

# EFFECTS OF NOVEL ANTIMICROBIALS ON THE QUALITY CHARACTERISTICS OF GROUND BEEF

by

SAMANTHA ELIZABETH BELANGER

(Under the Direction of Alexander M. Stelzleni)

## ABSTRACT

The control of shiga toxin-producing *E. coli* is of major concern for non-intact beef products such as ground beef. As novel antimicrobials are developed to reduce these pathogens, it is critical to understand their impact on meat quality. The objective of this study was to evaluate the effects of two novel antimicrobials, acidic electrolyzed oxidizing water (EO) and levulinic acid plus sodium dodecyl sulfate (LVASDS), on quality and shelf life characteristics of ground beef as compared to two industry standards, lactic acid (LA) and peroxyacetic acid (PAA). Beef trim was produced from whole boneless chuck rolls and treated with antimicrobial interventions then ground. Patties were formed, packaged into polyvinyl chloride (PVC) overwrap or vacuum packaging (VP), and placed in simulated retail display. In conclusion the results showed that EO and LVASDS could be used without negatively affecting quality and shelf life characteristics as compared to industry standards.

INDEX WORDS: Antimicrobials, ground beef, quality, sensory characteristics

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GROUND BEEF

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## DEDICATION

To my late grandfather, Robert G. Huetz Jr. and my late dog, Zeus Belanger.

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## **CHAPTER 1**

### **INTRODUCTION**

In the United States ground beef is the number one selling beef item in foodservice and retail facilities. Over 50% of the beef that is purchased by consumers at the retail level and 73% of the beef that is purchased at foodservice establishments are of the ground variety (NCBA, 2002). With the quantity of ground beef that is consumed it is important to provide a safe and wholesome product for the consumer. To ensure a product was safe, unadulterated, and wholesome the United States Department of Agriculture – Food Safety Inspection Service (USDA-FSIS) implemented the Hazard Analysis and Critical Control Points (HACCP) system in the 1996 Final Rule on Pathogen Reduction; HACCP System (FSIS-USDA, 1996). Protocols may include carcass washes, use of antimicrobials; such as lactic acid (Castillo et al., 2000), chemical dehairing, and spot-cleaning of carcasses by knife-trimming prior to evisceration, chilling, and fabrication. The need for these protocols is due to the possible contamination that can occur during the slaughter and fabrication processes. Product can become contaminated during the conversion of live animals to the fresh meat product from processing practices, fecal material, hide contact, and unsanitary facilities and employees (Lahr, 1996 & Galland, 1997). Studies have shown that using multiple decontamination technologies are more effective than a single process; this resulted in multiple hurdle technology systems (Huffman, 2002; Belk, 2001; & Kang, et al., 2000). Abusive conditions and contamination can lead to an adulterated product



from the presence of *Escherichia coli* O157:H7 (Sofos et al., 1999) which is the predominant pathogen of concern in ground beef. The most significant outbreak related to *E. coli* O157:H7 in ground beef was in 1993 when The Jack in the Box restaurants served undercooked hamburger that resulted in 720 people to become infected and the death of 4 children (CDC, 1993). This outbreak resulted in the USDA-FSIS providing more regulations and safety practices that are now implemented within processing and slaughter facilities. *Escherichia coli* O157:H7 produces a shiga toxin that causes acute hemorrhagic diarrhea which if not treated can lead to death (FSIS-USDA, 2013). Due to the increase in surveillance and concern, in 2011 six additional shiga toxin-producing *Escherichia coli* (STEC) serogroups known as the “Big Six”; O26, O103, O45, O111, O121, and O145 were added to the adulterant list (FSIS-USDA, 2011). These additional STEC do not cause the same clinical signs or exhibit the same mortality as *E. coli* O157:H7 but are responsible for about 1/3 of the *Enterohaemorrhagic Escherichia coli* (EHEC) cases in humans (Koochmaraie et al., 2005). Due to the severity and concerns associated with *E. coli* O157:H7 and other STEC the industry is under pressure to investigate both existing and novel antimicrobials for their effectiveness at reducing or eliminating STECs.

The Code of Federal Regulations 9 §319.15, (CFR, 2012) defines ground beef as chopped fresh and/or frozen beef with or without seasoning and without the addition of beef fat as such, shall not contain more than 30 percent fat, and shall not contain added water, phosphates, binders or extenders. The production of ground beef is quite simple; during carcass fabrication, boneless trim is produced and ground. While the muscle is intact the interior of meat is inherently sterile. However, once exposed as in the case of trim, there is the possibility for contamination (Belk, 2001). Furthermore, the trim from multiple animals, lots, production times, and sources may be combined before grinding. Because there can be so many sources going into the production of

ground beef it is likely unknown as to which pieces may or may not be contaminated with pathogens.

Shelf life of a product is an important aspect to the industry and extending the product life is a key goal (Delmore, 2009). Shelf life is the period of time for which a product will remain usable and safe for consumption. Factors that influence the effects of shelf life may include; distribution, type of display, microbial load, pH, temperature, and packaging. The type of packaging for ground beef can greatly affect the shelf life. Two common forms of packaging include: polyvinyl chloride (PVC) and vacuum packaging (VP). Retail stores produce bench trim that are combined with multiple sources, ground, and packaged in PVC for retail display where as VP product is typically cased-ready that is produced at the processors and shipped to retail stores. Polyvinyl chloride overwrap is oxygen permeable allows oxygen to react with the myoglobin of the ground beef, allowing the product to bloom to an oxymyoglobin state for a short period of time. However, PVC wrapped ground beef has a short color shelf life, approximately 3 days, before browning due to meat pigment oxidation and the formation of metmyoglobin (Cornforth & Hunt, 2008). Vacuum packaging on the other hand excludes oxygen while maintaining ground beef in a deoxymyoglobin state. The exclusion of oxygen may extend the shelf life of ground beef and decrease oxidation (Brooks, 2007). The purchasing decision of consumers is highly dependent on the color of ground beef (Mancini & Hunt, 2005). Therefore, as novel pathogen interventions are being validated as an antimicrobial it is also important to understand the effects these interventions will have on shelf life and quality characteristics. Due to the short shelf life of ground beef and the influence of packaging types, it is important to understand how novel pathogen interventions will affect, specifically the quality and shelf life characteristics. These interventions may be applied at the processor before grinding or with new

regulations for enhanced record keeping and control of ground beef product; there is potential interest to start applying pathogen interventions at the retail level prior to the final grind (FSIS-USDA, 2014).

As current and novel pathogen interventions are found to be effective against STECs it is also important to understand their effect on meat quality and shelf life characteristics. The most prevalent interventions used that have shown to be effective against pathogens are the organic acids; lactic acid (LA) and peroxyacetic acid (PAA). The beef industry is currently seeking new alternatives to the pre-existing antimicrobial interventions that will reduce microbial contamination as well as not have adverse effects on quality and sensory characteristics (Quilo et al., 2009b). Two recent technologies that have been shown to be effective against pathogens are acidic electrolyzed oxidizing (EO) water (Hung et al., 2010) and levulinic acid plus sodium dodecyl sulfate (LVASDS; Zhao, Zhao, & Doyle, 2009).

Therefore the objective of this research was to:

1. Evaluate EO water and LVASDS for their effect on ground beef quality and shelf life analysis of aerobic and lactic acid bacteria, subjective and objective color, sensory characteristics, lipid oxidation, and Kramer-shear force when compared to the industry standards LA and PAA.
2. Evaluate shelf life characteristics under two production and packaging types; application of antimicrobials on bench trim going out for aerobic packaging at the retail level and application of antimicrobials at the processor in case-ready vacuum packaged ground beef product.

## CHAPTER 2

### LITERATURE REVIEW

#### **Ground Beef - *Escherichia coli* 0157:H7**

Ground beef is commonly marketed at refrigerated temperatures (2-5°C); this can cause undesirable changes to the product including microbial growth and lipid oxidation, leading to spoilage and reduction in quality (Sallam & Samejima, 2004). The production of ground beef starts with the trimmings of carcasses that can be ground at the plant, packaged for storage and distribution, or is produced from bench trim at local retail stores. The production of ground beef is an area where one or several interventions are needed due to the high risk of pathogenic bacteria that can potentially contaminate the products (Quilo et al., 2009b). Microbial contamination and food borne illness are of major concern throughout the beef industry and among consumers. Raw products can become contaminated from many sources including; carcass hides, intestinal tracts, employees, and the environment. Because of this the industry has taken steps such as multiple hurdle technology to provide a safe product for consumers. These steps are measured from the involvement of the United States Department of Agriculture – Food Safety and Inspection Services (USDA-FSIS) and the implementation of Hazard Analysis and Critical Control Points (HACCP) from the 1996 Final Rule on Pathogen Reduction; HACCP System (FSIS-USDA, 1996) which is now required in all inspected facilities. Inspection plans are set in place to ensure plants are following critical control points during the slaughter, fabrication, and cooking process where contamination can occur and the proper action can be taken to improve the safety and wholesomeness of the resulting meat products for the consumer.

*Escherichia coli* O157:H7 was discovered in 1982, when there were two reported outbreaks of hemorrhagic colitis and was deemed a human pathogen (Doyle, M.P., Beuchat, L.R., & Montville, T.J., 1997). However, the most notorious outbreak of *E. coli* O157:H7 was in 1993 from the sale of undercooked hamburgers from Jack in the Box restaurants, where more than 720 people were infected and hospitalized and the death of 4 children (CDC, 1993). Under the Federal Meat Inspection Act (21 U.S.C. 601) *E. coli* O157:H7 was labeled as an adulterant in raw ground beef products unless the ground beef is further processed to destroy the pathogen. This led to the industry to take action and fund research on how to reduce *E. coli* O157:H7 in slaughter facilities and develop more sensitive testing and sampling for the detection of *E. coli* O157:H7 (Golan et al., 2004). The USDA FSIS will require the “hold and test” policy for ground beef, tenderized steaks and ready-to-eat products which will hold shipments of products until the test results come back negative for Shiga-toxin producing *E. coli* (Bottemiller, 2012 & FSIS USDA, 2013). The most recent case of *E. coli* contamination was the recall of 1.8 million pounds of ground beef from Wolverine Packaging Co, which was delivered to multiple states within the US (CDC, 2014). This resulted in 12 people becoming sick within 4 states, however, no one developed hemolytic uremic syndrome and no deaths were reported.

The CDC has reported confirmed cases of *E. coli* O157:H7 ranging across multiple food sources including; ground beef and other meat products, ready-to-eat salads, spinach and spring mix blends, lettuce, bologna, and hazelnuts (CDC, 2014). Reports of non-O157 STECs have contaminated products such as clover sprouts, frozen food products, lettuce, and beef products (CDC, 2014). Pathogenic *E. coli* is capable of producing large quantities of toxins, known as Shiga toxins, which can cause severe damage to the intestinal linings. Because of the known toxicity of shiga toxin-producing *E. coli* it is important to reduce or eliminate the pathogen so

that it will not come in contact with non-intact beef products such as ground beef. The presence of STEC and shiga-toxin producing *E. coli* is normally found in ruminant animals and causes no harm to the host. However, the pathogenic form of *E. coli* can be ingested due to contamination of food or water and this can cause severe, acute hemorrhagic diarrhea and the infectious dose for *E. coli* O157:H7 is ingesting anywhere from 10 – 100 cells (Feng, Weagant, & Jinneman, 2011). Elder et al. (2000) found that the prevalence of *E. coli* O157:H7 was found to be on 28% of feces and 11% of hides from beef cattle processing plants in the months of July and August of 1999. With the implementation of pathogen interventions after evisceration, Elder also found that this prevalence decreased to 2% positive when carcasses were tested. As stated prior, *Escherichia coli* O157:H7 and non-O157:H7 STECs are considered an adulterant in ground beef and if found products must be destroyed or sent to fully cooked facilities to destroy the pathogen before consumed.

### **Interventions:**

Now that *E. coli* O157:H7 and the Big Six *E. coli* are labeled as adulterants in meat products, the use of post-harvest interventions has been continually improved, with new attention to hide decontamination and innovative treatments of carcasses (Koohmaraie et al. 2005). Practices that are currently used within the industry range from single use or a combination of trimming visible contamination, heat treatments, hot water and organic acid rinses. Many of these practices are dependent on the temperature and the exposure time at which the intervention is applied to the surface of the meat. The use of interventions and removal of contaminants complies with the “zero-tolerance” policy in that no visible fecal material, ingesta, or milk is allowed to pass on any type of meat or poultry products. (FSIS-USDA, 2004).

*Hot water:* The use of hot water to rinse carcasses and meat products have been thoroughly examined to determine the efficacy on decreasing bacterial attachment, however, time, application to lean or fat tissues, and temperature all affect the results (Huffman, 2002). The decontamination of beef carcasses with hot water at a temperature of 95°C (causing the carcass surface temperature to reach 82°C, which can cause microbial destruction) for 10 seconds has been shown to reduce aerobic plate counts (APC) by 1.3 log<sub>10</sub>/cm<sup>2</sup> (Barkate, Acuff, Lucia, & Hale, 1993). Moreover, Barkate et al. (1993) found that the use of hot water was effective but it discolored the carcass, however the color did return to normal within 24 hours. Castillo et al. (1998) found that the placing beef carcasses in a spray cabinet and applying hot water at 95°C reduced inoculated *S. Typhimurium* and *E. coli* O157:H7 from 5.0 and 6.0 log CFU/cm<sup>2</sup> to 4.0 and less than 4.8 log CFU/cm<sup>2</sup>; respectively. In another study, Bosilevac et al. (2006) reported that the use of hot water (74°C) for 5.5 seconds in a commercial wash cabinet reduced aerobic plate counts and Enterobacteriaceae counts by 2.7 log CFU/100cm<sup>2</sup> on pre-evisceration carcasses. Furthermore, the hot water treatment reduced the prevalence of *E. coli* O157:H7 by 81% (Bosilevac et al., 2006).

*Organic Acids:* In order to prevent microbial growth and possibly extend the shelf life of ground beef the use of organic acids and other antimicrobials can be used. Organic acids have been widely used and studied within the industry to evaluate their efficacy of reducing pathogens pre- and post-evisceration, prior to chilling, and during further processing. Lactic acid (LA) has become one of the most commonly used pathogen intervention treatments applied to carcasses within slaughter facilities today (Koohmaraie et al., 2005 & Bosilevac et al., 2006). Bosilevac et al. (2006), found that the combination of lactic acid and hot water wash proved to be more effective than lactic acid alone. When beef carcasses were sprayed with 74°C hot water followed

by 2% LA (42°C) it reduced aerobic plate counts and Enterobacteriaceae by 2.2 and 2.5 log CFU/cm<sup>2</sup>, respectively, along with a 79% reduction in *E. coli* O157:H7. Gill and Badoni (2004) found that spraying 50 mL of either 4% lactic acid (LA) or 0.02% peroxyacetic acid (PAA) onto beef carcasses resulted in 1.5 and 1.0 log units reduction of *E. coli*, respectively, compared to a water wash. When 0.02% of PAA was applied to whole beef carcasses via a spray cabinet for 15 seconds there was a reduction of the inoculated *E. coli* Type I, coliforms, *E. coli* O157:H7, and *S. Typhimurium* by 1.6 log CFU/cm<sup>2</sup>, 1.6 log CFU/cm<sup>2</sup>, 1.8 log CFU/cm<sup>2</sup>, and 1.8 log CFU/cm<sup>2</sup>, respectively (King et al., 2005). However King et al. (2005), reported that during the 48 hour chilling period there was a 1.0 log increase in *E. coli* Type I and coliforms, likely due to storage temperatures of 7°C which is within the ideal growth range. Dorsa et al. (1998) collected beef carcass necks post slaughter and treated them with 2% LA, hot water at 70 ± 2°C, or water at 32 ± 2°C in a stainless steel wash cabinet for 15 seconds followed by inoculation with antibiotic-resistant strains of *E. coli* O157:H7 which had viable levels of 2 to 3 log CFU/cm<sup>2</sup>. Samples were ground and packaged in heat sealed bags then stored at 4 or 12°C. Dorsa et al. (1998) found that LA treated samples had no detection of *E. coli* O157:H7 after 3 and 21 days of storage when compared to untreated samples. From this Dorsa et al. (1998) states that the treatment of carcasses post slaughter is an effective decontamination step to the resulting ground beef products.

The greater log reduction of microbial loads is very dependent on the treatment method, application, exposure time, and temperature along with the pathogen itself (Harris et al., 2006). As novel antimicrobials are developed to reduce these pathogens, it is critical to understand their impact on meat quality.

### **Spoilage bacteria**



Spoilage bacteria can come in many forms. Spoilage of meat can be very subjective, however, the main factors associated with spoilage can include, color defects, changes in texture, development of off flavors, off odors, slime, or other organoleptic characteristics that may be considered undesirable for consumption (Doyle, Beuchat, & Montville, 1997). Aerobic and lactic acid bacteria are prevalent within refrigerated meat products. Aerobic bacteria, such as psychrotrophs, grow at 0°C to 40°C and can thrive in the presence or absence of oxygen (Doyle, Beuchat, & Montville, 1997); however, when oxygen is excluded from packaging the spoilage microflora of meat is dominated by lactic acid bacteria (Doyle, Beuchat, & Montville, 1997). The growth rate of bacteria under an anaerobic condition will typically be lower when compared to aerobic conditions. Meat products may be vacuum packaged or packaged in low oxygen permeable packaging to prolong the shelf life of the product. However, with this prolonged shelf life an increase in unfavorable organoleptic changes has been noted (Nassos, King, & Stafford, 1983). The anaerobic environment of vacuum packaged products and other low oxygen packaging enhances the growth of non-proteolytic lactic acid-producing bacteria, relating to the cause of undesirable flavor characteristics (Pierson, Collins-Thompson, & Ordal, 1970). Furthermore, Nassos et al., (1983) reported that coarsely ground beef packaged in low oxygen permeable casing (40 ml of O<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> atm<sup>-1</sup> at 22.8°C) stored at 7°C for 18 days had increased aerobic plate counts (9 x 10<sup>7</sup> bacterial cells per g) and lactic acid bacteria concentrations (800mg/100g). Nassos et al. (1983) also reported that odor acceptability decreased over days of display and from 81 to 24% acceptance as the lactic acid levels increased. Nassos et al. (1983) suggest that lactic acid concentrations of 725 mg/100g of meat would be unacceptable by odor evaluation at least 50% of the time. In a study done by Mancini et al. (2002), ground beef was packaged in chubs and stored at 0°C for six days then packaged in polyvinyl chloride overwrap

at 0°, 4°, and 7°C and placed in retail display for 0, 4, 8, and 12 days. Ground beef was analyzed for aerobic and lactic acid bacteria populations. On day 0 at 0°C there was an increase of aerobic bacteria and lactic acid bacteria, 0.1 and 0.4 log CFU/g, respectively. After 12 days at 7°C the aerobic and lactic acid bacteria counts increased to 7.6 and 6.6 log CFU/g, respectively. Furthermore, Mancini et al. (2002) reported that ground beef chubs stored at 0 and 4°C were similar but lower than when stored at 7°C for aerobic plate counts whereas the lactic acid bacteria numbers increased with each increase in storage temperature. However, on days 0 and 4 the population counts were similar and then decreased after 8 and 12 days of storage. The combination of cold storage followed by cold display will help minimize microbial growth, decrease off odors, and increase shelf life (Mancini et al., 2002). Bacterial counts and the presence of off flavors are two indicators that correspond to spoilage.

### **Current antimicrobial interventions**

*Lactic Acid:* Lactic acid (LA) is a liquid that is mixed with water to produce the desired concentrations prior to being applied to meat surfaces and cannot exceed 5% during processing at 55°C within the meat industry (FSIS Directive 7120.1, 2014). It has been widely used within the meat industry as a control for pathogenic bacteria, shelf life extender, and can be used to enhance and protect meat flavor. Lactic acid is an effective antimicrobial because it is able to reduce pH and lower water activity (Crozier-Dodson, B., Carter, M., and Zuoxing Zheng, Z., 2005). The major bacterial growth inhibitory factor caused by lactic acid is lowering the pH to levels at which bacteria cannot survive (Davidson, Sofos, & Brannen, 2005). Topical spray washes with lactic acid solutions are widely used as post-harvest interventions to reduce bacterial pathogen loads (Carpenter, Smith, and Broadbent 2011). Stivarius, Pohlman, McElyea, and Waldroup (2002) inoculated boneless cow beef trim with *E. coli* (ATCC #11775) to  $10^7$  log

CFU/ml and allowed microbial attachment for 12-14 hours at 4°C, trim was treated with 5% lactic acid in a tumbler for 3 minutes then ground and wrapped the product in polyvinyl chloride overwrap and placed it in retail display for 0, 1, 2, 3, and 7 days. Microbial samples were taken prior to grinding and Stivarius et al. (2002), reported that LA reduced *E. coli* (ATCC #11775) and aerobic plate counts (APC) by 0.66 and 0.64 log CFU/g, respectively compared to a hot water treatment. However, when samples were taken on 0, 1, 2, 3, and 7 days of display there were no difference from day 0 and day 7 for *E. coli* (ATCC #11775) and APC counts. Furthermore Stivarius et al. (2002) suggested that the application of 5% LA solutions onto beef trimmings prior to grinding would reduce *E. coli* to provide an added measure of safety during ground beef production. Similarly, Harris et al. (2012) used 2% and 5% LA treatments on beef trim that was inoculated with  $1 \times 10^5$  log CFU/g of *E. coli* O157:H7 and *Salmonella Typhimurium*. Trim was treated via spray application on a conveyor belt with an exposure time of 10 seconds following grinding, formed into patties, and packaged in vacuum packaged bags and stored at 4°C for 6 or 24 hours. After 6 hours aerobic plate counts (APC) were determined and did not differ between LA treatments and sterile water (4.8 to 5.1 log CFU/g), however at 24 hours the LA treatments decreased APC compared to sterile water treated ground beef (Harris et al., 2012). Furthermore, Harris et al. (2012) reported that the use of 5% LA on ground beef was more effective 6 hours after application and reduced *E. coli* O157:H7 by 0.8 logs compared to sterile water. There was also a significant reduction in *S. Typhimurium* at the 6 and 24 hours from the treatment of 2% and 5% LA when compared to ground beef treated with sterile water. Although there was minimal reduction, Harris et al. (2012) concludes that the use of 2% and 5% LA treatments could reduce *E. coli* O157:H7 and *S. Typhimurium* up to 0.5 and 0.6 log, respectively. According to Dorsa et al. (1998), LA treated beef tissue was significantly lower in

aerobic plate counts (APC) and lactic acid bacteria (LAB) populations when compared to an untreated control and there was no detection of *E. coli* O157:H7 on treated tissue prior to and following the grinding process. This was determined by beef necks which were inoculated with 2 to 3 log CFU/cm<sup>2</sup> of *E. coli* O157:H7 and allowed to dry 24 hours for microbial attachment. Beef necks were treated with 2% LA in a spray cabinet for 15 seconds, then ground and placed into heat sealed bags, stored at 4°C for 7, 14, or 21 days or 12°C for 1, 2, or 3 days. Dorsa et al. (1998), reported *E. coli* O157:H7 was not detected after treatment of 2% LA prior to grinding and *E. coli* O157:H7 was not detected when samples were ground and stored at both temperatures. From the research LA has the ability to reduce populations of aerobic bacteria, lactic acid bacteria, *S. Typhimurium*, *E. coli* species, and *E. coli* O157:H7.

