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# ANTIMICROBIAL INTERVENTIONS APPLIED TO BEEF SUB-PRIMALS FOR THE CONTROL OF ESCHERICHIA COLI AND THEIR IMPACT ON GROUND BEEF QUALITY

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**ANTIMICROBIAL INTERVENTIONS APPLIED TO BEEF SUB-PRIMALS FOR  
THE CONTROL OF *ESCHERICHIA COLI* AND THEIR IMPACT ON GROUND  
BEEF QUALITY**

by

Kelly Ann McCarty

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ANTIMICROBIAL INTERVENTIONS APPLIED TO BEEF SUB-PRIMALS FOR  
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University of Nebraska, 2016

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Due to their severe health implications, seven shiga toxin-producing *E. coli* (STEC) serotypes were declared adulterants by USDA-FSIS in raw, non-intact beef products. Small business beef processing facilities are being asked to implement an intervention on the beef sub-primals and trim they receive for ground beef production. This study evaluates the efficacy of reducing rifampicin-resistant *E. coli* (*E. coli*<sup>Rif</sup>) using 4.5% lactic acid (LA), 2.5% Beefxide™ (BX), and 380 ppm peroxyacetic acid (PAA) when applied by dip, spray or electrostatic spray (ESS) to the mostly fat lateral surface and mostly lean medial surface of beef shoulder clods (IMPS 114) and compared to an inoculated control on reducing the presence of *E. coli*<sup>Rif</sup> on beef sub-primals meant for ground beef. All organic acid treatments had a greater reduction ( $P < 0.01$ ) than the inoculated control using *E. coli* petrifilm. However, when using APC petrifilm, all ESS treatments were similar ( $P > 0.05$ ) to the control. In addition, LA had greater ( $P < 0.05$ ) *E. coli*<sup>Rif</sup> reductions on *E. coli* petrifilm than BX treatments. PAA had greater ( $P < 0.05$ ) *E. coli*<sup>Rif</sup> reductions using APC petrifilms than BX. Ground beef samples from electrostatic spray treatments had similar reductions ( $P > 0.05$ ) to that of the inoculated control and had lower reductions ( $P < 0.001$ ) than the dip or spray methods. A second

study was designed to determine the effects of the same acids and application methods on ground beef quality. Ground beef samples from the PAA spray were darker ( $P < 0.05$ ) than the control, PAA dip and electrostatic spray, BX spray and electrostatic spray, and LA spray. In addition, LA electrostatic spray was less yellow ( $P < 0.01$ ) than the control and BX dip and spray treatments. When looking at APC counts, LA electrostatic spray had higher CFU/g than PAA spray and dip, BX spray and electrostatic spray, and LA dip. TBARS values showed that LA treatments had greater ( $P < 0.01$ ) oxidation than PAA treatments. LA dip and electrostatic and BX spray had lower ( $P < 0.001$ ) pH values compared to the control. All treatments reduced *E. coli*<sup>Rif</sup> counts and had minimal effects on quality attributes.

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## INTRODUCTION

An estimated 9.4 million Americans get sick each year with 55,961 hospitalizations and 1,351 deaths from foodborne illnesses in 2006 (Scallan et al., 2011). These illnesses can be brought on by several different pathogenic microorganisms that are found on various foods. *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli* (STEC) are major pathogens when related to beef products, mainly ground beef. After a major foodborne outbreak in 1993 that resulted in numerous hospitalizations and several deaths, the Food Safety and Inspection (FSIS) has conducted testing for *E. coli* O157:H7 in raw ground beef products (FSIS, 1999). Multiple outbreaks involving other STEC have caused six other strains of *E. coli* to become adulterants in non-intact beef products (FSIS, 2012). Since 2010, recalls related to STEC have been between 5-13 recalls a year, with year 2011 experiencing the most. This has caused over 16 million pounds of ground beef to be recalled from 2010 to 2015 from STEC.

During the process of slaughter, microorganisms can be transferred to the beef carcass during the slaughter process as the intestinal tract is the primary reservoir of *E. coli* O157:H7. Consequently, the hide can become contaminated during the slaughter process and cause contamination on the beef carcass if appropriate sanitary and slaughter procedures are not followed. It is well documented that STEC can cause mild to severe illnesses which include diarrhea, bloody diarrhea, hemorrhagic colitis, hemolytic-uremic syndrome and even death (HUS; Brooks et al., 2005). However, the meat industry has worked to administer interventions to reduce the occurrence of STEC on beef.

Regardless of what intervention is selected, factors such as cost, effect on the food, and the legal limit of its use will be the major determining factors on which product

is more suitable for the meat processor (Wheeler et al., 2014). Small and very small processing facilities often have to buy beef sub-primals or beef trim for the production of ground beef. When doing so, it is hard to tell if there were any interventions already applied to the meat. Due to recent rules and regulations, this research was conducted to better understand the effects organic acid interventions on the reduction of *E. coli* and the effects on quality attributes of ground beef produced.

## LITERATURE REVIEW

### *Escherichia coli*

*Escherichia coli* can be normally found in the intestinal tract of most warm blooded animals such as cattle and humans. However, some strains of *E. coli* are known to be human pathogens due to their ability to survive various environments (Karmali, 2004; Meng et al., 2001). Acidic environments, such as the gastrointestinal tract of humans, are one of those environments that *E. coli* has to endure to cause illnesses (Bearson, Bearson, and Foster, 1997). *E. coli* are classified as Gram negative, rod-shaped, non-spore forming, peritrichous, motile and facultatively anaerobic organisms. Water activity, temperature and pH are the most important environmental factors that determine the growth, survival and multiplication of *E. coli* (ICMSF, 1996). Strains of *E. coli* are differentiated by surface antigens: O (somatic), H (flagella), and K (capsule). A total of 167 'O', 53 'H' and 74 'K' antigens have been identified and documented (Kaper, Nataro, and Mobley, 2004). However, during an outbreak for instance, only the O and H antigen are used to describe the serotype of an *E. coli* strain (Meng et al., 2001). *E. coli* are separated out into categories based upon their clinical symptoms, virulence properties, mechanisms of pathogenicity, and O:H serotypes (Meng et al., 2001). In 1982, *E. coli* O157:H7 was identified as the cause of two hemorrhagic colitis outbreaks causing Enterohemorrhagic *E. coli* (EHEC) to first be recognized as human pathogens. According to Meng et al (2001), EHEC cause the most severe illness when related to foodborne outbreaks. Some *E. coli* strains are known to produce Shiga toxins, and are therefore named shiga toxin-producing *E. coli* (STEC) (Meng et al., 2001). STEC infections are linked with a severe and sometimes fatal condition called hemolytic-uremic

syndrome (HUS), hemorrhagic colitis, and nonspecific diarrhea (Karmali et al., 1985; Karmali, 2004). Food and water sources as well as person to person transmission are viable sources STEC infections (Karmali, 2004). Contaminated foods such as raw or undercooked ground meat products, raw milk, and fecal contamination of vegetables are primary sources of EHEC outbreaks (Karmali, 2004; WHO, 2011).

*Escherichia coli* O157:H7 and non-O157 STEC have raised major concerns for the beef industry, health organizations and consumers. Recalls and outbreaks are still happening in spite of all the efforts made to control these pathogens. FSIS reported that the total number of STEC recalls from 2010 to 2015 were 31% of total beef recalls (Table 1).

**Table 1: STEC related beef recalls from 2010-2015**

Year	Number of STEC recalls	Number of beef recalls	lbs of STEC related recalls**
2010	12	28	2,313,423
2011	13	35	1,002,971
2012*	5	19	63,467
2013	9	20	10,771,539
2014	5	22	1,840,533
2015	8	41	215,593
Total	52	165	16,207,526

\*The year 2012 was when testing for non-O157 STEC began; FSIS (2012).

\*\*Total pounds of beef recalled from 2010 to 2015 was 39,569,843 lbs; FSIS (2015a; 2015b; 2015c; 2015d; 2015e; 2016b)

Since *E. coli* are a natural inhabitant in cattle intestinal tracts, there is a high potential that these microorganisms could be transferred to the carcass from hides and feces during the slaughter process (Liao et al., 2015). Research has indicated beef hides are the primary source of carcass contamination (Barkocy-Gallagher et al., 2003; Elder et al., 2000; Small et al., 2005; Wheeler et al., 2014). Contaminated beef products can support the growth of

STEC if not processed sufficiently, handled, and cooked properly, resulting in serious public health problems (Wheeler et al., 2014). The contamination level of pathogens on the hide, techniques used to minimize pathogen transfer from the hide to the carcass, and efficacy of interventions applied during various beef processing steps are all major factors that play a role in the risk of potential carcass contamination (Barkocy-Gallagher et al., 2003; Wheeler et al., 2014). Preventative measures such as animal cleaning and post-stunning hide decontamination, knife trimming of defined carcass areas, whole-carcass hot water and chemical washes or sprays, and effective carcass chilling are applied in combination as an attempt to increase meat safety (Wheeler et al., 2014). Arthur et al. (2004) tested the effectiveness of antimicrobial interventions in the slaughter process by testing the prevalence of *E. coli* O157 at different points throughout the process. With the two plants they tested, *E. coli* O157 was found on 75.7% of the cattle hides. When tested again at pre-evisceration and post-evisceration percentages dropped to 14.7% and 3.8%, respectively. Furthermore, when tested at post-intervention percentages dropped to 0.3% and were non-detectable after chilling.

### ***E. coli* O157:H7**

After being linked as the source for two outbreaks of hemorrhagic colitis in 1982, *E. coli* O157:H7 was recognized for as a human pathogen for the first time (Meng, 2001). According to the CDC (2015), and estimated 95,400 illnesses annually in the United States are caused by *E. coli* O157:H7. The FoodNet 2014 Surveillance Report has reported 444 laboratory confirmed cases including 155 hospitalizations and 3 deaths. The incidence of infection in 2014 was 0.91 per 100,000 Americans, which decreased from 2011 to 2013 by 19% (CDC, 2014). A very low infectious dose of *E. coli* O157:H7, 0.3

to 15 CFU per gram were enumerated from sources of outbreaks (Karmali, 2004; Meng et al., 2001). *E. coli* O157:H7 possess many virulence factors including shiga toxin (Stx) production, attaching and effacing (*eae*) gene, locus of enterocyte effacement (LEE), and tolerance to acidic environments. The *eae* gene is located in the central region of LEE encoding the adhesion and intimin of the organism (Karmali, 2004). After an incubation period of 3-5 days, these factors can cause symptoms such as stomach cramps, bloody diarrhea, and vomiting, as well as more severe symptoms such as hemorrhagic colitis, hemolytic uremic syndrome (HUS), or thrombotic thrombocytopenic purpura (TTP) (CDC, 2015; Karmali, 2004; Meng et al., 2001; Wang et al., 2012). The attaching-and-effacing (AE) mechanism causes the pathogen to colonize in the cecum or colon of orally infected animals (Karmali, 2004; Meng et al., 2001). The intimate attachment of bacteria to the intestinal cells, with effacement of the underlying microvilli and accumulation of filamentous actin (F-actin) in the cytoplasm characterize the AE lesion. The LEE island, which provides necessary proteins, has also been identified to assist with AE formation (Meng et al., 2001). Doyle and Schoeni (1984) and Meng et al., (2001) state that *E. coli* O157:H7 in ground beef have no unusual resistance to heat. However, Line et al. (1991) suggest that the presence of fat may protect *E. coli* O157:H7 causing D values with longer cook times.

The largest outbreak of *E. coli* O157:H7 happened in 1996 at several public elementary schools in Sakai City, Japan. More than 11,000 *E. coli* O157:H7 cases were reported that resulted in 11 deaths. Authorities never positively confirmed the source of this outbreak, but isolates from the outbreak suggested various origins of contamination (Meng et al., 2001; WHO, 1996).

The Jack in the Box outbreak that occurred in 1993 drastically revolutionized the meat and food safety industry. Over 700 cases were reported and 4 children died from *E. coli* O157:H7 infections. The outbreak was linked to undercooked beef patties in Washington, Idaho, California, and Nevada that were served at various Jack in the Box restaurants (Barkocy-Gallagher, 2003; FSIS, 1999; Meng et al., 2001). In October 1994, as a result to this outbreak, the Food Safety and Inspection Service of the United States Department of Agriculture (USDA-FSIS) declared *E. coli* O157:H7 to be an adulterant in raw ground beef products. Additionally, federally inspected plants and retail stores began testing for *E. coli* O157:H7 as part of the sampling program (FSIS, 1999; Meng et al., 2001; Wheeler et al., 2014). Currently, all meat and poultry plants are required to develop and implement a Hazard Analysis Critical Control Points (HACCP) program. HACCP is now used as one of the vital tools to prevent and control *E. coli* O157:H7 and non-O157 STEC contamination in the beef industry.

### ***Non-O157 STEC***

According to the CDC (2015), six serotypes of non-O157 shiga toxin-producing *E. coli* (STEC) are responsible for approximately 169,600 illnesses annually in the United States, nearly twice the amount of *E. coli* O157:H7. The most common non-O157 STEC strains linked to foodborne illnesses are O26, O45, O103, O111, O121, and O145. These six non-O157 serotypes account for approximately 70% of the non-O157 STEC (Wang et al., 2012) and were declared adulterants in raw non-intact beef products as of June 2012 along with *E. coli* O157:H7 (FSIS, 1999; FSIS, 2012; Wheeler et al., 2014). The FoodNet 2014 Surveillance Report has reported 697 laboratory confirmed cases including 106



hospitalizations and 2 deaths. The incidence of infection for 2014 was 1.43 per 100,000 Americans, which has increased from 2011 to 2013 by 22% (CDC, 2014).

### ***Bacterial Attachment to Beef Tissues***

Different bacterial species, serotypes or strains can respond to various environmental conditions and food matrix characteristics. Since beef cattle are a natural reservoir of STEC, there is a potential for meat surface contamination during the slaughter and fabrication process, especially from hides (Liao et al., 2015; Wheeler et al., 2014). The ability for bacteria to attach to food surfaces and their potential to multiply is a critical step in food contamination.

Understanding the mechanisms that are involved in bacterial attachment is needed to prevent and remove attached microorganisms. A conditioning film is formed on food contact surfaces from bacteria and other organic and inorganic molecules absorbing to the surface, leading to a higher concentration of nutrients. With increased nutrients, biofilm formation is favorable (Kumar and Anand, 1998). According to Kumar and Anand (1998), proteins such as casein and gelatin favored microbial attachment while albumin inhibited attachment of microbes to surfaces.

Attachment of organisms to the conditioned surface is the second step in biofilm formation, which partially depends on the motility and growth stage of the bacteria as well as the nutrient availability, pH, and temperature. Attachment of bacteria usually happens in two stages, reversible adhesion followed by irreversible adhesion. Reversible adhesion develops a weak interaction between the bacterial cells and conditioning surface, which can easily be reversed by processes like rinsing. During irreversible adhesion repulsive forces prevent direct contact with the surface, but allows appendages,

such as flagella or pili, to contact the surface. The bacterial cell and the conditioned surface form a bridge enabling the irreversible adhesion. Scrubbing or scrapping must occur to remove cells in this stage. (Kumar and Anand, 1998).

Once irreversible adhesion has occurred, bacteria will start to grow and divide using the nutrients from the conditioned surface which leads to the formation of microcolonies.

Additional polymer (EPS) is produced during this stage to anchor the cells to the surface and stabilize colonies. The bacterial cell's continuous attachment and growth to the conditioned surface forms the biofilm. Serious hygienic problems and food spoilage are some major issues related to bacterial attachment (Kumar and Anand, 1998).

### **Antimicrobial Interventions**

Interventions during the slaughter process, such as hide-on carcass wash and proper hide removal, can significantly reduce the level of bacteria on hides, which would reduce the risk of contamination to beef products. Additionally, physical removal of contamination from the carcass (knife trimming, steam-vacuuming, and water washing) before entering the cooler can also reduce microbial growth. Even with the above programs in place the risk of contamination is still present, and if contaminated, animal products can support the growth of bacteria if not properly handled (Wheeler et al., 2014). No single intervention is 100% effective and therefore a multi-hurdle approach would reduce the risk of STEC and other pathogens significantly.

Organic acid interventions can interfere with nutrient transport affecting microbial growth. Therefore, organic acid interventions during the fabrication process could greatly reduce the risk of STEC on the surface of beef products. Conner and Kotrola (1995) reported that temperature and pH of the acid solution played a major role in the ability to

inhibit growth of *E. coli* O157:H7. They found that at increasing incubation temperatures, *E. coli* was less likely to be inhibited. Similarly, with increasing pH values (from 4.0 to 7.0) *E. coli* had more growth. Therefore, by adding organic acids to meat products, pH is lowered and in turn the ability of *E. coli* to grow is reduced. In a review by Wheeler et al. (2014), greater than 1.0 log reduction of *E. coli* was found when using lactic acid, Beefxide™, and peroxyacetic acid.

### ***Lactic Acid***

Lactic acid and other organic acids are generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) as an antimicrobial for use on beef products up to 5% concentration by FSIS Directive 7120.1 Safe and Suitable Ingredients (FSIS, 2016a). The most commonly used organic acid in the meat industry is lactic acid because of low cost and effectiveness (Wheeler et al., 2014) and its ability to inhibit the growth of food spoilage organisms (Alakomi et al., 2000).

Lactic acid, when in its uncharged, protonated form diffuses across the cell membrane of *E. coli* and other enterobacteria. Once in the membrane, the weak acid dissociates which in turn lowers the internal pH of the cell and disrupts the transmembrane proton force. More acid will be able to diffuse across the cell membrane and dissociate inside the cell with a more acidic pH outside of the cell (Alakomi et al., 2000; Bearson, Bearson, and Foster, 1997). Alakomi et al. (2000), used NPN (hydrophobic probe) to test the effectiveness of lactic acid against *E. coli*, *P. aeruginosa*, and *Servoar Typhimurium*. An increase of NPN uptake within the cell indicates damage in the outer membrane. They found that lactic acid had had a greater uptake of NPN than that of the other substances they used (HCl and EDTA). They concluded that lactic acid was successful at

disintegrating the outer membrane by releasing the lipopolysaccharide (LPS) layer from the surface.

However, Youssef et al. (2012) suggest that the effectiveness of lactic acid can vary based on the concentration of the solution, the nature of the treated surface, the degree of contamination, and the volume of solution applied to the surface of the meat. To test this, they used an outer fat surface (outer round and brisket), a cut meat surface (sirloin tip primal cuts), and a membrane overlying muscle tissue (medial surface of flank). Each cut of meat was subjected to an inoculation at either 7.0, 4.0, or 1.0 log CFU/ml; a spray volume of 0.5, 0.1, and 0.02 ml of fluid per square centimeter (ml/cm<sup>2</sup>); and a treatment type of no treatment, water spray, or 5% lactic acid spray. When spraying on the fat surface, no reductions were found with the water spray treatment but applying lactic acid at 0.5 and 0.1 ml/cm<sup>2</sup> reduced *E. coli* counts by >0.9 log units whereas 0.02 ml/cm<sup>2</sup> lactic acid application only reduced *E. coli* counts by ≥0.4 log units. When spraying the cut muscle surface, reductions of <0.1 log units were found with the water spray treatment. When spraying 5% lactic acid solution, 0.5 ml/cm<sup>2</sup> volume applied reduced *E. coli* counts by >1 log unit, while 0.1 and 0.02 ml/cm<sup>2</sup> volumes only reduced *E. coli* counts by ≤0.7 log units. When using membrane covered muscle tissue, all volumes of 5% lactic acid had a reduction of >2 log units. With this study, Youssef, et al. (2012), concluded that with most meat surfaces a greater volume of 5% lactic acid applied will result in a larger reduction. They also stated that spraying 5% lactic acid at ≥0.1 ml/cm<sup>2</sup> would sufficiently reduce *E.coli* and presumably other pathogens by 0.5-1.0 log units.

Similarly, Hardin, et al. (1994) used subcutaneous fat and lean carcass tissues from four different hot carcass regions (inside and outside round, brisket, and clod) to model

decontamination of prechilled carcass surface regions. Each carcass tissue was trimmed, sprayed with water, or sprayed with water followed by 2% lactic or acetic acid.

Regardless of treatment, the outside round was the most difficult surface to decontaminate. However, they found that spraying with water followed by organic acid decontaminated surfaces better than trimming or water alone on all surfaces except the inside round. The decontamination of the inside round was equal from both trimming and water spray followed by organic acid. Additionally, lactic acid treatments had higher reductions than acetic acid treatments overall. However, all treatments significantly reduced pathogens from fecal contamination on all carcass surface regions. Gill and Badoni (2004) tested the effects on the natural microflora of chilled beef carcasses using 0.02% peroxyacetic acid, 0.16% acidified sodium chlorite, 2% lactic acid and 4% lactic acid. This study showed that acidified sodium chlorite and peroxyacetic acid had little effects on the microflora of beef carcasses and were less effective than 4% lactic acid. Gill and Badoni (2004) reported that a reduction of  $\geq 1.5$  log units and 1 log unit was achieved when using 4% lactic acid and 2% lactic acid, respectively. However, they state that the efficacies of these solutions may be inconsistent on chilled surfaces due to a reduction of  $\geq 2$  log units on distal surfaces and  $< 2$  log units on medial surfaces.

Harris, et al. (2006) tested lactic and acetic acids at 2% and 4% as well as acidified sodium chlorite at 1,200 ppm on the effectiveness of reducing *Salmonella* Typhimurium and *E. coli* O157:H7 at low (1.0 log CFU/g) and high (4.0 log CFU/g) inoculation levels from beef trim prior to grinding. They tested each sample 5 times throughout production and storage. All trim and ground beef samples at low levels of inoculation were reduced to non-detectable numbers. Regardless of treatment, all trim samples at high inoculation

levels were reduced by 1.5-2.0 log units. However, immediately after grinding both organic acids reduced pathogens more effectively than acidified sodium chlorite, but no differences were found in treatments after one day of storage. However, the effectiveness of 4% lactic acid, 200 ppm peroxyacetic acid, 1,000 ppm acidified sodium chlorite and 85°C hot water spray treatments were tested on the surface of fresh beef flanks. Hot water spray treatments resulted in the highest reductions (3.2-4.2 log CFU/cm<sup>2</sup>) followed by lactic acid (Kalchayanand et al., 2012).

Heller et al. (2007) inoculated beef subprimal cuts intended for mechanical tenderization with *E. coli* O157:H7. Inoculated outside round pieces were trimmed with a knife, sprayed with hot water (82°C) sprayed with 2.5% or 5.0% lactic acid (55°C), or sprayed with 2% activated lactoferrin followed by warm 5.0% lactic acid. After treatment, outside round pieces were subjected to either blade tenderization or moisture enhancement. Mean surface reductions of 0.93-1.10 log CFU/100 cm<sup>2</sup> of *E. coli* O157:H7 we found from all treatments. Blade tenderized steaks had internalized *E. coli* O157:H7 in 3 of 76 samples, while 73 of the 76 moisture enhancement samples had internalized *E. coli* O157:H7. They concluded that interventions before mechanical tenderization were effective in reducing the transfer of *E. coli* O157:H7 to the interior of beef subprimals.

