	1.09	1.12	1.12	1.10	1.10	0.07
WBSF, kgf[7]	2.85 [b]	3.28 [ab]	3.15 [b]	3.93 [a]	2.98 [b]	0.27
Cooking Characteristics						
Thaw Loss, %	2.80	2.39	2.96	2.23	2.36	0.35
Cook Loss, %	12.57[b]	15.62[ab]	16.96[a]	14.12[b]	13.46[b]	0.97

[ab] Least squares means within a row with different superscripts are different ($P < 0.05$).
[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]8 = extremely tender, 7 = very tender, 6 =moderately tender, 5 = slightly tender, 4 = slightly tough, 3 = moderately tough, 2 = very tough, and 1 = extremely tough.
[4]8 = extremely intense, 7 = very intense, 6 = moderately intense, 5 = slightly intense, 4 = slightly bland, 3 = moderately bland, 2 = very bland and 1 = extremely bland.
[5]8 = extremely juicy, 7 = very juicy, 6 = moderately juicy, 5 = slightly juicy, 4 = slightly dry, 3 = moderately dry, 2 = very dry, and 1 = extremely dry.
[6]6 = extreme off-flavor, 5 = very strong off-flavor, 4 = moderate off-flavor, 3 = slight off-flavor, 2 = threshold off-flavor, and 1 = non-detected.
[7]Warner-Bratzler Shear Force.

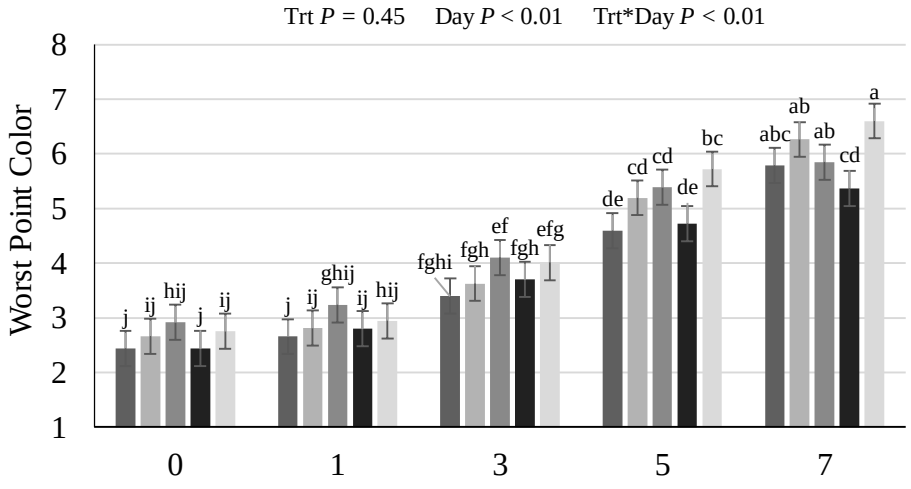
Figure 3.1

Least square means and standard errors for subjective color for PVC overwrapped steaks from beef loin subprimals subjected to antimicrobial intervention and blade tenderization. A) Overall appearance (8 = Extremely dark red, 7 = Dark red, 6 = Moderately dark red, 5 = Slightly dark cherry-red, 4 = Slightly bright cherry-red, 3 = Moderately bright cherry-red, 2 = Bright cherry-red, and 1 = Extremely bright cherry-red); B) Worst Point Color measures a single or combined area of at least 2-cm^2 used to evaluate average color; (8 = Extremely dark red, 7 = Dark red, 6 = Moderately dark red, 5 = Slight dark cherry-red, 4 = Slight bright cherry-red, 3 = Moderately bright cherry-red, 2 = Bright cherry-red and 1 = Extremely bright cherry-red); and C) Surface discoloration (8 = No discoloration, 7 = 1-5%, 6 = 6-10%, 5 = 11-25%, 4 = 26-50%, 3 = 51-75%, 2 = 76-90% and 1 = 91-100%). Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm) and LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate. Least squares means with different superscripts are different ($P < 0.05$).

A.


Trt P = 0.70 Day P < 0.01 Trt*Day P = 0.14
Overall Color
e
d
c
b
a
0
1
3
5
7

B.


Trt P = 0.45 Day P < 0.01 Trt*Day P < 0.01
Worst Point Color
0
1
3
5
7

C.

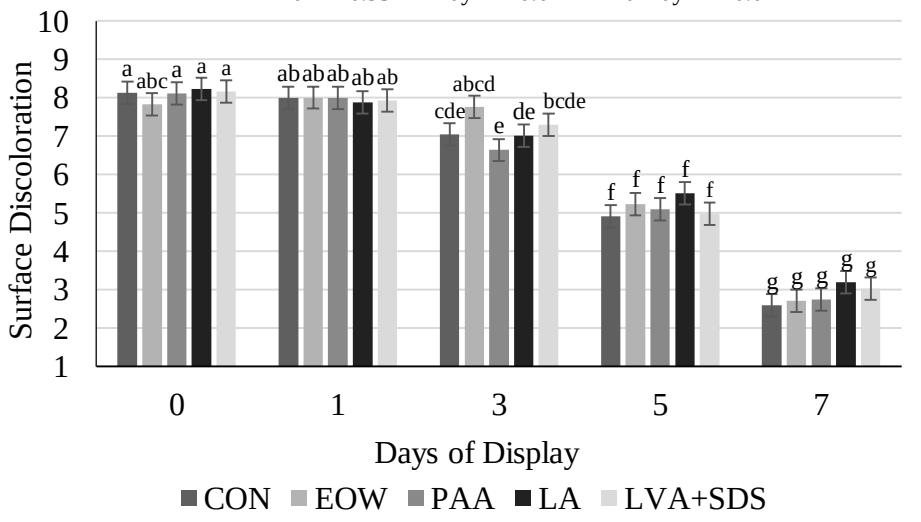
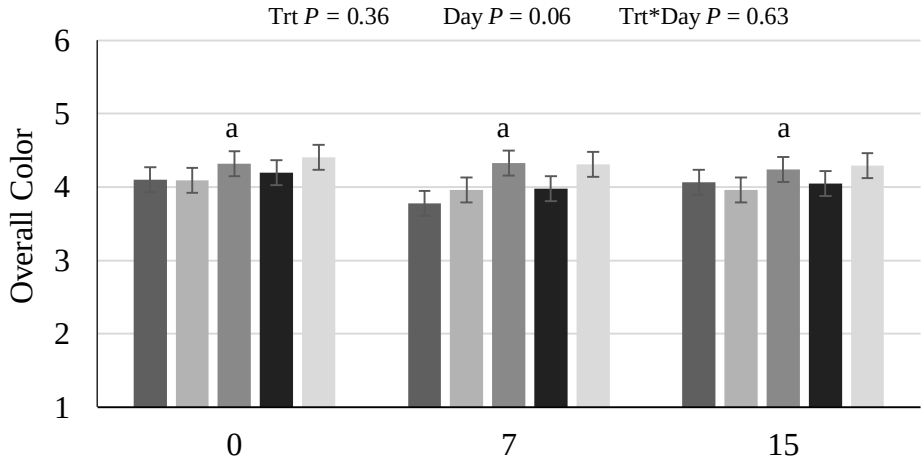

Trt P = 0.95 Day P < 0.01 Trt*Day P < 0.01
Surface Discoloration
0
1
3
5
7
Days of Display
CON EOW PAA LA LVA+SDS

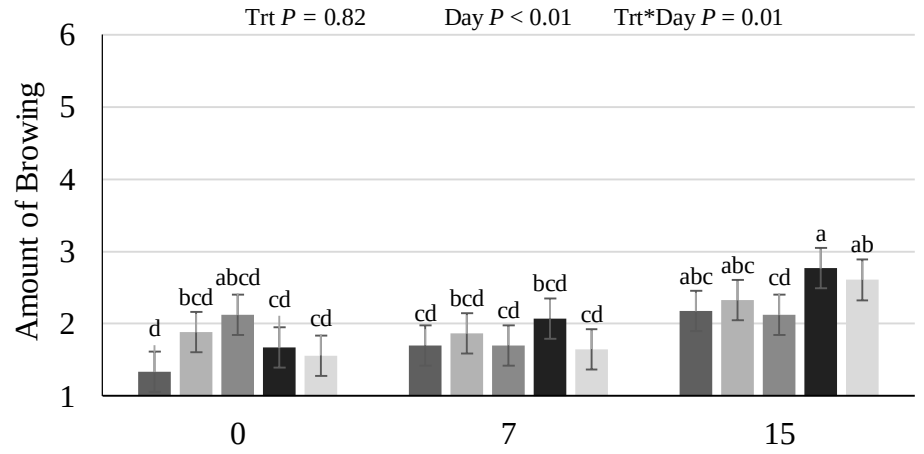
Figure 3.2

Least square means and standard error for subjective color for vacuum packaged steaks from beef strip loins subjected to antimicrobial intervention and blade tenderization. A) Overall color (5 = Bright Purple-red, 4 = Dull Purple-red, 3 = Slightly Brownish-red, 2 = Moderately Brownish-red, and 1 = Brown); B) Amount of Browning (6 = Dark brown, 5 = Brown, 4 = Brownish-grey, 3 = Grayish, 2 = Dull, and 1 = No evidence of browning); and C) Discoloration (5 = Extreme, 4 = Moderate, 3 = Small, 2 = Slight, and 1 = None). Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm) and LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate. Least squares means with different superscripts are different ($P < 0.05$).