*Peroxyacetic Acid:* Peroxyacetic acid (PAA), also known as peracetic acid, is a colorless liquid with strong oxidizing properties; it is used as a disinfectant and sanitizer within the food and beverage industry and has bactericidal effects. Peroxyacetic acid is produced from the binding of an oxygen molecule from hydrogen peroxide to the carboxyl carbon atom of acetic acid in an aqueous solution (Davidson, Sofos, & Branen, 2005). Peroxyacetic acid is an effective antimicrobial because of its high oxidizing properties that penetrate the cell membrane of organic matter releasing oxygen and causing the disruption of necessary cellular functions. Food Safety Inspection Service Directive 7120.1 (2014), states that no more than 400 ppm of PAA is allowed in processing water used for washing, rinsing, or cooling whole or cut meat including carcasses, parts, trim, and organs. Ellebracht et al. (2005) found that dipping beef trimmings into 200 ppm PAA solutions for 15 seconds reduced *E. coli* O157:H7 and *Salmonella* Typhimurium by 0.6 and 1.01 log CFU/cm<sup>2</sup>, respectively. Similarly Geornaras et al. (2012) reported that dipping beef trim into 200 ppm PAA for 30 seconds reduced initial counts of 3.4 to 3.9 log CFU/cm<sup>2</sup> of *E. coli*

O157:H7 and non-STEC serogroups by 0.6 to 1.0 log CFU/cm<sup>2</sup>. In a study done by Pohlman et al. (2009), beef trim was treated with 0.02% PAA in a meat tumbler for 3 minutes. Beef trim was ground, formed into patties, packaged in polyvinyl chloride overwrap and placed in simulated retail display for 0, 1, 2, 3, and 7 days. On day 0 0.02% PAA displayed greater than 1.0 log CFU/g reduction for *E. coli* (ATCC #11775) and aerobic plate counts compared to untreated ground beef patties (Pohlman et al., 2009). Treating beef trim prior to grinding with PAA reduced *E. coli* (ATCC #25922) counts by approximately 1 log and by day 2 of display there was a reduction of *E. coli* (ATCC #25922) and APC by approximately 2.0 log and 1.7 logs CFU/g, respectively (Mohan et al., 2012). Furthermore Mohan et al. (2012) suggests that treating beef trim prior to grinding can improve ground beef safety and improve shelf life.

### **Novel antimicrobial interventions**

*Electrolyzed oxidizing water:* With the advancement in antimicrobial technology, novel antimicrobials can be implemented as a single intervention method or into multi-hurdle technology systems as a way to reduce microbial load. A newly developed technology is the use of electrolyzed oxidizing water. Electrolyzed oxidizing water (EO) is produced by passing a diluted salt solution through an electrolytic cell having anode and cathode electrodes. It dissociates the salt solution into acidic electrolyzed water and alkaline electrolyzed water. For example, when NaCl is used it dissolves and dissociates into the negatively charged chlorine ( $\text{Cl}^-$ ) and positively charged sodium ( $\text{Na}^+$ ) ions, as well as the formation of hydroxide ( $\text{OH}^-$ ) and hydrogen ( $\text{H}^+$ ) ions. Negatively charged ions move to the anode to produce oxygen gas ( $\text{O}_2$ ), chlorine gas ( $\text{Cl}_2$ ), hypochlorite ions ( $\text{OCl}^-$ ), hypochlorous acid ( $\text{HOCl}$ ), and hydrochloric acid. The positive ions move to the cathode to become hydrogen gas ( $\text{H}_2$ ) and sodium hydroxide ( $\text{NaOH}$ ). The acidic EO has a pH range of 2 to 3 (Izumi, 1999) and an oxidation-reduction

potential of  $> 1,150$  mV along with an active chlorine content of 10 to 90 ppm (Kim, Hung, & Brackett, 2000). Due to federal regulations meat products that are treated with solutions containing chlorine cannot exceed 50 ppm calculated as free available chlorine measured prior to application (FSIS Directive 7120.1., 2014). Free chlorine is the availability of hypochlorous acid (HOCl) and hypochlorite ( $\text{OCl}^-$ ), these ions can be added to water systems as a disinfectant and the allotment in drinking water should not exceed 4.0 mg/L (EPA, 2013). Hung et al. (2010) stated that EO is advantageous to the food industry because it is easy to operate, relatively inexpensive, and environmentally friendly. It is only produced using water and sodium chloride so it does not require specific handling instructions for dangerous chemicals and it can be made on site. Electrolyzed water is a novel intervention that is becoming popular within the food industry. According to Hricova et al. (2008), EO is gaining popularity as a sanitizer in the food industry to reduce or eliminate bacterial populations on food products, food processing surfaces, and non-food contact surfaces. Acidic EO has a strong antimicrobial activity against a variety of microorganisms, yet it is still uncertain as to how it is effective. Parks et al. (2005) suggests that the low pH of acidic EO is believed to reduce the bacterial growth by destroying their outer membrane and allowing HOCl to enter the cell body. Hypochlorous acid has the ability to disrupt important functions of the bacteria cell and is most effective when the pH of the acidic EO is between 4.0 –5.0 (Hricova et al., 2008). Chlorine is the active ingredient within the solution that affects cells in multiple ways; 1) disruption of protein synthesis, 2) oxidizing decarboxylation of amino acids to nitrites and aldehydes, 3) reactions with nucleic acids, purines, and pyrimidines, 4) unbalanced metabolism after destruction of key enzymes, and 5) induction of DNA lesions (Hati et al., 2012). The oxidizing reduction potential (ORP) is another factor that can influence the efficacy of EO water against microorganisms. The ORP of a solution is an indicator of its

ability to reduce or oxidize (Hrivoca et al., 2008), the higher the ORP value the more oxidizing power it has. The oxidation potential of a solution causes the destruction of microorganisms' membranes and in turn shuts down the metabolic process of the cell. Bacteria generally grow in a pH range of 4-9 and the growth of aerobic bacteria is usually at an ORP range of 200 to 800 mV and anaerobic bacteria grow at +200 to +700 mV (Hati et al., 2012). The use of acidic electrolyzed water was the first type of electrolyzed water produced and found to be useful in killing bacteria on raw fish without changing the sensory characteristics (Hati 2012). Huang et al. (2006) reported that the immersion of tilapia in EO achieved a 0.76 and 5.61 log CFU/cm<sup>2</sup> reduction of *E. coli* and *V. parahaemolyticus*, respectively. When salmon fillets were inoculated with *E. coli* O157:H7 and treated with acidic EO water for 2, 4, 8, 16, 32, and 64 minutes at room temperature a reduction of 0.49 log CFU/g at 2 minutes and 1.07 log CFU/g after 64 minutes was realized (Ozer and Demirci 2006). Huang et al. (2006) also found that washing retailers' platforms with 50, 100, and 200 ppm chlorine produced from EO showed a significant reduction in bacterial counts. A disadvantage to acidic EO is that it does not have a very long shelf life. According to Hung et al. (2010), the efficacy of EO deteriorates over a couple of hours because the chlorine gas dissipates; therefore, once produced it needs to be used quickly to ensure effective antimicrobial activity. Another disadvantage is that it is best used when applied as an immersion solution and not spray application. Hung et al. (2010) reported that increasing soaking times for fresh cut produce reduced the bacterial population an additional 0.30 log CFU/g and 0.33 log CFU/g for strawberries and broccoli, respectively. Longer exposure times or higher residual chlorine concentrations for EO achieve additional microbial reductions, but chlorine concentrations cannot exceed 50 ppm within the meat industry. Issa-Zacharia et al. (2010) found that EO with a pH of 5.0-6.5 can effectively reduce populations of *E. coli* and *S.*

*aureus* by more than 5 log CFU/ml when exposed for 90 seconds. Spray applications of EO tend to be less effective because the HOCl is unable to react effectively with the microorganisms on organic matter. The immersion process allows constant contact with HOCl and chlorine ions allowing EO to break down cell membranes and to disrupt the metabolic functions of the cell.

*Levulinic Acid plus Sodium Dodecyl Sulfate:* Levulinic acid plus the addition of sodium dodecyl sulfate (LVASDS) is a newly introduced technology to the meat industry. Levulinic acid is an organic acid that is generally recognized as safe by the U.S. Food and Drug Administration (FDA) for direct addition to food as a flavor additive or adjunct (21 CFR, 172.515). Levulinic acid (LVA) is effective as an antimicrobial because its pKa is relative to that of lactic acid and acetic acid; it contains similar properties as lactic acid in that its effectiveness is in reducing the environment pH to where bacteria cannot survive (Carpenter, Smith, & Broadbent, 2011).

Sodium dodecyl sulfate (SDS) is a surfactant that is labeled as generally recognized as safe by the FDA as a food additive (21 CFR 172.822), SDS is effective against bacteria because it causes the cell to lyse, disrupting the cell membrane, and denatures proteins (Adamowicz, Kelley, & Nickerson, 1990). Recent studies have shown that the combination of levulinic acid (LVA) and sodium dodecyl sulfate (SDS) have proven to be highly effective in reducing pathogens across many mediums. Some research that has been done has evaluated the effects of using LVA and SDS separately. Zhao, Zhao, & Doyle (2009), used 0.1%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, and 3.0% levulinic acid, 0.05%, 0.5%, 1.0%, 1.5%, 2.0% SDS and a combination of 0.3/0.05%, 0.4/0.05%, 0.5/0.05%, 0.5/0.03% levulinic acid plus sodium dodecyl sulfate. Each treatment type was applied to chicken feces, feather, skin, and wings inoculated with *Salmonella Enteritidis*, *Salmonella Typhimurium* DT104, and *E. coli* O157:H7. Zhao, Zhao, & Doyle (2009), reported that the use of 0.5% LVA or 0.5% SDS used separately did not have a large killing effect for *E.*



*coli* O157:H7 or *Salmonella Enteritidis* within 30 minutes at 21°C, however, the combination of 0.5% LVA and 0.05% SDS provided a 7 log reduction of *Salmonella Enteritidis*, *Salmonella Typhimurium* DT104, and *E. coli* O157:H7 within 1 minute. Furthermore, *Salmonella* inoculated chicken skin treated with 0.3% LVA plus 0.05% SDS for 1 minute reduced *Salmonella Enteritidis* by 3.7 log CFU/cm<sup>2</sup>. Low concentrations of LVA plus SDS did not have a great effect on chicken feces in water. Zhao, Zhao, & Doyle (2009) reported that the use of 3% LVA and 2% SDS, used separately, greatly reduced *Salmonella* from chicken feces to undetectable levels (>7 log reduction) within 2 minutes of exposure. Lastly Zhao, Zhao, & Doyle (2009), reported the treatments of 2% LVA plus 1% SDS and 3% LVA plus 1% SDS reduced *Salmonella Enteritidis* populations on chicken wings by 2.6 and 4.0 log CFU/g, respectively. Carpenter, Smith, & Broadbent (2011) applied 2% LVA at temperatures of 55.4, 68.3, and 76.7°C to beef plates inoculated with *E. coli* O157:H7 and recovered counts of 5.45, 5.38, 5.34, 5.03 log CFU/g, respectively, and reported that the reduction was not significant and appeared to have no advantages compared to the industry standards lactic and acetic acids. However, Stelzleni, Ponrajan, & Harrison (2013) reported that the combination of 1.0% LVA plus 0.1% sodium dodecyl sulfate (SDS) applied to beef trim prior to grinding reduced *Salmonella* populations by 0.17 to 0.36 logs, although this is not a significant reduction, further research evaluating the use of higher concentration of LVA and SDS could result in greater log reduction. However, Zhao et al. (2014) reported the use of 3% LVA plus 2% SDS when applied by spray application on inoculated beef trim for 1 to 5 minutes reduced *E. coli* O157:H7 by 1.5 log CFU/cm<sup>2</sup>.

### **Effects of interventions on quality and sensory characteristics of ground beef**

Although pathogen interventions may prove beneficial from an antimicrobial perspective, it is also important to understand their effects on the quality and sensory characteristics of

ground beef. For instance, consumers base their buying decision of ground beef primarily from the color (Mancini & Hunt, 2005). Ground beef color is affected by cut surfaces, microbial growth, lipid oxidation, pathogen interventions, and many other endogenous and exogenous factors. The meat industry is currently evaluating a variety of antimicrobial solutions that are effective at reducing pathogenic microorganisms in meat as well as not having adverse effects on the sensory and color properties (Quilo et al., 2009).

### **Color attributes**

As stated prior, color is an important factor for consumers when purchasing meat products (Mancini & Hunt, 2005). Myoglobin is the main protein related to the color of meat. When exposed to or repressed from oxygen it can turn color from red, purple, or brown (Mancini & Hunt, 2005). When meat is exposed to oxygen, oxygenation occurs; it develops a bright cherry red color due to the reaction of oxygen and myoglobin (Mancini & Hunt, 2005). Consumers relate the bright cherry red color to freshness and high quality.

*Commission Internationale de l'Eclairage (CIE) L\*, a\*, b\**: The use of instrumentation to measure meat color is highly recognized. The most widely used measurements are Commission Internationale de l'Eclairage (CIE) L\*, a\*, and b\*. CIE L\* is used to measure the lightness and darkness while a\* is a measure of red to green, and b\* is a measure of yellow to blue. The CIE L\*, a\*, and b\* can also be used to evaluate the change of color over time ( $\Delta E$ ;  $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$ ) as well as the saturation index (vividness of products;  $(a^{*2} + b^{*2})^{0.5}$ ) and hue angle ( $\tan^{-1}(b^*/a^*)$ ). Another measure of redness is the reflectance ratio (630/580 nm), this is a measure of redness due to either the amount of oxymyoglobin or deoxymyoglobin; as the ratio reaches or becomes closer to 1 the product has more formation of metmyoglobin (AMSA, 2012).

Stivarius et al. (2002) reported using 5% LA prior to grinding maintained color stability of ground beef packaged in polyvinyl chloride overwrap during refrigerated retail display. Lactic acid (5%) treated ground beef was lighter in color (CIE L\*) and less red (630/580 nm) compared to ground beef treated with hot water (82°C). When evaluating days of display, Stivarius et al. (2002), reported that LA treated ground beef was less red (a\*) and less vivid (saturation index) on day 0, 1 and 7 compared to untreated ground beef. Harris et al. (2012) treated ground beef patties with 2% and 5% LA and evaluated that the CIE  $a^*$  values which did not differ after 6 hours, however, there was a significant change in redness after 24 hour compared to the two concentrations of LA and ground beef patties treated with sterile water. Additionally, patties treated with 2% LA were more red compared to those treated with 5% LA and the control patties. Patties treated with 2% LA were more uniform in color on day 1 of display compared to day 2 and 3 which did not differ (Harris et al., 2012). Jimenez-Villarreal et al. (2003c) treated beef trim with 2% LA then ground the trim and packaged it in polyvinyl chloride overwrap and placed the packages in simulated retail display for 0, 1, 2, 3, and 7 days. It was reported that LA treated patties were lighter in color (CIE L\*) and there was no difference in hue angle compared to untreated ground beef patties.

Pohlman et al. (2009) applied 200 ppm PAA to beef trim prior to grinding then packaged patties in polyvinyl chloride overwrap, and placed them in retail display for 0, 1, 2, 3, and 7 days. They reported that patties from trim treated with 200 ppm PAA had the largest L\* value compared to those treated with acidified sodium chlorite but were similar to untreated patties. Mohan et al. (2012) reported that ground beef treated with 200 ppm PAA had a lower hue angle value indicating less brown discoloration and greater CIE  $a^*$  and reflectance ratio (630 nm/580 nm) indicating that PAA treated ground beef patties had less redness compared to untreated

ground beef patties. Quilo et al. (2009b) reported that the use of 200 ppm PAA resulted in a greater CIE  $L^*$  values which means the product was lightest in color and had greater oxymyoglobin proportions compared to the untreated ground beef patties. Furthermore 200 ppm PAA treated patties were more vivid compared to the untreated ground beef patties on day 1 through 7 of display (Quilo et al., 2009a).

Being a novel intervention, there has been little research on levulinic acid plus sodium dodecyl sulfate (LVASDS) and its potential effects on quality characteristics in beef. A study done by Stelzleni et al. (2013) reported that 1.0% LVA plus 0.1% SDS caused ground beef patties to be less red as indicated by  $a^*$  and had an increased hue angle when compared to untreated ground beef patties after day 3 of display. The 630/580 ratio was also lower for LVASDS on day 3 of display, meaning it was less red compared to untreated ground beef patties.

*Subjective color:* There are two types of panelists used to evaluate meat color. The first are “consumer panelists” which are recruited within a predefined demographic and are given basic information about a study that is required to complete results (AMSA, 2012). The second type is a “trained descriptive visual color panel”, these panelists are screened and trained for their ability to discern color. These panelists must score a 50 or less on the Farnsworth-Munsell 100-Hue test in order to be successful panelists and then further training is done so the panelists will provide accurate and repeatable data, preliminary trials are usually preformed to make adjustments (AMSA, 2012). Panelists are trained based on the requirements of the specific research project. Color scales are used and adapted from the American Meat Science Association: Meat Color Measurement Guidelines (AMSA, 2012).

According to John et al. (2004), ground beef that was vacuum packaged received low visual scores because of surface browning after 14 and 21 days of storage at 2°C. Rahman et al.

(2012) reported that acidic EO water and the residual NaCl content within the solution caused chicken breasts to maintain a fresher color and scored better in sensory scores after 7 days of storage compared to untreated chicken breasts. Furthermore, Rahman et al. (2012) suggests that the EO water treatment can improve the sensory qualities and extend the shelf life of chicken meat during storage at 5°C. The use of EO water on ground beef and red meat quality has not been fully studied. Stivarius et al. (2002) reported the application of 5% LA on PVC packaged ground beef patties did not affect panelists' evaluation for worst point color, beef overall color, and percentage discoloration when compared to a hot water rinse (82°C). However when beef trim was vacuum tumbled with the application of 5% LA, ground, and overwrapped with PVC panelists found the 5% LA treated patties to have a higher bright purple red overall score and lower worst point color, as well as a lower percent discoloration compared to untreated ground beef patties (Stivarius et al., 2012) this suggests that LA treatment of 5% can be used to maintain color stability of ground beef on retail display. Likewise Harris et al. (2012) reported that panelists found no difference in beef color, percent discoloration or browning among 2% and 5% LA ground beef patties in simulated retail display compared to patties treated with sterile water. Pohlman et al. (2009) treated beef trim with 200 ppm PAA had a lower overall color score indicating less red color than untreated ground beef patties. Furthermore, PAA treated patties' worst point color was more red on day 2 of display compared to untreated patties and both had the same scores for browning by day 7 according to trained panelists (Pohlman et al., 2009). Evaluating the effects of LVASDS, trained panelists scored the LVASDS as having a prominent decrease in overall color and increase in discoloration as well as having the darkest worst point color score by day 3 of display (Stelzleni et al., 2013). More research is needed to fully understand the quality effects that LVASDS may have on ground beef and other meat products.

## **Lipid oxidation**

Lipid oxidation is the oxidative degradation of lipids within meat. The initiation of lipid oxidation occurs when a labile hydrogen atom is removed from the fatty acyl chain, causing free radical production, which quickly reacts with oxygen forming a peroxyradical (Ladikos & Lougovois, 1990). The rate of lipid oxidation development is influenced by the lipid depot (type of fatty acids present), diet, processing and storage conditions, and added ingredients; furthermore, lipid oxidation can cause off-flavor, off-odors, and color deterioration (Ladikos & Lougovois, 1990). Lipid oxidation can be measured using the thiobarbituric acid reactive substance analysis (TBARS) to quantify lipid hydroperoxides and aldehyde compounds in products due to oxidative stress. Values are typically reported in milligrams of malonaldehyde per kilogram of meat (Oxford, 2012). The sensory acceptability for lipid oxidation is below the threshold of 2.0 mg/kg of malonaldehyde, values above this threshold have been related to rancidity (Campo et al., 2006). A study done by Rahman et al. (2012) dipped chicken breasts in slightly acid low chlorine concentration electrolyzed water (SlALcEW; 10 mg/L of available chlorine) and strong acidic electrolyzed water (StAEW; 50 mg/L of residual chlorine) for 10 minutes at room temperature (22°C) and then packaged the breasts in polyethylene terephthalate containers stored at 5°C. The SlALcEW and StAEW increased lipid stability after 4 days of storage compared to the untreated chicken breast that reached 3.2 mg of malonaldehyde/ kg of meat (Rahman et al., 2012). Conversely, when low concentration electrolyzed water (LcEW; 10 mg/L of available chlorine) and strong acidic electrolyzed water (SaEW; 50 mg/L of residual chlorine) were used on fresh pork packaged in polyethylene air permeable bags and stored at 4°C for 12 days, LcEW and SaEW extended lipid shelf life up to 6 day, which was similar to the untreated pork samples, however after 8 to 12 day of storage TBARS values increased rapidly in

untreated pork samples (5.6 mg of MA/kg of meat by day 12; Rahman et al., 2013). Furthermore, 2% LA treated patties, wrapped in polyvinyl chloride overwrap, were evaluated on 0, 1, 2, 3 and 7 days of display and Jimenez-Villarreal et al. (2003c & 2003d) reported no difference in lipid oxidation values when compared to the untreated ground beef patties. According to Ellebracht et al. (1999) when beef trim was manufactured into ground beef and stored in chubs up to 42 days there was no difference in lipid oxidation values between trim treated with 2% LA and the untreated samples. Jimenez-Villarreal et al (2003a & 2003b) & Quilo et al. (2009a & 2009b) reported that PAA treated ground beef patties also had lower levels of lipid oxidation compared to untreated ground beef patties through 7 days of retail storage (Quilo et al., 2009b). Additionally, beef patties treated with 1.0% levulinic acid plus 0.1% sodium dodecyl sulfate were similar in lipid oxidation values compared to untreated control patties but by day 3 both products had reached the threshold of 2 mg of MDA/kg (Stelzleni et al., 2013).