### ***Peroxyacetic Acid***

Peroxyacetic acid is another organic acid that is generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) as an antimicrobial for use on beef products (FSIS, 2016a).

Peroxyacetic acid (0.02%), potassium lactate (3%) sodium metasilicate (4%) or acidified sodium chlorite (0.1%) were applied using a tumbler to beef trimmings inoculated with

*E. coli* and *Salmonella* Typhimurium. All treatments reduced *E. coli*, coliforms and aerobic plate counts up to 1 log and *Salmonella* Typhimurium to  $\geq 1.5$  log. This defends the hypothesis that peroxyacetic acid would be effective at reducing surface bacteria on beef trim meant for ground beef (Pohlman et al., 2009).

Contradictorily, King et al. (2005) tested the effectiveness of reducing *E. coli* O157:H7 and *Salmonella* Typhimurium using peroxyacetic acid at three concentrations (200, 600, and 1000 ppm) applied at two temperatures (45° and 55°C). They used a hand-pump sprayer for 15 seconds on meat pieces from the plate, brisket, and clod regions. The 4% lactic acid (55°C) solution was applied to meat pieces in the same manner for comparison. Peroxyacetic treatments at low concentrations had very little lethality at either temperature. Application of 1000 ppm peroxyacetic acid at 45°C showed numerically greater reductions, whereas, 1000 ppm peroxyacetic acid at 55°C had significantly greater reductions of both *E. coli* O157:H7 and *S. Typhimurium*. However, lactic acid reductions (2.7 log CFU/cm<sup>2</sup>) were numerically higher than all peroxyacetic acid treatments, but 1000 ppm peroxyacetic acid at 55°C (1.7 log CFU/cm<sup>2</sup>) was not significantly different. However, the authors of this study conclude that the reductions found from peroxyacetic acid treatments were not effective and further in plant validation studies should be conducted to verify this antimicrobial treatment. In addition, Ellebracht et al. (2005) concluded that 200 ppm peroxyacetic acid dip was less effective than 2% lactic acid dip in reducing pathogens (*E. coli* O157:H7 and *Salmonella* Typhimurium) on fresh beef trim.

### ***Beefxide™***

Beefxide is a blend of lactic acid and citric acid that can be used up to a 2.5% concentration for antimicrobial reduction on beef.

A study done by Hendricks et al. (2014) looked at the effectiveness of 2.9% lactic acid and 2.4% Beefxide™ applied to boneless strip loins, top sirloin butt-cap off, and bottom sirloin flap using a commercial spray cabinet. After application of an antimicrobial each product was passed through a blade tenderizer. Lactic acid treatments had a 1.3 log reduction while Beefxide™ treatments had a 1.4 log reduction. These researchers concluded that lactic acid and Beefxide™ were similar in the efficiency of reducing *E. coli* in the production of non-intact beef products. Similarly, in a validation study done by Laury et al. (2009), 2.5% Beefxide™ was applied to beef tips using a spray cabinet to test the reductions of generic *E. coli*, *E. coli* O157:H7 and *Salmonella*. In this study Beefxide™ reduced generic *E. coli* by 0.4 log CFU/cm<sup>2</sup>, *E. coli* O157:H7 by 1.4 log CFU/cm<sup>2</sup>, and *Salmonella* by 1.1 log CFU/cm<sup>2</sup>.

### **Antimicrobial Effects on Beef Quality**

Although antimicrobial interventions have been investigated on the efficacy to reduce pathogen growth, effects on beef quality are also important. Factors including the retention of an attractive, fresh appearance, retardation of bacterial spoilage, and minimization of exudative losses are principal factors described by Gill (1996) that must be addressed in the preservation of chilled meats. Ideally, antimicrobial interventions should have no impact or a positive impact on beef quality while still minimizing microbial growth.



## ***Color***

Color is an important factor in meat quality as consumers use color to decide freshness of ground beef quality (Gill, 1996; Pohlman et al., 2009). Therefore, improving color stability of meat and maximizing shelf life is a concern to retailers. Meat color is determined by the type of pigment molecule, its chemical state, and the quantity of the pigment (Bekbolet, 1989). When meat is exposed to oxygen for an extended amount of time—like that of retail display—oxymyoglobin and deoxymyoglobin meat pigments can oxidize to form metmyoglobin. This results in a brown colored meat that consumers associate with deterioration of meat quality (Gill, 1996). According to a review done by Mancini and Hunt (2005), many researchers have found both positive and negative color changes when applying organic acids. For instance, Ellebracht et al. (1999) found that hot water treatment and hot water followed by lactic acid both darkened trim samples compared to the untreated control. In this study they used mature and young beef trim and dipped them into a hot water or hot water followed by 2% lactic acid. Even though darker surfaces were found in treated trim, this was not the case in ground beef samples. They found that there was no difference in  $L^*$ ,  $a^*$ , or  $b^*$  values in ground beef samples. They stated that the color change was temporary, not sufficient enough to effect the final product, or was diluted when mixed with the unaffected inner lean tissue. Similarly, 0.02% peroxyacetic acid was applied by tumbler to beef trim for three minutes prior to grinding. In their analysis of color, they found that peroxyacetic acid had lighter color ( $L^*$  value) than the control. However, there were no differences in redness ( $a^*$  value), yellowness ( $b^*$  value), or oxymyoglobin proportions when compared with the control. The reason for the lighter colored ground beef could be attributed to the acidic

nature of peroxyacetic acid (pH = 5.2). These authors concluded that it is possible to maintain color and sensory properties while using antimicrobial interventions for ground beef (Pohlman et al., 2009). In early work, Kotula and Thelappurath (1994) dipped steaks into 0.6% or 1.2% lactic acid or acetic acid for 20 or 120 seconds. They found that all treated samples had lighter color than the untreated control. They stated that they believe this is due to leaching of the pigment during treatment. However, they found no differences in  $a^*$  or  $b^*$  values.

In addition, Stivarius et al. (2002) tumbled beef trimmings for three minutes using three different treatments: hot water (82°C), 5% lactic acid, or an untreated control. They found that the lactic acid treatment had lighter color and less oxymyoglobin content compared to the other two treatments. Lactic acid pH was 5.44 while pH for the control and hot water treatment were 6.06 and 6.01, respectively.

In addition, Quilo et al. (2009) found that ground beef patties from beef trim tumbled in a 0.02% peroxyacetic acid solution for three minutes were lighter colored than the untreated control. However, they also found that peroxyacetic acid treated samples had higher redness values than the control on days 0, 1, and 3 of retail display (sampled on days 0, 1, 2, 3, & 7). Additionally, oxymyoglobin content was higher in peroxyacetic acid samples than the control for days 0, 1 and 3. The decrease in oxymyoglobin values throughout retail display was similar to the decrease in  $a^*$  values. Peroxyacetic acid treated samples (pH = 5.6) had pH values similar to the control samples.

Additionally, Jimenez-Villarreal et al. (2003) tumbled beef trim in 2% lactic acid for 3 minutes before grinding and making ground beef patties. They found that ground beef patties exhibited lighter color compared to the untreated control. Additionally, they found

that lactic acid treatments were less red and had a lower pH but had similar oxymyoglobin content than the control. This shows that lactic acid had a considerable effect on pH which could have affected the  $L^*$  and  $a^*$  values.

### ***Lipid Oxidation***

When purchasing meat in stores, oxidized fat can detract a consumer from buying a product (Gill, 1996). Therefore, determining if an antimicrobial treatment has an impact on lipid oxidation is a major concern to retailers.

Quilo et al. (2009) found that ground beef patties from beef trim tumbled in a 0.02% peroxyacetic acid solution for three minutes had lower lipid oxidation values than the control sample throughout the 7 day retail display. In contrast, Jimenez-Villarreal et al. (2003) applied 2% lactic acid to beef trim using a meat tumbler. They found that in days 0-3 there were no differences in TBARS values, but on day 7 lactic acid treated samples had greater lipid oxidation than the untreated control. Additionally, Ellebracht et al. (1999) found no differences in lipid oxidation between young and mature beef samples when dipped in hot water or hot water followed by 2% lactic acid when compared to the control.

### ***Odor***

Meat with unappealing or off odors will deter consumers from purchasing meat products from stores. Therefore, determining if antimicrobials will form an unappealing or off odor would be beneficial to retailers.

Quilo et al. (2009), found that beef trim that was tumbled in 0.02% peroxyacetic acid for 3 minutes had similar or less off odors than untreated control samples. On days 0, 2, and 7 of retail display there were no differences in off odors. On days 1 and 3 of retail

display, peroxyacetic acid treated samples had less off odors than untreated control samples. In addition, Ellebracht et al. (1999) found that beef trim from either mature cattle or young cattle treated with hot water or hot water followed by 2% lactic acid had less of a soured and putrid odor than the untreated control. These two aromas are both related to bacterial spoilage, as soured smells increase as lactic acid bacteria increase and putrid smells increase with sulfur-producing spoilage microorganisms. In agreement, when Jimenez-Villarreal et al. (2003) tumbled beef trim in 2% lactic acid for three minutes. They found the sensory panelists did not find any odor characteristic differences throughout retail display. These studies indicate that peroxyacetic acid and lactic acid could be used as an antimicrobial intervention while still maintaining or improving odor of ground beef samples.

### ***Bacterial Spoilage***

The rate of bacterial spoilage can affect the quality of the meat product as certain bacteria cause unappealing odors and can cause pH fluctuations causing color changes. Therefore, it is important to note if antimicrobial interventions will decrease spoilage organisms and impact beef shelf life.

Kotula and Thelappurath (1994) dipped steaks in acetic or lactic acid at 0.6% or 1.2% for either 20 or 120 seconds. They found that steaks dipped for 120 seconds in 1.2% acetic acid had the lowest microbial counts (0.8 log CFU lower) when compared to the untreated control on day 0. Steaks dipped in 1.2% lactic acid for 120 seconds had a 0.4 log CFU lower than the untreated control on day 0. However, by day 9 of retail display 1.2% lactic acid dipped for 120 seconds had the greatest difference between the untreated control at 1.7 log CFU. They found that increasing concentration of the acid and

increasing length of time dipped proved to be more effective at reducing microbial populations.

Although Ellebracht et al. (1999) did not directly measure bacterial spoilage organisms, they found that soured and putrid smells were decreased with hot water followed by 2% lactic acid. Since soured aromatic are associated with lactic acid producing bacteria and putrid smells are associated with sulfur producing bacteria, one could conclude that with decrease in both of these smells could result in less spoilage bacteria.

### **Small and Very Small Processor Practices**

With numerous *E. coli* and *Salmonella* pathogen outbreaks, processors are under more pressure to ensure the safety of meat produced in their facilities. FSIS has required beef processors to evaluate their HACCP plans to determine if *E. coli* O157:H7 contamination is reasonably likely to occur. If the reassessment was likely, then this hazard was to be addressed in their HACCP plan. This resulted in all beef facilities to implement at least one intervention to serve as a critical control point (CCP). This is especially challenging for very small processing facilities, which have less than 10 employees and less than \$2.5 million in annual sales (Algino, Ingham, and Zhu, 2007). The very small processing facilities often do not have the resources or finances for mechanized intervention systems that large facilities have. Therefore, this research will examine the effects of implementing an antimicrobial intervention strategy for small processing facilities that could provide a cost-effective way to reduce pathogens to acceptable levels without affecting the quality attributes of the ground beef product.

## LITERATURE CITED

- Alakomi, H.-L., E. Skytta, M. Saarela, T. Mattila-Sandholm, K. Latva-Kala, and I.M. Helander. 2000. Lactic Acid Permeabilizes Gram-Negative Bacteria by Disrupting the Outer Membrane. *Applied and Environmental Microbiology*. 66:2001-2005.
- Algino, R.J., S.C. Ingham, and J. Zhu. 2007. Survey of Antimicrobial Effects of Beef Carcass Intervention Treatments in Very Small State-Inspected Slaughter Plants. *Journal of Food Safety: Food Microbiology and Safety*. 72:M173-M179.
- Arthur, T.M., J.M. Bosilevac, X. Nou, S.D. Shackelford, T.L. Wheeler, M.P. Kent, D. Jaroni, B. Pauling, D.M. Allen, and M. Koohmaraie. 2004. *Escherichia coli* O157 Prevalence and Enumeration of Aerobic Bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at Various Steps in commercial Beef Processing Plants. *Journal of Food Protection*. 67:658-665.
- Barkocy-Gallagher, G.A., T.M. Arthur, M. Rivera-Betancourt, X. Nou, S.D. Shackelford, T.L. Wheeler, and M. Koohmaraie. 2003. Seasonal Prevalence of Shiga Toxin-Producing *Escherichia coli*, including O157:H7 and Non-O157 Serotypes, and *Salmonella* in Commercial Beef Processing Plants. *Journal of Food Protection*. 66:1978-1986.
- Bearson, S., B. Bearson, and J.W. Foster. 1997. Acid Stress Responses in Enterobacteria. *Federation of European Microbiological Societies*. 147:173-180.
- Bekbolet, M. 1989. Light Effect on Food. *Journal of Food Protection*. 53:430-440.
- Brooks, J.T., E.G. Sowers, J.G. Wells, K.D. Greene, P.M. Griffin, R.M. Hoekstra, and N.A. Strockbine. 2005. Non-O157:H7 Shiga Toxin-Producing *Escherichia coli* Infections in the United States, 1983-2002. *Journal of Infectious Diseases*. 192:1422-1429.
- Centers for Disease Control and Prevention. 2014. Foodborne Diseases Active Surveillance Network: FoodNet 2014 Surveillance Report.
- Centers for Disease Control and Prevention. 2015. Available at: <http://www.cdc.gov/ecoli/general/index.html>. Accessed: July 25, 2016.
- Conner, D.E., and J.S. Kotrola. 1995. Growth and Survival of *Escherichia coli* O157:H7 under Acidic Conditions. *Applied and Environmental Microbiology*. 61:382-385.
- Doyle, M.P., and J.L. Schoeni. 1984. Survival and Growth Characteristics of *Escherichia coli* Associated with Hemorrhagic Colitis. *Applied and Environmental Microbiology*. 48:855-856.
- Elder, R.O., J.E. Keen, G.R. Siragusa, G.A. Barkocy-Gallagher, M. Koohmaraie, and W.W. Laegreid. 2000. Correlation of Enterohemorrhagic *Escherichia coli* O157 Prevalence in Feces, Hides, and Carcass of Beef Cattle During Processing. *Proceedings of the National Academy of Sciences of the United States of America*. 97:2999-3003.

- Ellebracht, E.A., A. Castillo, L.M. Lucia, R.K. Miller, and G.R. Acuff. 1999. Reduction of Pathogens Using Hot Water and Lactic Acid on Beef Trimmings. *Journal of Food Science*. 64:1094-1099.
- Ellebracht, J.W., D.A. King, A. Castillo, L.M. Lucia, G.R. Acuff, K.B. Harris, and J.W. Savell. 2005. Evaluation of Peroxyacetic Acid as a Potential Pre-grinding Treatment for Control of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on Beef Trimmings. *Meat Science*. 70:197-203.
- Food Safety and Inspection Service. 1999. Policy on Non-intact Raw Beef Products Contaminated with *E. coli* O157:H7. Washington, D.C. Available at: <http://www.fsis.usda.gov/Oa/background/O157policy.htm>. Accessed: July 27, 2016.
- Food Safety and Inspection Service. 2012. Shiga Toxin-Producing *Escherichia coli* in Certain Raw Beef Products: Rules and Regulations. *Federal Register*. 77:31975-31981
- Food Safety and Inspection Service. 2015a. Summary of Recall Cases in Calendar Year 2010. Available at: <http://www.fsis.usda.gov/wps/portal/fsis/topics/recalls-and-public-health-alerts/recall-summaries/recall-summaries-2010>. Accessed: August 10, 2016.
- Food Safety and Inspection Service. 2015b. Summary of Recall Cases in Calendar Year 2011. Available at: <http://www.fsis.usda.gov/wps/portal/fsis/topics/recalls-and-public-health-alerts/recall-summaries/recall-summaries-2011>. Accessed: August 10, 2016.
- Food Safety and Inspection Service. 2015c. Summary of Recall Cases in Calendar Year 2012. Available at: <http://www.fsis.usda.gov/wps/portal/fsis/topics/recalls-and-public-health-alerts/recall-summaries/recall-summaries-2012>. Accessed: August 10, 2016.
- Food Safety and Inspection Service. 2015d. Summary of Recall Cases in Calendar Year 2013. Available at: <http://www.fsis.usda.gov/wps/portal/fsis/topics/recalls-and-public-health-alerts/recall-summaries/recall-summaries-2013>. Accessed: August 10, 2016.
- Food Safety and Inspection Service. 2015e. Summary of Recall Cases in Calendar Year 2014. Available at: <http://www.fsis.usda.gov/wps/portal/fsis/topics/recalls-and-public-health-alerts/recall-summaries/recall-summaries-2014>. Accessed: August 10, 2016.
- Food Safety and Inspection Service. 2016a. Safe and Suitable Ingredients used in the Production of Meat, Poultry, and Egg Products. FSIS Directive 7120.1 Rev. 38.
- Food Safety and Inspection Service. 2016b. Summary of Recall Cases in Calendar Year 2015. Available at: <http://www.fsis.usda.gov/wps/portal/fsis/topics/recalls-and-public-health-alerts/recall-summaries>. Accessed: August 10, 2016.
- Gill, C.O. 1996. Extending the Storage Life of Raw Chilled Meats. *Meat Science* 43:S99-S109.

- Gill, C.O., and M. Badoni. 2004. Effects of Peroxyacetic Acid, Acidified Sodium Chlorite or Lactic Acid Solutions on the Microflora of Chilled Beef Carcasses. *International Journal of Food Microbiology*. 91:43-50.
- Hardin, M.D., G.R. Acuff, L.M. Lucia, L.S. Oman, and J.W. Savell. 1994. Comparison of Methods for Decontamination from Beef Carcass Surfaces. *Journal of Food Protection*. 58:368-374.
- Harris, K., M.F. Miller, G.H. Loneragan, and M.M. Brashears. 2006. Validation of the Use of Organic Acids and Acidified Sodium Chlorite to Reduce *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in Beef Trim and Ground Beef in a Simulated Processing Environment. *Journal of Food Protection*. 69:1802-1807.
- Heller, C.E., J.A. Scanga, J.N. Sofos, K.E. Belk, W. Warren-Serna, G.R. Bellinger, R.T. Bacon, M.L. Rossman, and G.C. Smith. 2007. Decontamination of Beef Subprimal Cuts Intended for Blade Tenderization or Moisture Enhancement. *Journal of Food Protection*. 70:1174-1180.
- Hendricks, K.M., A.N. Arnold, T.M. Taylor, K.B. Gehring, and J.W. Savell. 2014. In-Plant Validation of Two Antimicrobial Agents Applied During Production of Further Processed Beef Products. *International Congress of Meat Science and Technology*. 60:186-189.
- ICMSF. 1996. Microorganisms in Foods 5, p. 126-140. *Characteristics of Microbial Pathogens*. Blackie Academic & Professional, London.
- Jimenez-Villarreal, J.R., F.W. Pohlman, Z.B. Johnson, and A.H. Brown, Jr. 2003. Effects of Chlorine Dioxide, Cetylphridinium Chloride, Lactic Acid, and Trisodium Phosphate on Physical, Chemical and Sensory Properties of Ground Beef. *Meat Science* 65:1055-1062.
- Kalchayanand, N., T.M. Arthur, J.M. Bosilevac, J.W. Schmidt, R. Wang, S.D. Shackelford, and T.L. Wheeler. 2012. Evaluation of Commonly Used Antimicrobial Interventions for Fresh Beef Inoculated with Shiga Toxin-Producing *Escherichia coli* Serotypes O26, O45, O103, O111, O121, O145, and O157:H7. *Journal of Food Protection*. 75:1207-1212.
- Kaper, J.B., J.P. Nataro, and H.L.T. Mobley. 2004. Pathogenic *Escherichia coli*. *Nature Reviews Microbiology*. 2:123-140.
- Karmali, M.A. 2004. Infection by Shiga Toxin-Producing *Escherichia coli*: An Overview. *Molecular Biotechnology*. 26:117-122.
- Karmali, M.A., M. Petric, C. Lim, P.C. Fleming, G.S. Arbus, and H. Lior. 1985. The Association Between Idiopathic Hemolytic Uremic Syndrome and Infection by Verotoxin-producing *Escherichia coli*. *Journal of Food Protection*. 151:775-782.
- King, D.A., L.M. Lucia, A. Castillo, G.R. Acuff, K.B. Harris, and J.W. Savell. 2005. Evaluation of Peroxyacetic Acid as a Post-Chilling Intervention for Control of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on Beef Carcass Surfaces. *Meat Science* 69:401-407.



- Kotula, K.L., and R. Thelappurath. 1994. Microbiological and Sensory Attributes of Retail Cuts of Beef Treated with Acetic and Lactic Acid Solutions. *Journal of Food Protection*. 57:665-670.
- Kumar, C.G., and S.K. Anand. 1998. Significance of Microbial Biofilms in Food Industry: A Review. *International Journal of Food Microbiology*. 42:9-27.
- Laury, A.M., M.V. Alvarado, G. Nace, C.Z. Alvarado, J.C. Brooks, A. Echeverry, and M.M. Brashears. 2009. Validation of a Lactic Acid- and Citric Acid-Based Antimicrobial Product for the Reduction of *Escherichia coli* O157:H7 and *Salmonella* on Beef Tips and Whole Chicken Carcasses. *Journal of Food Protection* 72:2208-2211.
- Liao, Y.-T., J.C. Brooks, J.N. Martin, A. Echeverry, G.H. Loneragan, and M.M. Brashears. 2015. Antimicrobial Interventions for O157:H7 and Non-O157 Shiga Toxin-Producing *Escherichia coli* on Beef Pubprimal and Mechanically Tenderized Steaks. *Journal of Food Protection*. 78:511-517.
- Line, J.E., A.R. Fain, Jr., A.B. Moran, L.M. Martin, R.V. Lechowich, J.M. Carosella, and W.L. Brown. 1991. Lethality of Heat to *Escherichia coli* O157:H7: D-Value and Z-value Determinations in Ground Beef. *Journal of Infectious Diseases*. 54:762-766.
- Mancini, R.A., and M.C. Hunt. 2005. Current Research in Meat Color. *Meat Science* 71:100-121.
- Meng, J., M.P. Doyle, T. Zhao, and S. Zhao. 2001. Enterohemorrhagic *Escherichia coli*, p. 193-213. In *Food Microbiology: Fundamentals and Frontiers*, 2<sup>nd</sup> Ed. ASM Press, Washington, D.C.
- Pohlman, F.W., P.N. Dias-Morse, S.A. Quilo, A.H. Brown, Jr., P.G. Grandall, R.T. Baublits, R.P. Story, C. Bokina, and G. Rajaratnam. 2009. Microbial, Instrumental Color and Sensory Characteristics of Ground Beef Processed from Beef Trimmings Treated with Potassium Lactate, Sodium Metasilicate, Peroxyacetic Acid or Acidified Sodium Chlorite as Single Antimicrobial Interventions. *Journal of Muscle Foods*. 20:54-69.
- Quilo, S.A., F.W. Pohlman, A.H. Brown, P.G. Crandall, P.N. Dias-Morse, R.T. Baublits, and J.L. Aparicio. 2009. Effects of Potassium Lactate, Sodium Metasilicate, Peroxyacetic Acid, and Acidified Sodium Chlorite on Physical, Chemical, and Sensory Properties of Ground Beef Patties. *Meat Science* 82:44-52.
- Scallan, E., R.M. Hoekstra, F.J. Angulo, R.V. Tauxe, M.-A. Widdowson, S.L. Roy, J.L. Jones, and P.M. Griffin. 2011. Foodborne Illness Acquired in the United States—Major Pathogens. *Emerging Infectious Diseases*. 17:7-15.
- Small, A., B. Wells-Burr, and S. Buncic. 2005. An Evaluation of Selected Methods for the Decontamination of Cattle Hides Prior to Skinning. *Meat Science* 69:263-268.
- Stivarious, M.R., F.W. Pohlman, K.S. McElyea, and A.L. Waldroup. 2002. Effects of Hot Water and Lactic Acid Treatment of Beef Trimmings Prior to Grinding on Microbial, Instrumental Color and Sensory Properties of Ground Beef. *Meat Science* 60:327-334.