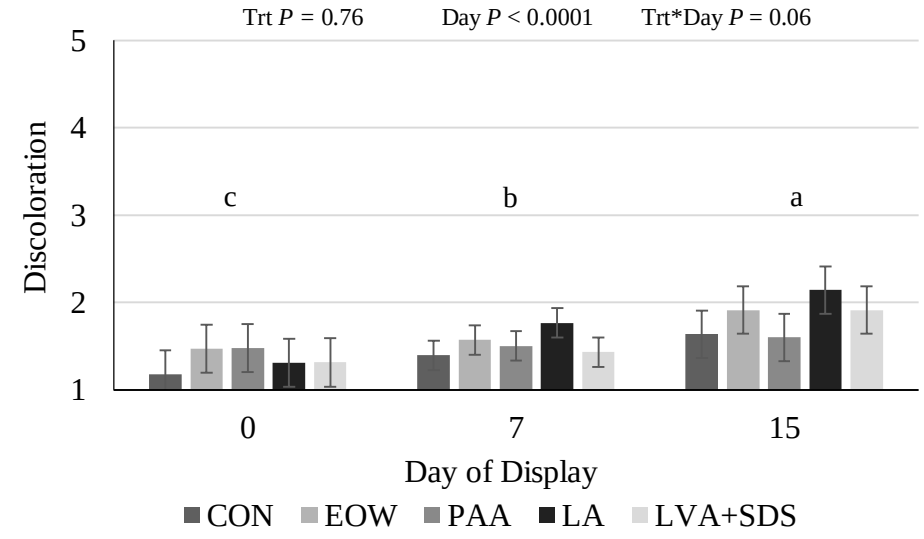
A.


Trt P = 0.36
Day P = 0.06
Trt*Day P = 0.63
Overall Color
6
5
4
3
2
1
a
a
a
0
7
15

B.


Trt P = 0.82
Day P < 0.01
Trt*Day P = 0.01
Amount of Browing
6
5
4
3
2
1
d
bcd
abcd
cd
cd
cd
bcd
cd
bcd
cd
abc
abc
cd
a
ab
0
7
15

C.


Trt P = 0.76
Day P < 0.0001
Trt*Day P = 0.06
Discoloration
5
4
3
2
1
c
b
a
0
7
15
Day of Display
CON
EOW
PAA
LA
LVA+SDS

CHAPTER 4

ANTIMICROBIAL INTERVENTIONS FOR BLADE TENDERIZED WHOLE MUSCLE NON-INTACT BEEF BONELESS TOP SIRLOIN: EVALUATION OF SHELF-LIFE AND SENSORY CHARACTERISTICS[1]

[1]Kersey, R.J., Hung, Y.C., and A.M.Stelzleni. To be submitted to *Meat Science*

Abstract

Quality and sensory characteristics of steaks from blade tenderized (BT) beef top sirloins subjected to antimicrobial interventions were investigated. Beef top sirloins (n=60) were assigned to one of five treatments: Electrolyzed oxidizing water (EOW), 0.2% levulinic acid + 0.02% sodium dodecyl sulfate (LVA+SDS), peroxyacetic acid (200 ppm) (PAA), 4.5% lactic acid (LA) and BT only (CON). Steaks were PVC overwrapped or vacuum packaged (VP) for shelf-life retail display. Two steaks were selected for Warner-Bratzler shear force (WBSF) and sensory. All treatments were similar ($P > 0.05$) for VP and PVC objective color, lipid oxidation, and psychrotrophic growth. Panelists detected differences in subjective color for VP and PVC steaks and sensory characteristics. Warner-Bratzler shear force values were similar ($P > 0.05$) across all treatments. Results suggest that LVA+SDS and EOW are acceptable as current industry standard antimicrobials for use on beef subprimals subjected to blade tenderization without detrimental effects to quality and shelf-life.

Keywords: Beef, Antimicrobial, Blade Tenderization

Introduction

Tenderness is one of the most influential factors that contribute to palatability and ultimately the overall perception of quality in beef products (Miller, Carr, Ramsey, Crockett, & Hoover, 2001; Savell et al., 1987). The assurance of acceptable tenderness is important not only to consumers but also to retail, foodservice, and restaurant industries. In the United States, beef top sirloin steaks are among the top 10 most popular steaks consumers purchase at retail, while also being one of the most cost efficient steaks served in restaurants (Lee, Apple, Yancey, Sawyer, & Johnson, 2008; NCBA, 2005). Consumers gravitate to the beef top sirloin steak because of its leanness and low price, although it has inherent inconsistencies in tenderness and color stability (Lee et al., 2008; O'Keeffe & Hood, 1982). To combat the tenderness inconsistencies in the top sirloin, processors and purveyors use commercial applications such as blade tenderization to create consistently tender products.

In 2003 North American Meat Processors and Food Safety Systems, LLC surveyed 200 processers and found that approximately 102 processors surveyed used blade tenderization, predominantly on beef rounds, chucks, strip loins and top sirloin butts (NCBA, 2005). Blade tenderization works by physically disrupting the connective tissue and myofibrillar contractile system by using closely placed blades that penetrate the muscle tissue cutting the muscle fibers into shorter segments. The advantages of blade tenderization are insurance of acceptable tenderness of normal table-grade cuts, equalized tenderness in portioned items containing two or more muscles that differed in tenderness, and its effects are more uniform and more easily controlled than enzyme treatments (Miller, 1975). Blade tenderization has shown to improve tenderness (Glover, Forrest, Johnson, Bramblett, & Judge, 1975; Hayward, Hunt, Kastner, & Kropf, 1980; Savell, Carpenter, & Smith, 1976) and confirmed by sensory panelist that have

noted improved tenderness and tenderness desirability (Davis, Smith, & Carpenter, 1977; Glover et al., 1975; Jeremiah, Gibson, & Cunningham, 1999; Savell et al., 1976).

Despite its benefits, mechanical tenderization may introduce pathogens into the interior of the meat as tenderizing blades act as a vehicle for surface pathogen to be carried to the inherently sterile interior. Whole muscle non-intact beef products can pose a health risk due to the cooking method and degree of doneness preferred by consumers, who are often unaware of their procurement of whole muscle, non-intact products. Unlike ground beef products usually cooked to an internal temperature of 71.1˚C or medium according to USDA (USDA-FSIS, 2013), some consumers prefer to cook whole muscle products to an internal temperature corresponding to a rare or medium rare degree of doneness. The internal temperatures of rare (57°C) and medium rare (62°C) doneness may not reach temperatures lethal to *Escherichia coli* O157:H7 and other pathogens (Gill, Moza, & Barbut, 2009; Luchansky, Phebus, Thippareddi, & Call, 2008; Luchansky et al., 2012).

Antimicrobial interventions that are clean label products and have U.S. Food and Drug Administration's Generally Recognized as Safe status are of interest to packers and processers due to the growing trend in consumer preference for clean-labeled products. Antimicrobial interventions should not affect quality or consumer perception and therefore, their effects on quality and sensory characteristics of meat need to be understood. The objective of this study was to evaluate the effect of two novel and two industry standard antimicrobial interventions on the quality and sensory characteristics of steaks from blade tenderized beef top sirloins.