### **Sensory and texture characteristics**

Antimicrobials can also affect the sensory and texture characteristics of ground beef products. The pH of ground beef is typically between 5.5 – 5.7 (Gill & Newton, 1982 & Doyle, Beuchat, & Montville, 1997). Water-holding capacity is the ability of meat to retain the water that naturally occurs within muscle proteins or water that may be added to meat products during the use of external force; this possible loss in water can affect color, texture, firmness, juiciness, and the bind of cooked meat (Aberle et al., 2012). When an acidic antimicrobial is used to treat meat it typically causes a drop in pH, and brings meat proteins closer to their isoelectric point. The isoelectric point of meat is 5.0-5.2, this is the point at which the positive and negative charges are relatively equal (Aberle et al., 2012). Additionally, the positive and negative charges are attracted to each other and bind tightly causing the muscle proteins to be more compact and

in turn having less capacity to hold and bind water (Aberle et al., 2012). This relates to the characteristics of sensory and texture for meat products. Therefore, the decrease in pH due to the addition of antimicrobials can cause the proteins to bind tighter causing water-holding capacity to decrease and reducing the sensory quality of raw and cooked ground beef. The decline in pH of the meat product can cause the amount of purge to increase while on retail shelves and influence thaw loss prior to cooking to increase, both of which could affect tenderness, juiciness, flavor, and texture. In a study conducted by Jimenez-Villarreal et al. (2003c) taste panelists were unable to detect off flavors in beef patties treated with 2% LA but these patties had lower bind compared to untreated patties. The sensory bind ratings of this study were further certified when shear force tests showed that LA patties required less peak force to shear (Jimenez-Villarreal et al., 2003c). Evaluating the application of 200 ppm PAA to patties, Quilo et al. (2003a) reported that PAA did not affect the pH of the meat when compared to the control, but was lower than other treatments. Quilo et al. (2003a) further suggested that lower pH contributed to color differences which could be related to increase in purge that were noted through 7 days of display. Contrarily, when beef trim was immersed into 200 ppm PAA for 30 seconds it did not cause a change to the pH when compared to untreated beef trim (Geornaras et al., 2012). The lowering of pH due to LA and PAA has been suggested to increase the percent cook loss, causing the product to have less juiciness and overall bind scores (Jimenez-Villarreal et al. 2003c & Quilo et al. 2003a). Additionally, patties treated with 1.0% levulinic acid plus 0.1% sodium dodecyl sulfate had lower pH values, more moisture loss during cooking, and had less bind than untreated patties (Stelzleni et al., 2013).



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### **CHAPTER 3**

## **THE EFFECTS OF ANTIMICROBIALS ON THE SHELF LIFE CHARACTERISTICS OF GROUND BEEF WHEN MANUFACTURED FROM BENCH TRIM AT THE RETAIL LEVEL<sup>1</sup>**

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<sup>1</sup> Belanger, S.E. and A.M. Stelzleni. To be submitted to *Meat Science*.

## Abstract

The objective of this study was to evaluate the effects of two novel antimicrobials on ground beef produced at the retail level on shelf life and quality characteristics. Beef trim was treated with either 50 ppm Cl from electrolyzed oxidizing water (EO), 4.5% lactic acid (LA), 200 ppm peroxyacetic acid, 2.0/0.2% levulinic acid plus sodium dodecyl sulfate (LVASDS), or left untreated (CON). Ground beef patties were formed, packaged and placed in retail display wrapped in polyvinyl chloride overwrap (PVC) for 0, 1, 2, 3, 4, or 5 d. All treatments decreased in redness over days of display. The LVASDS patties displayed less total color change ( $\Delta E$ ) than EO, LA, and PAA ( $P < 0.05$ ). The use of LVASDS maintained lower aerobic plate count populations compared to PAA ( $P < 0.05$ ). Lipid oxidation values were higher for EO and LA compared to CON or PAA patties ( $P < 0.05$ ). These results suggest that LVASDS could be used as an antimicrobial for bench trim packaged in PVC without negatively affecting shelf life.

Keywords: antimicrobials, color, ground beef, shelf life



## Introduction

More than 50% of beef consumed in the United States is of the ground variety (NCBA, 2002). The need to uphold safety and quality is important to producers and consumers. Consumers are becoming more aware of food safety issues and processes that are used to produce products such as ground beef (Stelzleni et al., 2013). Intact muscle is inherently sterile, however in the case of trim the interior is exposed which can facilitate contamination (Belk, 2001). *Escherichia coli* O157:H7, which is listed as an adulterant, is of primary concern during ground beef production. Furthermore, in 2011 six additional shiga toxin-producing *E. coli* (STEC) serogroups were added to the adulterant list; O26, O103, O45, O111, O121, and O145 (FSIS-USDA, 2011). Although these new STECs do not exhibit the same clinical signs as *E. coli* O157:H7 they are still responsible for about 1/3 of *Enterohaemorrhagic Escherichia coli* (EHEC) cases in humans (Koohmaraie et al., 2005).

Ground beef from bench trim is produced from a combination of lean and fat sources that are obtained from a variety of primals and subprimals at a location other than where it was originally produced, such as a retail market. This type of ground beef is then typically packaged in Styrofoam trays with polyvinyl chloride overwrap (PVC) for display. Recently new regulations have been proposed to enhance record keeping and control ground beef products (FSIS-USDA, 2014) at the retail level due to food safety concerns. With the new regulations there may be interest at the retail level to incorporate interventions to beef trim prior to grinding. Previous research has shown lactic acid and peroxyacetic acid to be effective at reducing contamination (Dorsa et al., 1998, King et al., 2004, & Harris et al., 2012) and could be easily be incorporated into the retail setting. However, it is also important to evaluate novel antimicrobials as advancements in technology occur.

Two novel antimicrobials that show promise for use in meat systems include acidic electrolyzed oxidizing (AEO) water and levulinic acid (LA) plus sodium dodecyl sulfate (SDS; LA+SDS). Acidic electrolyzed oxidizing water is produced from a sodium chloride solution that passes through an electrolytic cell (Jadeja & Hung, 2013). The production of acidic electrolyzed oxidizing water has a pH range of 2 to 3 (Izumi, 1999) and an oxidation-reduction potential of > 1,150 mV with an active chlorine content of 10 to 90 ppm (Kim, Hung, & Brackett, 2000). Due to federal regulations chlorine content cannot exceed 50 ppm when applied to meat products (FSIS Directive 7120.1 Rev. 22, 2014). However electrolyzed water is effective at reducing microbial growth because of its low pH and chlorine content (Parks et al., 2005 & Hricova et al., 2008). Huang et al. (2006) reported the emersion of tilapia in electrolyzed oxidizing water for 10 seconds achieved a 0.76 log CFU/cm<sup>2</sup> in *E. coli* O157:H7 and Kalchayanand et al. (2008) reported a 0.5 log reduction of *E. coli* O157:H7 on beef heads. Levulinic acid and sodium dodecyl sulfate are generally recognized as safe (GRAS; 21 CFR 172.515 and 21 CFR 172.822, respectively). Levulinic acid lowers the pH of the environment similar to that of lactic acid (Carpenter, Smith, & Broadbent, 2011) and SDS causes cells to lyse and denature proteins (Adamowicz, Kelley, & Nickerson, 1990). According to Zhao, Zhao, & Doyle (2009) the use of 0.5% levulinic acid plus 0.05% sodium dodecyl sulfate achieved a 7 log reduction of *E. coli* O157:H7 when applied to bacterial suspension. Furthermore, Zhao et al. (2014) reported the use of 3% levulinic acid plus 2% sodium dodecyl sulfate reduced *E. coli* O157:H7 by 1.5 log CFU/cm<sup>2</sup> when applied to inoculated beef trim, this reduction is lower even with the use of higher concentration because of the reaction of the proteins from the organic substance it was applied to

It is important to provide a safe, unadulterated, and wholesome product for consumers and research has shown that antimicrobials applied to beef trim can be effective at reducing or eliminating contamination. As new antimicrobials are being evaluated for use on consumer ready product, it is also important to ensure that they are not detrimental to meat quality and shelf life. Therefore, the objective of this study was to determine the effects of two novel antimicrobials when applied to bench trim before grinding on shelf life characteristics when compared to two industry standards.

## **Methods and Materials**

### **Preparation of antimicrobials**

Antimicrobial treatments included 50 ppm chlorine generated from acidic electrolyzed oxidizing water (EO), 4.5% lactic acid (LA; Birko Inc., Henderson, CO), 200 ppm peroxyacetic acid (PAA; EnviroTech, Modesto, CA), 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate (LVASDS; Sigma-Aldrich Corp., St Louis, MO. Acros Organics, Thermo Fisher Scientific, New Jersey, USA), and an untreated control (CON). For the production of electrolyzed oxidizing water the methods of Waters and Hung (2013) were followed. Briefly, a 12% food grade sodium chloride solution (Avantor Performance Materials Inc. Center Valley, PA) was prepared and made with the ROX-20TA electrolyzed water generator (Hoshizaki Electric Company Ltd., Toyooka, Aichi, Japan). The acidic phase was collected. The resulting acidic water had an average chlorine content 86.3 ppm of free chlorine which was diluted using deionized water to 50 ppm chlorine (Model 16900 Hach Digital Titration Kit, Hach Company, Loveland, CO.) and was brought to a final pH of 6.0-6.2 using NaOH.

### **Ground beef preparation and packaging**

Whole boneless chuck rolls (Arko Veal Co. Forest Park, GA) were processed into beef trim (85/15) to ensure known source and slaughter date. Beef trim was then combined and 15 kg of trim was randomly separated into 5 treatment bins. Bins were then randomly assigned to a treatment. The beef trim was placed into a spray cabinet conveyor and the treatment was applied by a 365° six nozzle sanitizing spray cabinet (Birko Chad Co., Olathe, KS.) to ensure each piece of trim received the treatment. The conveyor exposed trim to the interventions for 24 s at a flow rate of 0.55 L/min•nozzle<sup>-1</sup> and 290 kPa. After each treatment application the beef trim was ground (AFMG 50, Danials Food Equipment, Parkers Prairie, MN) through a 12.7-mm plate followed by a 6.4-mm plate. Approximately 90 patties (150 ± 2g, 13 mm thick) were produced (Patti-O-Matic Protégé, Farmingdale, NJ) for each treatment replicate combination. For aerobic shelf life analysis, thirty of the 90 patties per treatment were randomly selected, weighed, and packaged in white Styrofoam S2 trays (Koch, Kansas City, MO) with an absorbent pad (4.75" x 7" Dri-Loc, Sealed Air Cryovac, Duncan, SC.) and covered with oxygen permeable polyvinyl chloride (PVC) overwrap film (O<sub>2</sub> transmission = 23,250 ml/m<sup>2</sup>/24h, 72 gauge; Pro Pack Group, Oakland, NJ). The 30 patties were then randomly assigned to 0, 1, 2, 3, 4, and 5 d (5 patties/treatment•day<sup>-1</sup>) of retail display. Patties were placed in coffin style retail display cases (Husmann Corporation, Bridgeton, MO.) at 2 ± 2 °C (T&A Thermo Recorders TR-52i, Japan) under 24 h warm white fluorescent lighting (1851lx; Osram Sylvania, Danvers, MA.) for their respective shelf life days. All equipment was cleaned and sanitized between each application treatment to ensure there was no mixing of treatments. These procedures were followed for a total of 3 replicates.

### **Objective and subjective color**

Objective CIE  $L^*$ ,  $a^*$ ,  $b^*$  was measured on d 0, 1, 2, 3, 4, and 5 for each ground beef patty using a Hunter-Lab Miniscan XE Plus (Model 45, Hunter Associates Laboratory, Inc., Reston, WV). The colorimeter was standardized using white and black tiles and a saturated red tile was used as a physical standard. Samples were read using illuminant A/10° with a 2.54 cm aperture. Hue angle,  $(\tan^{-1}(b^*/a^*))$  and saturation index,  $[(a^{*2} + b^{*2})^{0.5}]$  were calculated (AMSA 2012) along with the reflectance ratio of 630/580 nm which was used to estimate the amount of redness due to either oxymyoglobin or deoxymyoglobin. Delta E was also calculated to determine the color change from day 0 to 5:  $[(L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2}$ . Three color readings were taken for each patty and the average was recorded by the colorimeter.

A 7 member trained color panel (AMSA, 2012) recorded subjective color on d 0, 1, 2, 3, 4, and 5 of retail display for initial color (8 = dark red, 7 = moderately dark red, 6 = slightly dark red, 5 = bright red, 4 = slightly bright red, 3 = light red, 2 = moderately light red, and 1 = very light red), product discoloration (8 = tan to brown, 7 = dark red to tan, 6 = dark red to tannish red, 5 = moderately dark red, 4 = slightly dark red, 3 = dull red, 2 = bright red, and 1 = very bright red) and percent surface discoloration (7 = 96 – 100%, 6 = 80 – 95%, 5 = 60 – 18%, 4 = 40 – 59%, 3 = 20 – 39%, 2 = 5 – 20%, 1 = 0 – 4%) adapted from AMSA (2012). All panelist recorded <50 for the total error score on the Farnsworth-Munsell 100 Hue Test (Xrite, Grandville, MI).

### **pH and percent purge**

On d 0, 1, 2, 3, 4, and 5 ground beef patties samples were removed from the retail packaging and were weighed to calculate purge loss. The pH was determined by homogenizing a 10 g sample from each patty in 100 mL of deionized water for 30 s and the pH (pH 11 series pH/mV/°C meter, Oakton Instruments, Vernon Hills, IL) was recorded.

## **Microbiology**

After post display weight and pH samples were collected, a 25 g sample of the ground beef patty was aseptically removed and placed in a sterile stomacher bag. Two hundred and twenty-five milliliters of 0.1% Buffered peptone water (Difco Laboratories, Detroit, MI) was added to the sample and stomached (Stomacher 400 Circulator, Seward Ltd.) for 2 min at 230 rpm. Serial dilutions were made for all samples using 9 ml of 0.1% peptone (Difco Laboratories, Detroit, MI) and the dilutions were plated onto Aerobic Count Plate Petrifilm (3M Corp., St. Paul, MN) in duplicate. Plated samples were incubated at 37°C in an incubation chamber (Thelco, Ontario, Canada) for  $48 \pm 2$  h. Psychotrophic bacteria counts were expressed as CFU/g. Remaining samples were vacuum packaged and froze for further analysis.

## **Lipid Oxidation**

Ground beef patties from each sample day of retail display were thawed in vacuum bags overnight in a cold room (4°C) to be used for thiobarbituric acid reactive substance analysis (TBARS), adopted from Ahn et al. (1998). After thawing the patty was thoroughly mixed and a 5 g sample was collected for analysis. The sample was placed into a 50 ml conical centrifuge tube and homogenized (Tissumizer Mark II, Tekmar Company, Cincinnati, OH) with 15 ml of deionized water for 30 s. The tubes were centrifuged at  $1850 \times g$  for 10 min at 24°C (CR 312, Jouan Inc., Winchester, VA). Two milliliters of the supernatant was removed and placed into disposable glass test tubes (13 x 100 mm) in duplicate. One hundred microliters of butylated hydroxyl toluene (7.2%) and 4 ml of thiobarbituric acid/trichloroacetic acid were added to the supernatant. All tubes were vortexed for 5 s and placed into a hot water bath (90°C) for 15 min then allowed to cool in a room temperature (24°C) water bath for 10 min. Samples were then centrifuged at  $3000 \times g$  for 15 min at 24°C. Patty lipid oxidation was measured as a function of

malonaldehyde (MDA) per kg of meat via spectrophotometric readings at 531 nm (DU Series 600, Beckman Instruments, Brea, Ca. V-630, Jasco Analytical Instruments, Easton, MD).

Malonaldehyde concentration was calculated by comparison to known standards.

### **Statistical Analysis**

Sample size was calculated according to the methods of Montgomery (2001) and Ferris, Grubs, and Weaver (1956) to guard against probability of making a Type II error. This research was conducted as a completely randomized design. Data was analyzed using the Mixed Procedures of SAS (V.9.1 SAS Inst. Inc., Carry, NC). When a treatment by day of display interaction occurred, data was reanalyzed by day. For shelf life data, the model included the fixed effects of treatment and day of display. Patty within treatment by rep was included as the random variable. Least squares means were generated and means were separated using the PDIFF option. Differences were considered significant at  $\alpha < 0.05$ .

## **Results and Discussion**

### **Shelf life characteristics**

#### **pH and purge**

There was not treatment by day interaction ( $P > 0.05$ ) for shelf life pH (Figure 3.1) or purge (Figure 3.2). Therefore the main effects are shown in Table 3.1 and Table 3.2 for treatment and day of display, respectively. Levulinic acid plus sodium dodecyl sulfate (LVASDS) treated patties had a greater pH than LA ( $P < 0.05$ ) but were lower than CON, EO, and PAA ( $P < 0.05$ ). Percent purge for LVASDS treated patties followed the same trend as pH. Polyvinyl chlorine overwrap patty pH decreased as time on display increased and percent purge increased as time on display increased. This increase in percent purge and decrease in pH for LVASDS and LA patties is related to the antimicrobial. The low pH resulting from the treatment is beneficial in reducing the microbial environment however (Crozier-Bodson, B., Cater, M., and Zuoxing

Zheng, Z., 2005); the low pH has detrimental effects on the quality of meat proteins and water holding capacity (Quilo et al., 2003a). As time on display increased percent purge increased, this suggests that the proteins were approaching closer to the isoelectric point (5.1-5.2) and losing the ability to hold water. Quilo et al. (2003a) found similar results in with the use of 200 ppm PAA ground beef patties were similar in pH to untreated patties and purge increased as days of display increased.

### **Objective and subjective color**

There was a treatment by day interaction ( $P < 0.05$ ) for all objective color characteristics (Figure 3.3). For CIE  $L^*$  the only differences that occurred between treatments were on d 0. Lactic acid treated patties were lighter in color (CIE  $L^*$ ) compared CON, PAA, and LVASDS ( $P < 0.05$ ) and EO was similar ( $P > 0.05$ ) to all treatments. Redness as indicated by CIE  $a^*$  decreased as time on display increased ( $P < 0.05$ ) with difference among treatments being evident on d 0, where LA, EO, and PAA were more red than the CON ( $P < 0.05$ ). There were no differences among treatments for d 1, 2, and 3. After d 4 of display EO treated patties were less red than LA and LVASDS ( $P < 0.05$ ) and LVASDS, LA, and CON patties were all similar ( $P > 0.05$ ). After 5 d of display, CON, EO, and LVASDS were less red than PAA ( $P < 0.05$ ). Similar to CIE  $L^*$  there were differences within treatments for CIE  $b^*$  on d 0. Lactic acid treated patties had more yellow characteristics than did CON, EO, and LVASDS ( $P < 0.05$ ) but similar to PAA ( $P > 0.05$ ). Additionally, CIE  $b^*$  decreased as time on display increased, however, there were no treatment within day differences for d 1 through 5.

Additional measures of redness, including reflectance ratio (630/580 nm) and hue angle show that all patties become less red as time on display increased, similar to the trend of CIE  $a^*$ . There were no differences on d 0 for 630/580 and hue angle among treatments ( $P > 0.05$ ; Figure



3.4). All treatments became less red as time on display increased. Differences within treatment occurred on d 1 where LA was less red than CON and LVASDS ( $P < 0.05$ ). There were no differences among treatments on d 2. By d 3 EO, LA, PAA were less red than the CON ( $P < 0.05$ ) and patties treated with LVASDS were similar to EO and CON ( $P > 0.05$ ). On d 4, EO was less red than CON or LVASDS ( $P < 0.05$ ) and LVASDS were redder than LA and PAA ( $P < 0.05$ ). By d 5 there was a jump in LVASDS hue angle and it became less red than LA and PAA ( $P < 0.05$ ) but was similar to EO and CON ( $P > 0.05$ ). There was a treatment by day interaction for saturation index. Patties treated with LA were more vivid than EO, LVASDS, and CON on d 0 ( $P < 0.05$ ). There were no differences within treatment on d 1, 2, and 3; however vividness decreased over time of display. On d 4, LA and LVASDS were more vivid than EO ( $P < 0.05$ ) however by d 5 PAA was more vivid than EO and CON ( $P < 0.05$ ). There was an increase in total color change ( $\Delta E$ ) for all samples as time on display increased. Within day there were no treatment differences for d 1 or 2. However, by d 3 and 4 LVASDS had less total color change than EO, LA, and PAA ( $P < 0.05$ ) and was similar to CON ( $P > 0.05$ ). After 5 days of display total color change among all treatments were similar again.

There was a treatment by day interaction for subjective color (Figure 3.5). On d 0 all patties were similar and averaged between bright red and slightly bright red. As time on display increased patties became darker in color for all treatments. However on d 1 through 5, LA maintained a brighter red color than the CON ( $P < 0.05$ ). Additionally, LVASDS maintained a brighter red color, compared to the CON on d 2 through 5 ( $P < 0.05$ ). After 5 days of display subjective panelists scored CON treated patties to be darker red than all other treatments ( $P < 0.05$ ). Discoloration and percent discoloration increased as time on display increased. Through 4 d of display there was a trend for EO and PAA treated patties, which were less red and showed

greater discoloration than LVASDS ( $P < 0.05$ ). By d 5 CON and EO were similar ( $P > 0.05$ ) and were less red and had more overall percent discoloration than did PAA and LA ( $P < 0.05$ ). For the use of LA on PVC patties, Jimenez-Villarreal et al. (2003d) reported that LA patties were less red than that of patties treated with 200 ppm chlorine dioxide. The current research suggests that LA patties were redder compared to EO water. Quilo et al. (2009a) reported similar results in that ground beef patties wrapped in PVC and treated with 200 ppm PAA had more yellow (CIE  $b^*$ ) and more red (CIE  $a^*$ ) than untreated control patties.

### **Lipid oxidation**

For lipid oxidation there was a treatment by day interaction (Figure 3.6). Overall lipid oxidation increased as time on display increased. After d 2 of display EO and LA treated patties exhibited more lipid oxidation than did CON or PAA treated patties ( $P < 0.05$ ). However all treatments regardless of day were below 2.0 mg/kg of malonaldehyde, which has been considered the threshold value for sensory acceptability (Campo et al., 2006). However, these results for PVC patties disagree with Quilo et al. (2009b) who reported 200 ppm PAA treated patties had lower lipid oxidation values compared to control patties, the current research shows that CON and PAA were similar across days of display.

### **Microbial analysis**

For psychotropic bacteria counts there was a treatment by day interaction (Figure 3.7). Within d 0 and 1 all treatments were similar to each other ( $P > 0.05$ ). After d 2 of display EO and LA had fewer APC populations (log CFU/g) than did PAA ( $P < 0.05$ ). After d 3, 4, and 5 LA and LVASDS treated patties were lower than EO and PAA ( $P < 0.05$ ). Although we did what we could to control the age and source of product, the handling of the product was unknown. All product started with an initial count of 3.4 log CFU, by the time 3 d of shelf life was reached,

EO, CON and PAA were reaching 7.0 log CFU/g which has been reported as the spoilage threshold (ICMSF, 1986). However, LA treated patties maintained APC populations below 6.0 log CFU/g. The shelf life of PVC packaged ground beef is typically 3 days, this short shelf life is due to the grinding process that allows the addition of oxygen to contact the meat surface (Brooks, 2007). Although this allows the meat to become a brighter red color it does also increase the surface area for spoilage to occur (Brooks, 2007). For the retail level it is important to understand ways to increase the shelf life and longevity of ground beef. This data demonstrates that the use of LVASDS may extend the shelf life of ground beef from an aerobic plate count standpoint compared to EO, PAA, or untreated ground beef, and provide an alternative to the use of lactic acid, especially through 3 d of retail display.

### **Conclusion**

The shelf life of products has increased due to the use of technologies and innovations in packaging and antimicrobials. Retail stores have used carcass primals and subprimals to produce in store ground beef to be packaged in Styrofoam trays wrapped in polyvinyl chloride. Consumers are very concerned with the safety and quality of products they purchase. It is important to provide and uphold quality products. With the procedures at retail level it is important to extend the shelf life of ground beef while maintaining color and reducing contamination and spoilage. Currently bench trim manufactured into ground beef has a typical shelf life of 3 d, applying an antimicrobial at the retail level may extend a product's life span. The use of levulinic acid plus sodium dodecyl sulfate can improve the color characteristics and reduce aerobic bacteria counts if applied in the retail store prior to grinding. Furthermore, the levulinic acid plus sodium dodecyl sulfate at the levels applied in the current research may be used as an alternative to industry standards for PVC package ground beef without negatively

affecting ground beef shelf life. However, further research is needed in order to fully understand these effect if the two novel antimicrobials are applied on bench trim at the retail level.