- Wang, R., J. L. Bono, N. Kalchayanand, S. Shackelford, and D. M. Harhay. 2012. Biofilm formation by Shiga Toxin-Producing *Escherichia coli* O157:H7 and Non-O157 Strains and Their Tolerance to Sanitizers Commonly Used in the Food Processing Environment. *Journal of Food Protection*. 75:1418-1428.
- Wheeler, T.L., N. Kalchayanand, and J.M. Bosilevac. 2014. Pre- and Post-Harvest Interventions to Reduce Pathogen Contamination in the U.S. Beef Industry. *Meat Science* 98:372-382.
- World Health Organization. 1996. Enterohaemorrhagic *Escherichia coli* in Japan – Update. Available at: [http://www.who.int/csr/don/1996\\_08\\_28/en/](http://www.who.int/csr/don/1996_08_28/en/). Accessed: July 27, 2016.
- World Health Organization. 2011. Enterohaemorrhagic *Escherichia coli*. Available at: <http://www.who.int/mediacentre/factsheets/fs125/en/>. Accessed: July 25, 2016
- Youssef, M.K., X. Yang, .Badoni, and C.O. Gill. 2012. Effects of Spray Volume, Type of Surface Tissue and Inoculum Level on the survival of *Escherichia coli* on Beef Sprayed with 5% Lactic Acid. *Food Control*. 25:717-722.

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## Abstract

The purpose of this study was to test the effectiveness of 4.5% lactic acid (LA), 2.5% Beefxide™ (BX), and 380 ppm peroxyacetic acid applied to beef shoulder clods using dip (15 sec), spray, (5 sec, 137,895 Pa), and electrostatic spray (10 sec). Also, this research tested the effectiveness of these antimicrobial interventions on the mostly fat covered lateral surface and mostly lean covered medial surface of the sub-primal. The outer fat or inner lean surfaces were inoculated with a five strain cocktail of rifampicin resistant *E. coli* (*E. coli*<sup>Rif</sup>; ~ 5.6 log CFU/cm<sup>2</sup>). Samples were enumerated on APC and *E. coli*/coliform Petrifilm. All antimicrobial treatments had less *E. coli*<sup>Rif</sup> ( $P < 0.001$ ) than the untreated control. However, dip and spray applications were more effective at reducing *E. coli*<sup>Rif</sup> populations than the electrostatic spray application. In addition, LA was more effective ( $P < 0.05$ ) at reducing *E. coli*<sup>Rif</sup> than the BX treatments when using *E. coli* Petrifilms and PAA was more effective ( $P < 0.05$ ) than BX using APC Petrifilms. *E. coli*<sup>Rif</sup> counts (CFU/g) in ground beef samples from the electrostatic spray treatments were similar ( $P > 0.05$ ) to the untreated control, and had a greater ( $P < 0.05$ ) survivor *E. coli*<sup>Rif</sup> populations than the spray or dip treatments. Sub-primal samples had a greater reduction ( $P < 0.001$ ) of *E. coli*<sup>Rif</sup> on the fat surface than the lean surface. In addition, ground beef samples had a higher ( $P < 0.001$ ) survival population in the lean samples compared to the fat samples. All treatments reduced *E. coli*<sup>Rif</sup> populations compared to the untreated control, so these antimicrobial methods could be used to reduce the risk of STEC on beef sub-primals meant for ground beef production.

## Introduction

*Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli* (STEC; O26, O45, O103, O111, O121, & O145) have been a major concern in raw, non-intact beef products because of severe illnesses that can arise from these pathogens. According to FoodNet 2014 Surveillance Report, *E. coli* O157:H7 caused 155 hospitalizations and 3 deaths while non-O157 STEC have caused 106 hospitalizations and 2 deaths in the United States. *E. coli* O157:H7 recalls for all foods have decreased prior to 2014; however, the number of non-O157:H7 STEC recalls have increase in years prior to 2014(CDC, 2014).

*E. coli* O157:H7 and non-O157 STEC are commonly found in beef cattle feces and hides. The risk of contaminating the beef carcass is high during slaughter (Arthur et al., 2004; Barkocy-Gallagher et al., 2003; Elder et al., 2000; Small et al., 2003). Arthur et al. (2004) found that multiple antimicrobial interventions are effective at reducing STEC during the slaughter process. However, the risk of STEC on the resulting beef sub-primals during fabrication is still of concern (Barkocy-Gallagher et al., 2003; Wheeler et al., 2014). Therefore, there is uncertainty of the microbial safety of beef sub-primals received from other processors.

Implementing an antimicrobial intervention upon receiving beef sub-primals could minimize the risk of STEC. A variety of studies have shown the effects of implementing antimicrobial treatments to inoculated meat products. Lactic acid solution has been found to be effective at reducing *E. coli* on the surface of meat cuts (Heller et al., 2007; Kalchayanand et al. 2012; Youssef et al., 2012). Additionally, Pohlman et al. (2009) found that peroxyacetic acid was effective in reducing *E. coli* populations.

However, King et al. (2005) found that peroxyacetic treatments had very little lethality and the lactic acid treatments were more effective at reducing *E. coli*. Beefxide™ was found to have similar reductions to lactic acid by Hendricks et al. (2014). From our experience, small processors are challenged with finding an effective solution to reduce STEC from beef products used for ground beef that is not costly or difficult to manage.

A variety of application methods can be used to apply antimicrobials to beef. The effects of dipping inoculated beef trim into 95°C water for 3 seconds followed by 2% lactic acid (55°C) for 11 seconds was studied. This treatment reduced *E. coli* O157:H7 and *Salmonella* Typhimurium (Ellebracht et al., 1999). A similar study tested the efficacy of spraying 2% and 4% lactic acid on inoculated beef trim. They found that low inoculation level samples treated with 2% and 4% lactic acid reduced *E. coli* O157:H7 and *Salmonella* to non-detectable levels (Harris et al., 2006).

An alternative method to these two application methods is electrostatic spraying. This system is new to the meat industry but has been evaluated on other food products. Massey et al. (2013) studied the effects electrostatic spraying would have on inoculated *E. coli* O157:H7 on fresh-cut cantaloupe using 2% lactic acid. Inoculated cantaloupe cubes were sprayed using an electrostatic spraying chamber. They found that 2% lactic acid had a reduction in *E. coli* of 0.6 log CFU/g after three days of storage. Another study found that the electrostatic spray system was more effective at reducing *Salmonella* Typhimurium in spinach (Ganesh et al., 2010).

This study is aimed to explore antimicrobial treatments for use by small business processors to minimize the risk of STEC present in their ground beef products. Specifically, this study will evaluate application methods and organic acids as antimicrobials applied to a beef sub-primal used for the production of ground beef.

## **Materials and Methods**

### *Experimental design.*

This study evaluated the effectiveness of three organic acid types combined with three application methods on the reduction of Rifampicin resistant *E. coli* (*E. coli*<sup>Rif</sup>) when compared to an inoculated control. This was tested on both the mostly fat covered lateral surface and the mostly lean covered medial surface of beef shoulder clods roasts. Treatments were randomly assigned to beef clods. Clods were then cut in half and the same treatment was applied to the lateral or medial surface areas. Three independent replicates were conducted.

### *Raw meat materials.*

Beef shoulder clods (IMPS 114) were obtained from a local distributor to replicate how small processors would receive their meat supply. Beef clods were randomly assigned to an organic acid type and application method treatment or to a control. Each replication used one clod per treatment. Preparation of the samples took place at the University of Nebraska Loeffel Meat Laboratory in Lincoln, Nebraska.

### *Culture Preparation*

A five strain cocktail of approximately equal parts of the following rifampicin-resistant *E. coli* were used to create the inoculum, (1) USDA-FSIS 011-82, (2) ATCC 43888, (3) ATCC 43889, (4) ATCC 43890, and (5) USDA-FSIS 45756. Each sample was

grown separately by combining a small amount of each frozen culture in 10 mL of tryptic soy broth (Bectin, Dickinson and Company, Sparks, MD) in 15 mL tubes and placed into an incubator (VWR 1535, Air-Jacketed Incubator) at 37°C for at least 24 hours. One mL of each culture was combined with 10 mL of tryptic soy broth containing 5 µL of rifampicin (100 µg/ml double distilled deionized water; Sigma Chemical Company, St. Louis, MO) and incubated for 24 hours. Just before inoculation, 7 mL of each culture was mixed together. After thoroughly mixing, 5 mL of the combined culture was mixed with 45 mL of peptone water (BBL Buffered Peptone Water, Becton, Dickinson, and Company, Sparks, MD) in 50 mL tubes. Fifteen mL of each sample was then transferred into 15 mL tubes and stored at < 2.5°C for inoculation.

#### *Inoculation.*

Meat surface temperature was measured with a calibrated thermometer immediately prior to inoculation. One side of each clod roast (~450 cm<sup>2</sup> surface area) was inoculated with 15 mL of a five strain cocktail of rifampicin resistant *E. coli* using a hand sprayer. Inoculated meat surfaces were left undisturbed for 20 minutes at ambient room temperatures (14-16°C) to allow for bacterial attachment prior to the organic acid intervention. Initial inoculation levels were measured by taking five surface core samples (25.3 cm<sup>2</sup>, < 0.75 cm depth) from the inoculated surface area after the 20 minute attachment period, placed in Whirl Pak Bags (Item # 01-812-5C, Fisher Scientific, Pittsburgh, PA) and stored at <2.5°C (36.5°F) until microbial analysis later the same day.

#### *Organic acid preparation/titration.*

Lactic acid (4.5%) was prepared by mixing 194 ml of concentrated lactic acid (88%, Birko, Henderson, CO) with 3.78 liters of water (~21°C, 3.78 kg). Beefxide™



(2.5%) was prepared by adding 94.6 ml of Beefxide™ to 3.78 liters of water.

Peroxyacetic acid (380 ppm) was prepared by using a transfer pipette to mix 8.45 ml of peroxyacetic acid with 3.78 liters of water. The concentration of each acid was tested by measuring the titratable acidity of the solution before application by using a titration kit provided by Birko. To confirm the solution was within acceptable range.

*Organic acid application.*

After the 20 minute inoculum attachment period, the surface temperature of the meat and the temperature of the solution was recorded using a calibrated meat thermometer. 3.78 liters of solution was made for each organic acid and 0.95 liters was reserved for spray and electrostatic spray treatments and the rest was reserved for dip treatments. Spray treatments were sprayed for 5 seconds at 25.4-35.5 cm from the inoculated surface using a stainless steel spray gun (Item # EW-98515-15, Cole-Parmer, Vernon Hills, IL) with a flat spray nozzle (Item # 3404K75, McMaster-Carr, Chicago, IL) and a pressure of 137,895 Pa delivering 1.1 liters per minute for a target coverage of 0.26 ml/cm<sup>2</sup>. Electrostatic spray treatments were sprayed for 10 seconds at 25.4-35.5 cm from the inoculated surface using an Electrostatic Sprayer XT-3 (Electrostatic Spraying Systems, Watkinsville, GA) for a target coverage of 0.045 ml/cm<sup>2</sup>. Dip treatments were submerged in 2.83 liters of solution for 15 seconds. The surface temperature of the beef clods and the temperature of the organic acid solution was recorded immediately following treatments.

Treated beef clods were allowed to drip for 2 minutes. After the drip period, five core samples (25.3 cm<sup>2</sup>, < 0.75 cm depth) were collected and stored at <2.5°C in Whirl Pak Bags (Item # 01-812-5C, Fisher Scientific, Pittsburgh, PA). Beef clods and core

samples were put into a refrigerated cooler ( $<2.5^{\circ}\text{C}$ ) immediately following the second sampling. Core samples were held for approximately 6-10 hours before analyzing them in the microbiology lab.

*Temperature procedures.*

Surface temperature was taken three times for each beef clod. This was taken with a calibrated Omega Thermocouple Thermometer (Type T) within 0.75 cm from the surface. The first temperature was taken immediately prior to inoculation on the surface that was intended to be inoculated. After the twenty minute wait period, the temperature was taken again on the inoculated surface. The product was then subjected to treatment and temperature was taken again within 5 minutes after treatment.

Temperature of the treatment solution was taken twice with a calibrated Omega Thermocouple Thermometer (Type T). Spray and electrostatic spray treatment solution temperatures were taken inside the spray tank immediately prior to solution application and immediately after application. In the same manner, dip treatment solution temperatures were taken in the dip tub immediately prior to dipping and immediately after. Each of these temperatures were taken in the middle of the solution.

*Grinding procedures.*

Inoculated and treated beef clods, stored overnight in a refrigerated cooler ( $<2.5^{\circ}\text{C}$ ), were ground through a 1.27 cm plate and then ground again using a 0.48 cm plate. When grinding with the 0.48 cm plate, 25 grams of ground beef was collected from random locations while grinding and placed in Whirl Pak Bags (Item # 01-812-5C, Fisher Scientific, Pittsburgh, PA) and stored at  $<4^{\circ}\text{C}$  to be used for microbial analysis.

The entire grinder disassembled, washed with hot soapy water, and then sanitized with a Clorox™ solution between each clod ground to avoid cross contamination.

*Microbial analysis.*

Microbial data for rifampicin resistant *E. coli* and aerobic microorganisms were determined by using *E. coli*/coliform Petrifilm™ and ACP Petrifilm™ (3M, St. Paul, MN). Each treatments core samples were combined with 25 ml peptone water (BBL Buffered Peptone Water, Becton, Dickinson, and Company, Sparks, MD) containing rifampicin (100 mg/ml of double distilled deionized water) and stomached (AES Laboratoire Stomacher, AES Laboratoire, Bruz, France) for 3 minutes. Reductions were calculated by subtracting sample 2 (treated samples) from sample 1 (inoculated samples).

Ground beef samples were transferred from Whirl Pak Bags to blender bags (Item # 14-258-201, Fisher Scientific, Pittsburgh, PA) with 25 ml of peptone water (BBL Buffered Peptone Water, Becton, Dickinson, and Company, Sparks, MD) containing rifampicin and stomached (AES Laboratoire Stomacher, AES Laboratoire, Bruz, France) for 3 minutes.

At least 6 ml was collected from each sample. One mL was used for serial dilutions and one mL was plated on each of the two ACP Petrifilms™ and two *E. coli*/coliform Petrifilms™. Plated samples were then incubated at 37°C for 24 hours and were then counted.

*Statistical analysis.*

Data was first analyzed with the inoculated control as a 10 x 2 factorial arrangement (treatment x fat/lean) for interactions and main effects to study treatment differences against the inoculated control. The data was then analyzed without the control

as a 3 x 3 x 2 factorial arrangement (organic acid x application method x fat/lean) for interactions and main effects using analysis of variance (ANOVA) through the PROC GLIMMIX procedure in SAS (v9.4, SAS Institute, Cary, NC). Least square means were generated using LSMEANS and separated with Tukey's HSD adjustment using the  $P$  value ( $P < 0.05$ ) and the PDIFF option of SAS.

## Results

When compared to the control, all treatments resulted in greater ( $P < 0.05$ ) *E. coli*<sup>Rif</sup> log count (CFU/cm<sup>2</sup>) reductions when plated on *E. coli*/coliform Petrifilm and are summarized in Table 1. When excluding the control, the electrostatic spray treatments had less of a reduction ( $P < 0.001$ ) than both the spray and dip treatments. In addition, LA treatments (0.86 log CFU/cm<sup>2</sup> reduction) were more efficient at reducing ( $P < 0.05$ ) *E. coli*<sup>Rif</sup> counts than the BX treatments (0.55 log CFU/cm<sup>2</sup> reduction). All treatments had greater log reductions ( $P < 0.05$ ) on the mostly fat covered surface (0.77 log CFU/cm<sup>2</sup>) than the mostly lean covered surface (0.51 log CFU/cm<sup>2</sup>) when using *E. coli* Petrifilm.

In ground beef samples, all the electrostatic spray treatments were similar ( $P > 0.05$ ) in *E. coli*<sup>Rif</sup> counts (CFU/g) to the control when plated on *E. coli* petrifilm (Table 2). The electrostatic spray (3.82 log CFU/g) application had greater ( $P < 0.001$ ) *E. coli*<sup>Rif</sup> counts than either the spray or dip methods (3.46 log CFU/g and 3.32 log CFU/g, respectively). Also, all of the ground beef samples had higher ( $P < 0.001$ ) *E. coli*<sup>Rif</sup> counts when the mostly lean covered surface (3.79 log CFU/g) was inoculated than when the mostly fat covered surface (3.36 log CFU/g) was inoculated.

When using ACP Petrifilm, which allows for damaged *E. coli* cells to grow, all electrostatic spray treatments had similar reductions ( $P > 0.05$ ) to the control that showed no reductions Table 3. When analyzed without the inoculated control, dip and spray

treatments (0.62 log CFU/cm<sup>2</sup> and 0.61 log CFU/cm<sup>2</sup>, respectively) were more efficient ( $P < 0.001$ ) at reducing *E. coli*<sup>Rif</sup> than the electrostatic spray treatments (0.12 log CFU/cm<sup>2</sup>). Additionally, PAA treatments (0.56 log CFU/cm<sup>2</sup>) had a greater reductions ( $P < 0.05$ ) than the BX treatments (0.29 log CFU/cm<sup>2</sup>).

When analyzing ground beef samples plated on APC Petrifilm, BX spray along with all the electrostatic spray treatments were not different ( $P > 0.05$ ) than the inoculated control (Table 4). Without the inoculated control, electrostatic spray treatments (4.15 log CFU/g) had greater ( $P < 0.01$ ) *E. coli*<sup>Rif</sup> log counts than the spray and dip treatments (3.91 log CFU/g and 3.79 log CFU/g, respectively). All of the ground beef samples had greater ( $P < 0.05$ ) *E. coli*<sup>Rif</sup> counts when the mostly lean covered surface (4.12 log CFU/g) was inoculated than when the mostly fat covered surface (3.83 log CFU/g) was inoculated.

Temperature of meat taken prior to inoculation had no differences ( $P > 0.05$ ) among treatments (Table 5). There was a difference in temperatures before treatment, with the inoculated control treatment having a lower ( $P < 0.01$ ) surface temperature than the PAA electrostatic treatment. However, after application of treatment LA dip had a higher ( $P < 0.001$ ) surface temperature than any of the other treatments.

The BX spray treatment had a higher ( $P < 0.001$ ) solution temperature than the electrostatic spray treatment solution before application to the beef clod (Table 5). After treatment, BX spray treatment solution had greater ( $P < 0.01$ ) solution temperature than LA dip and PAA dip.

## Discussion

When plating *E. coli*<sup>Rif</sup> on both APC and *E. coli* Petrifilms, electrostatic spray treatments were found to have less reduction than either the spray or the dip treatments.

This could be explained by Youssef, et al. (2012) who tested different volumes of organic acid applied to the surface of beef trim. They recommended that at least 0.1 ml/cm<sup>2</sup> of LA solution should be applied to receive a significant log reduction in *E. coli*<sup>Rif</sup>. In the present study, spray systems applied 0.26 ml/cm<sup>2</sup> organic acid solution while electrostatic spray systems only applied 0.045 ml/cm<sup>2</sup>. This could contribute to the electrostatic spray system not being as effective as the spray or dip systems. However, Ganesh et al. (2010) found that the electrostatic spray system was more effective than conventional spray systems when decontaminating spinach. Similarly, Massey et al. (2013) found the electrostatic spray system to be effective at reducing *E. coli* O157:H7 from fresh-cut cantaloupe cubes.

In this study, LA was more effective at reducing *E. coli*<sup>Rif</sup> counts on beef sub-primals than BX. This was not similar to a study done by Hendricks, et al. (2014), who found that 2.9% lactic acid and 2.5% Beefxide™ were similar in their efficiency to reduce *E. coli* in non-intact beef products. This could be explained by the difference in acid concentrations used. In the present study, LA (4.5%) was a much higher concentration than the BX (2.5%) treatment. The similar concentrations used by Hendrick et al. (2014) may explain why lactic acid and Beefxide™ treatments were similar in their study.

The mostly fat covered surface had greater *E. coli*<sup>Rif</sup> reduction than the mostly lean covered surface regardless of treatment or sample type. The lean surface would be expected to have a greater buffering capacity than the fat surface. The buffering capacity could reduce the effectiveness of the organic acids as antimicrobials. Similar results were found by Line, et al. (1991), showing that lean ground beef samples had higher rate of

survival of *E. coli* than fatty ground beef samples. Youssef et al. (2012) also found that *E. coli* counts were greater on cut muscle surfaces than on fat surfaces when sprayed with 5% lactic acid solution.

We found that BX spray had a slightly higher solution temperature than the LA and PAA dip solutions. Conner and Kotrola (1995) reported that increasing the temperature of the organic acid solution has a major role in the ability to inhibit growth of *E. coli*. However, the temperature span of their treatments were from 0.5°C to 37°C. The temperature difference in our study was < 2°C, which could be why we didn't see any correlation between temperature of the organic acid solution and reductions in *E. coli*<sup>Rif</sup>.

## **Conclusions**

Although all treatments reduced *E. coli*<sup>Rif</sup> on beef sub-primals, some were more effective than others. Organic acids applied using dip and spray methods were effective at reducing *E. coli*<sup>Rif</sup> log counts compared to the control. However, the efficacy of electrostatic spray method is questionable for reducing *E. coli*<sup>Rif</sup> log counts on the surface of beef sub-primals meant for ground beef production. In general, lactic acid and peroxyacetic acid treatments were more effective at reducing *E. coli*<sup>Rif</sup> counts than Beefxide™. However, application of lactic acid, Beefxide™, or peroxyacetic acid applied by dip or spray methods can be applied as antimicrobial interventions on beef sub-primals before the production of ground beef.