Methods and materials

Meat procurement and enhancement

Sixty boneless beef top sirloin butts, Institutional Meat Purchase Specifications (IMPS) 184 (*gluteus medias*) were purchased (Arko Veal company, Forest Park GA) across three replicates, approximately 10 ± 1 d postmortem and transported (0 ± 2˚C) to the University of Georgia Meat Science Technology Center (Athens, GA). Upon arrival, subprimals were stored (2 ± 1˚C) for 4 d. On the fifth day subprimals were unpacked, trimmed of all external fat and had the sirloin cap (biceps femoris) removed before being weighed and equally assigned to one of five treatments: BT only (CON), electrolyzed oxidizing water (50 ppm Cl; EOW), peroxyacetic acid (200 ppm; PAA), 4.5% lactic acid (LA), or 0.2% levulinic acid + 0.02% sodium dodecyl sulfate (LVA+SDS). Interventions were applied to subprimals dorsal side up using a six-nozzle sanitizing cabinet (Chad Co., Olathe, KS) equipped with an automated conveyor belt system. The automatic premixed spray treated all sides of the subprimal with nozzles located above and below the subprimal at a flow rate of 0.42 L/nozzle•min^{-1}, with a pressure of 275.79 kPa as the product advanced on conveyor belt. Following treatment application, subprimals continued on the conveyor belt (1.0 m/min) and made a single pass, through a mechanical tenderizer (model TC700MC, Ross manufacturing, Midland, VA). The tenderizer blade head consisted of seven alternating angle rows containing 32 perpendicular blades (3mm wide) and set 10 mm apart between rows and column. After tenderization, subprimals were halved perpendicular to the muscle orientation. Within each treatment, each top sirloin anterior and posterior halves were alternated and assigned to aerobic or vacuum package (VP) retail display to simulate retail or processor fabrication and packaging, respectively. The first two steaks from the medial portion of the VP halves were designated for Warner-Bratzler shear force (WBSF) and sensory analysis.

Sirloin halves designated for PVC shelf-life were vacuum packaged (30 to 50 mL of $O_2/m^2/24$ h; 101,325 Pa; 23°C; B-620 series, Cryovac Sealed Air Corporation, Duncan, SC), boxed and held in cooler (2 ± 2°C) for additional 4 d. From the subprimal halves designated for VP, three 2.54 cm thick steaks were cut and randomly assigned to 0, 7, or 15 d of simulated retail display. Steaks were individually vacuum packaged, boxed and held in the cooler for an additional 4 d. Additional holding time was to simulate transportation and storage time before further processing by the end users such as restaurants, retailers, or supermarkets (Guelker et al., 2013). Between each treatment, the spray cabinet, spray tank tenderizer cabinet, and tenderizer heard were rinsed and flushed with hot potable water (50°C) for 2 min and allowed to cool to room temperature (3 ± 1°C) before proceeding with the next treatment.

Following the 4 d storage period, samples designated for PVC packaging were cut into individual steaks (2.54 cm thick) and randomly assigned 0, 1, 3 or 5 d for simulated retail display. Steaks were placed on absorbent pads (Dri-Loc AC-40, Cryovac Sealed Air, Duncan, SC) in Styrofoam trays and then wrapped with an oxygen permeable polyvinylchloride (PVC) overwrap (O_2 transmission=23,250 $mL/m^2/24$ h, 72 gage; Pro Pack Group, Oakland, NJ, USA). Previously packaged VP steaks were removed from cold storage and put out for retail display. Steaks for retail display were placed in open-topped coffin chest display cases (Hussmann Bridgeton, MO) with 24 h continuous lighting (Octron/ECO; 3000K; F032/830/ECCO; Sylvania CO., Versailles, KY) between 1610-2000 lux. Steaks were rotated within case daily. Retail case temperature was monitored and recorded by continuous data loggers (TR-50U2, T&D Corp., Toyko, Japan). Steaks designated for WBSF and sensory characterization were frozen (-20˚C) until further analysis. Samples and treatments were equally represented in each replicate.

Preparation of treatments

Electrolyzed oxidizing water was produced using a generator (ROX-20TA-U, Hoshizaki Electric, Japan) by electrolysis of NaCl according to Park, Hung, and Brackett (2002) Briefly, a 9.4 % salt solution (CAS # 7647-14-5, J.T Baker, Center Valley, PA) and deionized water was simultaneously pumped through the generator at approximately 10 volts and 18 amps producing two types of water including electrolyzed oxidizing acidic water (pH= 2.3 - 2.7, ORP >1100 mV) from the anodic side containing approximately 50-90 ppm free chlorine concentration and alkaline water (pH >11, ORP -800 to -900 mV) from the cathodic side, were collected in separate sealable containers the morning of experiment. Immediately before use, alkaline and acidic water were mixed to produce approximately 57 L with a pH of 6.2-6.5 and have an oxidation-reduction potential (ORP) between +800 to + 850 mV. Free chlorine concentration and ORP were determined using a pH meter and an ORP single junction ion electrode (model WD-35649-50, Oakion Instruments, Vermon Hills, IL). Free chlorine content was determined following the Hach DPD-FEAS (diethyl-*p*-phenylenediamine - 0.00564 *N*-ferrous ethylenediammonium sulfate) titration method (Hach CO., Loveland, CO). Briefly, the sample was diluted by two 10-fold dilutions before 25 mL was transferred into a 50-mL beaker. Then, a DPD free chlorine powder pillow was added to the sample and was swirled to mix. The sample was titrated using a digital titrator (product # 1690001, Hach CO., Loveland CO) with FEAS to a colorless endpoint. Free chlorine concentration was calculated from the number obtained following titration, inclusive of the dilution factor (1:100).

A 4.5% lactic acid solution was prepared by diluting 88% lactic acid (Birko Company, Henderson, CO) with water in the automated spray tank by bringing it to volume (56.78-L) with tap water.

A concentrated peroxyacetic acid solution was prepared by mixing PAA (Envirotec, Perason MP-2, Modesto, CA) with 1L water in a glass amber screw top bottle the morning of the experiment. Immediately before use, the PAA concentrate was added to approximately 32.85 L of water in spray tank, and then brought to volume (56.78 L).

The morning of experiment 113-mL of levulinic acid (LVA) (CAS #123-76-2, Acros Organics, New Jersey, U.S.) and 11.3-g of sodium dodecyl sulfate (SDS) (CAS #151-21-3, Sigma Aldrich, St. Louis, MO) mixed with approximately 4-L of tap water. Immediately before use, LVA+SDS was added to approximately 26.5 L of tap water in the automatic spray tank and brought to volume (56.78-L) with of tap water to create 0.2% LVA + 0.02% SDS solution.

Retail display color

Objective color was collected on d 0, 1, 3 and 5 for PVC and 0, 7 and 15 for VP steaks as a repeated measure of d 5 and 15 d unopened packaged steaks with a Hunter-Lab MiniScan XE Spectrocolorimeter (Hunter Associates Laboratory, Reston, West Virginia, USA) using illuminant A with a 10˚ viewing angle and standardized using a white tile, black tile, and saturated red tile as a working standard before each use. Three objective color readings were recorded for each steak on each day and averaged for *Commission Internationale de l'Eclairage* (CIE) L^*a^*, and b^* color values. Hue angle ($\tan^{-1}(b^*/a^*)$), chroma ($(a^{*2} + b^{*2})^{0.5}$), and $\Delta E = ([(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{0.5})$ were calculated. In addition, the visible spectrum from 630/580nm was determined and reflectance measurements were measured and calculated to estimate the redness due to either oxymyoglobin or deoxymyoglobin fraction of the myoglobin pigment (AMSA, 2012).

A six member trained subjective color panel evaluated steaks on d 0, 1, 3 and 5 for PVC and 0, 7 and 15 for VP unopened steak packages respectively. Panelists evaluated PVC overwrapped steaks for overall color (8 = Extremely dark red, 7 = Dark red, 6 = Moderately dark red, 5 = Slightly dark cherry-red, 4 = Slightly bright cherry-red, 3 = Moderately bright cherry-red, 2 = Bright cherry-red, and 1 = Extremely bright cherry-red), worst point color **(**8 = Extremely dark red, 7 = Dark red, 6 = Moderately dark red, 5 = Slight dark cherry-red, 4 = Slight bright cherry-red, 3 = Moderately bright cherry-red, 2 = Bright cherry-red and 1 = Extremely bright cherry-red), and surface discoloration (8 = No discoloration, 7 = 1-5%, 6 = 6-10%, 5 = 11-25%, 4 = 26-50%, 3 = 51-75%, 2 = 76-90% and 1 = 91-100%) and VP steaks for overall color (5 = Bright purple-red, 4 = Dull purple-red, 3 = Slightly brownish-red, 2 = Moderately brownish-red, and 1 = Brown), amount of browning (6 = Dark brown, 5 = Brown, 4 = Brownish-grey, 3 = Grayish, 2 = Dull, and 1 = No evidence of browning), and surface discoloration (5 = Extreme, 4 = Moderate, 3 = Small, 2 = Slight, and 1 = None) according to (AMSA, 2012).