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Table 3.1. Least squares means for the main effect of antimicrobial treatments on pH and percent purge of polyvinyl chloride overwrapped ground beef patties through 5 days of retail display.

| Traits   | Treatments <sup>1</sup> |                   |                   |                   |                   | SEM <sup>2</sup> |
|----------|-------------------------|-------------------|-------------------|-------------------|-------------------|------------------|
|          | CON                     | EO                | LA                | PAA               | LVASDS            |                  |
| pH       | 5.67 <sup>a</sup>       | 5.70 <sup>a</sup> | 5.16 <sup>c</sup> | 5.71 <sup>a</sup> | 5.50 <sup>b</sup> | 0.03             |
| Purge, % | 1.69 <sup>c</sup>       | 1.77 <sup>c</sup> | 2.43 <sup>a</sup> | 1.77 <sup>c</sup> | 2.01 <sup>b</sup> | 0.08             |

<sup>abc</sup>Denotes least squares means within a row with different superscripts are different ( $P < 0.05$ ).

<sup>1</sup>CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate.

<sup>2</sup>SEM = standard error of the mean.

Table 3.2. Least squares means of the main effect of day of retail display for pH and percent purge for polyvinyl chloride overwrapped ground beef patties manufactured with antimicrobials<sup>1</sup>.

| Traits   | Day of display    |                   |                   |                   |                   |                   | SEM <sup>2</sup> |
|----------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------------|
|          | 0                 | 1                 | 2                 | 3                 | 4                 | 5                 |                  |
| pH       | 5.55 <sup>b</sup> | 5.64 <sup>a</sup> | 5.56 <sup>b</sup> | 5.56 <sup>b</sup> | 5.37 <sup>c</sup> | 5.53 <sup>c</sup> | 0.03             |
| Purge, % | 0.29 <sup>f</sup> | 0.76 <sup>e</sup> | 1.47 <sup>d</sup> | 2.14 <sup>c</sup> | 2.92 <sup>b</sup> | 4.02 <sup>a</sup> | 0.09             |

<sup>abcdef</sup> Denotes least squares means within a row with different superscripts are different ( $P < 0.05$ ).

<sup>1</sup>CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate.

<sup>2</sup>SEM = standard error of the mean.

Figure 3.1. Day of display by antimicrobial treatment interaction ( $P = 0.24$ ; main effect of antimicrobial,  $P < 0.0001$ ; main effect of day of display,  $P < 0.0001$ ) on pH (least squares means  $\pm$  S.E.) for polyvinyl chloride overwrapped ground beef patties through 5 days of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate.

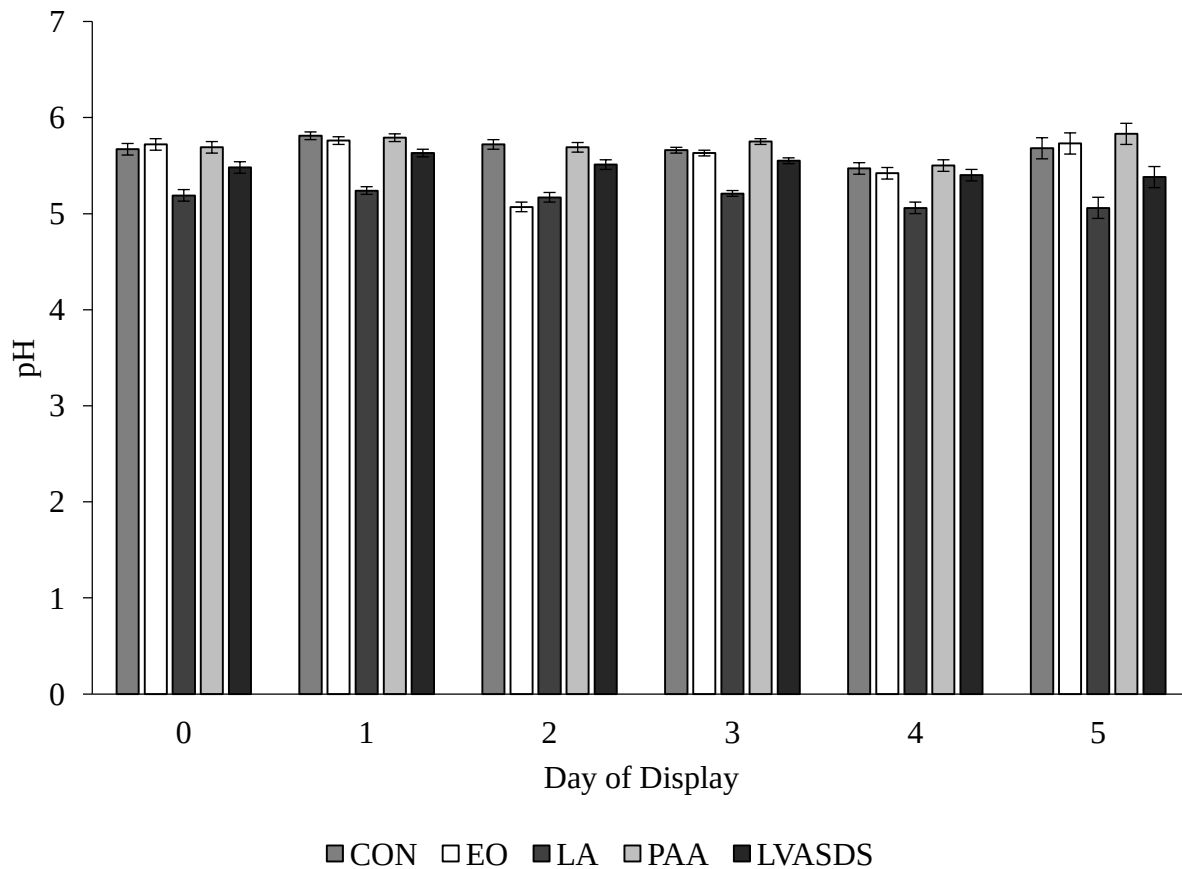


Figure 3.2. Day of display by antimicrobial treatment interaction ( $P = 0.31$ ; main effect of antimicrobial,  $P < 0.0001$ ; main effect of day of display,  $P < 0.0001$ ) on percent purge (least squares means  $\pm$  S.E.) for polyvinyl chloride overwrapped ground beef patties through 5 days of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate.

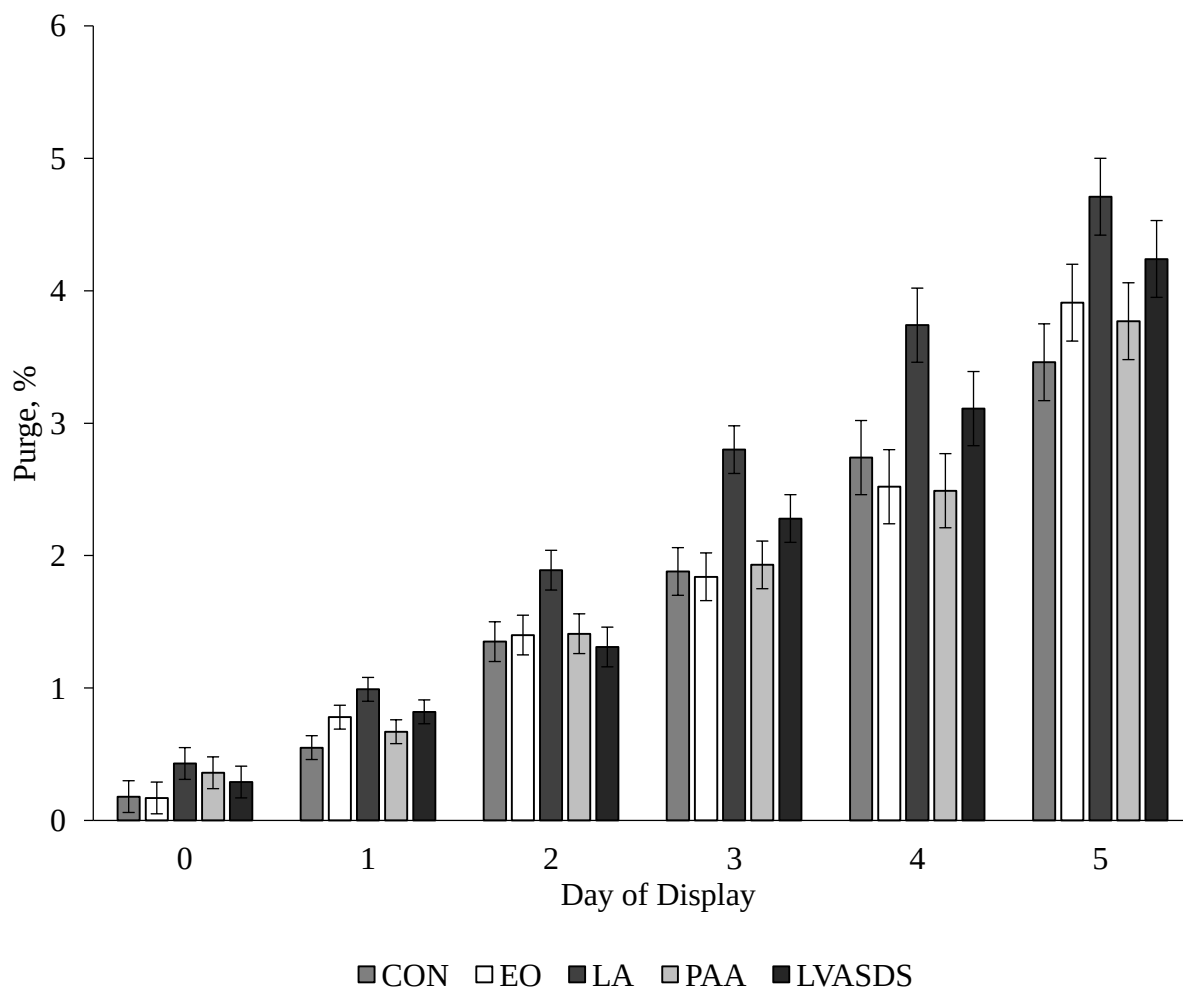


Figure 3.3. Day of display by antimicrobial treatment interaction for objective CIE L\*, a\*, and b\* color (least squares means  $\pm$  S.E.) for polyvinyl chloride overwrapped ground beef patties through 5 days of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate. A. CIE L\* - 0 = black to 100 = white. B. CIE a\* - measures the green to red color spectrum; higher values indicate more red color. C. CIE b\* - measures the yellow to blue color spectrum; higher values indicate more yellow color. Least square means within a day of display with different superscripts are different ( $P < 0.05$ ).



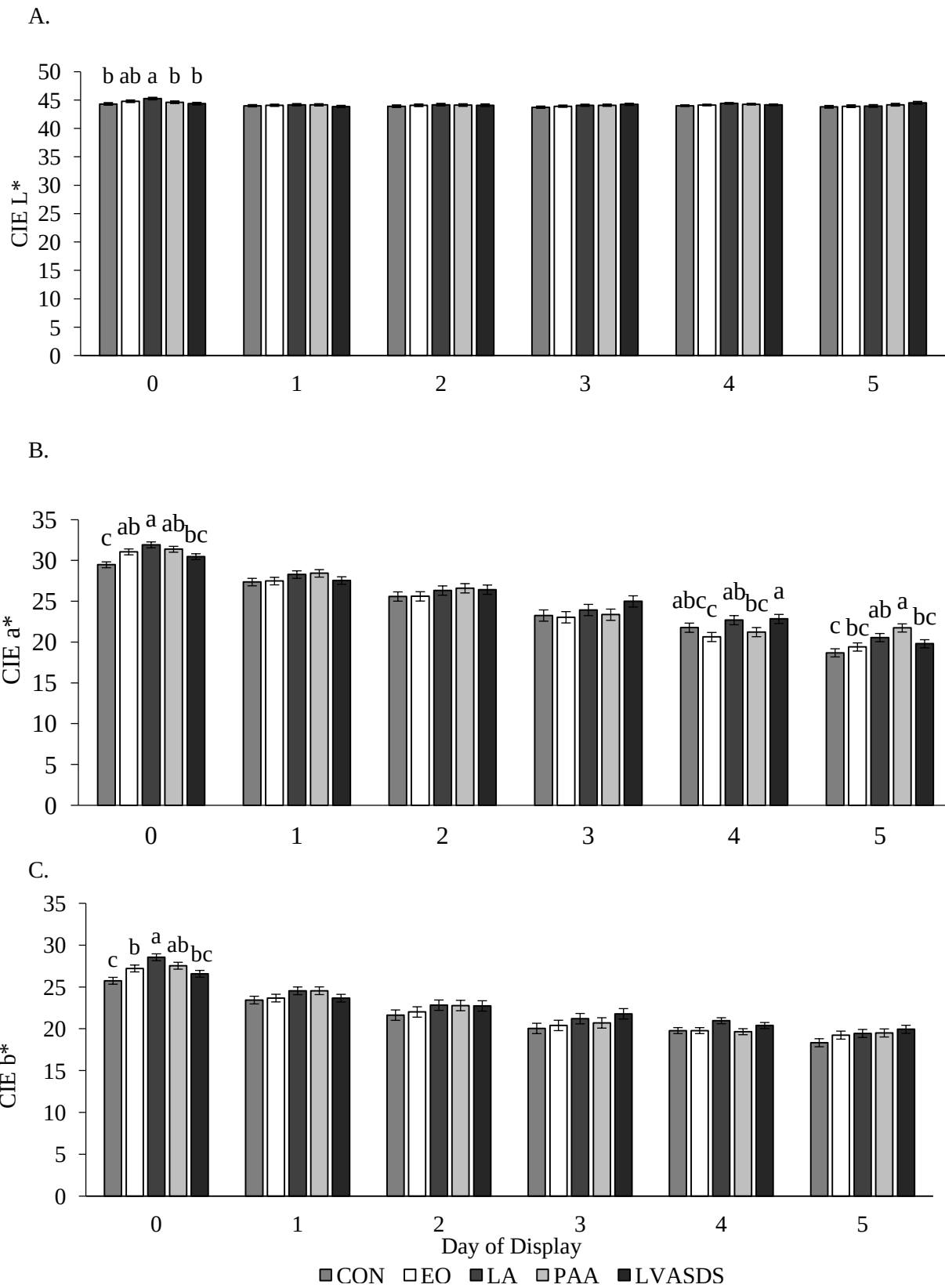
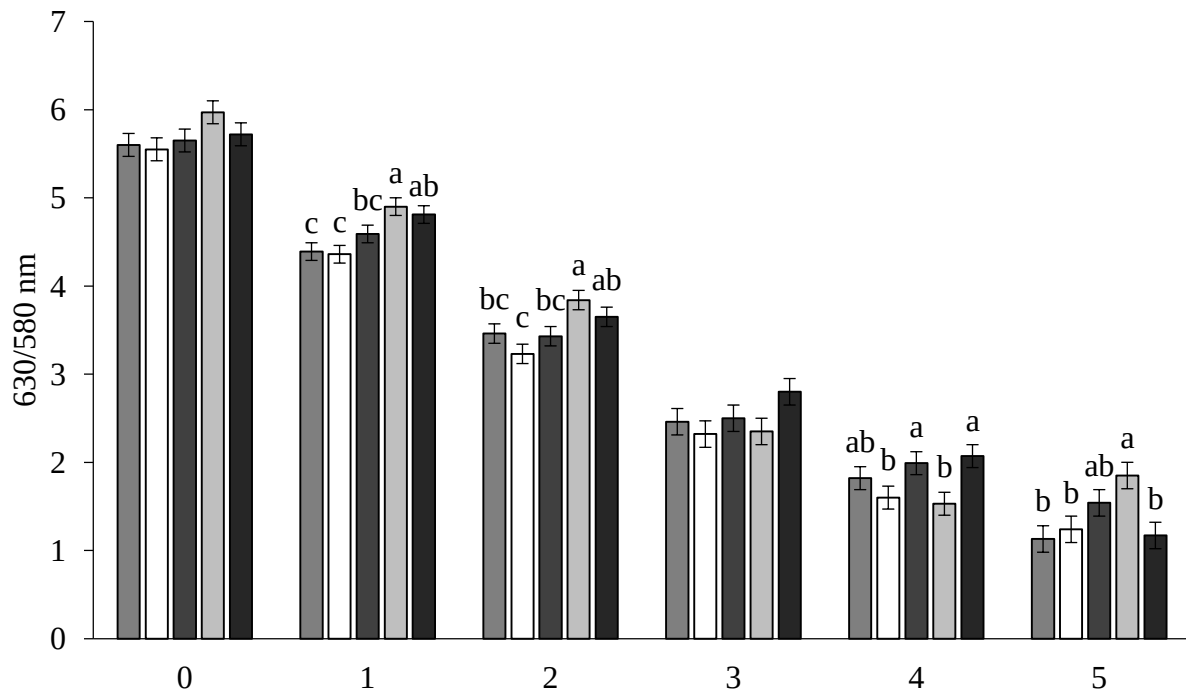
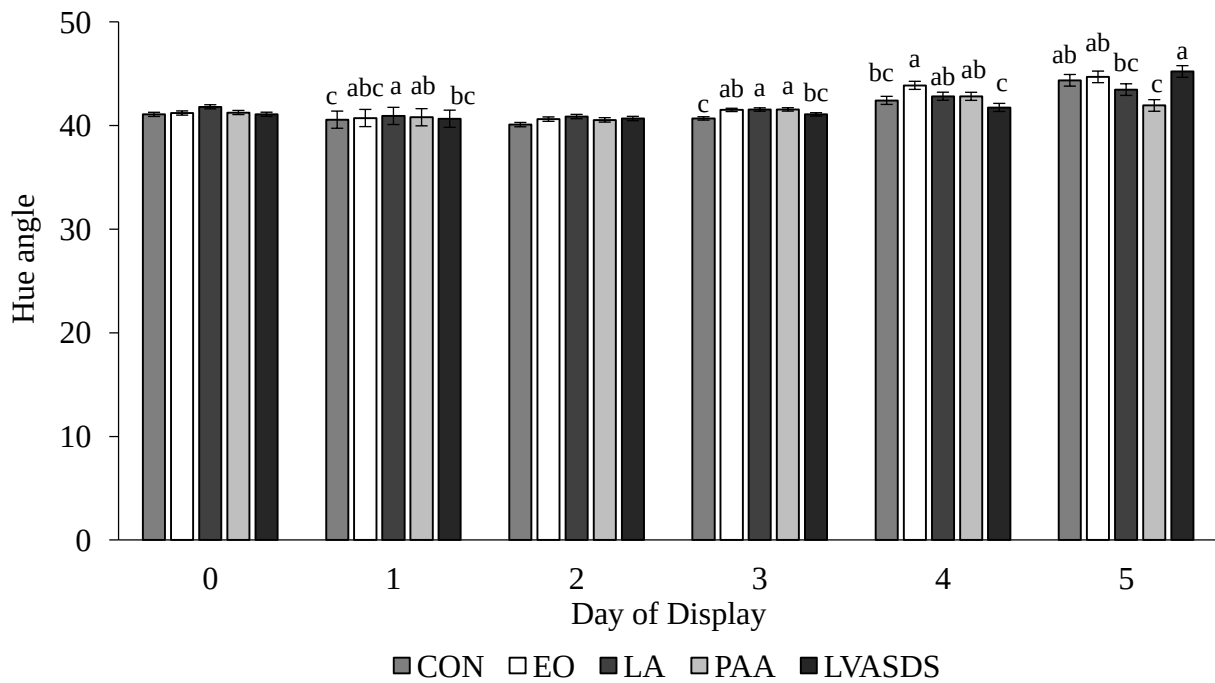


Figure 3.4. Day of display by antimicrobial treatment interaction for 630/580 nm reflectance ratio, saturation index, hue angle, and Delta E (least square means  $\pm$  S.E.) for polyvinyl chloride overwrapped ground beef patties through 5 days of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate. A. 630/580 nm reflectance ratio – larger ratio indicates more redness. B. Hue angle – lower values indicate redder color, calculated as  $\tan^{-1}(b^*/a^*)$ . C. Saturation index – higher values indicate more red saturation, calculated as  $(a^{*2} + b^{*2})^{0.5}$ . D. Delta E ( $\Delta E$ ) – overall color change when compared to d 0, calculated as  $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$ . Least squares means within a day of display with different superscripts are different ( $P < 0.05$ ).

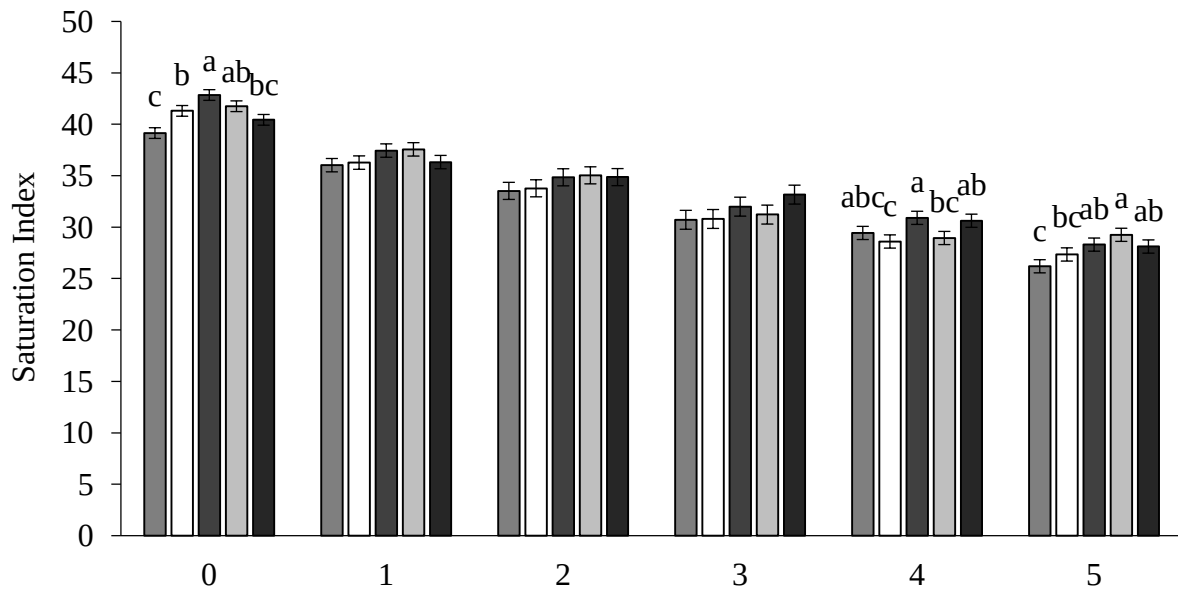
A.



B.



C.



D.