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## References

- Arthur, T.M., J.M. Bosilevac, X. Nou, S.D. Shackelford, T.L. Wheeler, M.P. Kent, D. Jaroni, B. Pauling, D.M. Allen, and M. Koohmaraie. 2004. *Escherichia coli* O157 Prevalence and Enumeration of Aerobic Bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at Various Steps in commercial Beef Processing Plants. *Journal of Food Protection*. 67:658-665.
- Barkocy-Gallagher, G.A., T.M. Arthur, M. Rivera-Betancourt, X. Nou, S.D. Shackelford, T.L. Wheeler, and M. Koohmaraie. 2003. Seasonal Prevalence of Shiga Toxin-Producing *Escherichia coli*, including O157:H7 and Non-O157 Serotypes, and *Salmonella* in Commercial Beef Processing Plants. *Journal of Food Protection*. 66:1978-1986.
- Centers for Disease Control and Prevention. 2014. Foodborne Diseases Active Surveillance Network: FoodNet 2014 Surveillance Report.
- Conner, D.E., and J.S. Kotrola. 1995. Growth and Survival of *Escherichia coli* O157:H7 under Acidic Conditions. *Applied and Environmental Microbiology*. 61:382-385.
- Elder, R.O., J.E. Keen, G.R. Siragusa, G.A. Barkocy-Gallagher, M. Koohmaraie, and W.W. Laegreid. 2000. Correlation of Enterohemorrhagic *Escherichia coli* O157 Prevalence in Feces, Hides, and Carcass of Beef Cattle During Processing. *Proceedings of the National Academy of Sciences of the United States of America*. 97:2999-3003.
- Ellebracht, E.A., A. Castillo, L.M. Lucia, R.K. Miller, and G.R. Acuff. 1999. Reduction of Pathogens Using Hot Water and Lactic Acid on Beef Trimmings. *Journal of Food Science*. 64:1094-1099.
- Ganesh, V., N.S. Hettiarachchy, M. Ravichandran, M.G. Johnson, C.L. Griffis, E.M. Martin, J.-F. Meullenet, and S.C. Ricke. 2010. Electrostatic Sprays of Food-Grade Acids and Plant Extracts are More Effective than Conventional Sprays in Decontaminating *Salmonella* Typhimurium on Spinach. *Journal of Food Science*. 75:M574-M579.
- Harris, K., M.F. Miller, G.H. Loneragan, and M.M. Brashears. 2006. Validation of the Use of Organic Acids and Acidified Sodium Chlorite to Reduce *Escherichia coli* O157 and *Salmonella* Typhimurium in Beef Trim and Ground Beef in a Simulated Processing Environment. *Journal of Food Protection*. 69:1802-1807.
- Heller, C.E., J.A. Scanga, J.N. Sofos, K.E. Belk, W. Warren-Serna, G.R. Bellinger, R.T. Bacon, M.L. Rossman, and G.C. Smith. 2007. Decontamination of Beef Subprimal Cuts Intended for Blade Tenderization or Moisture Enhancement. *Journal of Food Protection*. 70:1174-1180.
- Hendricks, K.M., A.N. Arnold, T.M. Taylor, K.B. Gehring, and J.W. Savell. 2014. In-Plant Validation of Two Antimicrobial Agents Applied During Production of Further Processed Beef Products. *International Congress of Meat Science and Technology*. 60:186-189.



- Kalchayanand, N., T.M. Arthur, J.M. Bosilevac, J.W. Schmidt, R. Wang, S.D. Shackelford, and T.L. Wheeler. 2012. Evaluation of Commonly Used Antimicrobial Interventions for Fresh Beef Inoculated with Shiga Toxin-Producing *Escherichia coli* Serotypes O26, O45, O103, O111, O121, O145, and O157:H7. *Journal of Food Protection*. 75:1207-1212.
- King, D.A., L.M. Lucia, A. Castillo, G.R. Acuff, K.B. Harris, and J.W. Savell. 2005. Evaluation of Peroxyacetic Acid as a Post-Chilling Intervention for Control of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on Beef Carcass Surfaces. *Meat Science* 69:401-407.
- Laury, A.M., M.V. Alvarado, G. Nace, C.Z. Alvarado, J.C. Brooks, A. Echiverry, and M.M. Brashears. 2009. Validation of a Lactic Acid- and Citric Acid-Based Antimicrobial Product for the Reduction of *Escherichia coli* O157:H7 and *Salmonella* on Beef Tips and Whole Chicken Carcasses. *Journal of Food Protection* 72:2208-2211.
- Line, J.E., A.R. Fain, Jr., A.B. Moran, L.M. Martin, R.V. Lechowich, J.M. Carosella, and W.L. Brown. 1991. Lethality of Heat to *Escherichia coli* O157:H7: D-Value and Z-value Determinations in Ground Beef. *Journal of Infectious Diseases*. 54:762-766.
- Massey, L.M., N.S. Hettiarachchy, E.M. Martin, and S.C. Ricke. 2013. Electrostatic Spray of Food-Grade Organic Acids and Plant Extract to Reduce *Escherichia coli* O157:H7 on Fresh-Cut Cantaloupe Cubes. *Journal of Food Safety*. 33:71-78.
- Pohlman, F.W., P.N. Dias-Morse, S.A. Quilo, A.H. Brown, Jr., P.G. Grandall, R.T. Baublits, R.P. Story, C. Bokina, and G. Rajaratnam. 2009. Microbial, Instrumental Color and Sensory Characteristics of Ground Beef Processed from Beef Trimmings Treated with Potassium Lactate, Sodium Metasilicate, Peroxyacetic Acid or Acidified Sodium Chlorite as Single Antimicrobial Interventions. *Journal of Muscle Foods*. 20:54-69.
- Small, A., B. Wells-Burr, and S. Buncic. 2005. An Evaluation of Selected Methods for the Decontamination of Cattle Hides Prior to Skinning. *Meat Science* 69:263-268.
- Wheeler, T.L., N. Kalchayanand, and J.M. Bosilevac. 2014. Pre- and Post-Harvest Interventions to Reduce Pathogen Contamination in the U.S. Beef Industry. *Meat Science* 98:372-382.
- Youssef, M.K., X. Yang, .Badoni, and C.O. Gill. 2012. Effects of Spray Volume, Type of Surface Tissue and Inoculum Level on the survival of *Escherichia coli* on Beef Sprayed with 5% Lactic Acid. *Food Control*. 25:717-722.

## Tables

**Table 1: Organic acid and application method treatment effects on *E. coli*<sup>Rif</sup> counts (log CFU/cm<sup>2</sup>) using *E. coli*/coliform Petrifilm with the inoculated control**

Treatment <sup>1</sup>	Pre-treated	Post-treated	Reduction
Control	5.50	5.64 <sup>a</sup>	-0.14 <sup>a</sup>
LA Electrostatic Spray	5.70	5.31 <sup>ab</sup>	0.39 <sup>ab</sup>
BX Electrostatic Spray	5.39	4.99 <sup>bc</sup>	0.40 <sup>ab</sup>
BX Spray	5.56	4.96 <sup>bc</sup>	0.60 <sup>bc</sup>
PAA Electrostatic Spray	5.52	4.90 <sup>bc</sup>	0.62 <sup>bc</sup>
BX Dip	5.63	4.98 <sup>bc</sup>	0.65 <sup>bc</sup>
PAA Spray	5.56	4.73 <sup>c</sup>	0.83 <sup>bc</sup>
PAA Dip	5.54	4.70 <sup>c</sup>	0.84 <sup>bc</sup>
LA Spray	5.68	4.62 <sup>c</sup>	1.06 <sup>c</sup>
LA Dip	5.80	4.67 <sup>c</sup>	1.13 <sup>c</sup>
SEM	0.1332	0.1369	0.1774
<i>P</i> value	0.1708	< 0.0001	< 0.0001

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>abc</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 2: Organic acid and application method treatment effects on *E. coli*<sup>Rif</sup> counts (log CFU/g) in ground beef using *E. coli*/coliform Petrifilm with the inoculated control**

<b>Treatment<sup>1</sup></b>	<b>Ground Beef</b>
Control	3.97 <sup>a</sup>
LA Electrostatic Spray	3.90 <sup>ab</sup>
BX Electrostatic Spray	3.80 <sup>abc</sup>
PAA Electrostatic Spray	3.76 <sup>abc</sup>
BX Spray	3.57 <sup>abc</sup>
PAA Spray	3.43 <sup>bc</sup>
BX Dip	3.39 <sup>bc</sup>
LA Spray	3.36 <sup>c</sup>
PAA Dip	3.31 <sup>c</sup>
LA Dip	3.27 <sup>c</sup>
SEM	0.1599
<i>P</i> value	0.0001

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>abc</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 3: Organic acid and application method treatment effects on *E. coli*<sup>Rif</sup> counts (log CFU/cm<sup>2</sup>) using aerobic count plate Petrifilm with the inoculated control**

<b>Treatment<sup>1</sup></b>	<b>Pre-treated</b>	<b>Post-treated</b>	<b>Reduction</b>
Control	5.65	5.73 <sup>a</sup>	-0.08 <sup>a</sup>
BX Electrostatic Spray	5.50	5.45 <sup>ab</sup>	0.05 <sup>a</sup>
LA Electrostatic Spray	5.74	5.66 <sup>a</sup>	0.08 <sup>a</sup>
PAA Electrostatic Spray	5.62	5.41 <sup>abc</sup>	0.21 <sup>ab</sup>
BX Dip	5.80	5.41 <sup>abc</sup>	0.39 <sup>abc</sup>
BX Spray	5.72	5.30 <sup>abcd</sup>	0.42 <sup>abc</sup>
LA Dip	5.91	5.25 <sup>abcd</sup>	0.66 <sup>bc</sup>
PAA Spray	5.60	4.94 <sup>cd</sup>	0.66 <sup>bc</sup>
LA Spray	5.82	5.08 <sup>bcd</sup>	0.74 <sup>bc</sup>
PAA Dip	5.67	4.86 <sup>d</sup>	0.81 <sup>c</sup>
SEM	0.1300	0.1528	0.1686
<i>P</i> value	0.1355	< 0.0001	< 0.0001

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>abcd</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 4: Organic acid and application method treatment effects on *E. coli*<sup>Rif</sup> counts (log CFU/g) in ground beef using aerobic count plate Petrifilm with the inoculated control**

<b>Treatment<sup>1</sup></b>	<b>Ground Beef</b>
Control	4.20 <sup>ab</sup>
LA Electrostatic Spray	4.21 <sup>a</sup>
PAA Electrostatic Spray	4.14 <sup>ab</sup>
BX Electrostatic Spray	4.09 <sup>ab</sup>
BX Spray	3.98 <sup>abc</sup>
LA Spray	3.93 <sup>abc</sup>
BX Dip	3.91 <sup>abc</sup>
LA Dip	3.86 <sup>abc</sup>
PAA Spray	3.82 <sup>bc</sup>
PAA Dip	3.63 <sup>c</sup>
SEM	0.1135
<i>P</i> value	< 0.0001

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>abc</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 5: Organic acid and application method treatment temperatures of meat surface and solutions.**

Treatment <sup>1</sup>	Meat Temperature (°C)			Solution Temperature (°C)	
	<u>Inoculation</u>	<u>Pre-treatment</u>	<u>Post-treatment</u>	<u>Before Application</u>	<u>After Application</u>
Control	3.44	3.67 <sup>a</sup>	5.00 <sup>b</sup>	-	-
BX Dip	3.78	5.39 <sup>bc</sup>	5.39 <sup>b</sup>	21.83 <sup>abc</sup>	20.56 <sup>bc</sup>
BX Electrostatic	2.89	5.44 <sup>bc</sup>	5.67 <sup>b</sup>	21.83 <sup>abc</sup>	21.67 <sup>ab</sup>
BX Spray	3.17	4.72 <sup>abc</sup>	5.28 <sup>b</sup>	22.61 <sup>a</sup>	22.22 <sup>a</sup>
LA Dip	2.50	4.56 <sup>abc</sup>	8.61 <sup>a</sup>	21.11 <sup>bc</sup>	20.11 <sup>c</sup>
LA Electrostatic	3.17	5.28 <sup>abc</sup>	5.11 <sup>b</sup>	20.72 <sup>c</sup>	20.56 <sup>bc</sup>
LA Spray	2.94	4.44 <sup>ac</sup>	5.00 <sup>b</sup>	21.67 <sup>abc</sup>	20.94 <sup>abc</sup>
PAA Dip	2.78	5.39 <sup>bc</sup>	5.56 <sup>b</sup>	21.50 <sup>abc</sup>	20.17 <sup>c</sup>
PAA Electrostatic	3.17	5.72 <sup>c</sup>	5.39 <sup>b</sup>	21.67 <sup>abc</sup>	21.11 <sup>abc</sup>
PAA Spray	3.17	4.44 <sup>ab</sup>	4.50 <sup>b</sup>	22.06 <sup>ab</sup>	21.83 <sup>ab</sup>
SEM	0.7422	0.6599	1.0716	0.6227	0.8037
<i>P</i> value	0.2471	0.0006	< 0.0001	0.0006	0.0001

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>abc</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**COMPARISON OF ELECTROSTATIC SPRAY, SPRAY, OR DIP USING  
LACTIC ACID, PEROXYACETIC ACID, OR BEEFXIDE™ ON THE EFFECTS  
OF COLOR AND AEROBIC PLATE COUNTS**

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Prepared using the style of *Meat Science*

## Abstract

The use of antimicrobials can minimize the risk of shiga toxin-producing *E. coli* on the surface of beef sub-primals meant for ground beef production. However, little is known on the quality effects this could have for ground beef produced from treated meat. The impact of using three organic acids, 2.5% Beefxide™ (BX), 4.5% lactic acid (LA), and 380 ppm peroxyacetic acid (PAA) applied by dip (15 sec), spray (11 sec/side, 137,895 Pa), or electrostatic spray (10 sec/side) to beef shoulder clods were used to determine the effects on subjective and objective color, aerobic plate counts, meat pH, and lipid oxidation. Beef clods were ground following application of organic acids, formed into one pound portions using a Colosimo™ press and placed onto trays overwrapped with oxygen permeable film. Ground beef samples were then placed in retail display for seven days. All treatments were similar ( $P > 0.05$ ) to the untreated control for  $L^*$ ,  $a^*$ ,  $b^*$ , delta E, or percent discoloration. All treatments decreased ( $P < 0.001$ ) in  $L^*$  and  $a^*$  values and increased ( $P < 0.001$ ) in  $b^*$ , delta E, and discoloration from day 0 to day 7 of retail display. Additionally, treatment aerobic plate counts and oxidation values were not different ( $P > 0.05$ ) from the untreated control. However, among treated samples alone, the LA electrostatic spray treatment had the highest ( $P < 0.05$ ) aerobic counts (log CFU/g) of all treatments. Both aerobic plate counts and lipid oxidation values increased ( $P < 0.001$ ) throughout retail display. LA electrostatic spray and dip, and BX spray had lower ( $P < 0.05$ ) meat pH values than the control. Meat pH values also decreased with increasing days of retail display. Minimal changes were found throughout retail display of meat treatments compared to the untreated control. Therefore,



the use of these antimicrobials and application methods can be used to reduce shiga toxin-producing *E. coli* on the surface of beef sub-primals meant for ground beef production without adverse effects on ground beef quality.

## **Introduction**

*Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli* (STEC) have caused 261 hospitalizations and 5 deaths in 2014 alone (CDC, 2014). Beef plants have been investigated and it has been reported that *E. coli* O157 was found on hides at 75.7%. Positive samples were found at 14.7% pre-evisceration and 3.8% post-evisceration. In this particular study, only 0.3% of carcasses tested positive for *E. coli* O157 post-intervention and none of the carcasses tested positive after chilling (Arthur et al., 2004). However, there are still risks associated with STEC in beef sub-primals and trim. An additional antimicrobial intervention can be implemented on beef sub-primals or trim to reduce the risk of STEC found in ground beef produced.

Previous research has found that antimicrobial treatments applied to beef sub-primals and trim can be effective in the reduction of *E. coli* (Wheeler et al., 2014). However, there are both positive and negative color change in meat. Quilo et al. (2009) found lighter colored ground beef patties when treated with peroxyacetic acid. Stivarius et al. (2002) had the same results when using lactic acid. In contrast, no color changes were found from lactic acid in ground beef samples in a study done by Ellebracht et al. (1999).

Some studies have reported the effects that antimicrobials have on bacterial populations. Kotula and Thelappurath (1994) found that steaks dipped in lactic or acetic

acid lowered the microbial counts when compared to the untreated control. Ellebracht et al. (1999) did not measure microbial counts directly, but found that odors released by spoilage organisms were reduced with the application of lactic acid.

A variety of application methods can be used to apply antimicrobials to beef. The effects of dipping inoculated beef trim into 95°C water for 3 seconds followed by 2% lactic acid (55°C) for 11 seconds was studied. They found that this treatment had a darker surface area in the trim samples but did not have any color differences in the ground beef samples. Additionally, there was less of a soured or putrid smell in the ground beef samples that were treated with lactic acid (Ellebracht et al., 1999). Another study tested the sensory effects of spraying 2% and 4% lactic acid on beef trim. Trained sensory panelists did not find any differences between treated and untreated samples (Harris et al., 2006). Electrostatic spray systems have been effective at reducing pathogens on cantaloupe (Massey et al., 2013) and spinach (Ganesh et al., 2010) but research on quality effects have yet to be published.

The objective of this study was to evaluate the impact of applying lactic acid, Beefxide™, or peroxyacetic acid applied by electrostatic spraying, spraying or dipping of beef shoulder clods used for the production of ground beef for small business processors. Characteristics such as objective and subjective color, aerobic plate counts, meat pH, and oxidation values were collected to determine these effects.

## **Materials and Methods**

### *Experimental design.*

This experiment was to determine the effectiveness of three organic acid types combined with three application methods on the effects of quality including color, meat pH, lipid oxidation and microbial counts. Treatments were randomly assigned to beef clods and applied to the entire surface area as an antimicrobial treatment. Is experiment was designed using a completely randomized design with a factorial treatment arrangement. Six independent replicates were conducted.

*Raw meat materials.*

Beef shoulder clods (IMPS 114) were obtained from a local distributor to replicate how a small processor would receive meat for ground beef production. Boxes of beef clods were stored at  $<2.5^{\circ}\text{C}$  for less than one week until production. Beef clods were randomly assigned to an organic acid type and application method or the control. Each replication used one clod per treatment. Preparation on the samples took place at the University of Nebraska Loeffel Meat laboratory in Lincoln, Nebraska.

*Organic acid preparation/titration.*

All organic acids were from Birko (Henderson, CO). Lactic acid (4.5%) was prepared by mixing 776 ml of concentrated lactic acid (88%, Birko) with 15.14 liters of water (15.15 kg;  $\sim 21.1^{\circ}\text{C}$ ). Beefxide™ (2.5%) was prepared by adding 378.4 ml of Beefxide™ to 15.14 liters of water (15.15 kg;  $\sim 21.1^{\circ}\text{C}$ ). Peroxyacetic acid (380 ppm) was prepared by using a transfer pipette to mix 33.8 ml of peroxyacetic acid with 15.14 liters of water (15.15 kg;  $\sim 21.1^{\circ}\text{C}$ ). The concentration of lactic acid and Beefxide™ was tested by measuring the titratable acidity of the solution before application by using a lactic acid

titration kit provided by Birko. The titratable acidity of peroxyacetic acid was measured using a peracidic acid titration kit to confirm the proper dilution was achieved.

*Organic acid application.*

The surface temperature of the meat and the temperature of the solution was recorded using a calibrated meat thermometer immediately before treatments. Spray treatments were sprayed for 11 seconds per side at 25.4-35.6 cm from the surface using a stainless steel spray gun (Item # EW-98515-15, Cole-Parmer, Vernon Hills, IL) with a flat spray nozzle (Item # 3404K75, McMaster-Carr, Chicago, IL) and a pressure of 137,895 Pa (20 psi) delivering 1.14 liters per minute for a target application rate of 0.26 ml/cm<sup>2</sup>. Electrostatic spray treatments were sprayed for 10 seconds per side at 25.4-35.6 cm from the surface using an Electrostatic Sprayer XT-3 (Electrostatic Spraying Systems, Watkinsville, GA) for a target application rate of 0.045 ml/cm<sup>2</sup>. Dip treatments were submerged in solution for 15 seconds in four gallons of solution. The surface temperature of beef clods and the temperature of the organic acid solution were recorded immediately following treatments.

Treated beef clods were allowed to drip for 2 minutes. After the drip period, beef clods were put into a cooler (<2.5°C).

*Temperature procedures.*

Surface temperature was taken two times on each beef clod. This was taken with an Omega Thermocouple Thermometer (Type T) within .75 cm from the surface. The temperature was taken prior to the treatment on the surface of the clod. The product was

then subjected to treatment and temperature was taken again within 5 minutes after treatment.

Temperature of the treatment solution was taken twice with an Omega Thermocouple Thermometer (Type T). Spray and electrostatic spray treatment temperatures were taken inside the spray tank immediately prior to solution application and immediately after application. In the same manner, dip temperatures were taken in the dip tub immediately prior to dipping and immediately after. Each of these temperatures were taken in the middle of the solution.

#### *Grinding procedures.*

On the same day of treatment, the treated beef clods were ground through a 1.27 cm plate and then ground again using a 0.48 cm plate. When grinding with the 0.48 cm plate, 125 grams of ground beef was collected from random locations while grinding. Whirl Pak Bags (Item # 01-812-5C, Fisher Scientific, Pittsburgh, PA) were used to store 25 grams of ground beef at  $<2.5^{\circ}\text{C}$  to be used for microbial analysis. The other 100 grams were packaged into vacuum bags and stored at  $-62.2^{\circ}\text{C}$  until preparation for lipid oxidation and pH analysis. The rest of the ground beef was formed into approximately one pound portions using a Colosimo press attached to a piston stuffer and placed onto trays (StyroTech, Denver, CO) and overwrapped with oxygen permeable film (PSM 18, Prime Source, St. Louis, MO). Six overwrapped ground beef samples were placed on a table for simulated retail display where the temperatures were maintained at 0 to  $2^{\circ}\text{C}$  under continuous 1,000 to 1,800 Lux warm white fluorescence lighting (PHILIPS F32T8/TL741 ALTO 700 Series, 32 WATT B7, Royal Philips Electronics, Amsterdam,

Netherlands) where objective color and subjective color was taken. Further collection days for microbial analysis, lipid oxidation, and pH (days 0, 1, 3, 5, and 7) were done using the extra samples.

*Microbial analysis.*

Microbial data for total aerobic microorganisms was determined by using ACP Petrifilm™ (3M, St. Paul, MN). Each 25 g ground beef sample was transferred from Whirl Pak Bags to blender bags (Item # 14-258-201, Fisher Scientific, Pittsburgh, PA) and combined with 25 ml peptone water (BBL Buffered Peptone Water, Becton, Dickinson, and Company, Sparks, MD) and stomached (AES Laboratoire Stomacher, AES Laboratoire, Bruz, France) for 3 minutes. At least 4 ml was collected from each sample. One ml was used for serial dilutions and one ml was plated on to each of two ACP Petrifilms™. Plated samples were then incubated at ~37°C for 24 hours and were then counted according to manufacturer's directions.