Psychrotrophic growth

Following the USDA-FSIS Microbiology Laboratory Guidebook 3.01 method (USDA-FSIS, 1998), aerobic plate count (APC) was conducted on d 0, 1, 3 and 5 of PVC steaks. To sample each steak, a sterile 5 x 5 cm^2 metal template was used to create an impression on the steak before cutting. The 5 x 5 cm^2 surface area of the steak was aseptically excised to a depth of approximately 0.2-cm using a sterile scalpel handle and blade. Samples were placed in a sterile stomacher bags and stomached for 2.5 min at 230 rpm. All utensils were sterilized by flame, rinsed with diluted chlorine and wiped free of residual chlorine. Serial dilutions were made for all samples using 9 ml of 0.1% peptone. One milliliter of each sample dilution was plated 3M Petrifilm (3M Manufacture, St. Paul, MN) following the manufacturer's instructions. Petrifilm

plates were incubated at 35 ± 1˚C for 48 ± 2 h. Plates were counted and reported in CFU/g of meat.

As well, the USDA-FSIS Microbiology Laboratory Guidebook 3.01 method (USDA-FSIS, 1998) for plating and enumeration of lactic acid bacteria was followed for samples for VP shelf-life d 0, 7 and 15. The same aseptic techniques described in the previous paragraph. Serial dilutions were made for all samples using 9 ml of 0.1% peptone. A combination of 0.5-mL from the sample dilution and 0.5-mL lactic acid bacteria MRS broth was plated on the Petrifilm following the manufacturer's instructions (3M Manufacture, ST. Paul, MN). Petrifilm plates were incubated anaerobically at 35 ± 1˚C for 48 ± 2h. Plates were counted and reported in CFU/g of meat.

Lipid oxidation

Thiobarbituric acid reactive substance analysis (TBARS) was adopted from Ahn et al. (1998). Briefly, steak samples were thawed (4 ± 1˚C) overnight, homogenized and a 5 g sample was placed in a 50 mL centrifuge tube with 15 mL deionized water and homogenized for 30 sec then centrifuged at 3077 x *g* (CR 312, Jouan INC., Winchester, VA) for 10 min. One milliliter of supernatant was transferred to a disposable glass test tube (13 x 100 mm). Fifty microliters of butylated hydroxyanisole (7.2%) and 2 mL of thiobarbituric acid/trichloroacetic acid were added to the homogenate. Samples were vortexed and incubated in a hot water bath (90˚C) for 15 min. After color development, the sample was placed in a cool water (20°C) bath for 10 min. The samples were then centrifuged at 3077 x *g* (CR 312, Jouan INC., Winchester, VA USA) for 15 min and the supernatant was separated for spectrophotometric analysis (model DU 640; Beckman Coulter, Indianapolis, IN, USA;). The absorbance of the supernatant was measured at

531 nm and fitted against a standard curve. Lipid oxidation values were expressed as milligrams of malonaldehyde (MDA) per kilogram of meat.

Sensory analysis

Steaks for sensory analysis were thawed (4 ± 1˚C) for 18 h. Steaks were cooked to an internal temperature of 71˚C on preheated clamshell grills (George Formen, Saltotn Inc., Miramar, FL) and were monitored by a Digi-Sense 12-Channel Scanning thermometer with copper-constantan thermocouples inserted into the geometric center of each steak. After the steaks are cooked they were served in warmed yogurt makers (Euro Cuisine, Inc., Los Angeles, CA) to an eight member trained sensory panel according to AMSA (1995). Panelists evaluated two cubes per steak (1.27-cm^3) and evaluated seven steaks per session, with two sessions per day. The panelists received samples from each treatment randomly at each session. The loaded yogurt makers were passed through a breadbasket door from the sensory kitchen to the sensory analysis room, equipped with negative pressure ventilation and eight individual booths with red lighting to minimize panelist influence and mask differences in cook steak color. Panelists evaluated each sample for initial tenderness (8 = Extremely tender, 7 = Very tender, 6 = Moderately tender, 5 = Slightly tender, 4 = Slightly tough, 3 = Moderately tough, 2 = Very tough, and 1 = Extremely tough), sustained tenderness (8 = Extremely juicy, 7 = Very juicy, 6 = Moderately juicy, 5 = Slightly juicy, 4 = Slightly dry, 3 = Moderately dry, 2 = Very dry, and 1 = Extremely dry), beef intensity flavor (8 = Extremely intense, 7 = Very intense, 6 = Moderately intense, 5 = Slightly intense, 4 = Slightly bland, 3 = Moderately bland, 2 = Very bland, and 1 = Extremely bland), overall juiciness (8 = Extremely juicy, 7 = Very juicy, 6 = Moderately juicy, 5 = Slightly juicy, 4 = Slightly dry, 3 = Moderately dry, 2 = Very dry, and 1 = Extremely dry), and

off-flavor (6 = Extreme off-flavor, 5 = Very strong off-flavor, 4 = Moderate off-flavor, 3 = Slight off-flavor, 2 = Threshold off-flavor, and 1 = None detected).

Warner-Bratzler shear force

Steaks that were previously designated and frozen for WBSF were thawed and cooked following the same procedures used for sensory. Cooked samples were cooled to room temperature, covered in plastic wrap, and chilled overnight (4 ± 1˚C). Six 1.27-cm diameter cores were removed parallel to the longitudinal orientation of the muscle fibers of each steak using a hand held coring device. Cores were sheared perpendicular to the longitudinal orientation of the muscle fibers using a Universal Testing Machine (Instron Dual Column Model 3365, Instron corp., Norwood, MA) equipped with a Warner-Bratzler shear head with a 51 kgf load cell with a cross head speed of 25 cm/min. The peak shear force (kgf) for each core was recorded (Bluehill software, Instron Corp.) and averaged for the shear force value of each steak.

Statistical analysis

Data were analyzed using Proc Mixed of SAS (version 9.3) as a completely randomized split-plot were subprimal was the whole-plot and steak within subprimal as the sub-plot. Subprimal identification within replication by treatment was included as the random variable. Subprimal was considered the experimental unit and steak was considered the observational unit. Main effects and all treatment by day interactions were tested when applicable. Differences were considered significant at $\alpha \leq 0.05$

Results and discussion

Simulated retail display color

There were no main effect differences ($P > 0.05$) among treatments for PVC (Table 4.1) or VP steaks (Table 4.2). However, L^*, a^*, b^*, chroma and hue were different ($P < 0.05$) on d 3 when compared to d 0 and d 1 of PVC steaks (Table 4.3). On d 3, L^* values increased ($P < 0.05$) while a^*, b^* and chroma values decreased ($P < 0.05$) compared to d 0 and d 1 indicating PVC steaks became darker and were more red before becoming lighter and less red and less vivid by day 5. Exposure to oxygen converts deoxymyoglobin to oxymyoglobin and creates an initial oxymyoglobin layer on the surface of the meat with subsequent layers of metmyoglobin and deoxymyoglobin. This reaction describes the darker L^* values see on d 0 and d 1 as compared to d 3, as the subsequent layers of metmyoglobin and deoxymyoglobin played a role in the color reading. After continuous exposure and further oxidation of myoglobin the oxymyoglobin on the surface of the meat begins to "fade" (as reflected in a^* values seen in this study) the underlying metmyoglobin layer begin to thicken and begins to encroach on the oxymyoglobin layer, the merging of the layers near the surface of the meat play into the lighter L^* values seen on d 3 (Mancini & Hunt, 2005). Figure 4.1 shows the interaction of treatment by day for hue ($P < 0.01$). Hue increased as days of display increased and by d 3 CON was similar ($P > 0.05$) to EOW and PAA but different ($P < 0.05$) from LA and LVA+SDS which were more red. However, by d 5 LVA+SDS were similar ($P > 0.05$) across all days of display. Furthermore, for PVC steaks the 630:580 nm ratio decreased ($P < 0.05$) as day of display increased indicating that steaks became less red over day of display. Similar trends were seen over days of display by King, Shackelford, Kalchayanand, and Wheeler (2012) where a^* and hue were slow to change at beginning of display but towards end of display changes were of greater magnitude. Changes in b^* and chroma reported

by King et al. (2012) were similar to the current study as beginning of display had smaller change in *b** and chroma values followed by a noticeable decline.