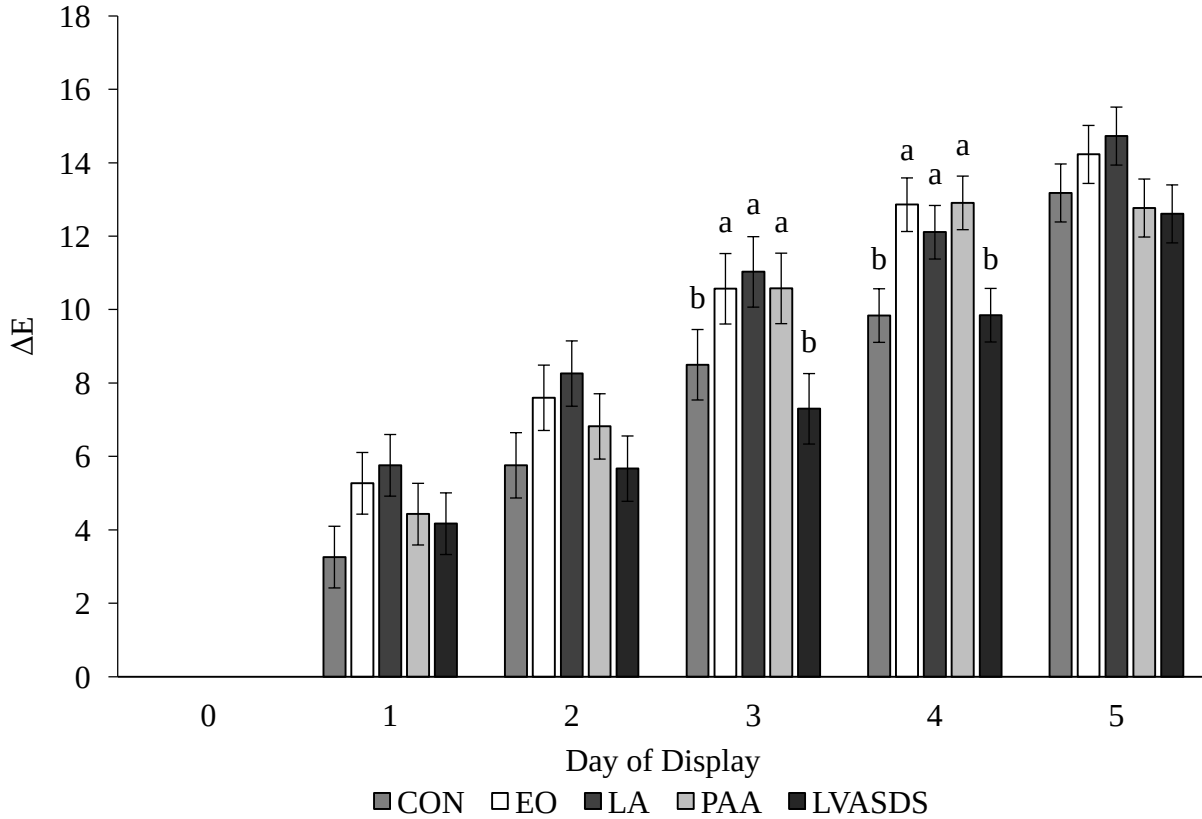


Figure 3.5. Day of display by antimicrobial treatment interaction for subjective color (least squares means  $\pm$  S.E.) for polyvinyl chloride overwrapped ground beef patties through 5 days of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate. A. Initial color - 8 = dark red, 7 = moderately dark red, 6 = slightly dark red, 5 = bright red, 4 = slightly bright red, 3 = light red, 2 = moderately light red, and 1 = very light red. B. Discoloration - 8 = tan to brown, 7 = dark red to tan, 6 = dark red to tannish red, 5 = moderately dark red, 4 = slightly dark red, 3 = dull red, 2 = bright red, and 1 = very bright red. C. Percent discoloration - 7 = 96 – 100%, 6 = 80 – 95%, 5 = 60 – 18%, 4 = 40 – 59%, 3 = 20 – 39%, 2 = 5 – 20%, 1 = 0 – 4% (adapted from AMSA, 2012). Least squares means within a day of display with different superscripts are different ( $P < 0.05$ ).

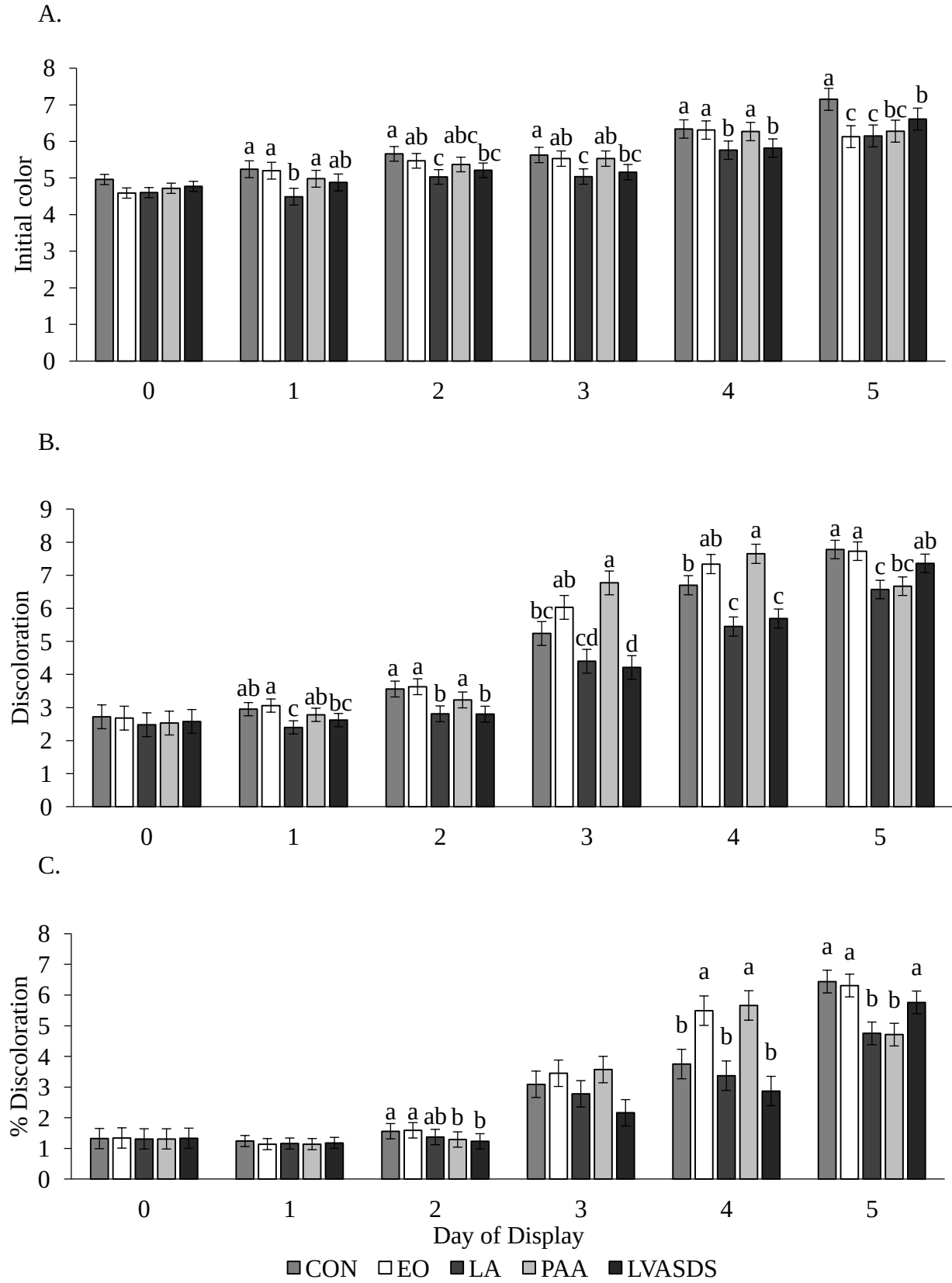


Figure 3.6. Day of display by antimicrobial treatment interaction on thiobarbituric acid reactive substance (mg malonaldehyde (MDA)/kg meat; least squares means  $\pm$  S.E.) for polyvinyl chloride overwrapped ground beef patties through 5 days of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate. Least squares means within a day of display with different superscripts are different ( $P < 0.05$ ).

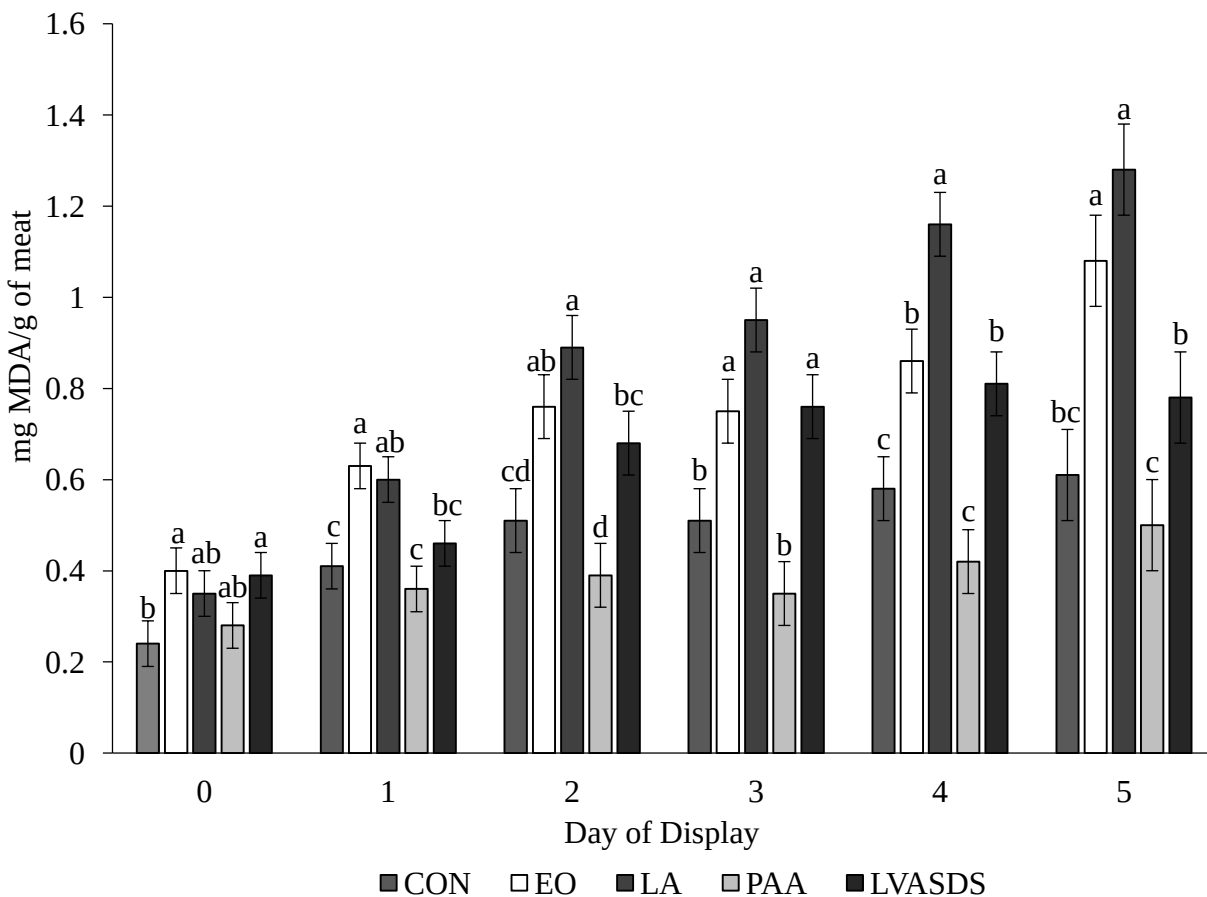
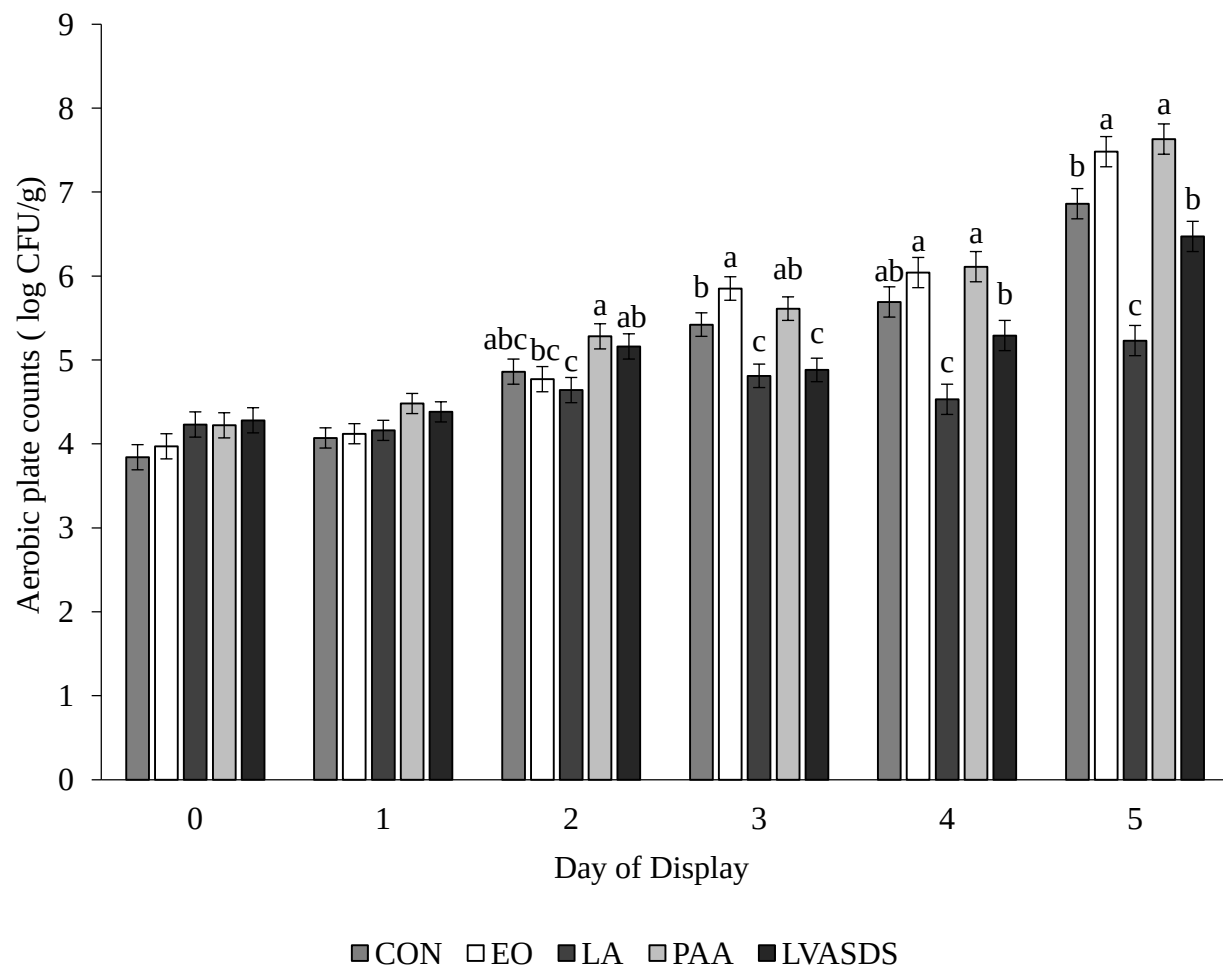


Figure 3.7. Day of display by antimicrobial treatment interaction for aerobic plate count bacteria populations (log CFU/g; least squares means  $\pm$  S.E.) for polyvinyl chloride overwrapped ground beef patties through 5 days of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate. Least squares means within a day of display with different superscripts are different ( $P < 0.05$ ).





## **CHAPTER 4**

### **THE EFFECTS OF NOVEL ANTIMICROBIALS ON THE QUALITY AND SHELF LIFE CHARACTERISTICS OF GROUND BEEF WHEN MANUFACTURED FROM TRIM AT THE PROCESSOR LEVEL<sup>2</sup>**

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<sup>2</sup> Belanger, S.E. and A.M. Stelzleni. To be submitted to *Meat Science*.

## Abstract

The effects of two novel antimicrobials were used to evaluate their effects on ground beef quality and shelf life characteristics. Beef trim was treated with 50 ppm chlorine from acidic electrolyzed oxidizing water (EO), 4.5% lactic acid (LA), 200 ppm peroxyacetic acid, 2.0/0.2% levulinic acid plus sodium dodecyl sulfate, or left untreated (CON). Trim was ground, formed into patties, vacuum packaged, stored for 4 days and then placed in retail display for 0, 3, 6, 9, 12, 15, and 18 days. The LA patties had the greatest percent purge and increased in pH compared to all other treatments ( $P < 0.05$ ). All treatments decreased in redness over time. The LVASDS and EO patties maintained more red color ( $a^*$ ) than LA ( $P < 0.05$ ). Aerobic and lactic acid counts were lower for LA patties ( $P < 0.05$ ). Novel antimicrobials had little effect on sensory characteristics and lipid oxidation ( $P > 0.05$ ). Acidic electrolyzed oxidizing water or levulinic acid plus sodium dodecyl sulfate can be used in place of lactic acid or peroxyacetic acid without being detrimental to shelf life and quality characteristics.

Keywords: antimicrobials, quality, shelf life, ground beef, color, sensory

## Introduction

Today in the United States ground beef is the number one selling beef item in the foodservice and within retail facilities (NCBA, 2013). During ground beef production steps are taken to ensure a product is safe, unadulterated, and wholesome through implementation of the Hazard Analysis and Critical Control Points (HACCP) system from the 1996 Final Rule on Pathogen Reduction; HACCP System (FSIS-USDA, 1996). The HACCP system includes protocols that may include carcass washes and use of antimicrobials on trim prior to being further processed. These preventative measures are important because product may become contaminated during the slaughter and fabrication processes (Lahr, 1996 & Galland, 1997). Contact with contaminated material may lead to an adulterated product from the presence of *Escherichia coli* O157:H7 (Sofos et al., 1999). This has been a predominant pathogen of concern for ground beef because it produces a shiga toxin that causes acute hemorrhagic diarrhea which if not treated can lead to death (FSIS-USDA, 2013; CDC, 1993). Increased *E. coli* surveillance has led to six additional shiga toxin-producing *E. coli* (STEC) serogroups to be added to the adulterant list (FSIS-USDA, 2011). Although the added serogroups do not cause the same clinical signs or mortality as *E. coli* O157:H7, they are responsible for about 1/3 of the *Enterohaemorrhagic Escherichia coli* (EHEC) cases in humans (Koohmaraie et al., 2005). Due to possible STEC and EHEC contamination, it is important to continue to investigate the effectiveness of existing and novel antimicrobials.

Research has shown that the use of antimicrobials on beef trim prior to grinding to be effective at reducing microorganisms (Stivarius, Pohlman, McElyea, and Waldroup, 2002; Geornaras et al., 2012; Mohan et al., 2012) and is a common practice during ground beef processing. Once the pathogen intervention has been applied the product is ground and may be

vacuum packaged as a case-ready product. The process of vacuum packaging ground beef extends shelf life (Brooks, 2007) beyond comparative aerobic packaging systems. Vacuum packaged ground beef has a refrigerated shelf life of up to 21 days (Brooks, 2007). Furthermore, the extension of a products shelf life is of economic importance and is a key goal of the industry (Delmore, 2009). An array of factors have an influence on the shelf life of a including distribution, storage, temperature, and microbial load. According to Martin, et al. (2013) storage temperatures that are not kept at proper conditions can cause an increase in microbial growth and enzymatic activity, in turn reducing shelf life and quality.

More research is needed to evaluate the effects of antimicrobials at the processing facility in the production of case-ready ground beef. Therefore, the objective of this research was to evaluate electrolyzed oxidizing water and levulinic acid plus sodium dodecyl sulfate on vacuum packaged ground beef quality and shelf life compared to that of the industry standards of lactic acid and peroxyacetic acid.

## **Methods and Materials**

### **Preparation of antimicrobials**

The treatments included 50 ppm chlorine generated from acidic electrolyzed oxidizing water (EO), 4.5% lactic acid (LA; Birko Inc., Henderson, CO), 200 ppm peroxyacetic acid (PAA; EnviroTech, Modesto, CA), 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate (LVASDS; Sigma-Aldrich Corp., St Louis, MO. Acros Organics, Thermo Fisher Scientific, New Jersey, USA), and untreated patties (CON). For the production of acidic electrolyzed oxidizing water the methods of Waters and Hung (2013) were followed. Briefly, a 12% food grade sodium chloride solution (Avantor Performance Materials Inc. Center Valley, PA) was prepared and mixed with water using the ROX-20TA electrolyzed water generator (Hoshizaki Electric

Company Ltd., Toyooka, Aichi, Japan). The acidic phase was collected. The resulting acidic water had an average chlorine content of 86.3 ppm of free chlorine, therefore, it was diluted using deionized water to obtain a final concentration of 50 ppm chlorine (Model 16900 Hach Digital Titration Kit, Hach Company, Loveland, CO.) and was brought to a final pH of 6.0-6.2 using NaOH.

### **Ground beef preparation**

Whole boneless chuck rolls (Arko Veal Co. Forest Park, GA) were used to ensure known source and slaughter date. The whole boneless chuck rolls were processed into beef trim (85/15) and then combined to acquire 18 kg of trim which was randomly separated into 5 treatment bins. The bins were then randomly assigned to a treatment and individual trim pieces were manually placed onto a spray cabinet conveyor to ensure full coverage and the intervention treatment was applied by a 365° six nozzle sanitizing spray cabinet (Birko Chad Co., Olathe, KS.). The conveyor exposed trim to each intervention for 24 s with a flow rate of 0.55 L/min•nozzle<sup>-1</sup> at 290 kPa. After treatment application the beef trim was ground (AFMG 50, Danials Food Equipment, Parkers Prairie, MN) through a 12.7-mm plate followed by a 6.4-mm plate. Approximately 90 patties (150 ± 2g, 13 mm thick) were produced (Patti-O-Matic Protégé, Farmingdale, NJ). Forty of the 90 ground beef patties per treatment were randomly selected, weighed, vacuum packaged (30 to 50 mL of O<sub>2</sub>/m<sup>2</sup>/24h; 101,325Pa; 23°C; B-620 series, Cryovac Sealed Air Corporation, Duncan, SC) and held for four days in dark cold storage (4°C) to simulate storage and transportation times and then were placed in coffin style retail display cases (Husmann Corporation, Bridgeton, MO.) for 0, 3, 6, 9, 12, 15, and 18 d (5 patties/treatment•day<sup>-1</sup>) at 2 ± 2°C (T&A Thermo Recorders TR-52i, Japan) with 24 h warm white fluorescent lighting (1851lx; Osram Sylvania, Danvers, MA.). An additional 10 patties

from each treatment were randomly selected, vacuum packaged, and stored ( $-28 \pm 2^{\circ}\text{C}$ ). From these patties, two were used for sensory evaluation and the remaining 5 for Kramer shear force analysis. All equipment was cleaned and sanitized between each application treatment to ensure there was no mixing of treatments. All procedures were followed for a total of three replicates.