*Objective color analysis.*

Overwrapped ground beef samples were placed under simulated retail display with the only light coming from continuous 1,000 to 1,800 Lux warm white fluorescence lighting (PHILIPS F32T8/TL741 ALTO 700 Series, 32 WATT B7, Royal Philips Electronics, Amsterdam, Netherlands). Samples were rotated daily to avoid any effects from differences in light. Objective color measurements were taken every day using a Minolta Colorimeter (Chroma Meter CR-400, Konica Minolta Sensing Americas, Inc., Ramsey, NJ) using a 2° standard observer and a D65 illuminate with an 8 mm aperture and recorded using L\*, a\*, and b\* values. The colorimeter was calibrated with the same

oxygen permeable film the samples were overwrapped with against a white tile. Six measurements were taken on two samples (total of twelve measurements per treatment) of each treatments and were averaged to obtain values for each treatment. Color was measured on days 0, 1, 2, 3, 4, 5, and 7.

$L^*$ ,  $a^*$ , and  $b^*$  values were used to calculate Delta E values using the formula:  $\Delta E = \sqrt{[(L_1-L_0)^2 + (a_1-a_0)^2 + (b_1-b_0)^2]}$  using day 0 from each treatment as the initial values.

#### *Subjective color analysis.*

Subjective color was recorded by 8-10 panelists containing graduate students in the Department of Animal Science at the University of Nebraska-Lincoln. Each panelist evaluated discoloration of each ground beef sample as a percentage (0-100%) of total surface area. Sample location in the display tables were rotated to minimize variation in lighting or temperatures within the room.

#### *pH analysis.*

Ground beef from each treatment used in pH determination was powdered using liquid nitrogen. A 10 gram sample was weighed into a beaker and mixed with 90 ml of distilled, deionized water. A stir plate (Thermolyne® Cimarec®-top stirring hotplate, Barnstead Thermolyne, Dubuque, IA) was used to mix the samples using a stir bar. Sample pH was read from the stirring sample with a pH meter, which was calibrated with standards of 4.0 and 7.0 (Orion 410Aplus, ThermoFisher Scientific, Waltham, MA). Measurements were conducted in duplicate.

### *Lipid Oxidation.*

The Thiobarbituric Acid Assay used was from Buege and Aust (1978), modified by Ahn et al. (1998), found in Appendix V.

Thiobarbituric acid values were determined for ground beef pulled on days 0, 1, 3, 5, and 7 of retail display. Fourteen mL of double distilled water was added to 5 g of powdered sample and 1.0 mL of butylated hydroxyanisole (10% stock solution dissolved in 90% ethanol). The mixture was homogenized for 15 seconds and then centrifuged for 2000 x g for 5 minutes. Two mL of TBA/TCA was added to the 1 mL of homogenate was vortexed and then incubated in a 70°C water bath for 30 minutes for color to develop. Samples were then allowed to cool in a cold water bath (13°C) for 10 minutes before centrifuging at 3000 x g for 15 minutes. Duplicate samples of 200 µL were transferred to a 96 well plate. Absorbance was read at 540 nm on a Dynatech Laboratories MR5000 plate reader (Chantilly, VA) and analyzed by BioLinx assay management software (Dynatech Laboratories). Results were expressed as mg of malonaldehyde per kg of sample.

### *Statistical analysis.*

The data was first analyzed with the control to study treatment differences against the untreated control. Data were then analyzed in a 3 x 3 (application by acid) factorial treatment arrangement excluding the control to detect main effects of application method main effects of organic acids, application methods, day and their interactions. Day was considered a repeated measure using a Toeplitz covariance structure. Data were analyzed using Proc GLIMMIX procedure in SAS (v9.4, SAS Institute, Cary, NC). The



experiment was replicated six times. Least square means were generated using LSMEANS and separated with Tukey's HSD adjustment using the  $P$  value ( $P < 0.05$ ) and the PDIFF option of SAS.

## Results

Results from statistical analysis conducted with the untreated control is reported in Table 1. The antimicrobial treatment by application method interaction results from the analysis without the control is reported in Table 2. For objective color, the PAA spray treatment resulted in darker ( $P < 0.01$ ) ground beef than the untreated control. When analyzed without the control, the  $L^*$  value had an organic acid by application method interaction ( $P < 0.001$ , Figure 1). This showed that the PAA spray had darker ( $P < 0.05$ ) surface than PAA dip and electrostatic spray, LA spray, and BX spray and electrostatic spray. Organic acid or application method did not impact ( $P > 0.05$ )  $L^*$  values throughout retail display. However, there was a day effect that showed all treatments darkened ( $P < 0.001$ ) with the increase of retail display.

For  $a^*$  there was a treatment effect, but with Tukey's adjustment no treatment differences were identified. In addition, when excluding the control, there was an effect of application method found for  $a^*$  values, but similarly there were no means separation when applying Tukey's adjustment. All treatments decreased ( $P < 0.001$ ) in redness with increased days in retail display.

The  $b^*$  data showed LA electrostatic spray was less yellow ( $P < 0.01$ ) compared to the untreated control. When analyzing the  $b^*$  data without the control, there was an organic acid by application method interaction (Figure 2). This showed that BX dip and

spray treatments had more yellow color ( $P < 0.05$ ) than the LA electrostatic spray treatment in ground beef samples. There was no interaction ( $P > 0.05$ ) between day and organic acid or application method. All treatments decreased ( $P < 0.001$ ) in yellowness with the increase in retail display.

For the comparison of treatments to the control, there was a significant treatment main effect ( $P > 0.05$ ) for delta E, however, no means separation occurred when Tukey's adjustment was applied. Among antimicrobial treatments alone, there was an organic acid by application method interaction with Delta E values, but had no means separation once Tukey's adjustment was applied. No organic acid or application method by day of retail display interaction for delta E ( $P > 0.05$ ) was identified but, delta E values increased ( $P < 0.001$ ) with increased days of retail display independent of organic acid or application method.

No differences ( $P > 0.05$ ) in discoloration of ground beef were identified for any organic acid, application method, or interaction among treated samples or compared to the control. In agreement with the objective color measures, discoloration of ground beef increased ( $P < 0.001$ ) with increased days of retail display.

Aerobic plate counts were determined from each ground beef sample. Aerobic plate counts were not affected ( $P > 0.05$ ) by organic acid or application method during retail display. The control samples were similar ( $P > 0.05$ ) in aerobic plate counts to all of the treated samples. Without the control, an organic acid by application interaction was found that showed LA electrostatic spray treated ground beef had greater ( $P < 0.05$ ) aerobic plate counts (CFU/g) than those treated by PAA spray and dip, BX spray and

electrostatic spray, and LA dip (Figure 3). As expected, day 7 of retail display had the greatest ( $P < 0.001$ ) aerobic plate counts while days 0 & 1 had the least.

Organic acid and application method did not impact ( $P > 0.05$ ) the pH values throughout days of retail display, however, pH values declined ( $P < 0.001$ ) with the increase of retail display. The untreated control had higher ( $P < 0.05$ ) pH values than LA electrostatic spray, BX spray, and LA dip treatments. An organic acid by application method interaction indicated that LA dip had lower ( $P < 0.05$ ) pH values than LA spray, PAA spray, dip, and electrostatic spray, and BX dip and electrostatic spray.

TBA values were not affected ( $P > 0.05$ ) by organic acid or application method by days of retail display interaction. TBA values were not different ( $P > 0.05$ ) between the treated samples and the untreated control. However, when only looking at the antimicrobial treatments, the LA treatments did have greater lipid oxidation ( $P < 0.05$ ) than PAA treatments. There was an increase ( $P < 0.001$ ) in oxidation with increased days of retail display.

## **Discussion**

Organic acid and application method did not affect subjective color values ( $L^*$ ,  $a^*$ , and  $b^*$ ) compared to the untreated control. This was partially supported by Ellebracht, et al. (1999), who found that trimmings treated with 2% lactic acid had a darker appearance before grinding, but ground beef samples had no color differences when compared to the untreated control. This could have been due the amount of the treated surface being much smaller than the amount of the whole piece of meat. This could

explain why there were no differences found in meat color compared to the untreated control in this experiment.

However,  $L^*$  values were greater in ground beef from PAA spray treated samples than for PAA electrostatic spray and dip, LA spray, and BX spray and electrostatic spray treated samples. Pohlman, et al. (2009) partially support these results with their research that indicated PAA treated beef trim had lighter ground beef samples. Additionally, Jimenez-Villarreal, et al. (2003) found that beef trim treated with LA resulted in lighter colored ground beef patties where as in the present study no differences were found due to lactic acid treatments.

No differences were found in  $a^*$  values, which was supported by research done by Pohlman, et al. (2009) and Stivarius, et al. (2002). However, Quilo et al. (2009) found that beef trim treated with PAA had increased  $a^*$  values in ground beef.

Yellowness ( $b^*$ ) was also greater for BX dip and spray and lower for LA ESS. These are statistically different, but are numerically close in value ( $< 0.5$  unit difference) that there would likely be no noticeable differences. Pohlman, et al. (2009) and Stivarius et al. (2002) both found that there were no differences in yellowness between organic acid treatments and the control treatment.

Organic acid treatments did not differ from the control in aerobic plate counts. However, LA electrostatic spray treatment had greater log CFU/g than PAA spray and dip, BX spray and electrostatic spray, and LA dip.

LA dip treatments had the lowest pH values and the highest TBA values. This could in part be due to LA being at the highest concentration of the three acids combined

with the dip which has a constant exposure time to the acid. The combination of these two elements could be what caused the pH of the LA dip treatments to be lower than the others. The combination of the acid concentration and the exposure time could have also contributed to the lipid oxidation values. These two elements could have contributed to expediting the auto-oxidation.

Meat pH values for all treatments were lower than the untreated control. This was expected as organic acid pH is lower than typical meat pH. This was also seen by Quilo, et al. (2009) who stated that pH levels were at 5.6 for PAA, and Stivarius et al. (2002) who found that meat treated with LA had a pH of 5.44 while the untreated control and hot water treatment were 6.06 and 6.01, respectively.

## **Conclusions**

The use of lactic acid, Beefxide™, and peroxyacetic acid as antimicrobial treatment of beef sub-primals for the production of ground beef could maintain the same instrumental color, aerobic plate counts, and oxidation characteristics. Minimal changes were found in subjective color and objective color, along with limited changes in aerobic plate counts and oxidation values were found with all treated samples. Therefore, the application of these antimicrobials using these methods can be applied to beef sub-primals with minimal effects on ground beef quality.

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## References

- Arthur, T.M., J.M. Bosilevac, X. Nou, S.D. Shackelford, T.L. Wheeler, M.P. Kent, D. Jaroni, B. Pauling, D.M. Allen, and M. Koohmaraie. 2004. *Escherichia coli* O157 Prevalence and Enumeration of Aerobic Bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at Various Steps in commercial Beef Processing Plants. *Journal of Food Protection*. 67:658-665.
- Centers for Disease Control and Prevention. 2014. Foodborne Diseases Active Surveillance Network: FoodNet 2014 Surveillance Report.
- Ellebracht, E.A., A. Castillo, L.M. Lucia, R.K. Miller, and G.R. Acuff. 1999. Reduction of Pathogens Using Hot Water and Lactic Acid on Beef Trimmings. *Journal of Food Science*. 64:1094-1099.
- Ganesh, V., N.S. Hettiarachchy, M. Ravichandran, M.G. Johnson, C.L. Griffis, E.M. Martin, J.-F. Meullenet, and S.C. Ricke. 2010. Electrostatic Sprays of Food-Grade Acids and Plant Extracts are More Effective than Conventional Sprays in Decontaminating *Salmonella* Typhimurium on Spinach. *Journal of Food Science*. 75:M574-M579.
- Harris, K., M.F. Miller, G.H. Loneragan, and M.M. Brashears. 2006. Validation of the Use of Organic Acids and Acidified Sodium Chlorite to Reduce *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in Beef Trim and Ground Beef in a Simulated Processing Environment. *Journal of Food Protection*. 69:1802-1807.
- Jimenez-Villarreal, J.R., F.W. Pohlman, Z.B. Johnson, and A.H. Brown, Jr. 2003. Effects of Chlorine Dioxide, Cetylphridinium Chloride, Lactic Acid, and Trisodium Phosphate on Physical, Chemical and Sensory Properties of Ground Beef. *Meat Science* 65:1055-1062.
- Kotula, K.L., and R. Thelappurath. 1994. Microbiological and Sensory Attributes of Retail Cuts of Beef Treated with Acetic and Lactic Acid Solutions. *Journal of Food Protection*. 57:665-670.
- Mancini, R.A., and M.C. Hunt. 2005. Current Research in Meat Color. *Meat Science* 71:100-121.
- Massey, L.M., N.S. Hettiarachchy, E.M. Martin, and S.C. Ricke. 2013. Electrostatic Spray of Food-grade Organic Acids and Plant Extract to Reduce *Escherichia coli* O157:H7 on Fresh-Cut Cantaloupe Cubes. *Journal of Food Safety*. 33:71-78.
- Pohlman, F.W., P.N. Dias-Morse, S.A. Quilo, A.H. Brown, Jr., P.G. Grandall, R.T. Baublits, R.P. Story, C. Bokina, and G. Rajaratnam. 2009. Microbial, Instrumental Color and Sensory Characteristics of Ground Beef Processed from Beef Trimmings Treated with Potassium Lactate, Sodium Metasilicate, Peroxyacetic Acid or Acidified Sodium Chlorite as Single Antimicrobial Interventions. *Journal of Muscle Foods*. 20:54-69.

- Quilo, S.A., F.W. Pohlman, A.H. Brown, P.G. Crandall, P.N. Dias-Morse, R.T. Baublits, and J.L. Aparicio. 2009. Effects of Potassium Lactate, Sodium Metasilicate, Peroxyacetic Acid, and Acidified Sodium Chlorite on Physical, Chemical, and Sensory Properties of Ground Beef Patties. *Meat Science* 82:44-52.
- Stivarious, M.R., F.W. Pohlman, K.S. McElyea, and A.L. Waldroup. 2002. Effects of Hot Water and Lactic Acid Treatment of Beef Trimmings Prior to Grinding on Microbial, Instrumental Color and Sensory Porperties of Ground Beef. *Meat Science* 60:327-334.
- Wheeler, T.L., N. Kalchayanand, J.M. Bosilevac. 2014. Pre- and Post-Harvest Interventions to Reduce Pathogen Contamination in the U.S. Beef Industry. *Meat Science* 98:372-382.

## Tables and Figures

**Table 1: L\*, a\*, and b\*, delta E, discoloration, APC, pH, and TBA values of organic acid and application method treatments compared to the untreated control.**

Treatments <sup>1</sup>	L*	a*	b*	Delta E	Discoloration	APC	pH	TBA
Control	49.84 <sup>a</sup>	15.96	10.89 <sup>a</sup>	11.35	30.91	3.05 <sup>ab</sup>	5.94 <sup>a</sup>	2.41 <sup>ab</sup>
BX Dip	48.98 <sup>ab</sup>	17.22	10.89 <sup>a</sup>	10.37	23.80	3.00 <sup>ab</sup>	5.83 <sup>abc</sup>	2.09 <sup>ab</sup>
BX Electrostatic	49.77 <sup>a</sup>	16.69	10.74 <sup>ab</sup>	10.59	25.38	2.77 <sup>b</sup>	5.82 <sup>abc</sup>	2.19 <sup>ab</sup>
BX Spray	49.91 <sup>a</sup>	16.93	10.87 <sup>a</sup>	10.97	24.19	2.72 <sup>b</sup>	5.80 <sup>bc</sup>	2.09 <sup>ab</sup>
LA Dip	48.99 <sup>ab</sup>	17.01	10.74 <sup>ab</sup>	10.86	25.82	2.81 <sup>b</sup>	5.79 <sup>c</sup>	3.10 <sup>a</sup>
LA Electrostatic	49.41 <sup>ab</sup>	16.15	10.41 <sup>b</sup>	10.90	27.47	3.51 <sup>a</sup>	5.80 <sup>bc</sup>	2.21 <sup>ab</sup>
LA Spray	49.88 <sup>a</sup>	16.93	10.86 <sup>ab</sup>	10.07	26.68	2.97 <sup>ab</sup>	5.87 <sup>ab</sup>	2.22 <sup>ab</sup>
PAA Dip	49.96 <sup>a</sup>	16.93	10.61 <sup>ab</sup>	9.81	22.71	2.54 <sup>b</sup>	5.85 <sup>ab</sup>	1.92 <sup>b</sup>
PAA Electrostatic	49.51 <sup>ab</sup>	16.88	10.82 <sup>ab</sup>	10.28	26.51	2.95 <sup>ab</sup>	5.84 <sup>ab</sup>	1.95 <sup>b</sup>
PAA Spray	48.23 <sup>b</sup>	16.09	10.48 <sup>ab</sup>	11.28	32.04	2.89 <sup>ab</sup>	5.87 <sup>ab</sup>	1.93 <sup>b</sup>
SEM	0.4029	0.4094	0.1426	0.4849	3.62	0.1932	0.0426	0.3236
<i>P</i> value	< 0.0001	0.0137	0.0020	0.0202	0.2068	0.0004	< 0.0001	0.0162

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>abc</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).



**Table 2: L\*, a\*, and b\*, delta E, discoloration, APC, pH, and TBA values of organic acid and application method treatments.**

<b>Treatment<sup>1</sup></b>	<b>L*</b>	<b>a*<sup>2</sup></b>	<b>b*</b>	<b>Delta E<sup>3</sup></b>	<b>Discoloration<sup>2</sup></b>	<b>APC</b>	<b>pH</b>	<b>TBA</b>
BX Dip	48.98 <sup>ab</sup>	17.22	10.89 <sup>a</sup>	10.37	23.78	2.99 <sup>ab</sup>	5.83 <sup>a</sup>	2.09
BX Electrostatic	49.77 <sup>a</sup>	16.69	10.74 <sup>ab</sup>	10.59	25.36	2.77 <sup>b</sup>	5.82 <sup>ab</sup>	2.19
BX Spray	49.91 <sup>a</sup>	16.93	10.87 <sup>a</sup>	10.97	24.18	2.72 <sup>b</sup>	5.80 <sup>ab</sup>	2.09
LA Dip	48.99 <sup>ab</sup>	17.14	10.74 <sup>ab</sup>	10.86	25.80	2.81 <sup>b</sup>	5.69 <sup>b</sup>	3.10
LA Electrostatic	49.41 <sup>ab</sup>	16.15	10.41 <sup>b</sup>	10.90	27.46	3.51 <sup>a</sup>	5.80 <sup>ab</sup>	2.21
LA Spray	49.88 <sup>a</sup>	16.93	10.86 <sup>ab</sup>	10.07	26.69	2.97 <sup>ab</sup>	5.87 <sup>a</sup>	2.22
PAA Dip	49.96 <sup>a</sup>	16.93	10.61 <sup>ab</sup>	9.81	22.68	2.54 <sup>b</sup>	5.85 <sup>a</sup>	1.92
PAA Electrostatic	49.51 <sup>a</sup>	16.89	10.82 <sup>ab</sup>	10.29	26.52	2.95 <sup>ab</sup>	5.84 <sup>a</sup>	1.95
PAA Spray	48.23 <sup>b</sup>	16.09	10.48 <sup>ab</sup>	11.28	32.06	2.89 <sup>b</sup>	5.87 <sup>a</sup>	1.93
SEM	0.4032	0.4142	0.1461	0.4895	3.66	0.1880	0.4059	0.3233
<i>P</i> value	< 0.0001	0.1173	0.0050	0.0163	0.3954	0.0052	0.0100	0.1283

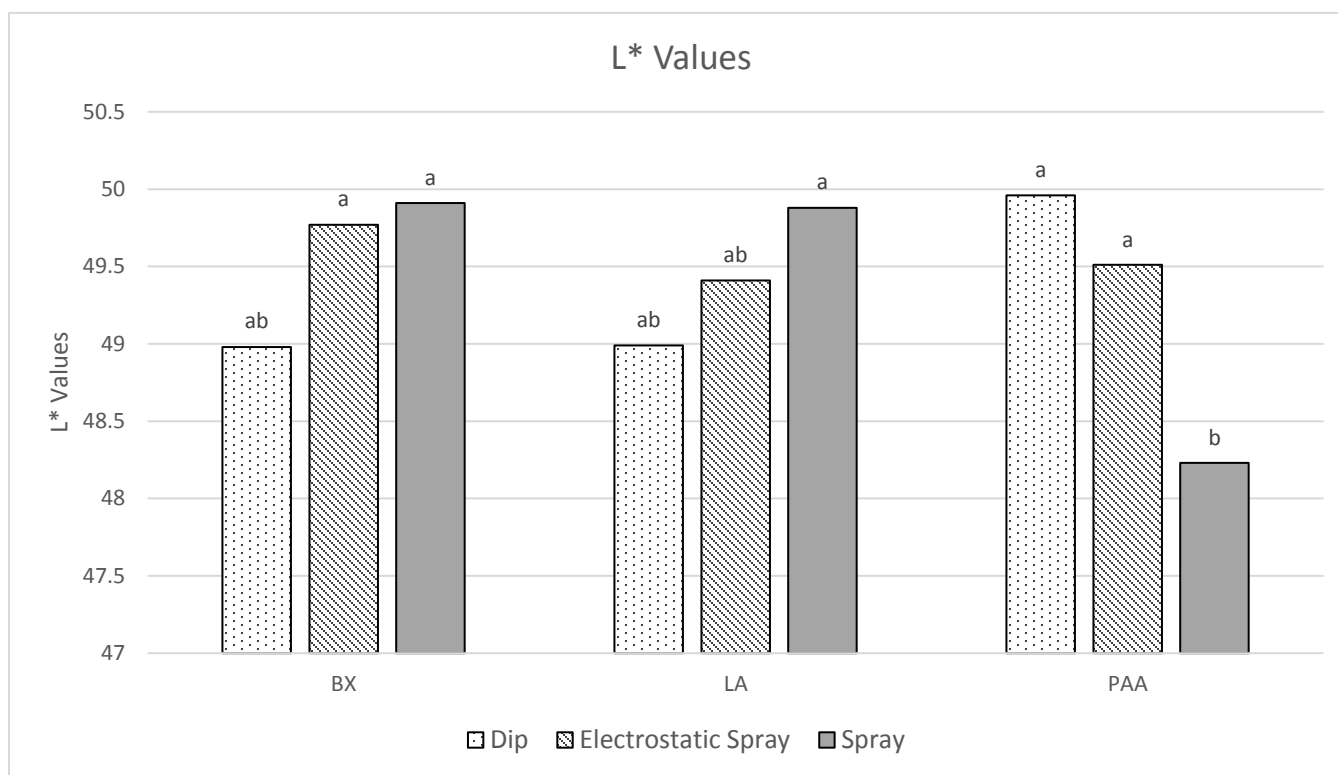
<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>2</sup> No significant main effects were identified ( $P > 0.05$ )

<sup>3</sup> No means separation were found once Tukey's adjustment was applied.