For VP steaks there was not a treatment by day of display interaction ($P > 0.05$) for objective color measures. The day effect for VP top sirloin steaks is shown in Table 4.4. *L** and *a** were not affected ($P > 0.05$) by time in display. However, on d 7, steaks were bluer ($P < 0.05$) and less red ($P < 0.05$) as indicated by *b** and hue respectively. Vacuum packaged steaks also became less vivid ($P < 0.05$) after 15 d compared to d 0 and 7. Though not many studies have evaluated objective color on steaks while still in vacuum packaging, Nanke, Sebranek, and Olson (1998) conducted a 12 wk study investigating objective color on vacuum packaged steaks subjected to various irradiation doses and different trends were seen for *L**, *a** and *b** as compared to the current study. Unlike the current study, the author reports *a** values to decrease similarly for all irradiation doses across display period, whereas, *b** remained relatively unchanged and similar for all radiation treatments, unlike *b** values in the current study. Furthermore, *L** values increased as time of display increased, which is not seen in the present study.

Figure 4.2 describes the interactions of treatment by day for overall color ($P < 0.01$), worst point color ($P < 0.01$) and surface discoloration ($P < 0.01$) in PVC steaks. Overall color increased as days of display increased ($P < 0.05$), however, 4.5% lactic acid, EOW and PAA were similar ($P > 0.05$) within day across all days of display while CON was different ($P < 0.05$) across days of display. On d 0, LVA+SDS was similar to all treatments and remained similar ($P > 0.05$) across days of display. As day of display increased, worst point color increased from moderately bright cherry red to slightly bright cherry red, however all treatments were similar ($P > 0.05$) across d 0 and 1. By d 5 CON and EOW had greater ($P < 0.05$) worst point color scores

than PAA, which was similar ($P > 0.05$) to LA and LVA+SDS. Between d 3 and 5 LVA+SDS and PAA maintained a slightly dark cherry-red worst point color while CON and EOW was closer to moderately dark red in color. There was no difference between treatments on d 0 and d 1 for surface discoloration. The percentage of surface discoloration was less for LVA+SDS than PAA, however by d 5 all treatments were similar ($P > 0.05$) in percentage of surface discoloration. Stelzleni, Ponrajan, and Harrison (2013) found that 1% levulinic acid plus 0.1% SDS ground beef patties had a decrease in overall color and discoloration when compared to non-treated patties. Ground beef treated with 200-ppm peroxyacetic acid has also been reported to have extensive discoloration by d 7 of display (Jimenez-Villarreal, Pohlman, Johnson, & Brown, 2003).

Figure 4.3 describes the interactions of treatment by day for overall color ($P < 0.02$), amount of browning ($P < 0.05$) and discoloration ($P < 0.01$) for VP steaks. As days of display increased, scores for overall color decreased ($P < 0.05$). On d 0 LA was similar ($P > 0.05$) to EOW and LVA+SDS but was brighter ($P < 0.05$) purple than CON or PAA. By d 15 all treatments were similar ($P > 0.05$) to each other and LVA+SDS was similar ($P > 0.05$) to that of EOW of d 0 and d 7 and LVA+SDS of d 0. Amount of browning increased as day of display increased ($P < 0.05$). All treatments were the similar ($P > 0.05$) on d 0 and d 7 except for LA on d 7 which had a greater ($P < 0.05$) amount of browning. Peroxyacetic acid (200 ppm) had less browning ($P < 0.05$) than LA on d 15 but was similar ($P > 0.05$) to all other treatments across days of display. John et al. (2005) reported that vacuum packaged top sirloin steaks had low redness value, low hue angle and a dark purple appearance. However, by d 14 the surface of the steaks were brown and had increased b^* values, and at d 21 the author notes some steaks were brown while others were purple. The author goes on to explain that partial browning and surface

metmyoglobin formation in some of the vacuum packaged steaks was probably due to residues of oxygen in the package. This could be a possible explanation as to why amount of browning scores in the present study are similar on d 0 and d 15. Percentage of surface discoloration increased and day of display increased, however all treatments were similar ($P > 0.05$) between d 0 and d 7 to respective treatments except LA.

Aerobic plate count and lactic acid producing bacteria

There was no treatment by day interaction for either psychrotrophic organism growth for PVC ($P = 0.83$) or VP steaks ($P = 0.38$) or lipid oxidation for PVC ($P = 0.82$) or VP steaks ($P = 0.14$) thus main effects of treatment and day are discussed. For both PVC (Table 4.5) and VP steaks (Table 4.6) psychrotrophic organism growth increased ($P < 0.05$) as day of display increased. However, there was no difference in psychrotrophic organisms growth between treatments in PVC ($P > 0.05$; Table 4.7) or VP ($P > 0.05$; Table 4.8) steaks. However, findings by Gill and Badoni (2004) revealed that 4% lactic acid significantly reduced microbial growth on chilled beef carcasses. However in the same study, PAA sprayed on chilled beef carcasses had little effect on aerobes, coliforms or *E. coli*. The lack of effectiveness for LVA+SDS and EOW were unexpected. It has been previously shown that 1% levulinic acid plus 0.1% sodium dodecyl sulfate were effective as an antimicrobial agent against *Listeria monocytogenes* and *E. coli* O157:H7 by Chen, Zhao, and Doyle (2014) and against psychrotrophic organisms in treat beef patties by Stelzleni et al. (2013). Additionally, EOW was found to be effective against *Campylobacter jejuni* by Park et al. (2002). Aerobic plate count in PVC steaks increased ($P < 0.05$) as days of display increased however, d 0 and d 1 psychrotrophic organism growth was similar ($P > 0.05$). In vacuum packaged steak lactic acid bacterial counts increased as days of display increased ($P < 0.05$). Similar findings were reported by King et al. (2005) who reported

that aerobic plate count increased during display however lactic acid bacteria stayed the same across days of display. However, by the end of simulated retail display, psychrotrophic growth for all treatments were still below the commonly held spoilage level of 10^6 CFU/g of meat, which is the normal indicator of sensory changes indicative of spoilage (Bruhn et al., 2004; Gill & Gill, 2005).

Lipid oxidation

Though the presence of microorganisms can affect change in the color of meat, lipid oxidation has also been found to play an important role (Faustman, Sun, Mancini, & Suman, 2010). Lipid oxidation values increased ($P < 0.05$) after 3 and 5 d for PVC steaks (Table 4.5). For VP steaks, there were no significant differences ($P > 0.05$) in lipid oxidation across 15 d display. Lipid oxidation levels in the current study were below the threshold of sensory acceptability (2 mg per kg MDA) for both PVC and VP steaks as defined by Campo et al. (2006) and Jayasingh, Cornforth, Brennand, Carpenter, and Whittier (2002).There was no difference ($P < 0.05$) in lipid oxidation between treatments for PVC steaks (Table 4.7) or VP (Table 4.6). Although hydroxide and hypochlorous acid found in EOW are considered antioxidants (Rahman, Park, Wang, & Oh, 2012), the use of EOW in the current study did not have significant impact on beef top sirloin steaks.

Sensory and cooking characteristics

Sensory and cooking characteristics main effects of treatment are discussed (Table 4.9). Although there was no difference ($P > 0.05$) between in initial tenderness or WBSF between treatments, panelist regarded PAA as slightly tender for sustained tenderness and was significantly different ($P < 0.05$) from LVA+SDS. However; both PAA and LVA+SDS were

similar ($P > 0.05$) to CON, EOW and LA. Warner-Bratzler shear force was similar ($P > 0.05$) among all treatment and all treatments had WBSF values lower that 4.4 kgf which qualify for USDA guaranteed tenderness (USDA-AMS, 2012). These values would be expected as steaks in this study were subjected to blade tenderization. Savell et al. (1976) reports that blade tenderization (1 pass) decreased the shear force by 1kgf for lower quality grade *gluteus medias* muscles. Peroxyacetic acid was significantly different ($P < 0.05$) from CON as panelist perceived a slightly more bland beef intensity flavor in PAA treated steaks. However, both PAA and CON were similar ($P > 0.05$) to EOW, LA and LVA+SDS where LVA+SDS had a slightly intense beef intensity flavor. Additionally, PAA had a higher percentage of cook and thaw loss and was significantly different ($P < 0.05$) from CON for cook loss and EOW for thaw loss.