### **Objective and subjective color**

Objective CIE  $L^*$ ,  $a^*$ ,  $b^*$  was measured on d 0, 3, 6, 9, 12, 15, and 18 using a Hunter-Lab Miniscan XE Plus (Model 45, Hunter Associates Laboratory, Inc., Reston, WV). The colorimeter was standardized using white and black tiles and a saturated red tile was used as a physical standard. Samples were read using illuminant A/10° with a 2.54-cm aperture. The reflectance ratio of 630/580 nm was used to estimate the amount of redness due to either oxymyoglobin or deoxymyoglobin and hue angle ( $\tan^{-1}(b^*/a^*)$ ) and saturation index  $[(a^{*2} + b^{*2})^{0.5}]$  were calculated (AMSA 2012). Three color readings were taken and the average was recorded by the colorimeter.

A 7 member trained color panel (AMSA, 2012) recorded subjective color on d 0, 3, 6, 9, 12, 15, and 18 d for initial color (8 = extremely dark purple, 7 = dark purple, 6 = moderately dark purple, 5 = slightly dark purple, 4 = slightly purple-red, 3 = moderately bright purple, 2 = bright purple-red, and 1 = extremely bright purple-red), amount of browning (6 = dark brown, 5 = brown, 4 = brownish-gray, 3 = grayish, 2 = dull, and 1 = no evidence of browning), and percent surface discoloration (7 = 96 – 100%, 6 = 80 – 95%, 5 = 60 – 80%, 4 = 40 – 59%, 3 = 20 – 39%, 2 = 5 – 20%, 1 = 0 – 4%). All panelists scored <50 for the total error score on the Farnsworth-Munsell 100 Hue Test (Xrite, Grandville, MI).

### **pH and percent purge**

On d -4, 0, 3, 6, 9, 12, 15, and 18 samples were removed from the vacuum packaging and weighed to calculate purge loss. pH was determined by homogenizing a 10 g sample from each patty in 100 mL of deionized water for 30 s and the pH (pH 11 series pH/mV/°C meter, Oakton Instruments, Vernon Hills, IL) was recorded.

### **Microbiology**

After each day of display aerobic plate count and lactic acid bacteria were determined once the post display weight and pH were evaluated. A 25 g sample was removed aseptically from each ground beef patty and placed into a sterile stomacher bag. Two hundred and twenty-five milliliters of 0.1% Buffered peptone water (Difco Laboratories, Detroit, MI) was added to the sample and stomached (Stomacher 400 Circulator, Seward Ltd.) for 2 min at 230 rpm. Serial dilutions were made for all samples using 9 ml of 0.1% peptone (Difco Laboratories, Detroit, MI) and the dilutions were plated onto Aerobic Count Plate Petrifilm (3M Corp., St. Paul, MN) in duplicate. Plated samples were incubated at 37°C (Thelco, Ontario, Canada) for  $48 \pm 2$  h. *Lactobacilli* determination was done using MRS broth (Sigma-Aldrich Corp., St. Louis, MO, USA) following the methods of 3M Microbiology (2006). Briefly, the aerobic plate count film was placed into an anaerobic chamber (GasPak EZ Incubation Containers, BD Corp., Franklin Lakes, NJ) equipped with oxygen scavengers (GasPak EZ Anaerobe Container System Sachets, BD Corp., Franklin Lakes, NJ). The samples were incubated at 37°C in an incubation chamber (Thelco, Ontario, Canada) for  $48 \pm 2$  h. Bacteria counts for LAB and APC were expressed as CFU/g (USDA, 2010). The remainder of each sample was individually vacuum packaged and stored at  $-20 \pm 2^\circ\text{C}$  for lipid oxidation analysis.

### **Lipid Oxidation**

Thiobarbituric acid reactive substance analysis (TBARS) was adopted from Ahn et al. (1998). Ground beef patties from each sample day of retail display were thawed in vacuum bags overnight in a cold room (4°C). After thawing the patty was thoroughly mixed and a 5 g sample was collected for analysis. The sample was placed into 50 ml conical centrifuge tube and homogenized (Tissumizer Mark II, Tekmar Company, Cincinnati, OH) with 15 ml of deionized water for 30 s. The tubes were centrifuged at 1850 x *g* for 10 min at 24°C (CR 312, Jouan Inc., Winchester, VA). Two milliliters of the supernatant was removed and placed into disposable glass test tubes (13 x 100 mm) in duplicate. One hundred microliters of butylated hydroxyl toluene (7.2%) and 4 ml of thiobarbituric acid/trichloroacetic acid were added to the supernatant. All tubes were vortexed for 5 s and placed into a hot water bath (90°C) for 15 min then allowed to cool in a room temperature (24°C) water bath for 10 min. Samples were then centrifuged at 3000 x *g* for 15 min at 24°C. Patty lipid oxidation was measured as a function of malonaldehyde (MDA) per kg of meat via spectrophotometric readings at 531 nm (Jasco Analytical Instruments, Easton, MD). Malonaldehyde concentration was calculated by comparison to known standards.

### **Sensory panelist evaluation**

An 8 member trained sensory taste panel (AMSA, 1995) was used to evaluate the sensory properties of the ground beef patties. For each sensory session 7 patties were randomly selected and thawed (24 hr) prior to being cooked on clamshell grills (George Foreman Grill, Spectrum Brands Inc., Madison, WI). Patties were cooked to an internal temperature of 71°C (AMSA, 1995). Internal temperature was monitored using copper-constantan thermocouples placed into the geometric center of each patty attached to a Digi-Sense 12-channel scanning thermometer (Cole-Palmer, Vernon Hills, IL). The cooked patties were then covered in foil and allowed to rest under a heat lamp for 5 min. Patties were sliced into 8 equal pie shaped pieces and served to



panelists in warmed yogurt makers (Euro cuisine, Inc., Los Angeles, CA). Panelists were in a negative air pressure room, in individual booths under a red light to avoid bias while evaluating samples for cohesiveness, beef flavor, and juiciness on an 8-point scale (8 = extremely cohesive, intense, juicy, 7=very cohesive, intense, juicy, 6 = moderately cohesive, intense, juicy, 5 = slightly cohesive, intense, juicy, 4 = slightly fragile, bland, dry, 3 = moderately fragile, bland, dry, 2 = very fragile, bland, dry, 1= extremely fragile, bland, dry). Panelists also evaluated off flavor intensity on a 5-point scale (5 = extreme off flavor, 4 = moderate off flavor, 3 = small off flavor, 2 = slight off flavor, 1 = no off flavor).

### **Kramer Shear Force**

Patties were thawed and cooked to an internal temperature of 71°C following the same methods outlined for sensory analysis. After cooking, the patties were allowed to cool to room temperature (24°C) and a 5x5 cm section was removed from the center of the patty (Quilo et al. 2009). The section was then sheared using a Kramer shear head attached to a Universal Testing Machine (Instron Dual Column Model 3365, Instron Corp., Norwood, MA) with a 5K N load cell at a crosshead speed of 25 cm/min. The peak shear force (kgf) for each sample was recorded (Bluehill software, Instron Corp.) and analyzed as kgf/g.

### **Statistical Analysis**

The sample size was calculated according to the methods of Montgomery (2001) and Ferris, Grubbs, and Weaver (1946) to guard against probability of making a Type II error. This research was conducted as a completely randomized design. The data was analyzed using the Mixed Procedures of SAS (V.9.1 SAS Inst. Inc., Cary, NC). When a treatment by day of display interaction occurred, data was reanalyzed by day. For shelf life data, the model included the fixed effects of treatment and day of display. For sensory and shear analysis the fixed effect was

treatment. Patty within treatment by rep was included as the random variable. Least squares means were generated and means were separated using the PDIFF option. Differences were considered significant at  $\alpha < 0.05$ .

## **Results and Discussion**

### **Shelf life characteristics**

#### **pH and purge**

For vacuum packaged (VP) ground beef patties there was no interaction ( $P > 0.05$ ; Figure 4.1) for pH therefore, the main effects are presented in Table 4.1 and Table 4.2 for treatment and day of display, respectively. There was a treatment by day interaction for percent purge ( $P < 0.05$ ) as shown in Figure 4.2. For the main effect of treatment, lactic acid (LA) treated patties had the lowest pH compared to all other treatments ( $P < 0.05$ ) and pH decreased as time on display increased. Correspondingly, percent purge was greater for LA treated patties on d 0, 3, 6, 9, and 12 compared to all other treatments ( $P < 0.05$ ). Jimenez-Villarreal et al. (2003c) suggests that the decline in pH of meat can cause the amount of purge to increase while on retail shelves which later on will influence thaw loss prior to cooking affecting the texture, tenderness, and juiciness.

#### **Objective and subjective color**

Vacuum packaged ground beef patties were similar ( $P < 0.05$ ) for CIE  $L^*$  and saturation index regardless of treatment (Table 4.1, Figure 4.3 and Figure 4.4). However, LA treated patties exhibited lower CIE  $a^*$  and a greater hue angle ( $P < 0.05$ ). Furthermore, the reflectance ratio (630/580 nm) decreased as time on display increased, with LA being lower than all other treatments on all days (Figure 4.3) indicating that LA patties were less red than any other treatment ( $P < 0.05$ ). There was a treatment by day interaction for CIE  $b^*$  ( $P < 0.05$ ; Figure 4.4) where on d 0, the CIE  $b^*$  values for electrolyzed oxidizing water (EO) was lower than all other

treatments, but on d 6, 9, and 12 CIE  $b^*$  was greater for LA treated patties, this indicated an increase in yellow hues. Examining CIE  $L^*$  across days of display, there was a decrease from d 0 and d 3 ( $P < 0.05$ ; Table 4.1, Table 4.2, and Figure 4.4) then an increase from d 3 to d 6 ( $P < 0.05$ ) and the CIE  $L^*$  remained fairly constant through d 18. Redness as indicated by CIE  $a^*$  and hue angle and the vividness of the patties (saturation index) all followed a similar trend where there was a slight increase ( $P < 0.05$ ; Table 4.1, Table 4.2, and Figure 4.4) in redness and vividness for d 0 and d 3, furthermore the redness and vividness decreased ( $P < 0.05$ ) from d 3 to d 6 and remained constant through d 15.

There was a treatment by day interaction ( $P < 0.05$ ) the subjective color score of initial color, amount of browning, and percent of browning (Figure 4.5 A, B, and C, respectively). Over time, panelists rated patties as becoming darker in color. Corresponding to the objective change in redness noticed for LA treated patties, LA patties were darker than all other treatments from d 9 through d 18 ( $P < 0.05$ ). Additionally, amount of browning and percent of browning also increased as time on display increased and LA patties exhibited more ( $P < 0.05$ ) brown color and a greater ( $P < 0.05$ ) percent of brown color than did all other treatments on all days of display. Vacuum packaging removes oxygen and is typically related to meat being purple in color. Many consumers are not yet accustomed to this color, but it is becoming more popular since it aids in extending the shelf life of meat (Brooks, 2007). This research has shown that using EO and levulinic acid plus sodium dodecyl sulfate (LVASDS) exhibited more red color from an instrumental values and panelists scores. This would conclude that patties treated with the novel interventions would allow vacuum packaged ground beef to be more acceptable to consumers because it maintains a more red color vs the darker purple and brown colors noted in LA patties. This project is unique in the fact that the ground beef patties were constantly evaluated under

vacuum package conditions. It is one of the few that evaluated the antimicrobial treated ground beef and the quality characteristics that were evaluated.

### **Lipid oxidation**

There was no treatment by day interaction ( $P > 0.05$ ; Figure 4.6) for lipid oxidation of ground beef patties; therefore, the main effect data is shown in Table 4.1 and 4.2 for treatment and day of display, respectively. There were no differences between treatments ( $P > 0.05$ ) or days of display ( $P > 0.05$ ). The results shown for this research on lipid oxidation for VP patties is conducive to the nature of vacuum packaged products, Brooks (2007) explains that the shelf life of vacuum packaged ground beef is increased because there is a decrease in oxidation.

### **Microbial analysis**

There was not an antimicrobial by day of display interaction ( $P > 0.05$ ) for APC or LAB counts (Figure 4.7 and 4.8, respectively), therefore, main effects of antimicrobial treatments and day of display are shown in Table 4.3 and Table 4.4, respectively. Lactic acid treated patties had lower counts (log CFU/g) of APC and LAB populations compared to all other treatments ( $P < 0.05$ ). As expected APC and LAB populations (log CFU/g) increased as time on display increased. Although attempts to control source and age of product, the beef was on average 18 d old before patties could be placed in the retail cases. This was due to uncontrollable weather patterns occurring where the product was sourced. The age of the beef upon receiving can partially explain the higher than expected bacterial counts at the start of shelf life.

### **Sensory and shear characteristics**

The effect of antimicrobial treatment on sensory and shear characteristics is shown in Table 4.5. Patties treated with LVSADS were greater in percent thaw loss numerically, however statistically were similar to LA ( $P > 0.05$ ). There were no differences in Kramer shear force

among patties; however, trained panelists detected a difference ( $P < 0.05$ ) between where CON patties had more bind than LA, PAA, and LVASDS ( $P < 0.05$ ) with EO patties being similar to all treatments ( $P > 0.05$ ). However, all patties were rated between slightly cohesive and slightly fragile with a maximum difference of 0.5 on the 8-point scale. Additionally, there was no differences detected by panelists for beef intensity and juiciness, but LA had slightly more off flavor ( $P < 0.05$ ) than all other treatments. However all treatments were rated between no off flavor and slight off flavor. Due to LVASDS and LA having lower pH, it resulted in the final meat pH to decline and bringing the product closer to the isoelectric point. The closer to the isoelectric point the less water hold capacity there is between meat proteins and contributed to the increase in percent thaw loss seen in LVASDS and LA treated ground beef patties. Although numbers show there was a different in thaw and cook loss there was no detection by panelists. The current research is similar to Jimenez-Villarreal et al. (2003c) when 2% LA resulted in a lower pH and had less bind compared to the untreated ground beef patties. Furthermore, the 2% LA patties did not differ in Kramer shear force values compared to untreated controls which is analogous to the current research. Similar to the current research, Quilo et al. (2009b) reported that sensory panelists scored untreated patties to have more bind than ground beef patties treated with 200 ppm PAA. Stelzleni et al. (2013) reported similar results for a lower concentration for LVASDS (1.0/0.1%) the percent thaw loss was greater and there was no difference in Kramer shear force values compared to untreated patties. Also sensory panelists scored patties between slightly cohesive and slightly fragile regardless of treatment (Stelzleni et al., 2013).

### **Conclusion**

Case-ready ground beef provides the consumers with a longer lasting product due to the nature of the packaging by removal of oxygen and reducing microbial growth. The addition of an

antimicrobial has shown that the aerobic and lactic acid bacteria can be reduced or maintain acceptable levels of spoilage. An added benefit of an antimicrobial is that it can improve the color stability without affecting sensory characteristics. Furthermore, this research has shown that acidic electrolyzed water and levulinic acid plus sodium dodecyl sulfate at the levels used in the current study can be used without causing negative effects to quality and shelf life. Further research should be conducted to evaluate these characteristics more closely concerning other types of vacuum packaging processes for ground beef at the processor facilities.

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Table 4.1. Least squares means for the main effect of antimicrobial treatment on objective color, pH, and lipid oxidation values for vacuum packaged ground beef patties through 18 days of retail display.

| Traits                        | Treatment <sup>1</sup> |                    |                    |                    |                    | SEM <sup>2</sup> |
|-------------------------------|------------------------|--------------------|--------------------|--------------------|--------------------|------------------|
|                               | CON                    | EO                 | LA                 | PAA                | LVASDS             |                  |
| pH                            | 5.56 <sup>a</sup>      | 5.60 <sup>a</sup>  | 5.19 <sup>b</sup>  | 5.54 <sup>a</sup>  | 5.53 <sup>a</sup>  | 0.05             |
| CIE $L^*$ <sup>3</sup>        | 37.61                  | 37.81              | 37.59              | 37.92              | 37.77              | 0.20             |
| CIE $a^*$ <sup>4</sup>        | 29.50 <sup>a</sup>     | 29.36 <sup>a</sup> | 28.61 <sup>b</sup> | 29.24 <sup>a</sup> | 29.41 <sup>a</sup> | 0.19             |
| Saturation Index <sup>5</sup> | 34.52                  | 34.30              | 33.97              | 34.32              | 34.42              | 0.14             |
| Hue <sup>6</sup>              | 31.32 <sup>b</sup>     | 31.16 <sup>b</sup> | 32.66 <sup>a</sup> | 31.61 <sup>b</sup> | 31.34 <sup>b</sup> | 0.28             |
| Lipid Oxidation <sup>7</sup>  | 0.35                   | 0.37               | 0.32               | 0.30               | 0.41               | 0.03             |

<sup>ab</sup>Denotes least squares means within a row with different superscripts are different ( $P < 0.05$ ).

<sup>1</sup>CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate.

<sup>2</sup> SEM = standard error of the mean.

<sup>3</sup> CIE  $L^*$  - 0 = black to 100 = white.

<sup>4</sup> CIE  $a^*$  - measures the green to red color spectrum, higher values indicate more red color.

<sup>5</sup> Saturation Index was calculated as  $(a^{*2} + b^{*2})^{0.5}$ .

<sup>6</sup> Hue was calculated as  $\tan^{-1}(b^*/a^*)$ .

<sup>7</sup> Miligrams of malonaldehyde (MDA)/kg of meat.

Table 4.2. Least squares means for the main effects of day of retail display for objective color, pH, and lipid oxidation values on vacuum packaged ground beef patties treated with antimicrobials<sup>1</sup>.

| Traits                        | Day of Display    |                    |                    |                     |                     |                      |                     |                    | SEM <sup>2</sup> |
|-------------------------------|-------------------|--------------------|--------------------|---------------------|---------------------|----------------------|---------------------|--------------------|------------------|
|                               | -4                | 0                  | 3                  | 6                   | 9                   | 12                   | 15                  | 18                 |                  |
| pH                            | 5.89 <sup>a</sup> | 5.79 <sup>a</sup>  | 5.57 <sup>b</sup>  | 5.55 <sup>b</sup>   | 5.52 <sup>bc</sup>  | 5.40 <sup>c</sup>    | 4.97 <sup>e</sup>   | 5.18 <sup>d</sup>  | 0.05             |
| CIE L* <sup>3</sup>           | -                 | 37.41 <sup>c</sup> | 35.93 <sup>d</sup> | 38.16 <sup>ab</sup> | 38.23 <sup>ab</sup> | 38.07 <sup>abc</sup> | 37.84 <sup>bc</sup> | 38.55 <sup>a</sup> | 0.23             |
| CIE a* <sup>3</sup>           | -                 | 28.41 <sup>c</sup> | 30.61 <sup>a</sup> | 29.64 <sup>b</sup>  | 29.57 <sup>b</sup>  | 29.61 <sup>b</sup>   | 29.61 <sup>b</sup>  | 27.10 <sup>d</sup> | 0.23             |
| Saturation Index <sup>3</sup> | -                 | 33.37 <sup>d</sup> | 35.38 <sup>a</sup> | 34.62 <sup>bc</sup> | 34.56 <sup>c</sup>  | 34.58 <sup>bc</sup>  | 34.63 <sup>b</sup>  | 32.99 <sup>d</sup> | 0.16             |
| Hue <sup>3</sup>              | -                 | 31.71 <sup>b</sup> | 30.12 <sup>c</sup> | 31.14 <sup>b</sup>  | 31.18 <sup>b</sup>  | 31.13 <sup>b</sup>   | 31.23 <sup>b</sup>  | 34.81 <sup>a</sup> | 0.32             |
| Lipid Oxidation <sup>4</sup>  | 0.27              | 0.25               | 0.40               | 0.36                | 0.36                | 0.31                 | 0.33                | 0.41               | 0.03             |

<sup>abcde</sup> Denotes least squares means within a row with different superscripts are different ( $P < 0.05$ ).

<sup>1</sup>CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate.

<sup>2</sup> SEM = standard error of the mean.

<sup>3</sup> CIE L\* - 0 = black to 100 = white; CIE a\* - measures the green to red color spectrum with higher values indicate more red color;

Saturation Index was calculated as  $((a^{*2} + b^{*2})^{0.5})$ ; Hue was calculated as  $(\tan^{-1}(b^*/a^*))$ .

<sup>4</sup>Miligrams of malonaldehyde (MDA)/kg of meat.



Table 4.3. Least squares means for the main effects of antimicrobial treatment on aerobic plate count (APC) and lactic acid bacteria (LAB) populations (log CFU/g) in vacuum packaged ground beef patties through 18 days of retail display.

| Traits | Treatment <sup>1</sup> |                   |                   |                   |                   | SEM <sup>2</sup> |
|--------|------------------------|-------------------|-------------------|-------------------|-------------------|------------------|
|        | CON                    | EO                | LA                | PAA               | LVASDS            |                  |
| APC    | 6.40 <sup>a</sup>      | 6.52 <sup>a</sup> | 5.53 <sup>b</sup> | 6.41 <sup>a</sup> | 6.17 <sup>a</sup> | 0.14             |
| LAB    | 6.25 <sup>a</sup>      | 6.25 <sup>a</sup> | 5.55 <sup>b</sup> | 6.23 <sup>a</sup> | 6.12 <sup>a</sup> | 0.14             |

<sup>ab</sup>Denotes least squares means within a row with different superscripts are different ( $P < 0.05$ ).

<sup>1</sup>CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate.

<sup>2</sup>SEM = standard error of the mean.

Table 4.4. Least squares means for the main effect of day of retail display for aerobic plate count (APC) and lactic acid bacteria (LAB) populations (log CFU/g) on vacuum packaged ground beef patties treated with antimicrobials<sup>1</sup>.

| Traits | Day of Display    |                   |                   |                   |                    |                   |                    |                    | SEM <sup>2</sup> |
|--------|-------------------|-------------------|-------------------|-------------------|--------------------|-------------------|--------------------|--------------------|------------------|
|        | -4                | 0                 | 3                 | 6                 | 9                  | 12                | 15                 | 18                 |                  |
| APC    | 4.26 <sup>f</sup> | 4.78 <sup>e</sup> | 6.01 <sup>d</sup> | 6.57 <sup>c</sup> | 6.81 <sup>bc</sup> | 7.19 <sup>a</sup> | 6.95 <sup>ab</sup> | 7.06 <sup>ab</sup> | 0.10             |
| LAB    | 3.99 <sup>f</sup> | 4.60 <sup>e</sup> | 5.87 <sup>d</sup> | 6.18 <sup>c</sup> | 6.79 <sup>b</sup>  | 7.30 <sup>a</sup> | 6.88 <sup>b</sup>  | 7.03 <sup>ab</sup> | 0.10             |

<sup>abcdef</sup> Denotes least squares means within a row with different superscripts are different ( $P < 0.05$ ).

<sup>1</sup>CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate.

<sup>2</sup>SEM = standard error of the mean.