<sup>ab</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Figure 1: Organic acid by application method treatment<sup>1</sup> differences for L\* values without the untreated control**

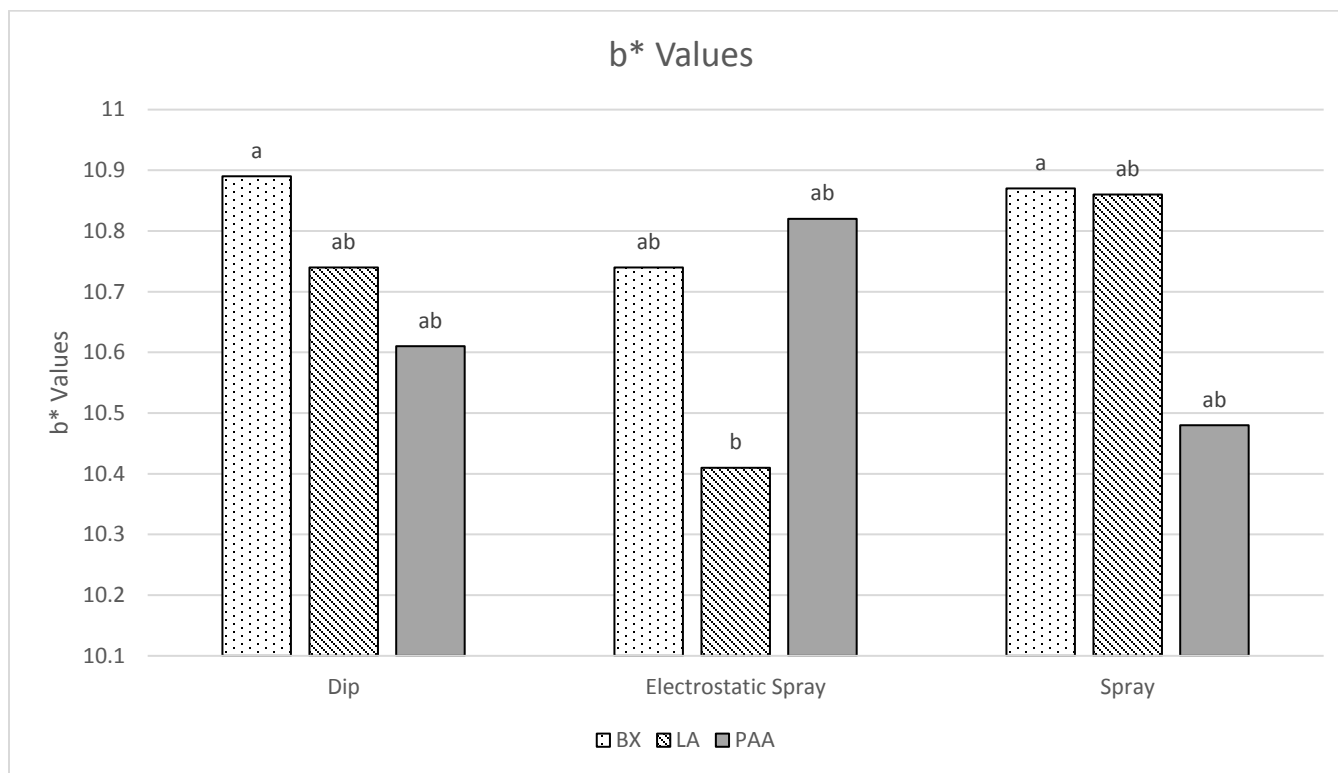


Acid\*app interaction ( $P < 0.001$ , SEM = 0.4032)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid, Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>ab</sup> Treatments with different superscripts indicate significant differences.

**Figure 2: Organic acid by application method treatment<sup>1</sup> differences for b\* values without the untreated control**

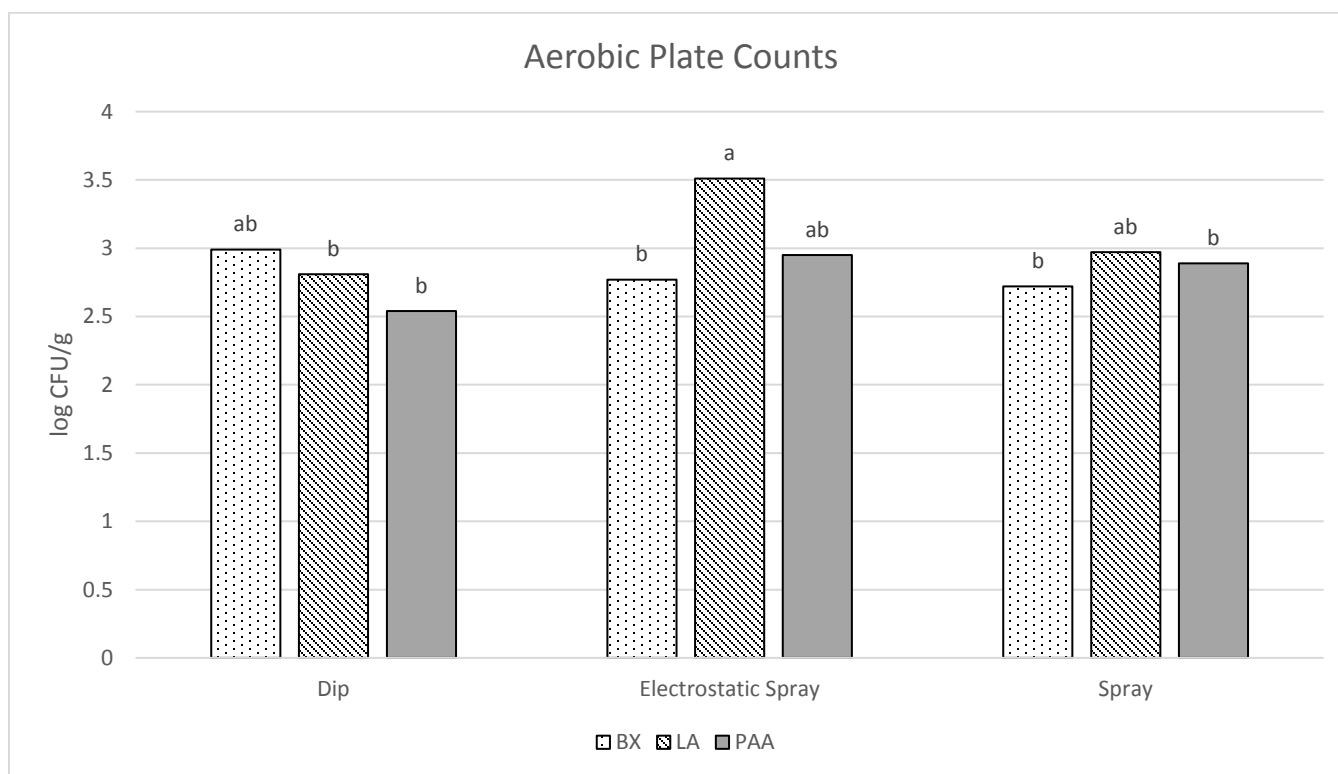


Acid\*app interaction ( $P < 0.01$ , SEM = 0.1461)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>ab</sup> Treatments with different superscripts indicate significant differences.

**Figure 3: Organic acid by application method treatment<sup>1</sup> differences for aerobic plate counts (log CFU/g) without the untreated control**



Acid\*app interaction ( $P < 0.01$ , SEM = 0.1880)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>ab</sup> Treatments with different superscripts indicate significant differences.

## **STANDARD OPERATING PROCEDURE FOR SPRAY APPLICATION OF LACTIC ACID SOLUTION TO BEEF SUBPRIMALS TO BE USED FOR RAW GROUND BEEF OR NON- INTACT BEEF PRODUCTION**

Each year, foodborne illnesses cause thousands of illnesses and deaths. One particular group of pathogens which cause concern are the shiga toxin-producing *E. coli* or STEC. These organisms can cause flu-like symptoms that can range from mild to life-threatening. Serious complications can arise such as hemolytic uremic syndrome which can result in kidney failure and death. While STEC can be found in a number of food borne sources, the USDA has declared 7 serotypes adulterants when found in raw, non-intact beef. They are: *E. coli* O157:H7, O26, O45, O103, O111, O121, and O145.

Beef processors have implemented food safety and processing controls in order to reduce the risk of STEC in their products. In particular, control measures have been taken for ground beef and non-intact beef production. These products require grinding, cubing, or other tenderization methods which break the surface of the meat and can redistribute STEC within the product. One control measure that can be used is the spray application of organic acids to beef subprimals to reduce the food safety risk of STEC in the finished product. The procedure described here is specifically targeted for small beef processors who will be applying the lactic acid intervention with a hand sprayer.

### **1. Purpose**

- a. Define a procedure for applying lactic acid antimicrobial intervention to beef subprimal cuts via hand spraying method.
- b. Define a generalized spraying process which can be adapted to other organic acid interventions and their supporting documents, with the appropriate changes to critical parameters.

### **2. Important Notes**

- a. Review the Safety Data Sheet (SDS) for any antimicrobial intervention product prior to use.
  - i. The SDS can be obtained by contacting the organic acid manufacturer or supplier. Some SDS documents may be available on the manufacturer's website.
- b. Wear all required personal protective equipment (PPE) as required by the SDS.
- c. Work in a well ventilated area.
- d. Follow all applicable good manufacturing procedures for preventing cross contamination of edible products, equipment and working surfaces.
- e. When selecting working surfaces and equipment, make sure to select those that will withstand the corrosive nature of organic acids over prolonged exposure.
  - i. Avoid contact with alkali metals and painted surfaces as they are more susceptible to corrosion.

- ii. Stainless steel and heavy food grade plastics are examples of surfaces that could be used.

### **3. Equipment & Supplies**

- a. 88% lactic acid concentrate
- b. Scale appropriate for weighing the needed amount of water
- c. Personal protective equipment, which may include:
  - i. Chemical resistant rubber gloves
  - ii. Splash proof goggles
  - iii. Apron or frock
  - iv. Chemical resistant rubber boots may be appropriate
- d. Lactic acid titration kit (available from the lactic acid manufacturer or supplier)
- e. Graduated cylinder for measuring 88% lactic acid
- f. Large spoon or other food grade utensil for stirring the lactic acid solution
- g. Spraying equipment
  - i. Pressurized, stainless steel sprayer appropriate for the volume of lactic acid solution to be used (generally a 1-3 gallon sprayer would be recommended) and fitted with stainless steel fittings
  - ii. Hand held, stainless steel spray gun (maximum gallons per minute 10.5)
  - iii. Fan shaped, stainless steel spraying nozzle (30° fan size, 0.05” orifice, flow rate of 0.3 gallons per minute @ 20 pounds per square inch pressure)
  - iv. Calibrated thermometer
  - v. In-house compressed air line access or air compressor with a pressure regulator
- h. Food grade work surface that allows drainage from both the top surface and underneath the treated subprimals

### **4. Mixing lactic acid solution**

- a. The legal maximum concentration for lactic acid antimicrobial application is 5.0%. The target for this procedure is 4.5% lactic acid solution or greater.
- b. Determine the amount of water needed. It is recommended that only the amount of solution needed for one day’s production be mixed at a time.
  - i. Target application is greater than or equal to 0.26 milliliters of lactic acid solution per square centimeter of product area.
  - ii. In UNL work with clods, approximately 1 gallon of solution is mixed per 4 clods.
- c. Allow the water to run until it reaches between 70°F and 75°F.
- d. Measure out the amount of water needed. Water should be weighed to ensure accurate measurement.
  - i. One gallon of water weighs approximately 8.34 pounds at 70°F. The weight of one gallon of water will vary slightly based on water temperature (higher temperature results in a lighter weight).
  - ii. Verify the water temperature with a clean, calibrated thermometer.

- iii. After weighing the needed amount of water, use a clean container to reserve some of the water. This will be used at a later step to rinse the graduated cylinder, as the lactic acid concentrate is thick and may not pour completely out of the graduated cylinder.
- e. Refer to the dilution chart to determine the amount of 88% lactic acid needed per gallon to reach the desired concentration.
  - i. In the UNL research, to reach 4.5% concentration of lactic acid, 194 milliliters of 88% lactic acid is needed for 1 gallon of water.
  - ii. It is recommended, for safety, to always add the acid to the water. Do not add water to the acid, which can cause splashing of concentrated lactic acid.
- f. Measure out the needed 88% lactic acid concentrate. This should be measured using a graduated cylinder to ensure accuracy.
- g. Add the lactic acid to the water.
- h. Use the water reserved earlier to rinse the graduated cylinder and ensure all measured lactic acid has been added to the solution. Pour the rinse water into the previously made solution. Add any remaining reserved water as well.
- i. Carefully, stir the solution, without splashing, to ensure it is mixed thoroughly.
- j. Follow the instructions provided in the titration test kit to verify that the proper dilution has been achieved.
  - i. Record important titration information to keep with production records. Examples would be concentration of lactic acid solution, time and date of mixing, plant lot number for grinding, lactic acid lot number, number of gallons prepared, and initials of the person completing the titration.
  - ii. It is important to hold the titration test kit bottles straight up and down when adding drops to the sample. This will help ensure uniform drops and a more accurate result.
- k. If concentration meets target, the solution can be put in the sprayer and be sealed. If concentration does not meet target, adjust with either the addition of water or lactic acid concentrate.

## **5. Hand Spraying Method**

- a. Prepare sprayer by attaching the small hand held spraying nozzle, without pressure on the tank. Be careful during all steps of the process to keep the tip of the spray nozzle away from any source of cross contamination and off the floor.
- b. Mix lactic acid solution using the procedure in section 4.
- c. Attach an air hose to either an in-house air line or air compressor tank. With air flow off, attach the air hose to the sprayer tank.
- d. Adjust the regulator on the air line so that pressure is at 20 pounds per square inch (psi). This is an important factor in determining the application rate of the lactic acid intervention.

- e. Lay out the subprimals to be treated on a working surface that is clean and allows for lactic acid solution drainage from both on top of and underneath the product. Ensure the entire surface of the beef is exposed, with no folds or overlapping.
- f. Hold the hand sprayer nozzle and pressurize the spray tank.
- g. With the spraying nozzle held approximately 10-12 inches above the product, apply the lactic acid solution to the subprimal allowing the solution to thinly cover the entire exposed surface. It is important to spray all sides, as well as the larger surfaces.
  - i. Based on the UNL research with clods, a surface area of approximately 800 square centimeters resulted in an approximately 11 second spray per side of the clod.
  - ii. Distance from the product may vary depending on the sprayer nozzle being used. It is important to achieve a full fan pattern for complete coverage.
  - iii. Target coverage is 0.26 milliliters of lactic acid solution per square centimeter, or greater.
- h. Turn over each cut so the opposite surfaces are exposed.
- i. Repeat the spraying procedure until all product has been treated.
- j. Allow all subprimals to drain until excess lactic acid solution and any pooling has been removed. In the UNL study, this took approximately 60 seconds on clods.
  - i. Treated subprimals should not be allowed to sit in a pool of lactic acid.
  - ii. Failure to allow adequate drainage could result in over application of lactic acid and potential quality defects due to overexposure.
- k. Once sufficient drainage has been allowed, cuts may be moved to the next step of the production process.

## **6. Equipment Sanitation**

- a. Remaining mixed lactic acid solution may be stored based on manufacturer's recommendations.
  - i. If storage is allowed, the solution will need to be titrated prior to each use to ensure it is still at proper concentration.
- b. Make sure to rinse all equipment thoroughly as failure to do so could result in corrosion. This includes the sprayer, hose to the spray gun, spray gun, and nozzle.
- c. Clean all tools and equipment according to plant sanitation standard operating procedures.

## **7. Supporting Documentation**

- a. 88% Lactic Acid Safety Data Sheet (provided by manufacturer)
- b. 88% Lactic Acid Dilution Chart (provided by manufacturer or chemical vendor)



- c. McCarty, K. A. (2016). *The Effect of Antimicrobial Treatment of Beef Sub-Primals on Reduction of E. coli and Ground Beef Quality*. [Antimicrobial Treatment of Beef Sub-Primals for *E. coli* and Ground Beef Quality]. Unpublished raw data.
- d. Kalchayanand, N.; Arthur, T.; Bosilevac, J.; Schmidt, J.; Wang, R.; Shackelford, S.; Wheeler, T. 2012. Evaluation of Commonly Used Antimicrobial Interventions for Fresh Beef Inoculated with Shiga Toxin-Producing *Escherichia coli* Serotypes O26, O45, O103, O111, O121, O145, and O157:H7. *J Food Prot* 75:1207-1212.
- e. Youssef, M.K.; Yang, X.; Badoni, M.; Gill, C.O. 2013. Survival of Acid-Adapted *Escherichia coli* O157:H7 and Not-Adapted *E. coli* on Beef Treated with 2% or 5% Lactic Acid. *Food Control* 34:13-18.
- f. M.K. Youssef, X. Yang, M. Badoni, C.O. Gill, 2012. Effects of spray volume, type of surface tissue and inoculum level on the survival of *Escherichia coli* on beef sprayed with 5% lactic acid. *Food Control* 25:717-722
- g. US Center for Disease Control and Prevention. *Escherichia coli* General Information. Updated Nov. 6, 2015.  
<http://www.cdc.gov/ecoli/general/index.html>
- h. Mayo Clinic. Hemolytic Uremic Syndrome. Updated July 7, 2016.  
<http://www.mayoclinic.org/diseases-conditions/hemolytic-uremic-syndrome/home/ovc-20204140>

## Appendix I

### Preparation of Rifampicin-resistant *E. coli*

1. Pipet 10 mL Tryptic Soy Broth into five individual labeled 15 mL tubes.
2. Scrape frozen (-80°C) *E. coli*<sup>Rif</sup> and combine with step 1—keeping each strain separate.
3. Incubate (Step 1 & 2) at least 24 at 37°C.
4. Pipet 10 mL Tryptic Soy Broth into individual labeled 15 mL tubes.
5. Pipet 5 µL Rifampicin into each 15 mL tube.
6. Pipet 1 mL of each culture (Step 3) into individual labeled 15 mL tubes creating a 10:1 dilution.
7. Incubate (Steps 4-6) for 24 hours at 37°C.
8. Mix a 1:1 ratio of culture from each 15 mL tube together.
9. Combine (Step 8) with peptone buffer to create a 9:1 ratio (Buffer : culture).
10. Pipet 15 mL of mixture (Step 9) into 15 mL tubes for inoculation.
11. Store in <4.4°C until inoculation.

## Appendix II

### Preparing the organic acid solution

1. Measure the specified amount of water (3.78 liters= 3.78 kg water).
2. Measure the specified amount of organic acid according to the manufactures instructions.
3. Gently pour the organic acid into the water to prevent splashing.
4. Thoroughly mix the solution to assure that the acid is evenly dispersed.

### Appendix III

#### Inoculation/Treatment

1. Cut clod in half width wise to form two roasts.
2. Inoculate each roast with 15 mL prepared culture (Appendix I).
3. Allow for a 20 minute wait period for *E. coli*<sup>Rif</sup> to attach to the meat surface.
4. Take 5 cores (25.3 cm<sup>2</sup> surface area) from the inoculated meat surface for Sample 1.
5. Apply designated treatment described below.
6. After treatment, take 5 cores from the inoculated and treated surface for Sample 2.
7. Store samples at <4.4°C.
8. Store roasts in individual bags at <4.4°C.

#### Spray Treatment

After taking Sample 1, spray inoculated surface for 5 seconds at ~25.4-35.6 cm from the meat surface using a 20° flat spray nozzle at 137,895 Pa.

#### Dip Treatment

After taking Sample 1, submerge entire roast in treatment solution for 15 seconds.

#### Electrostatic Spray Treatment

After taking Sample 1, spray inoculated surface for 10 seconds at ~25.4-35.6 cm from the meat surface.

## Appendix IV

### Grinding

1. Wait until immediately before to take the roast out of the cooler.
2. Grind using the 1.27 cm plate.
3. Grind the meat again using the .48 cm plate.
4. Disassemble the grinder and thoroughly clean all parts with Clorox before grinding another sample.

## Appendix V

### pH Procedure

1. Weigh out 10 g of raw sample in duplicate into small beakers.
2. Add 90 ml of distilled, deionized water to each.
3. Homogenize the solution for 30 seconds using a Polytron at 10,800 rpm.
4. Place a stir bar in the solution after homogenizing and while stirring the solution,
5. Read the pH using a pH meter which has been calibrated using 7.0 Buffer and 4.0 Buffer.
6. Be sure to keep electrode clean by rinsing well between samples.

Appendix VI  
**Thiobarbituric Acid Assay**  
 Buege and Aust (1978), Modified by Ahn et al. 1998

**TEP Solution (1,1,3,3-Tetraethoxypropane)** (Make new weekly)

Stock Solution: Dilute 99  $\mu$ l TEP (97%) bring volume to 100 mL ddH<sub>2</sub>O

Working Solution: Dilute stock solution to 1:3 (TEP Solution:ddH<sub>2</sub>O) ( $1 \times 10^{-3}$ M)

**TBA/TCA (2-Thiobarbituric Acid/Trichloroacetic Acid) Stock Solution: 1L**

15% TCA (w/v) and 20 mM TBA (MW 144.5) reagent in ddH<sub>2</sub>O.

Dissolve 2.88 g TBA in warm ddH<sub>2</sub>O first, then add TCA (150g) and ddH<sub>2</sub>O to 1L

**BHA (ButylatedHydroxyAnisole) Stock Solution:**

Make 10% stock solution by dissolving in 90% ethanol.

10g BHA dissolved in 90 mL ethanol (90%) + 5mL ddH<sub>2</sub>O

**Standards: In duplicate**

Blank:	1 ml ddH <sub>2</sub> O	<u><b>Moles of TEP</b></u>
Standard 5:	100 $\mu$ l working TEP + 1.90 mL ddH <sub>2</sub> O	( $5 \times 10^{-5}$ M)
Standard 4:	1 mL Std. 5 + 1 mL ddH <sub>2</sub> O	( $2.5 \times 10^{-5}$ M)
Standard 3:	1 mL Std. 4 + 1 mL ddH <sub>2</sub> O	( $1.25 \times 10^{-5}$ M)
Standard 2:	1 mL Std. 3 + 1 mL ddH <sub>2</sub> O	( $.625 \times 10^{-5}$ M)
Standard 1:	1 mL Std. 2 + 1 ml ddH <sub>2</sub> O	( $.3125 \times 10^{-5}$ M)
Remove 1 mL of Standard 1 and discard it, leaving 1 mL behind.		

**Procedure**

- Mix all reagents and standards before beginning.
- Transfer 5 g of powdered sample into a 50 ml conical tube, add 14 ml of ddH<sub>2</sub>O and 1.0 mL of BHA (Butylated hydroxyanisole).
- Homogenize for 15 sec with a polytron
- Centrifuge for 2000xg for 5 minutes.
- Transfer 1 ml of homogenate or standard to 15 ml conical tube
- Add 2 ml of TBA/TCA solution, vortex.
- Incubate in a 70°C water bath for 30 min to develop color.
- Cool samples in a cold water bath for 10 min.
- Centrifuge tubes at 2000xg for 15 min.
- Transfer duplicate aliquots of 200  $\mu$ l from each tube into wells on a 96 well plate.
- Read absorbance at 540nm.