Conclusion

In conclusion, these results suggest that along with LA and PAA the two novel antimicrobial interventions, EOW and LVA+SDS would be acceptable to use on beef top sirloin subprimals subjected to blade tenderization without detrimental effects to quality and shelf-life. Additional research should be conducted to investigate higher concentrations of levulinic acid and sodium dodecyl sulfate and various properties of electrolyzed oxidizing water on shelf-life and quality characteristics of beef subprimals and trimmings.

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Table 4.1
Least squares means and standard errors for objective color main effects by treatment for PVC overwrapped steaks from beef top sirloins subjected to antimicrobial intervention and blade tenderization

	Treatment[1]					
Trait	CON	EOW	PAA	LA	LVA+SDS	SEM[2]
L^*[3]	37.78	36.51	35.82	38.31	38.71	2.97
a^*[3]	20.81	20.83	21.27	21.67	22.07	0.82
b^*[3]	20.48	20.07	19.90	20.36	20.24	0.73
Hue[3]	45.44	44.70	43.93	43.67	42.64	1.11
Chroma[3]	29.45	29.12	29.30	29.91	30.08	0.98
630/580 nm[3]	6.10	5.88	5.08	5.10	4.77	0.61
ΔE[3]	27.13	30.02	23.89	26.00	28.46	2.12

[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM = standard error of means.
[3]L^* = 0 = black to 100 = white; a^* = measurement of green to red on color spectrum, high values indicate more red; b^* = measurement of yellow to blue on color spectrum, higher values indicate more yellow; Hue = lower values indicate redder color; Chroma = higher value indicates more red saturation.

Table 4.2
Least squares means and standard errors for objective color main effects by treatment for vacuum packaged steaks from beef top sirloins subjected to antimicrobial intervention and blade tenderization

	Treatment[1]					
Trait	CON	EOW	PAA	LA	LVA+SDS	SEM[2]
*L**[3]	33.98	34.15	32.12	32.31	33.25	1.14
*a**[3]	26.66	27.04	27.72	28.15	27.68	0.77
*b**[3]	20.58	20.71	20.08	20.57	20.64	0.30
Hue[3]	37.99	37.69	36.22	36.70	37.28	0.79
Chroma[3]	33.85	34.22	34.41	35.01	34.68	0.61

[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]*L** = 0 = black to 100 = white; *a** = measurement of green to red on color spectrum, high values indicate more red; *b** = measurement of yellow to blue on color spectrum, higher values indicate more yellow; Hue = lower values indicate redder color; Chroma = higher value indicates more red saturation.

Table 4.3
Least squares means and standard errors for objective color main effects by day for PVC overwrapped steaks from beef top sirloins subjected to antimicrobial intervention and blade tenderization

	Day of Display[1]				
Trait	0	1	3	5	SEM[2]
L^*[3]	38.37[b]	38.19[b]	48.78[a]	23.72[c]	2.29
a^*[3]	27.71[a]	26.27[a]	16.85[b]	14.49[c]	0.67
b^*[3]	24.04[a]	22.70[a]	19.43[b]	14.68[c]	0.60
Hue[3]	40.05[c]	40.96[c]	49.37[a]	45.92[b]	0.78
Chroma[3]	36.81[a]	34.79[a]	25.93[b]	20.76[c]	0.82
630/580 nm[3]	9.35[a]	6.12[b]	3.76[c]	2.31[d]	0.36
ΔE[3]	-	17.9[b]	32.50[a]	30.88[a]	1.54

[abc] Least squares means within row with different superscripts are different ($P < 0.05$).
[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]L^* = 0 = black to 100 = white; a^* = measurement of green to red on color spectrum, high values indicate more red; b^* = measurement of yellow to blue on color spectrum, higher values indicate more yellow; Hue = lower values indicate redder color; Chroma = higher value indicates more red saturation.

Table 4.4
Least squares means and standard errors for objective color main effects by day for vacuum packaged steaks from beef top sirloins subjected to antimicrobial intervention and blade tenderization

	Day of Display[1]			
Trait	0	7	15	SEM[2]
*L**[3]	34.40	32.37	32.72	0.73
*a**[3]	26.94	27.70	27.70	0.44
*b**[3]	20.70[a]	19.93[b]	20.92[a]	0.23
Hue[3]	37.89[a]	36.12[b]	37.51[a]	0.59
Chroma[3]	34.10[b]	34.29[b]	34.90[a]	0.32

[ab] Least squares means within row with different superscripts are different ($P < 0.05$).
[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]*L** = 0 = black to 100 = white; *a** = measurement of green to red on color spectrum, high values indicate more red; *b** = measurement of yellow to blue on color spectrum, higher values indicate more yellow; Hue = lower values indicate redder color; Chroma = higher value indicates more red saturation.

Table 4.5
Least squares means and standard errors for aerobic plate count and thiobarbituric acid reactive substance analysis (TBARS) main effects by day for PVC overwrapped steaks from beef top sirloins subjected to antimicrobial intervention and blade tenderization

	Day of Display[1]				
Trait	0	1	3	5	SEM[2]
Aerobic Plate Count[3]	2.45[c]	2.90[b]	3.06[b]	4.38[a]	0.17
TBARS[4]	0.39[c]	0.44[c]	0.73[b]	1.00[a]	0.06

[abc] Least squares means within row with different superscripts are different ($P < 0.05$).
[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]Values are reported as CFU/g.
[4]Values are reported in mg MDA/kg meat.

Table 4.6
Least squares means and standard errors for lactic acid bacteria and thiobarbituric acid reactive substance analysis (TBARS) main effects by day for vacuum packaged steaks from beef top sirloins subjected to antimicrobial intervention and blade tenderization

	Day of Display[1]			
Trait	0	7	15	SEM[2]
Lactic Acid Bacteria[3]	2.57[c]	4.36[b]	5.71[a]	0.17
TBARS[4]	0.38[a]	0.37[a]	0.45[a]	0.04

[abc] Least squares means within row with different superscripts are different ($P < 0.05$).
[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]Values are reported as CFU/g.
[4]Values are reported in mg MDA/kg meat.

Table 4.7
Least squares means and standard errors for aerobic plate count and thiobarbituric acid reactive substance analysis (TBARS) main effects by treatment for PVC overwrapped steaks from beef top sirloins subjected to antimicrobial intervention and blade tenderization

	Treatment[1]					
Trait	CON	EOW	PAA	LA	LVA+SDS	SEM[2]
Aerobic Plate Count[3]	3.53	3.26	2.79	3.07	3.33	0.32
TBARS[4]	0.64	0.69	0.70	0.55	0.62	0.11

[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]Values are reported as CFU/g.
[4]Values are reported in mg MDA/kg meat.

Table 4.8
Least squares means and standard errors for lactic acid bacteria and thiobarbituric acid reactive substance analysis (TBARS) main effects by treatment for vacuum packaged steaks from beef top sirloins subjected to antimicrobial intervention and blade tenderization

	Treatment[1]					
Trait	CON	EOW	PAA	LA	LVA+SDS	SEM[2]
Lactic Acid Bacteria[3]	4.27	4.06	4.17	4.24	4.33	0.36
TBARS[4]	0.45	0.40	0.41	0.36	0.37	0.06

[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm); LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]Values are reported as CFU/g.
[4]Values are reported in mg MDA/kg meat.

Table 4.9
Least squares means and standard error for main effects for sensory and cooking characteristics for steaks from beef top sirloin subjected to antimicrobial intervention and blade tenderization

	Treatments[1]					
Trait	CON	EOW	PAA	LA	LVA+SDS	SEM[2]
Sensory Characteristics						
Initial Tenderness[3]	5.01	5.50	5.37	5.12	4.97	0.33
Sustained Tenderness[3]	4.62^{ab}	4.87^{ab}	5.24^{a}	4.61^{ab}	4.45^{b}	0.30
Beef Flavor Intensity[4]	4.48^{b}	4.63^{ab}	4.71^{a}	4.80^{ab}	5.11^{ab}	0.21
Juiciness[5]	3.92^{b}	4.29^{ab}	4.84^{a}	4.29^{b}	4.05^{b}	0.27
Off-Flavor[6]	1.57	1.39	1.35	1.55	1.25	0.12
WBSF, kgf[7]	3.15	3.47	3.37	3.33	3.56	0.22
Cooking Characteristics						
Thaw Loss, %	4.94^{ab}	4.39^{b}	5.75^{a}	5.46^{ab}	4.69^{ab}	0.49
Cook Loss, %	18.23^{b}	20.07^{ab}	22.37^{a}	19.11^{ab}	19.00^{ab}	1.44

^{ab}Least squares means within a row with different superscripts are different ($P < 0.05$).
[1]Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm) and LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate.
[2]SEM= standard error of means.
[3]8 = extremely tender, 7 = very tender, 6 = moderately tender, 5 = slightly tender, 4 = slightly tough, 3 = moderately tough, 2 = very tough and 1 = extremely tough.
[4]8 = extremely intense, 7 = very intense, 6 = moderately intense, 5 = slightly intense, 4 = slightly bland, 3 = moderately bland, 2 = very bland and 1 = extremely bland.
[5]8 = extremely juicy, 7 = very juicy, 6 = moderately juicy, 5 = slightly juicy, 4 = slightly dry, 3 = moderately dry, 2 = very dry and 1 = extremely dry.
[6]6 = extreme off-flavor, 5 = very strong off-flavor, 4 = moderate off-flavor, 3 = slight off-flavor, 2 = threshold off-flavor and 1 = non-detected.
[7]Warner-Bratzler Shear Force.