Table 4.5. Least squares means for the main effect of antimicrobial treatment on cooking, texture, and sensory characteristic values for ground beef patties treated with antimicrobials.

|                                | Treatments <sup>1</sup> |                    |                    |                     |                    | SEM <sup>2</sup> |
|--------------------------------|-------------------------|--------------------|--------------------|---------------------|--------------------|------------------|
| Traits                         | CON                     | EO                 | LA                 | PAA                 | LVASDS             |                  |
| <i>Cooking Characteristics</i> |                         |                    |                    |                     |                    |                  |
| Thaw Loss, %                   | 0.04 <sup>b</sup>       | 0.05 <sup>b</sup>  | 0.29 <sup>ab</sup> | 0.09 <sup>b</sup>   | 0.49 <sup>a</sup>  | 0.11             |
| Cook Loss, %                   | 24.82 <sup>b</sup>      | 27.28 <sup>a</sup> | 27.81 <sup>a</sup> | 26.26 <sup>ab</sup> | 25.15 <sup>b</sup> | 0.69             |
| <i>Sensory Characteristics</i> |                         |                    |                    |                     |                    |                  |
| Kramer Shear, kgf/g            | 2.38                    | 2.33               | 2.48               | 2.36                | 2.43               | 0.06             |
| Bind <sup>3</sup>              | 5.03 <sup>a</sup>       | 4.77 <sup>ab</sup> | 4.55 <sup>b</sup>  | 4.72 <sup>b</sup>   | 4.58 <sup>b</sup>  | 0.09             |
| Beef Intensity <sup>4</sup>    | 4.66                    | 4.61               | 4.81               | 4.65                | 4.80               | 0.08             |
| Juiciness <sup>5</sup>         | 4.44                    | 4.55               | 4.52               | 4.76                | 4.81               | 0.11             |
| Off-flavor <sup>6</sup>        | 1.06 <sup>b</sup>       | 1.07 <sup>b</sup>  | 1.39 <sup>a</sup>  | 1.04 <sup>b</sup>   | 1.11 <sup>b</sup>  | 0.04             |

<sup>ab</sup>Denotes least squares means within a row with different superscripts are different ( $P < 0.05$ ).

<sup>1</sup>CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate.

<sup>2</sup>SEM = standard error of the mean.

<sup>3</sup>Bind: 8 = extremely cohesive, 7 = very cohesive, 6 = moderately cohesive, 5 = slightly cohesive, 4 = slightly fragile, 3 = moderately fragile, 2 = very fragile, 1 = extremely fragile.

<sup>4</sup>Beef Intensity: 8 = extremely intense, 7 = very intense, 6 = moderately intense, 5 = slightly intense, 4 = slightly bland, 3 = moderately bland, 2 = very bland, 1 = extremely bland.

<sup>5</sup>Juiciness: 8 = extremely juicy, 7 = very juicy, 6 = moderately juicy, 5 = slightly juicy, 4 = slightly dry, 3 = moderately dry, 2 = very dry, 1 = extremely dry.

<sup>6</sup>Off-flavor: 5 = extreme off flavor, 4 = moderate off flavor, 3 = small off flavor, 2 = slight off flavor, 1 = no off flavor.

Figure 4.1. Day of display by antimicrobial treatment interaction ( $P > 0.05$ ; main effect of antimicrobial  $P < 0.05$ ; main effect of day of display  $P < 0.05$ ) on pH (least squares means  $\pm$  S.E.) for vacuum packaged ground beef patties through 18 d of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate.

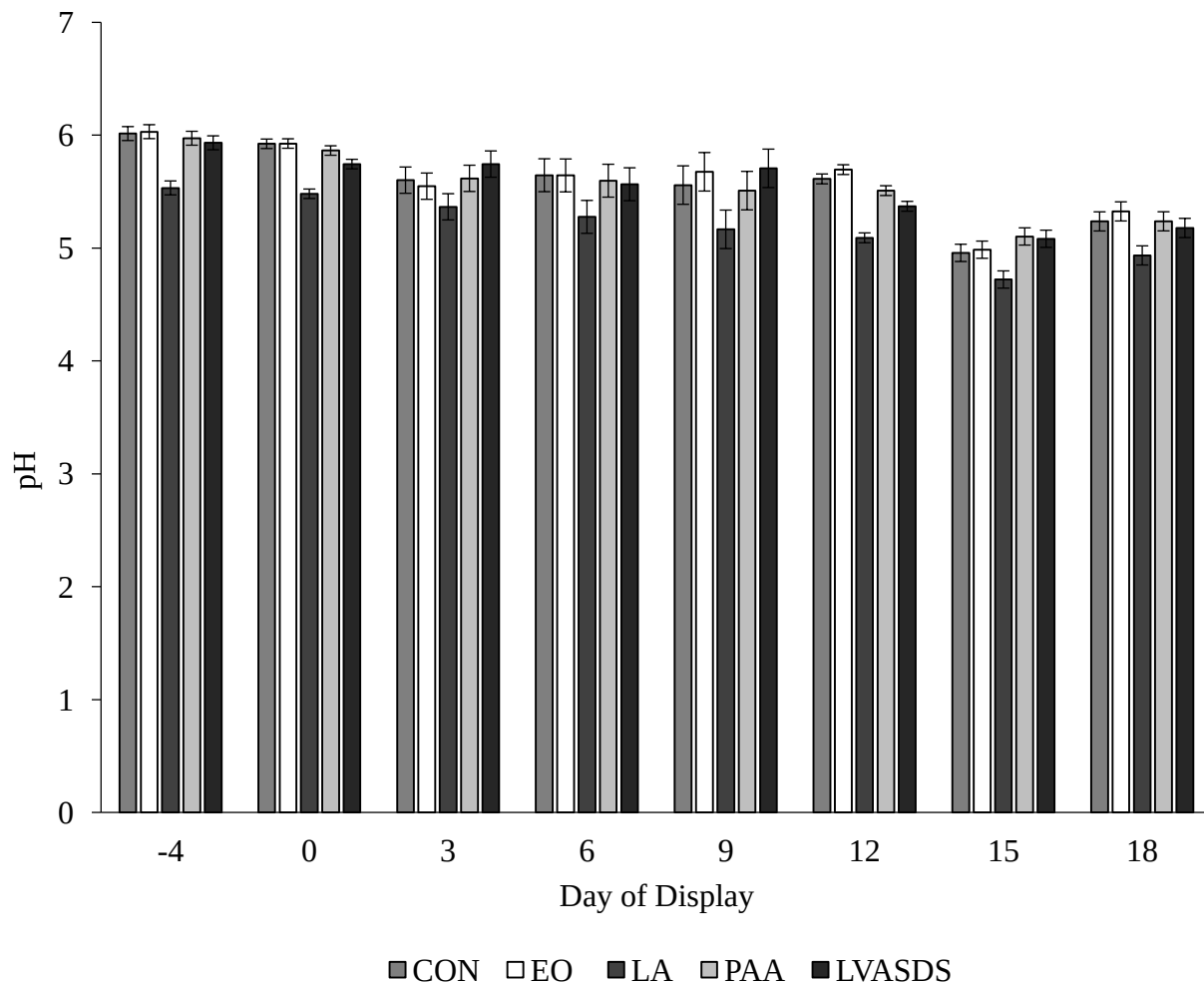


Figure 4.2. Day of display by antimicrobial treatment interaction on percent purge (least squares means  $\pm$  S.E.) for vacuum packaged ground beef patties through 18 d of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate. Least squares means within a day of display with different superscripts are different ( $P < 0.05$ ).

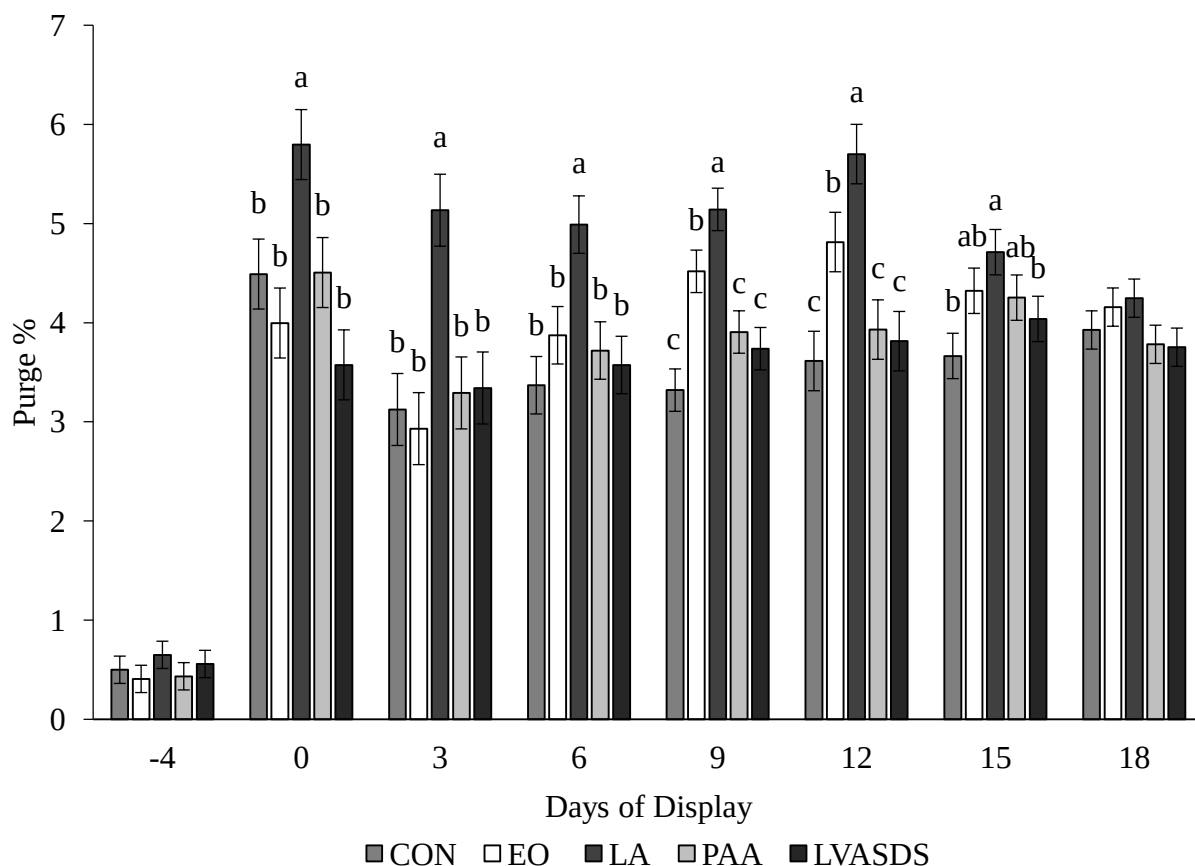
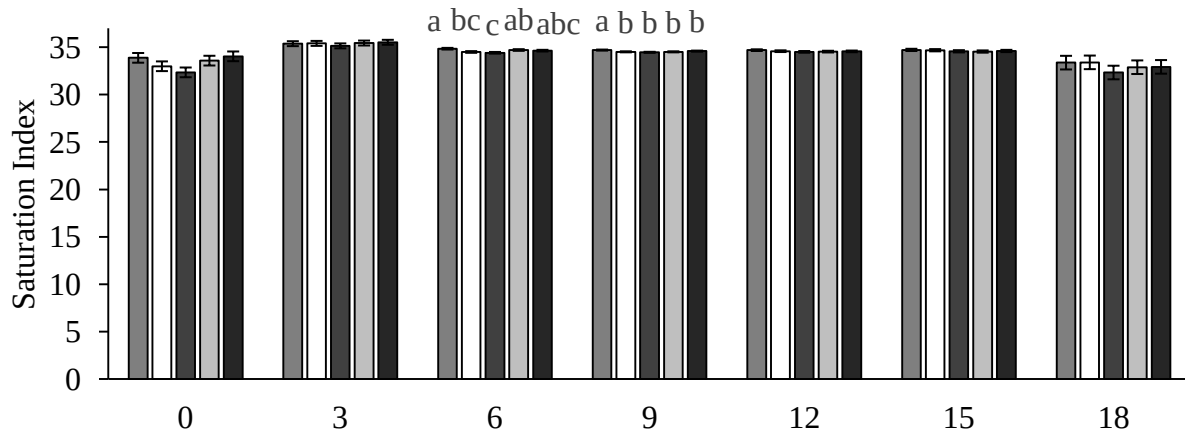
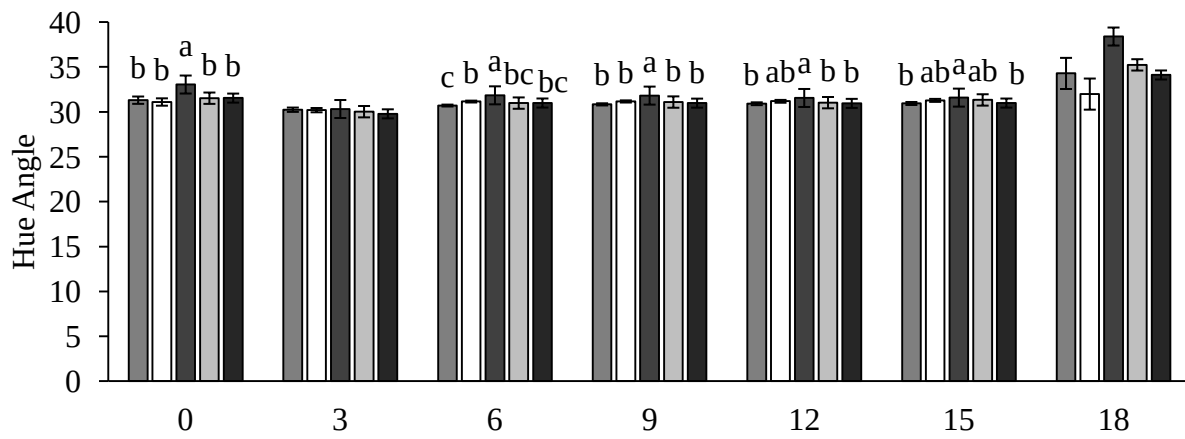


Figure 4.3. Day of display by antimicrobial treatment interaction for saturation index, hue angle, and 630/580 nm reflectance ratio (least square means  $\pm$  S.E.) for vacuum packaged ground beef patties through 18 d of retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate. A. Saturation index – higher values indicate more red saturation, calculated as  $(a^{*2} + b^{*2})^{0.5}$ . B. Hue angle – lower values indicate redder color, calculated as  $\tan^{-1}(b^*/a^*)$ . C. The 630/580 nm reflectance ratio – larger ratio indicates more redness. Least squares means within a day of display with different superscripts are different ( $P < 0.05$ ).

A.



B.



C.

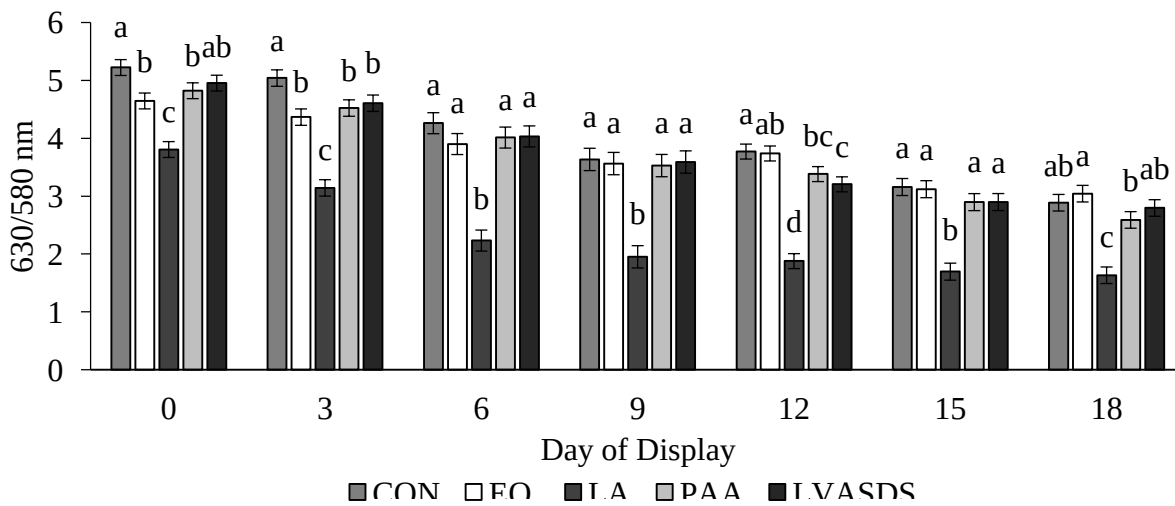
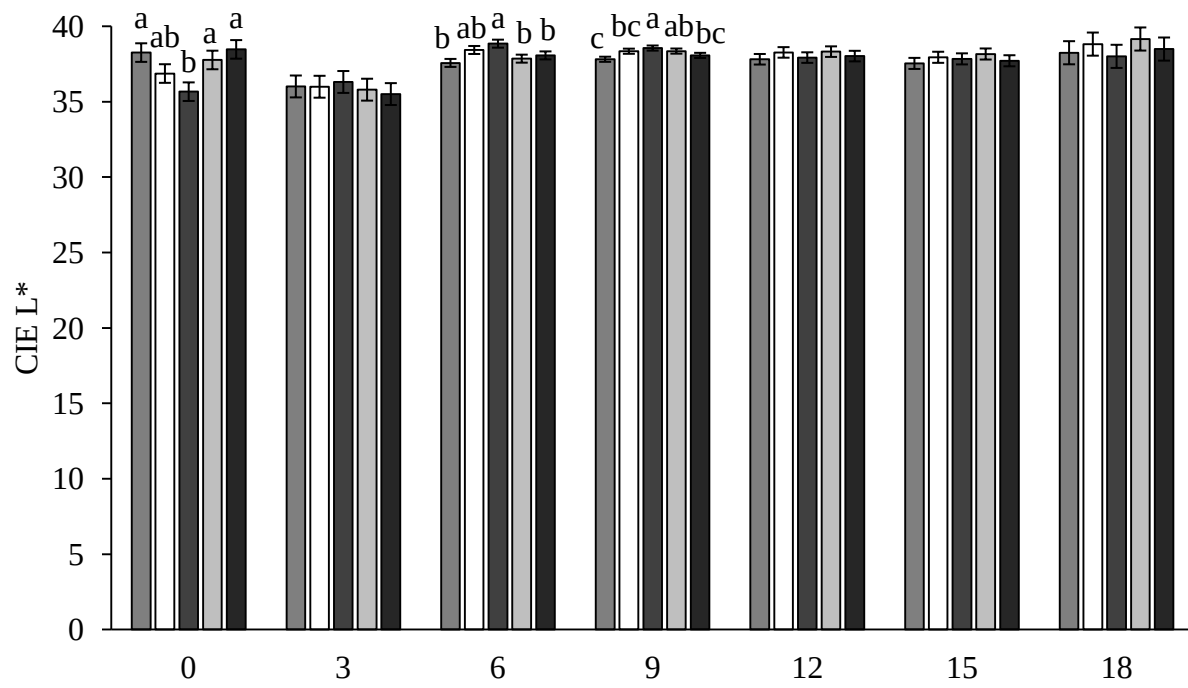


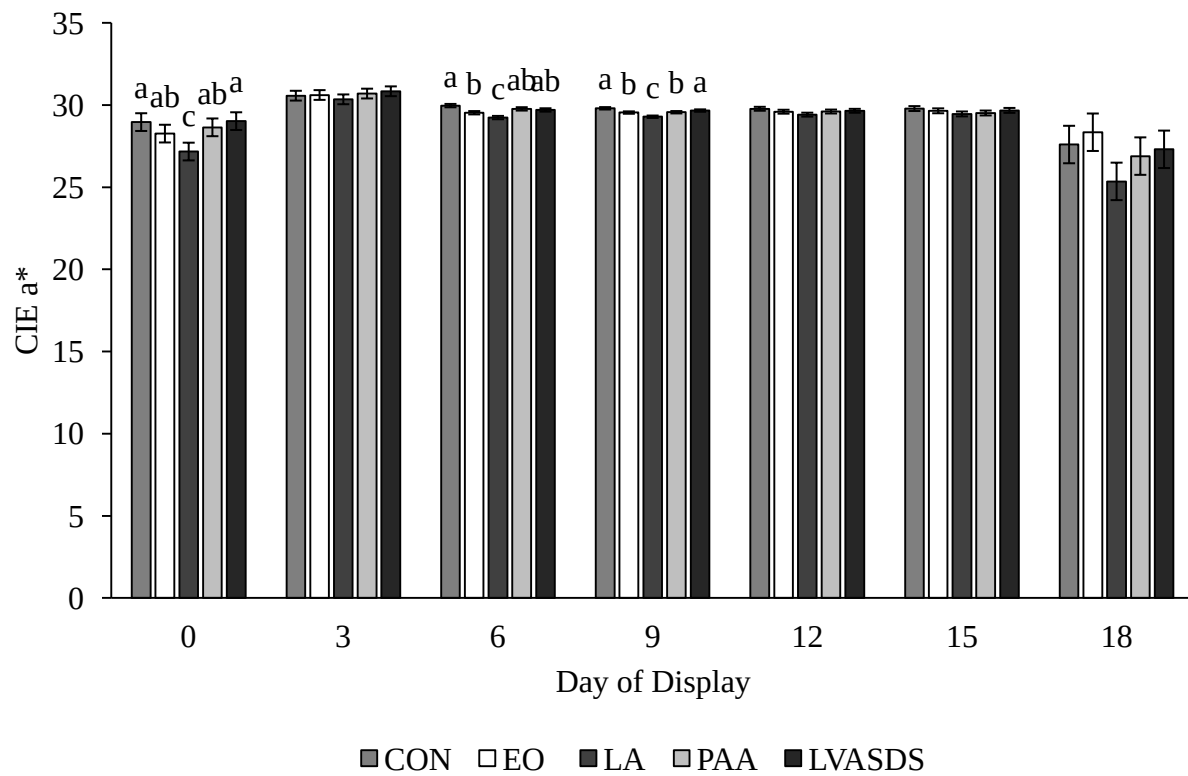


Figure 4.4. Day of display by antimicrobial treatment interaction for objective CIE L\*, a\*, and b\* color (least squares means  $\pm$  S.E.) for vacuum packaged ground beef patties through 18 d of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate. A. CIE L\* - 0 = black to 100 = white. B. CIE a\* - measures the green to red color spectrum; higher values indicate more red color. C. CIE b\* - measures the yellow to blue color spectrum; higher values indicate more yellow color. Least square means within a day of display with different superscripts are different ( $P < 0.05$ ).

A.



B.



C.