**Calculations: mgs of malonaldehyde/kg of tissue**

$K(\text{extraction}) = (S/A) \times MW \times (10^6/E) \times 100$

Where S=Standard concentration ( $1 \times 10^{-8}$  moles 1,1,3,3-tetraethoxypropane)/5ml.

A=Absorbance of standard      MW=MW of malonaldehyde (72.063 g/mole)

E= sample equivalent (1)      P=Percent recovery

**Final calculation: .012 x concentration x 72.063x10<sup>6</sup> = mgs Malonaldehyde/kg of tissue**

Reagents (Sigma): TBA- T5500; TCA- T9159; TEP- T9889; BHA- B1253

## Appendix VII

### ADDITIONAL TABLES AND FIGURES FOR STUDY 1

**Table 1: Organic acid and application method treatment effects on *E. coli*<sup>Rif</sup> counts (log CFU/cm<sup>2</sup>) using *E. coli*/coliform Petrifilm with the inoculated control**

Treatment <sup>1</sup>	Pre-treated	Post-treated	Reduction
Control	5.50	5.64 <sup>a</sup>	-0.14 <sup>a</sup>
LA Electrostatic Spray	5.70	5.31 <sup>ab</sup>	0.39 <sup>ab</sup>
BX Electrostatic Spray	5.39	4.99 <sup>bc</sup>	0.40 <sup>ab</sup>
BX Spray	5.56	4.96 <sup>bc</sup>	0.60 <sup>bc</sup>
PAA Electrostatic Spray	5.52	4.90 <sup>bc</sup>	0.62 <sup>bc</sup>
BX Dip	5.63	4.98 <sup>bc</sup>	0.65 <sup>bc</sup>
PAA Spray	5.56	4.73 <sup>c</sup>	0.83 <sup>bc</sup>
PAA Dip	5.54	4.70 <sup>c</sup>	0.84 <sup>bc</sup>
LA Spray	5.68	4.62 <sup>c</sup>	1.06 <sup>c</sup>
LA Dip	5.80	4.67 <sup>c</sup>	1.13 <sup>c</sup>
SEM	0.1332	0.1369	0.1774
<i>P</i> value	0.1708	< 0.0001	< 0.0001

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>abc</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).



**Table 2: Application method<sup>1</sup> effects on *E. coli*<sup>Rif</sup> counts (log CFU/cm<sup>2</sup>) using *E. coli*/coliform Petrifilm**

	Pre-treated	Post-treated	Reduction
Dip	5.66	4.78 <sup>a</sup>	0.88 <sup>a</sup>
Spray	5.60	4.77 <sup>a</sup>	0.83 <sup>a</sup>
Electrostatic Spray	5.53	5.07 <sup>b</sup>	0.46 <sup>b</sup>
SEM	0.0777	0.0802	0.1063
<i>P</i> value	0.2915	0.0009	0.0008

<sup>1</sup> Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>ab</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 3: Organic acid<sup>1</sup> effects on *E. coli*<sup>Rif</sup> counts (log CFU/cm<sup>2</sup>) using *E. coli*/coliform Petrifilm**

	Pre-treated	Post-treated	Reduction
LA	5.72 <sup>a</sup>	4.86 <sup>ab</sup>	0.86 <sup>a</sup>
PAA	5.54 <sup>ab</sup>	4.78 <sup>a</sup>	0.76 <sup>ab</sup>
BX	5.53 <sup>b</sup>	4.98 <sup>b</sup>	0.55 <sup>b</sup>
SEM	0.0777	0.0802	0.1063
<i>P</i> value	0.0251	0.0537	0.0168

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid

<sup>ab</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 4: Fat and lean effects on *E. coli*<sup>Rif</sup> counts (log CFU/cm<sup>2</sup>) using *E. coli*/coliform Petrifilm**

	Pre-treated	Post-treated	Reduction
Fat surface	5.63	4.78 <sup>a</sup>	0.85 <sup>a</sup>
Lean surface	5.55	4.96 <sup>b</sup>	0.59 <sup>b</sup>
SEM	0.0421	0.0433	0.0561
<i>P</i> value	0.1431	0.0069	0.0019

<sup>ab</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 5: Organic acid and application method treatment effects on *E. coli*<sup>Rif</sup> counts (log CFU/g) in ground beef using *E. coli*/coliform Petrifilm with the inoculated control**

Treatment <sup>1</sup>	Ground Beef
Control	3.97 <sup>a</sup>
LA Electrostatic Spray	3.90 <sup>ab</sup>
BX Electrostatic Spray	3.80 <sup>abc</sup>
PAA Electrostatic Spray	3.76 <sup>abc</sup>
BX Spray	3.57 <sup>abc</sup>
PAA Spray	3.43 <sup>bc</sup>
BX Dip	3.39 <sup>bc</sup>
LA Spray	3.36 <sup>c</sup>
PAA Dip	3.31 <sup>c</sup>
LA Dip	3.27 <sup>c</sup>
SEM	0.1599
<i>P</i> value	0.0001

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>abc</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 6: Application method<sup>1</sup> effects on *E. coli*<sup>Rif</sup> counts (log CFU/g) in ground beef using *E. coli*/coliform Petrifilm**

Ground Beef	
Dip	3.32 <sup>a</sup>
Spray	3.45 <sup>a</sup>
Electrostatic Spray	3.82 <sup>b</sup>
SEM	0.0867
<i>P</i> value	< 0.0001

<sup>1</sup> Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>ab</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 7: Organic acid<sup>1</sup> effects on *E. coli*<sup>Rif</sup> counts (log CFU/g) in ground beef using *E. coli*/coliform Petrifilm**

	Ground Beef
PAA	3.50
LA	3.51
BX	3.59
SEM	0.0867
<i>P</i> value	0.5707

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid

**Table 8: Fat and lean effects on *E. coli*<sup>Rif</sup> counts (log CFU/g) in ground beef using *E. coli*/coliform Petrifilm**

	Ground Beef
Fat surface	3.32 <sup>a</sup>
Lean surface	3.74 <sup>b</sup>
SEM	0.0500
<i>P</i> value	< 0.0001

<sup>abc</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 9: Organic acid and application method treatment effects on *E. coli*<sup>Rif</sup> counts (log CFU/cm<sup>2</sup>) using aerobic count plate Petrifilm with the inoculated control**

Treatment <sup>1</sup>	Pre-treated	Post-treated	Reduction
Control	5.65	5.73 <sup>a</sup>	-0.08 <sup>a</sup>
BX Electrostatic Spray	5.50	5.45 <sup>ab</sup>	0.05 <sup>a</sup>
LA Electrostatic Spray	5.74	5.66 <sup>a</sup>	0.08 <sup>a</sup>
PAA Electrostatic Spray	5.62	5.41 <sup>abc</sup>	0.21 <sup>ab</sup>
BX Dip	5.80	5.41 <sup>abc</sup>	0.39 <sup>abc</sup>
BX Spray	5.72	5.30 <sup>abcd</sup>	0.42 <sup>abc</sup>
LA Dip	5.91	5.25 <sup>abcd</sup>	0.66 <sup>bc</sup>
PAA Spray	5.60	4.94 <sup>cd</sup>	0.66 <sup>bc</sup>
LA Spray	5.82	5.08 <sup>bcd</sup>	0.74 <sup>bc</sup>
PAA Dip	5.67	4.86 <sup>d</sup>	0.81 <sup>c</sup>
SEM	0.1300	0.1528	0.1686
<i>P</i> value	0.1355	< 0.0001	< 0.0001

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>abcd</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).



**Table 10: Application method<sup>1</sup> effects on *E. coli*<sup>Rif</sup> counts (log CFU/cm<sup>2</sup>) using aerobic count plate Petrifilm**

	Pre-treated	Post-treated	Reduction
Dip	5.79	5.17 <sup>a</sup>	0.62 <sup>a</sup>
Spray	5.71	5.11 <sup>a</sup>	0.60 <sup>a</sup>
Electrostatic Spray	5.62	5.51 <sup>b</sup>	0.11 <sup>b</sup>
SEM	0.0746	0.0897	0.1000
<i>P</i> value	0.0883	0.0002	< 0.0001

<sup>1</sup> Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>abc</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 11: Organic acid<sup>1</sup> effects on *E. coli*<sup>Rif</sup> counts (log CFU/cm<sup>2</sup>) using aerobic count plate Petrifilm**

	Pre-treated	Post-treated	Reduction
PAA	5.63 <sup>b</sup>	5.07 <sup>a</sup>	0.56 <sup>a</sup>
LA	5.82 <sup>a</sup>	5.33 <sup>b</sup>	0.49 <sup>ab</sup>
BX	5.67 <sup>ab</sup>	5.39 <sup>b</sup>	0.28 <sup>b</sup>
SEM	0.0746	0.0897	0.1000
<i>P</i> value	0.0382	0.0027	0.0260

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid

<sup>ab</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 12: Fat and lean effects on *E. coli*<sup>Rif</sup> counts (log CFU/cm<sup>2</sup>) using aerobic count plate Petrifilm**

	Pre-treated	Post-treated	Reduction
Fat	5.74	5.27	0.47
Lean	5.68	5.26	0.42
SEM	0.0581	0.0049	0.0754
<i>P</i> value	0.2214	0.9434	0.3772

**Table 13: Organic acid and application method treatment<sup>1</sup> effects on *E. coli*<sup>Rif</sup> counts (log CFU/g) in ground beef using aerobic count plate Petrifilm with the inoculated control**

	Ground Beef
Control	4.20 <sup>ab</sup>
LA Electrostatic Spray	4.21 <sup>a</sup>
PAA Electrostatic Spray	4.14 <sup>ab</sup>
BX Electrostatic Spray	4.09 <sup>ab</sup>
BX Spray	3.98 <sup>abc</sup>
LA Spray	3.93 <sup>abc</sup>
BX Dip	3.91 <sup>abc</sup>
LA Dip	3.86 <sup>abc</sup>
PAA Spray	3.82 <sup>bc</sup>
PAA Dip	3.63 <sup>c</sup>
SEM	0.1135
<i>P</i> value	< 0.0001

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>abc</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 14: Application method<sup>1</sup> effects on *E. coli*<sup>Rif</sup> counts in ground beef (log CFU/g) using aerobic count plate Petrifilm**

	Ground Beef
Dip	3.80 <sup>a</sup>
Spray	3.91 <sup>a</sup>
Electrostatic Spray	4.15 <sup>b</sup>
SEM	0.0650
<i>P</i> value	< 0.0001

<sup>1</sup> Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>ab</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 15: Organic acid<sup>1</sup> effects on *E. coli*<sup>Rif</sup> counts (log CFU/g) in ground beef using aerobic count plate Petrifilm**

Ground Beef	
LA	4.00
BX	3.99
PAA	3.87
SEM	0.0650
<i>P</i> value	0.0774

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid

**Table 16: Fat and lean effects on *E. coli*<sup>Rif</sup> counts (log CFU/g) in ground beef using aerobic count plate Petrifilm**

Ground Beef	
Fat	3.82 <sup>a</sup>
Lean	4.09 <sup>b</sup>
SEM	0.0508
<i>P</i> value	< 0.0001

<sup>ab</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Table 17: Organic acid and application method treatment<sup>1</sup> effects for temperature of meat and solutions**

Treatment <sup>1</sup>	Meat Temperature (°C)			Solution Temperature (°C)	
	<u>Inoculation</u>	<u>Pre-treatment</u>	<u>Post-treatment</u>	<u>Before Application</u>	<u>After Application</u>
Control	3.44	3.67 <sup>a</sup>	5.00 <sup>b</sup>	-	-
BX Dip	3.78	5.39 <sup>bc</sup>	5.39 <sup>b</sup>	21.83 <sup>abc</sup>	20.56 <sup>bc</sup>
BX Electrostatic	2.89	5.44 <sup>bc</sup>	5.67 <sup>b</sup>	21.83 <sup>abc</sup>	21.67 <sup>ab</sup>
BX Spray	3.17	4.72 <sup>abc</sup>	5.28 <sup>b</sup>	22.61 <sup>a</sup>	22.22 <sup>a</sup>
LA Dip	2.50	4.56 <sup>abc</sup>	8.61 <sup>a</sup>	21.11 <sup>bc</sup>	20.11 <sup>c</sup>
LA Electrostatic	3.17	5.28 <sup>abc</sup>	5.11 <sup>b</sup>	20.72 <sup>c</sup>	20.56 <sup>bc</sup>
LA Spray	2.94	4.44 <sup>ac</sup>	5.00 <sup>b</sup>	21.67 <sup>abc</sup>	20.94 <sup>abc</sup>
PAA Dip	2.78	5.39 <sup>bc</sup>	5.56 <sup>b</sup>	21.50 <sup>abc</sup>	20.17 <sup>c</sup>
PAA Electrostatic	3.17	5.72 <sup>c</sup>	5.39 <sup>b</sup>	21.67 <sup>abc</sup>	21.11 <sup>abc</sup>
PAA Spray	3.17	4.44 <sup>ab</sup>	4.50 <sup>b</sup>	22.06 <sup>ab</sup>	21.83 <sup>ab</sup>
SEM	0.7422	0.6599	1.0716	0.6227	0.8037
<i>P</i> value	0.2471	0.0006	< 0.0001	0.0006	0.0001

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>abc</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).



## Appendix VIII

### ADDITIONAL TABLES AND FIGURES FOR STUDY 2

**Table 18: L\*, a\*, and b\*, delta E, discoloration, APC, pH, and TBA values of organic acid and application method treatments compared to the untreated control.**

Treatment <sup>1</sup>	L*	a* <sup>2</sup>	b*	Delta E <sup>3</sup>	Discoloration <sup>2</sup>	APC	pH	TBA
BX Dip	48.98 <sup>ab</sup>	17.22	10.89 <sup>a</sup>	10.37	23.78	2.99 <sup>ab</sup>	5.83 <sup>a</sup>	2.09
BX Electrostatic	49.77 <sup>a</sup>	16.69	10.74 <sup>ab</sup>	10.59	25.36	2.77 <sup>b</sup>	5.82 <sup>ab</sup>	2.19
BX Spray	49.91 <sup>a</sup>	16.93	10.87 <sup>a</sup>	10.97	24.18	2.72 <sup>b</sup>	5.80 <sup>ab</sup>	2.09
LA Dip	48.99 <sup>ab</sup>	17.14	10.74 <sup>ab</sup>	10.86	25.80	2.81 <sup>b</sup>	5.69 <sup>b</sup>	3.10
LA Electrostatic	49.41 <sup>ab</sup>	16.15	10.41 <sup>b</sup>	10.90	27.46	3.51 <sup>a</sup>	5.80 <sup>ab</sup>	2.21
LA Spray	49.88 <sup>a</sup>	16.93	10.86 <sup>ab</sup>	10.07	26.69	2.97 <sup>ab</sup>	5.87 <sup>a</sup>	2.22
PAA Dip	49.96 <sup>a</sup>	16.93	10.61 <sup>ab</sup>	9.81	22.68	2.54 <sup>b</sup>	5.85 <sup>a</sup>	1.92
PAA Electrostatic	49.51 <sup>a</sup>	16.89	10.82 <sup>ab</sup>	10.29	26.52	2.95 <sup>ab</sup>	5.84 <sup>a</sup>	1.95
PAA Spray	48.23 <sup>b</sup>	16.09	10.48 <sup>ab</sup>	11.28	32.06	2.89 <sup>b</sup>	5.87 <sup>a</sup>	1.93
SEM	0.4032	0.4142	0.1461	0.4895	3.66	0.1880	0.4059	0.3233
P value	< 0.0001	0.1173	0.0050	0.0163	0.3954	0.0052	0.0100	0.1283

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>2</sup> No significant main effects were identified ( $P > 0.05$ )

<sup>3</sup> No means separation were found once Tukey's adjustment was applied.

<sup>ab</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

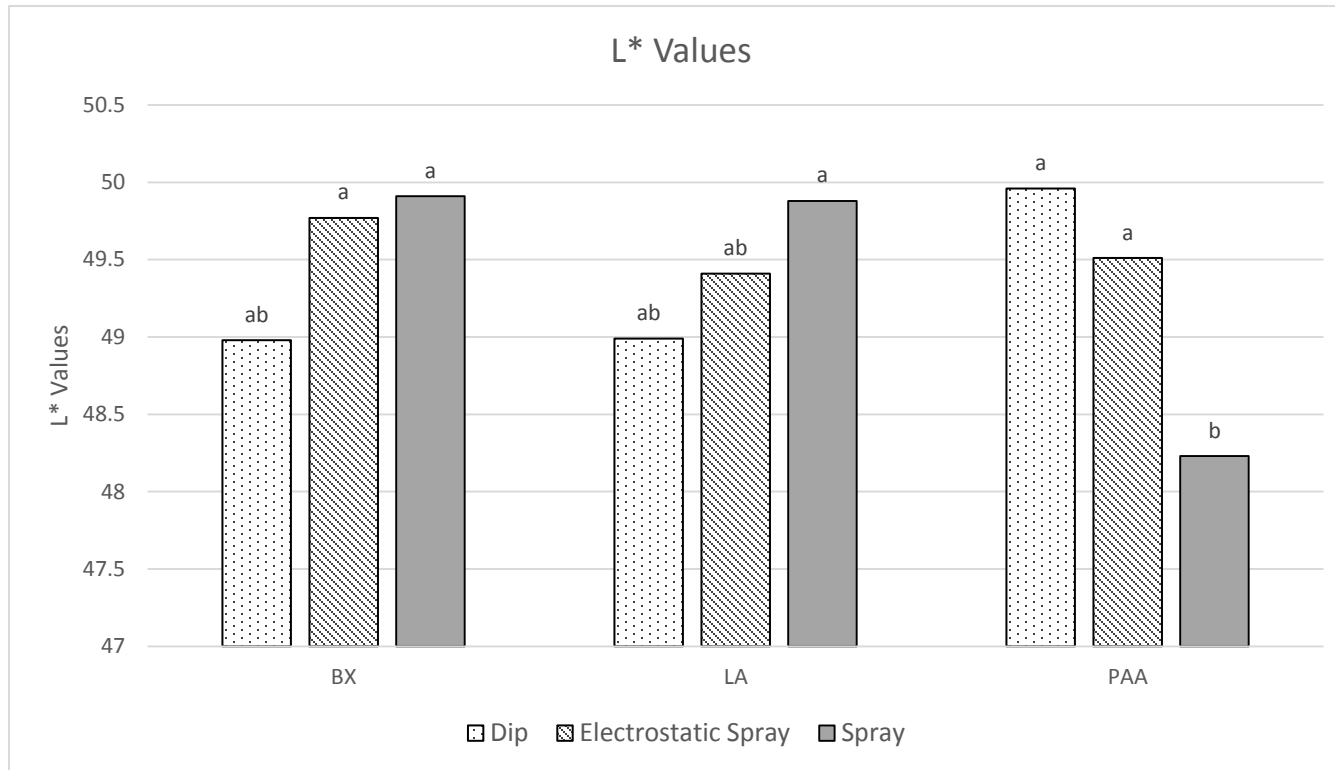
**Table 19: L\*, a\*, and b\*, delta E, discoloration, APC, pH, and TBA values of organic acid and application method treatments<sup>1</sup>.**

	L*	a*	b*	Delta E	Discoloration	APC	pH	TBA
BX Dip	48.98 <sup>ab</sup>	17.22	10.89 <sup>a</sup>	10.37	23.78	2.99 <sup>ab</sup>	5.83 <sup>a</sup>	2.09
BX Electrostatic	49.77 <sup>a</sup>	16.69	10.74 <sup>ab</sup>	10.59	25.36	2.77 <sup>b</sup>	5.82 <sup>ab</sup>	2.19
BX Spray	49.91 <sup>a</sup>	16.93	10.87 <sup>a</sup>	10.97	24.18	2.72 <sup>b</sup>	5.80 <sup>ab</sup>	2.09
LA Dip	48.99 <sup>ab</sup>	17.14	10.74 <sup>ab</sup>	10.86	25.80	2.81 <sup>b</sup>	5.69 <sup>b</sup>	3.10
LA Electrostatic	49.41 <sup>ab</sup>	16.15	10.41 <sup>b</sup>	10.90	27.46	3.51 <sup>a</sup>	5.80 <sup>ab</sup>	2.21
LA Spray	49.88 <sup>a</sup>	16.93	10.86 <sup>ab</sup>	10.07	26.69	2.97 <sup>ab</sup>	5.87 <sup>a</sup>	2.22
PAA Dip	49.96 <sup>a</sup>	16.93	10.61 <sup>ab</sup>	9.81	22.68	2.54 <sup>b</sup>	5.85 <sup>a</sup>	1.92
PAA Electrostatic	49.51 <sup>a</sup>	16.89	10.82 <sup>ab</sup>	10.29	26.52	2.95 <sup>ab</sup>	5.84 <sup>a</sup>	1.95
PAA Spray	48.23 <sup>b</sup>	16.09	10.48 <sup>ab</sup>	11.28	32.06	2.89 <sup>b</sup>	5.87 <sup>a</sup>	1.93
SEM	0.4032	0.4142	0.1461	0.4895	3.66	0.1880	0.0406	0.3233
<i>P</i> value	< 0.0001	0.1173	0.0050	0.0163	0.3954	0.0052	0.0100	0.1283

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>ab</sup> Treatments with different superscripts within a column indicate significant differences ( $P < 0.05$ ).