Figure 4.1

Least squares means and standard error for treatment by day interaction for Hue in PVC overwrapped steaks from beef top sirloin subjected to antimicrobial intervention and blade tenderization Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm) and LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate. Least squares means with different superscripts are different ($P < 0.05$).

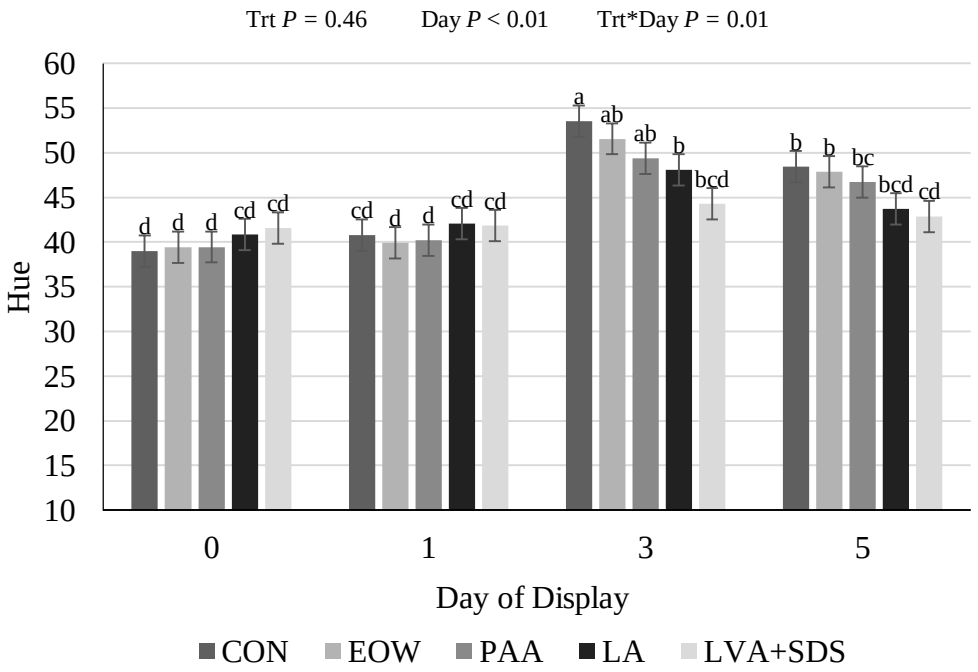
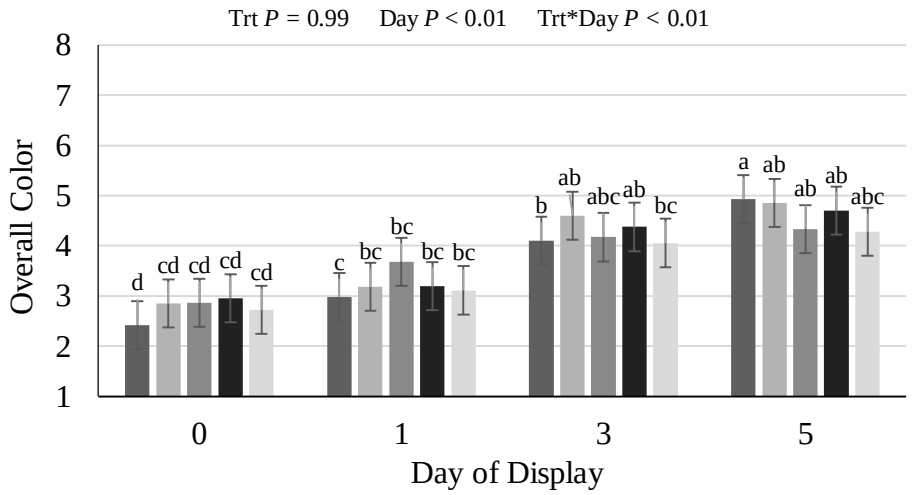


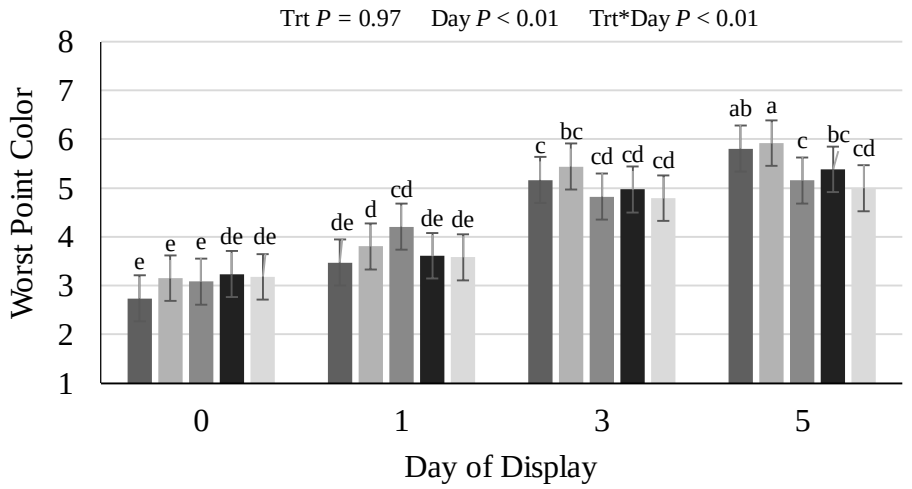
Figure 4.2

Least square means and standard errors for subjective color for PVC overwrapped steaks from beef top sirloin subprimals subjected to blade tenderization and antimicrobial interventions. A) Overall appearance (8 = Extremely dark red, 7 = Dark red, 6 = Moderately dark red, 5 = Slightly dark cherry-red, 4 = Slightly bright cherry-red, 3 = Moderately bright cherry-red, 2 = Bright cherry-red, and 1 = Extremely bright cherry-red); B) Worst Point Color measures a single or combined area of at least 2-cm^2 used to evaluate average color; (8 = Extremely dark red, 7 = Dark red, 6 = Moderately dark red, 5 = Slight dark cherry-red, 4 = Slight bright cherry-red, 3 = Moderately bright cherry-red, 2 = Bright cherry-red and 1 = Extremely bright cherry-red); and C) Surface discoloration (8 = No discoloration, 7 = 1-5%, 6 = 6-10%, 5 = 11-25%, 4 = 26-50%, 3 = 51-75%, 2 = 76-90% and 1 = 91-100%). Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm) and LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate. Least squares means with different superscripts are different ($P < 0.05$).

A.


Trt P = 0.99 Day P < 0.01 Trt*Day P < 0.01
Overall Color
Day of Display
0
1
3
5
d cd cd cd cd
c bc bc bc bc
b ab abc ab bc
a ab ab ab abc

B.


Trt P = 0.97 Day P < 0.01 Trt*Day P < 0.01
Worst Point Color
Day of Display
0
1
3
5
e e e de de
de d cd de de
c bc cd cd cd
ab a c bc cd

C.