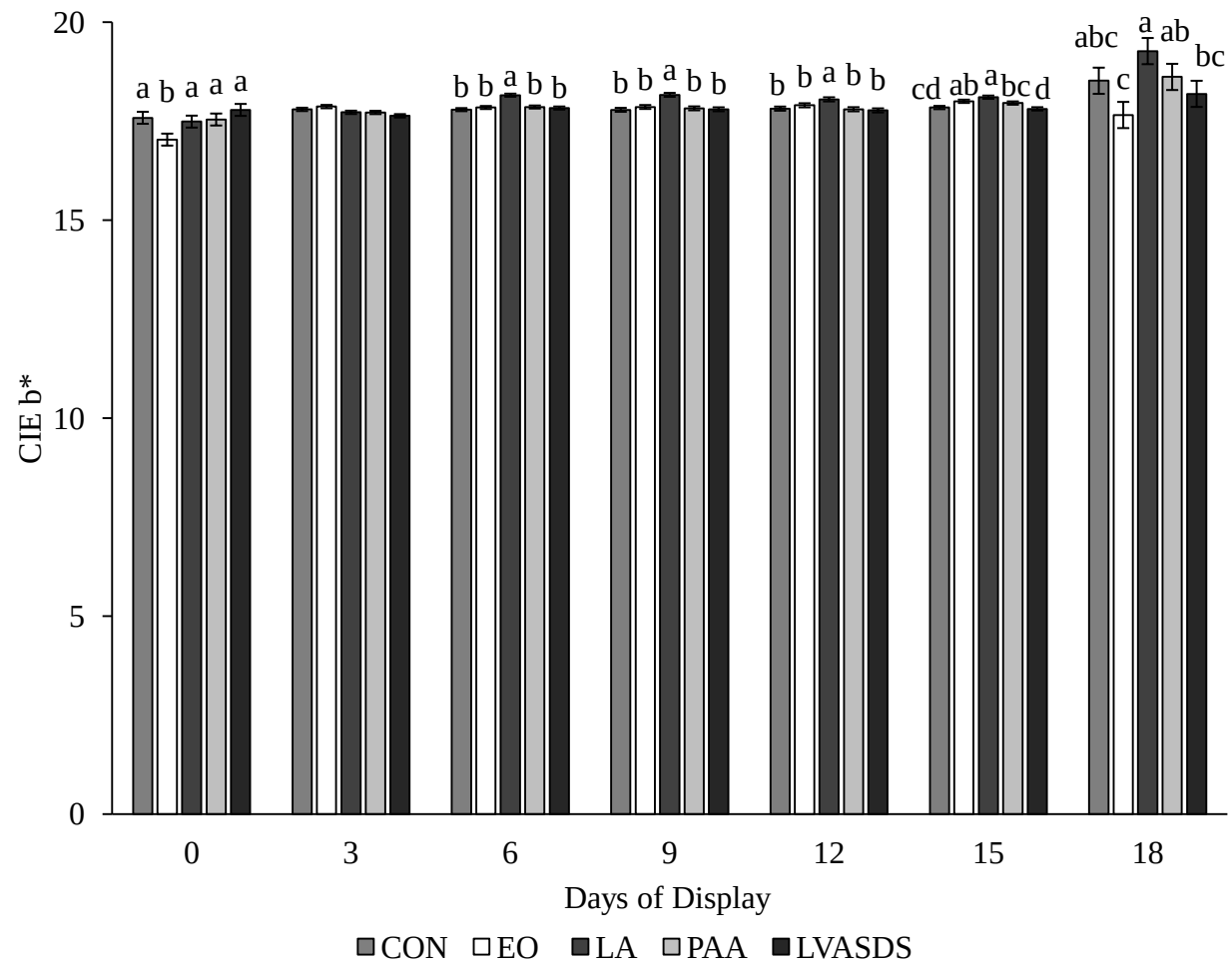
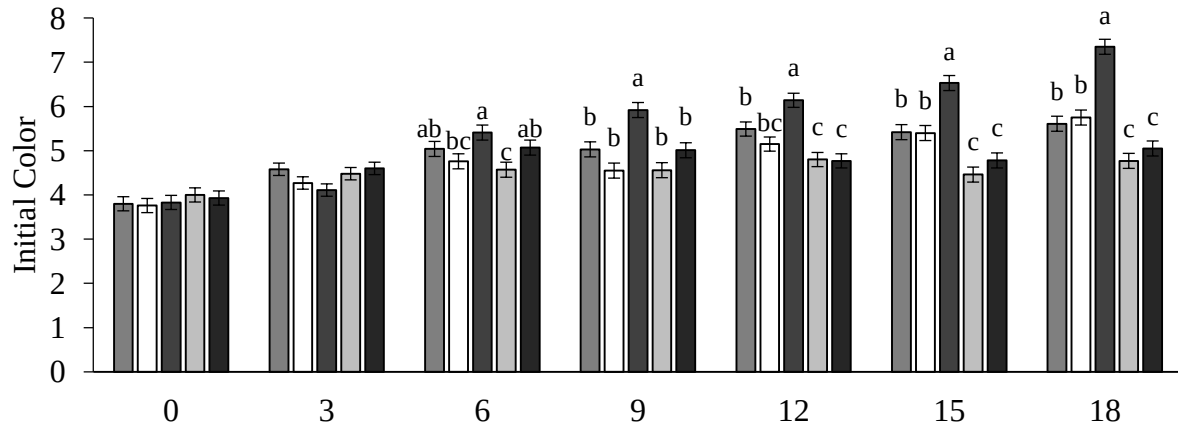
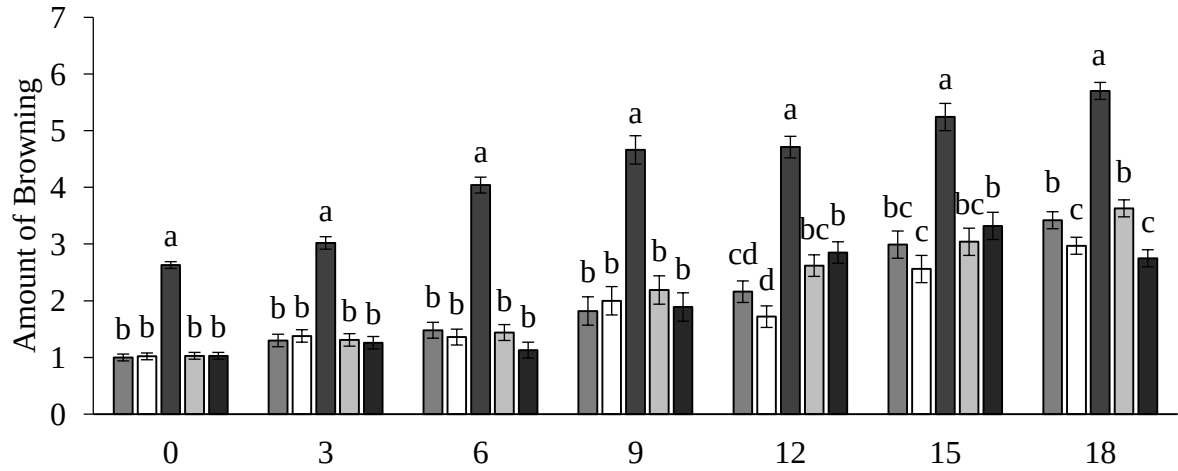


Figure 4.5. Day of display by antimicrobial treatment interaction for subjective color (least squares means  $\pm$  S.E.) for vacuum packaged ground beef patties through 18 d of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate. A. Initial color - 8 = extremely dark purple, 7 = dark purple, 6 = moderately dark purple, 5 = slightly dark purple, 4 = slightly purple-red, 3 = moderately bright purple, 2 = bright purple-red, and 1 = extremely bright purple-red. B. Amount of browning - 6 = dark brown, 5 = brown, 4 = brownish-gray, 3 = grayish, 2 = dull, and 1 = no evidence of browning. C. Percent of browning - 7 = 96 – 100%, 6 = 80 – 95%, 5 = 60 – 18%, 4 = 40 – 59%, 3 = 20 – 39%, 2 = 5 – 20%, 1 = 0 – 4% (Adapted from AMSA, 2012). Least squares means within a day of display with different superscripts are different ( $P < 0.05$ ).

A.



B.



C.

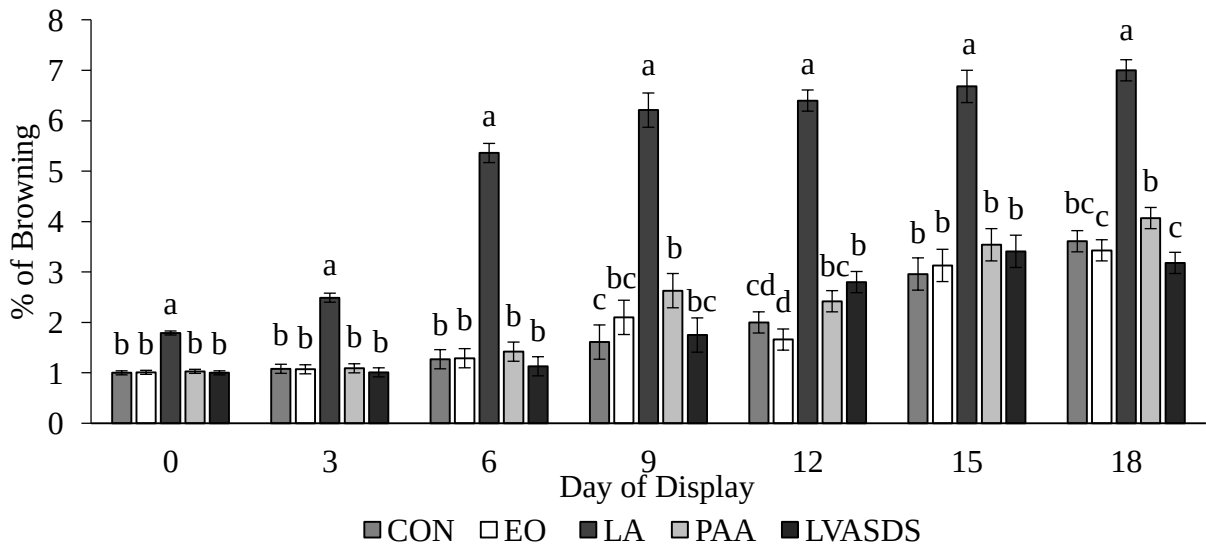


Figure 4.6. Day of display by antimicrobial treatment interaction ( $P = 0.26$ ; main effect of antimicrobial  $P = 0.10$ ; main effect of day of display  $P = 0.08$ ) on thiobarbituric acid reactive substance (mg malonaldehyde (MDA)/kg meat; least squares means  $\pm$  S.E.) for vacuum packaged ground beef patties through 18 days of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate.

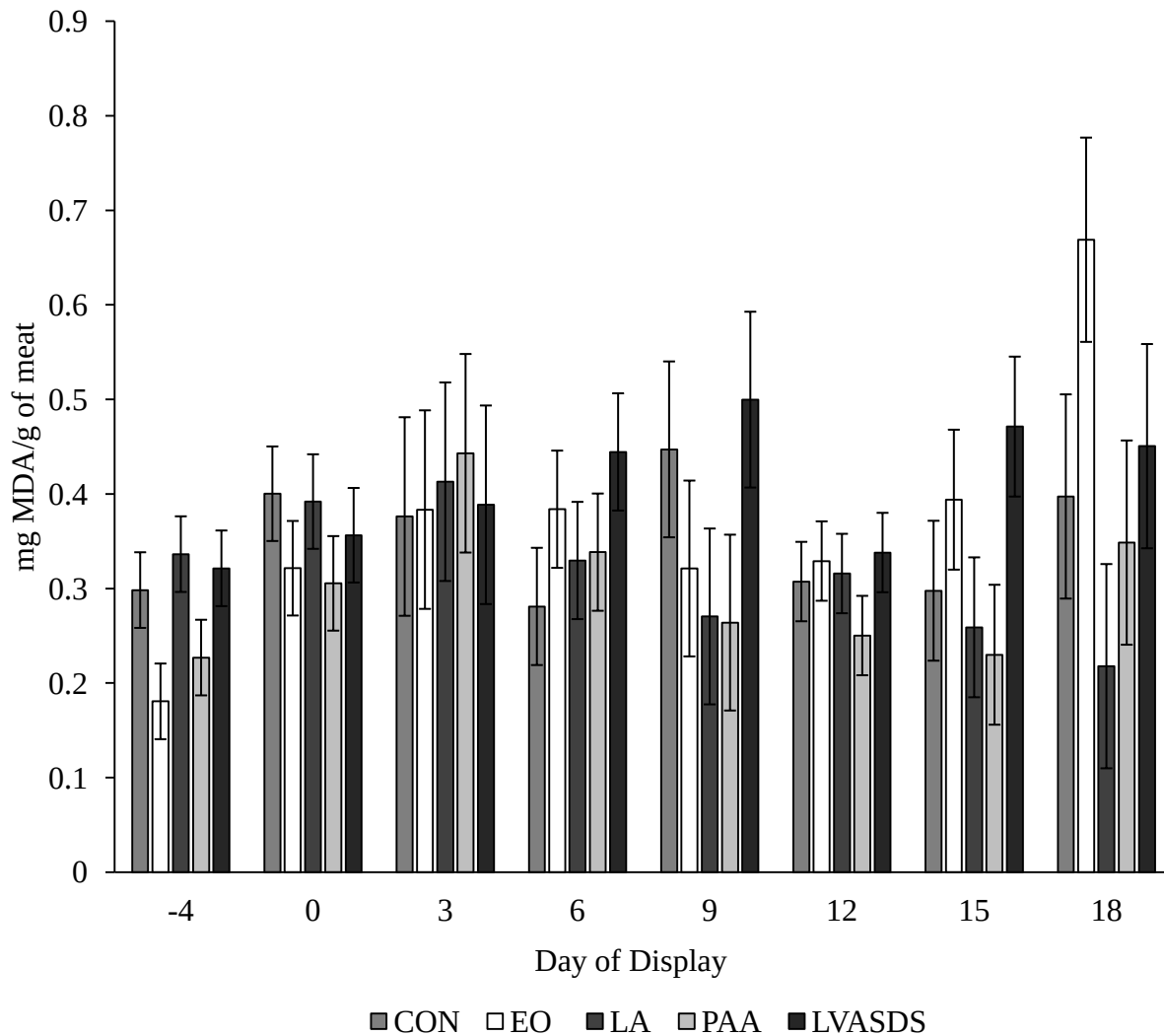


Figure 4.7. Day of display by antimicrobial treatment interaction ( $P = 0.26$ ; main effect of antimicrobial  $P < 0.0001$ ; main effect of day of display  $P < 0.0001$ ) for aerobic plate count (least squares means  $\pm$  S.E.) for vacuum packaged ground beef patties through 18 d of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate.

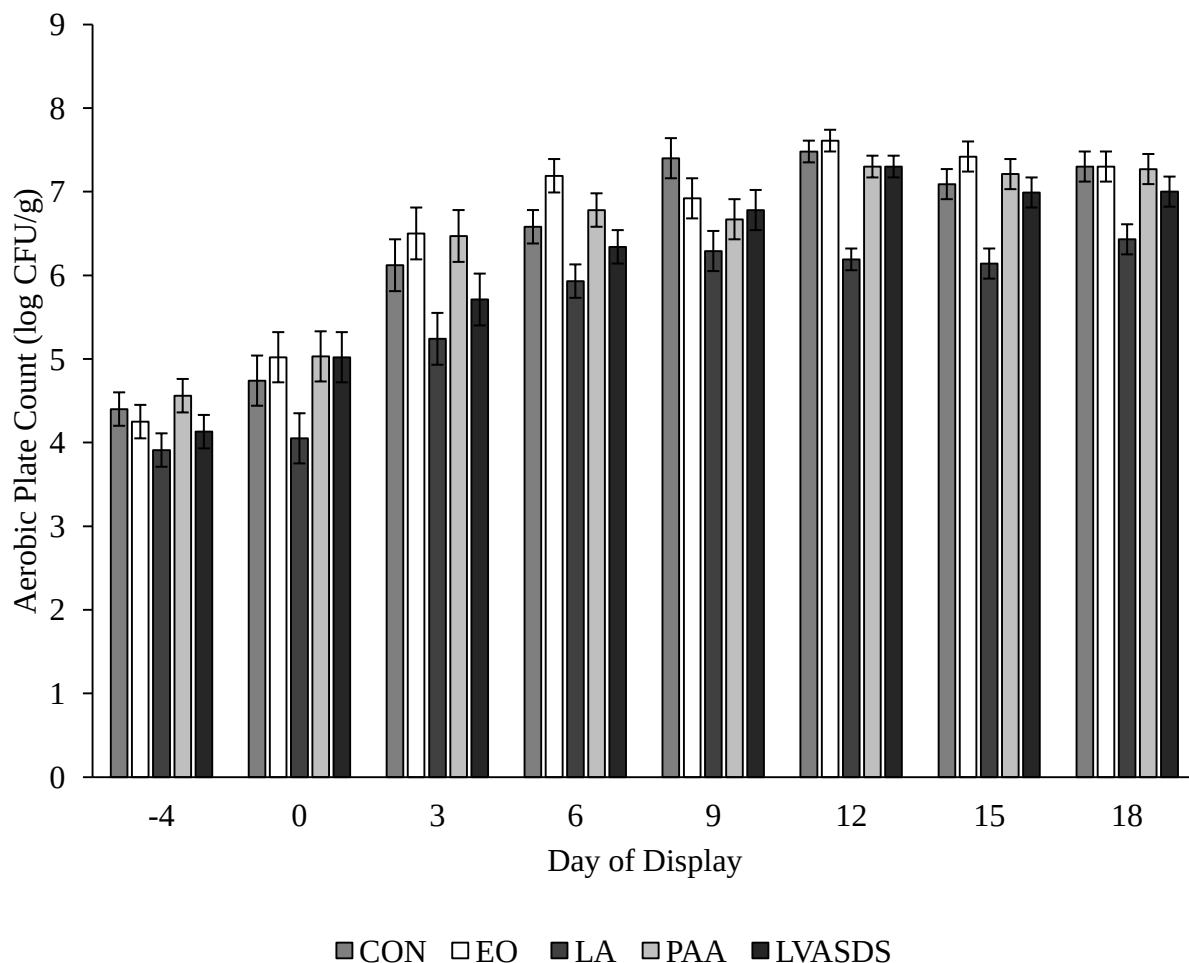
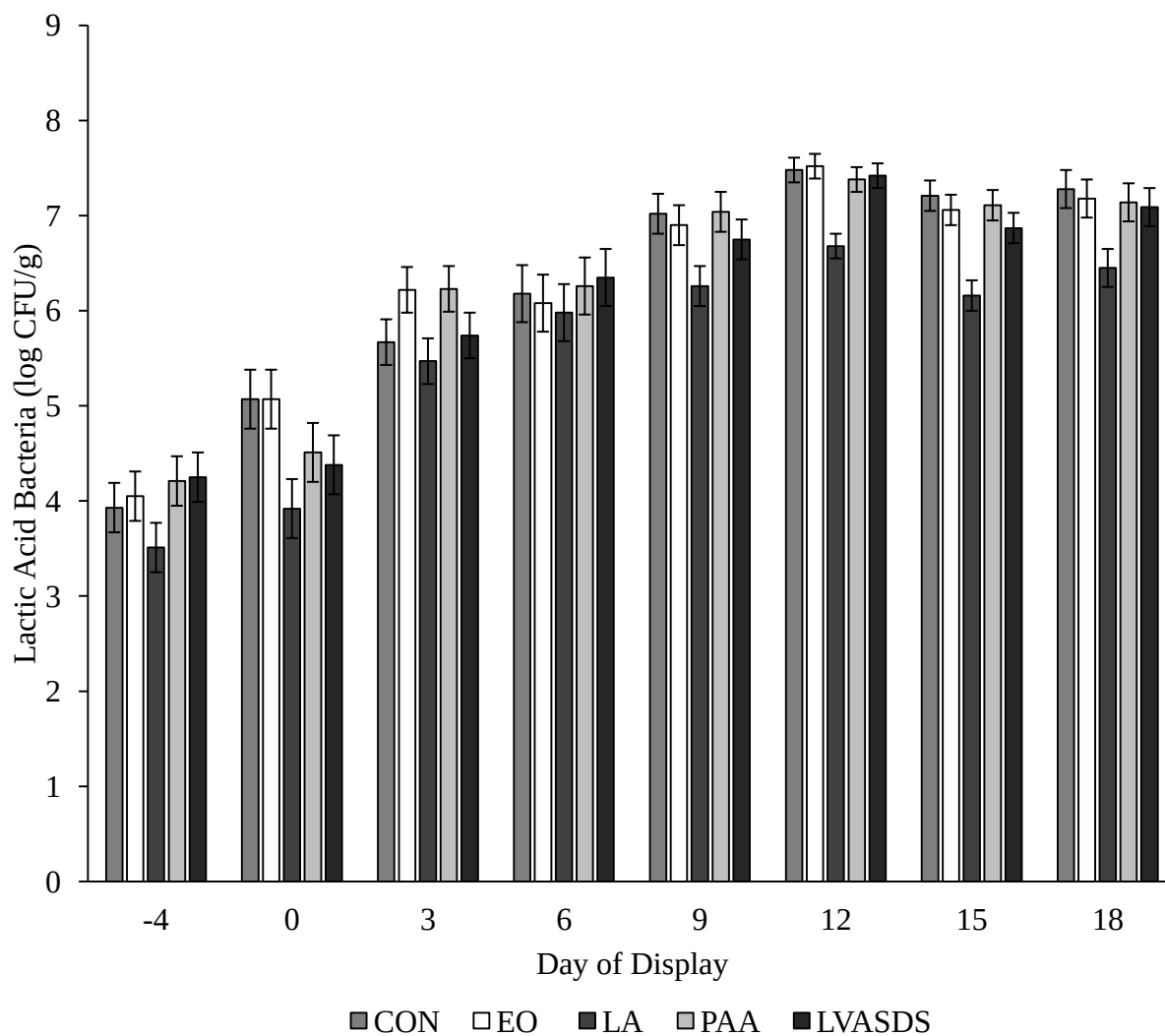


Figure 4.8. Day of display by antimicrobial treatment interaction ( $P = 0.53$ ; main effect of antimicrobial  $P < 0.0027$ ; main effect of day of display  $P < 0.0001$ ) for lactic acid bacteria counts (least squares means  $\pm$  S.E.) for vacuum packaged ground beef patties through 18 days of simulated retail storage. CON = control beef patties with no antimicrobial; EO = beef patties with 50 ppm chlorine generated from acidic electrolyzed oxidizing water; LA = beef patties with 4.5% lactic acid; PAA = beef patties with 200 ppm peroxyacetic acid; and LVASDS = beef patties with 2.0% levulinic acid plus 0.2% sodium dodecyl sulfate.





## **CHAPTER 5**

### **CONCLUSION**

In conclusion, ground beef is one of the most consumed products within the United States and providing a safe and wholesome product is important to the meat industry. The use of existing pathogen interventions and the production of novel interventions are an important technology to be used within ground beef production. Meat quality is affected by the growth of microorganisms and lipid oxidation. Antimicrobial technology has the ability to enhance the quality and prolong shelf life of ground beef. The use of levulinic acid plus sodium dodecyl sulfate has shown to be able to increase shelf life due to retarding the growth of microorganisms. Additionally, this study displayed that the use of current and novel antimicrobials can be applied without having negative effects on sensory characteristics.

Another important factor in meat acceptability is the color. Ground beef traditionally packaged in polyvinyl chloride overwrap is bright cherry red which relates to freshness and high quality. However, the use of antimicrobials can cause different effects upon the type of packaging a product is placed on display. Consumers approve of the bright cherry red color that is produced from oxygenated ground beef wrapped in polyvinyl chloride overwrap and are less accepting of product in vacuum packaged case-ready packages which are typically purple-red color. The use of levulinic acid plus sodium dodecyl sulfate and electrolyzed oxidizing water have shown that they can increase consumer acceptance, by causing the vacuum packaged product to be more red compared to the current industry standards. The color stability related to the use of these novel antimicrobials is beneficial to the beef industry.

The current research has shown that the use of the two novel antimicrobials, levulinic acid plus sodium dodecyl sulfate and electrolyzed oxidizing water, can improve and maintain meat quality and shelf life characteristics without causing negative effects when compared to the two industry standards. However, further research is required to test different concentrations and intervals of shelf life for both polyvinyl chloride overwrap and vacuum packaged ground beef patties.