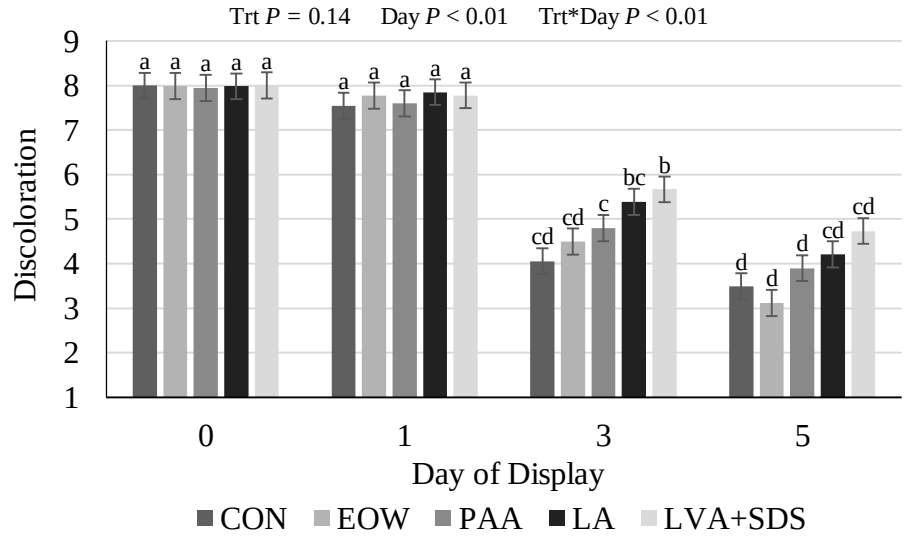
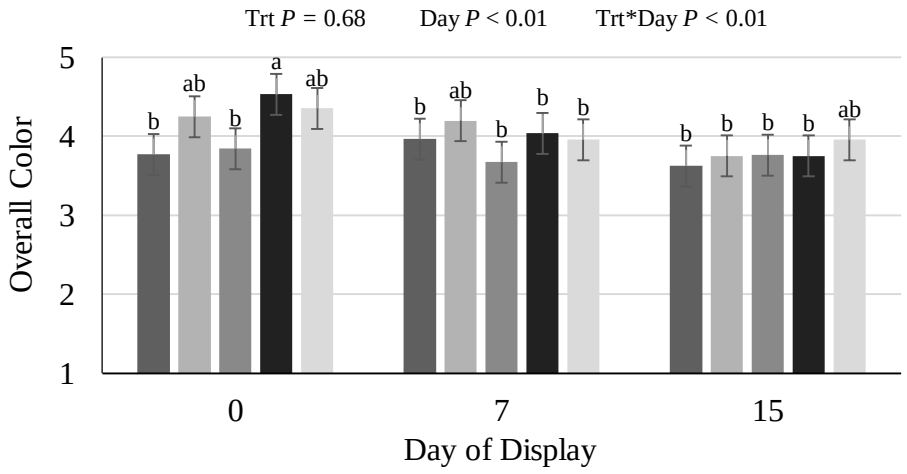

Trt P = 0.14 Day P < 0.01 Trt*Day P < 0.01
Discoloration
Day of Display
0
1
3
5
a a a a a
a a a a a
cd cd c bc b
d d d cd cd
CON
EOW
PAA
LA
LVA+SDS

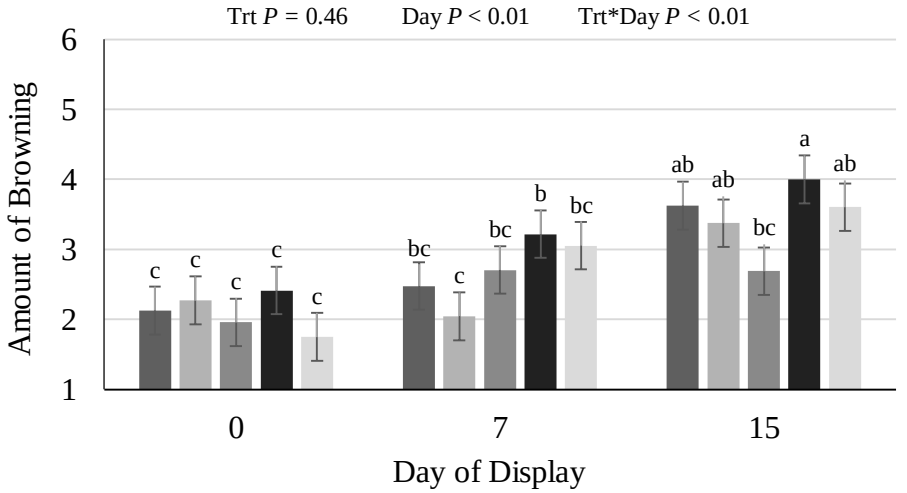
Figure 4.3

Least square means and standard errors for subjective color for vacuum packaged steaks from beef top sirloin subprimals subjected to antimicrobial interventions and blade tenderization. A) Overall color (5 = Bright Purple-red, 4 = Dull Purple-red, 3 = Slightly Brownish-red, 2 = Moderately Brownish-red, and 1 = Brown); B) Amount of Browning (6 = Dark brown, 5 = Brown, 4 = Brownish-grey, 3 = Grayish, 2 = Dull, and 1 = No evidence of browning); and C) Discoloration (5 = Extreme, 4 = Moderate, 3 = Small, 2 = Slight, and 1 = None). Antimicrobial intervention: CON = control; blade tenderization only; EOW = electrolyzed oxidizing water; PAA = peroxyacetic acid (200 ppm) and LA = 4.5% lactic acid and LVA+SDS = 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate. Least squares means with different superscripts are different ($P < 0.05$).

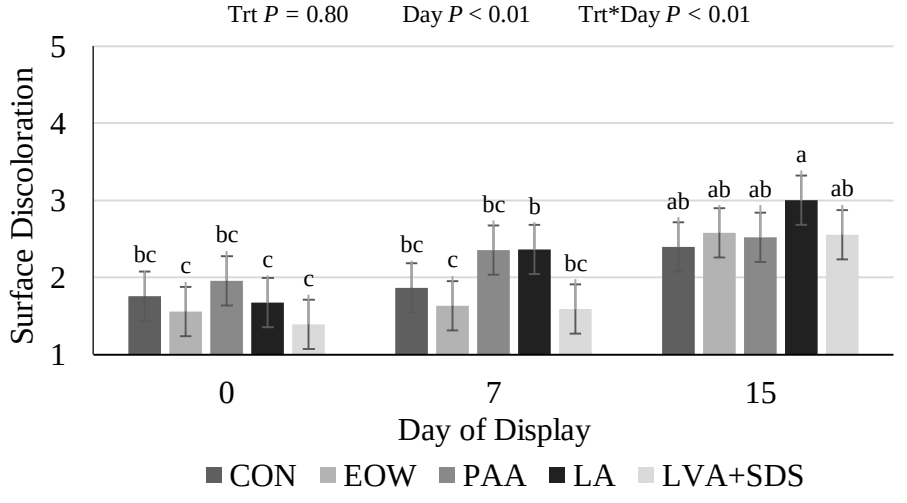
A.


Trt P = 0.68
Day P < 0.01
Trt*Day P < 0.01
Overall Color
5
4
3
2
1
b ab b a ab
b ab b b b
b b b b ab
0
7
15
Day of Display

B.


Trt P = 0.46
Day P < 0.01
Trt*Day P < 0.01
Amount of Browning
6
5
4
3
2
1
c c c c c
bc c bc b bc
ab ab bc a ab
0
7
15
Day of Display

C.


Trt P = 0.80
Day P < 0.01
Trt*Day P < 0.01
Surface Discoloration
5
4
3
2
1
bc c bc c c
bc c bc b bc
ab ab ab a ab
0
7
15
Day of Display
CON
EOW
PAA
LA
LVA+SDS

CHAPTER 5

CONCLUSION

Translocation of pathogenic bacteria by blade tenderization has an inherently low risk. However, due do to consumer safety antimicrobial interventions continue to be developed and investigated to aid in reducing the microbial risk and improve shelf life and quality characteristics in beef products. Commonly used antimicrobials, LA and PAA hindered psychrotrophic growth more than novel antimicrobials, LVA+SDS, EOW and the control in PVC overwrapped beef strip steaks. However, by the end of shelf-life display psychrotrophic growth for all treatments in both PVC and VP beef strip steaks and top sirloin steaks was below spoilage level of 10^6 CFU/g of meat which sensory changes indicative of spoilage are noticeable. Applied antimicrobial did not affect objective color regardless of packaging, or subprimal cut. There were no substantial adverse effects from antimicrobial treatments on sensory characteristics for beef strip loin or top sirloin steaks.

These results suggest that along with LA and PAA the two novel antimicrobial interventions, EOW and LVA+SDS would be acceptable to use on beef strip loin and top sirloin subprimals subjected to blade tenderization without detrimental effects to quality and shelf-life. Further research should be conducted to investigate higher concentrations of levulinic acid and sodium dodecyl sulfate and various properties of electrolyzed oxidizing water on shelf-life and quality characteristics of beef subprimals and trimmings.