**Figure 1: Organic acid and application method treatment<sup>1</sup> differences for L\* values without the untreated control**

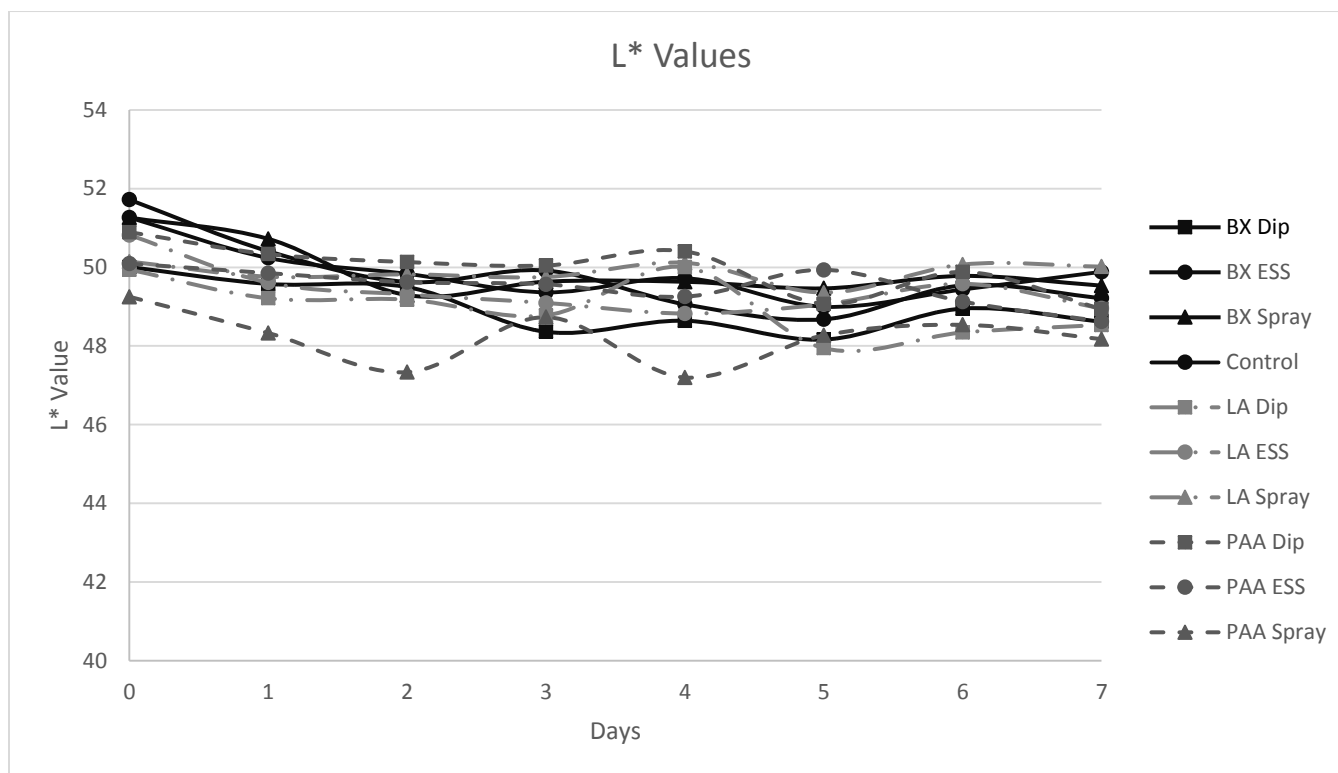


Acid\*app interaction ( $P < 0.001$ , SEM = 0.4032)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>ab</sup> Treatments with different superscripts indicate significant differences.

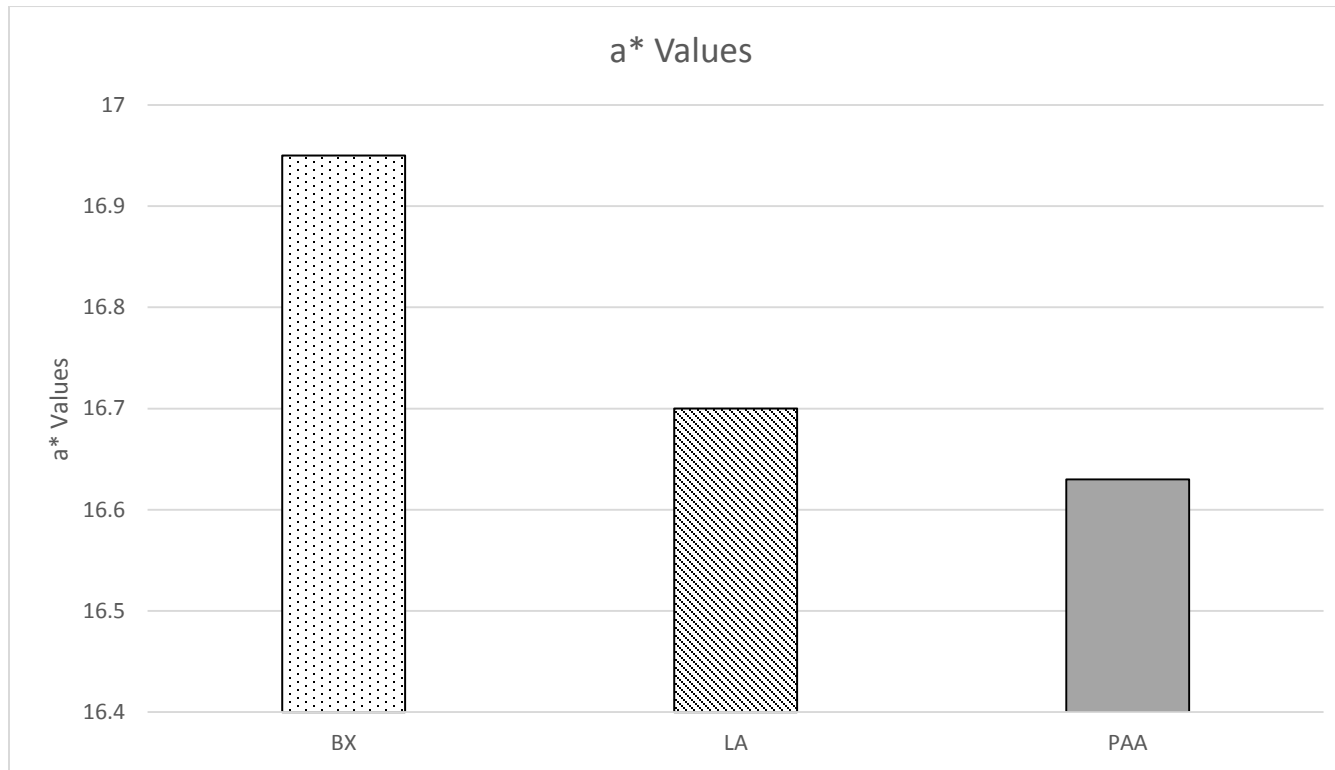
**Figure 2: Day effect for L\* values with organic acid and application method treatments<sup>1</sup> and the untreated control**



Day Effect ( $P = 0.0013$ ,  $SEM = 0.3603$ )

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; ESS = Electrostatic spray, 10 sec/side

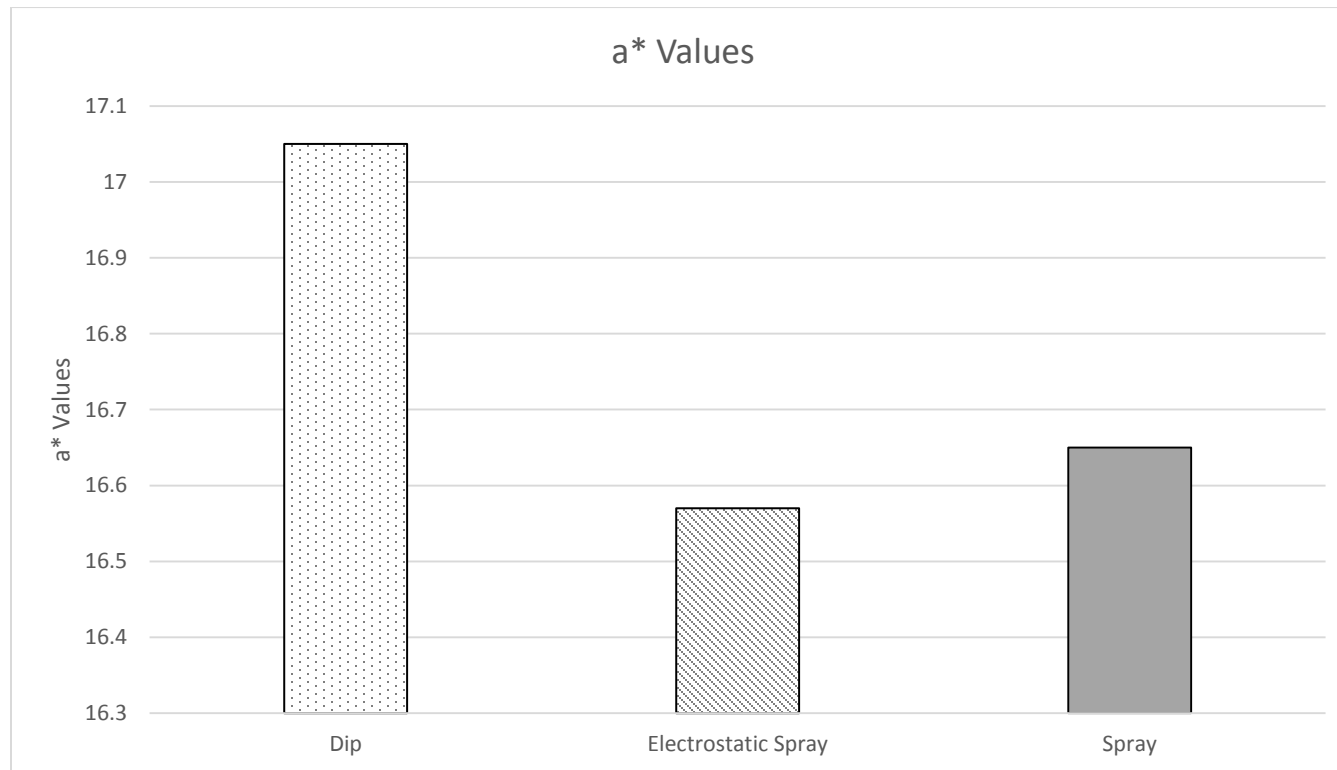
**Figure 3: Organic acid<sup>1</sup> effects for a\* values without the untreated control**



( $P = 0.3884$ , SEM = 0.2391)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid

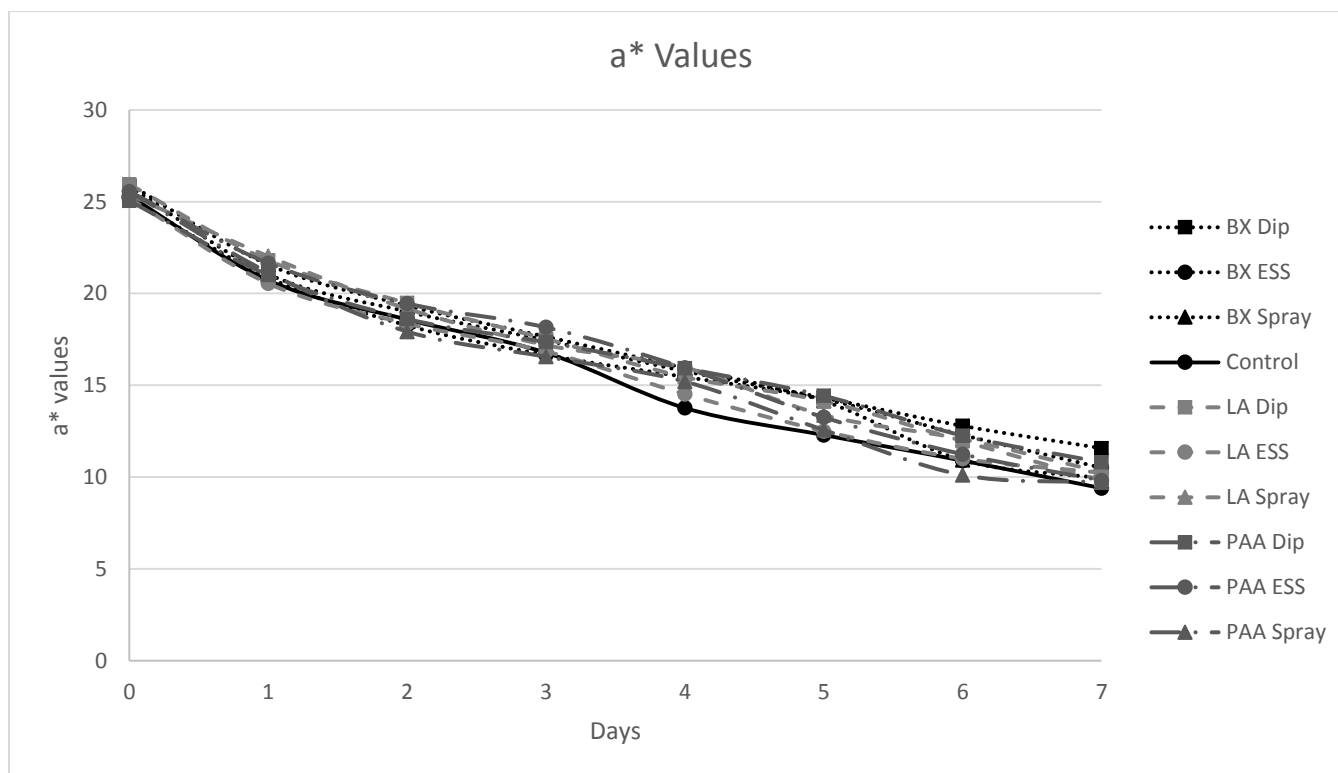
**Figure 4: Application method<sup>1</sup> effects for a\* values without the untreated control**



( $P = 0.0972$ , SEM = 0.1691)

<sup>1</sup> Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

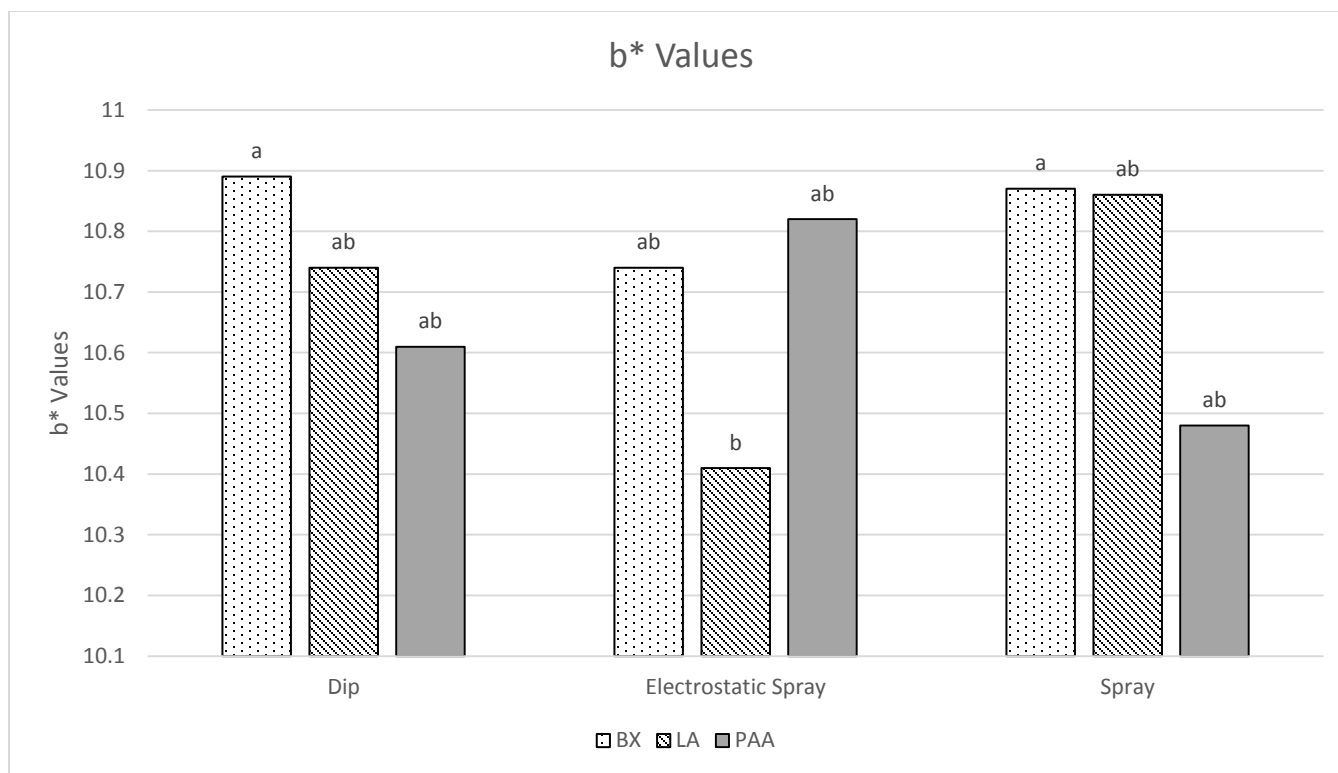
**Figure 5: Day effect for a\* values with organic acid and application method treatments<sup>1</sup> and the untreated control**



Day Effect ( $P < 0.0001$ , SEM = 0.3662)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; ESS= Electrostatic spray, 10 sec/side

**Figure 6: Organic acid and application method treatment<sup>1</sup> differences for b\* values without the untreated control**



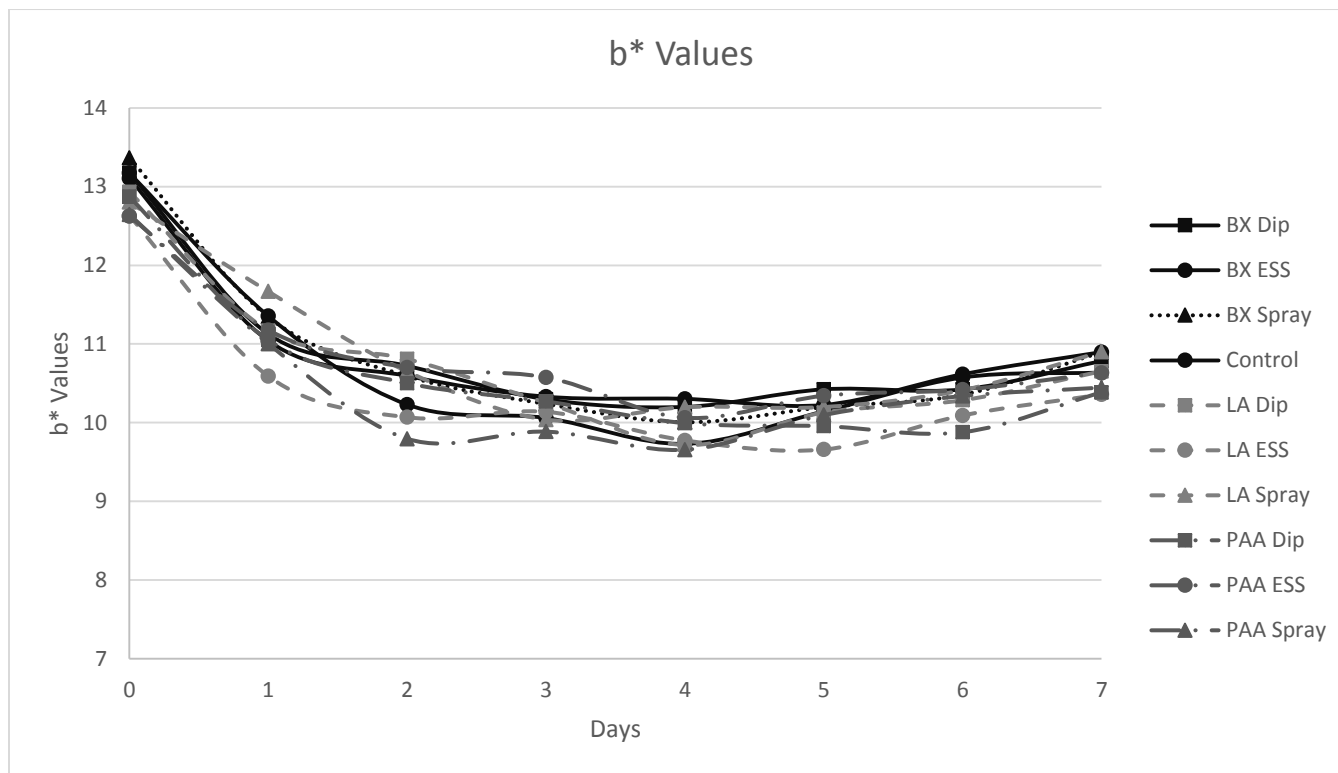
Acid\*app interaction ( $P = 0.0050$ , SEM = 0.1461)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>ab</sup> Treatments with different superscripts indicate significant differences.



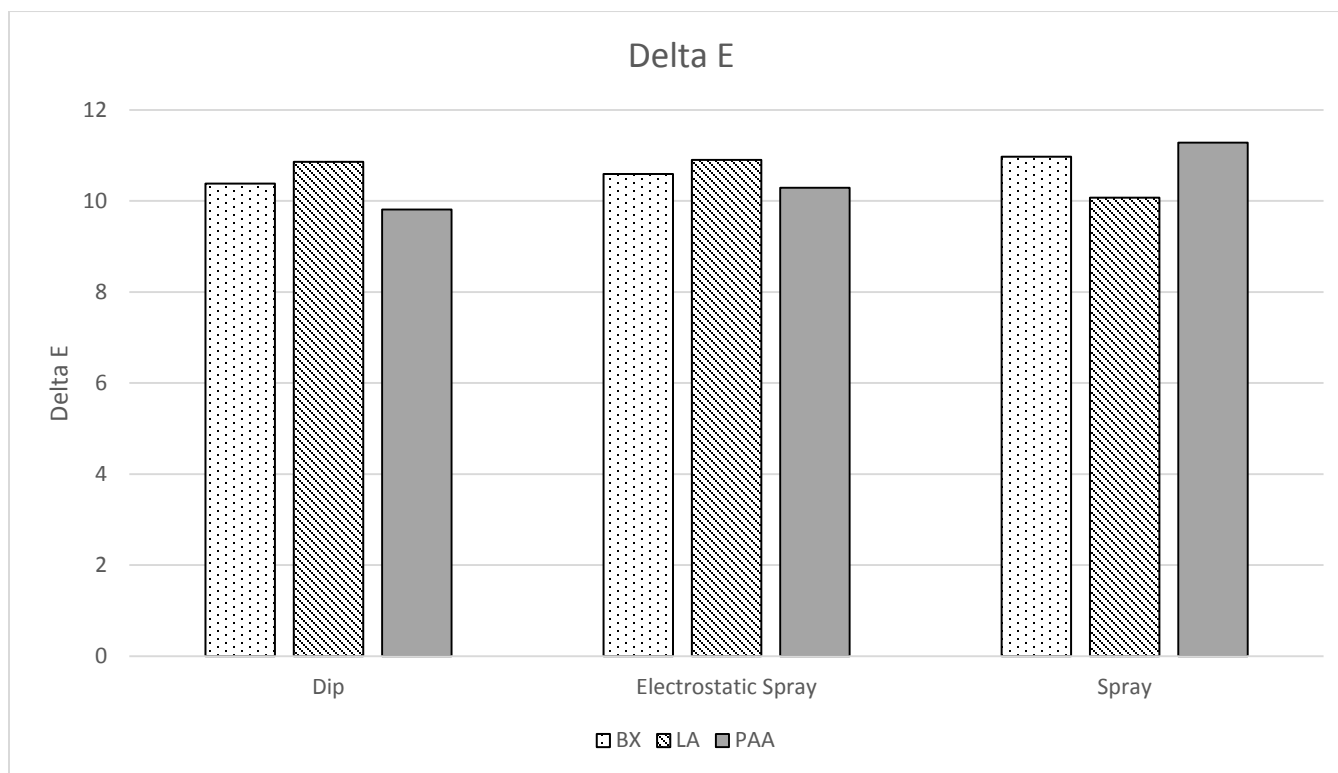
**Figure 7: Day effect for b\* values with organic acid and application method treatments<sup>1</sup> and the untreated control**



Day Effect ( $P < 0.0001$ , SEM = 0.1275)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; ESS= Electrostatic spray, 10 sec/side

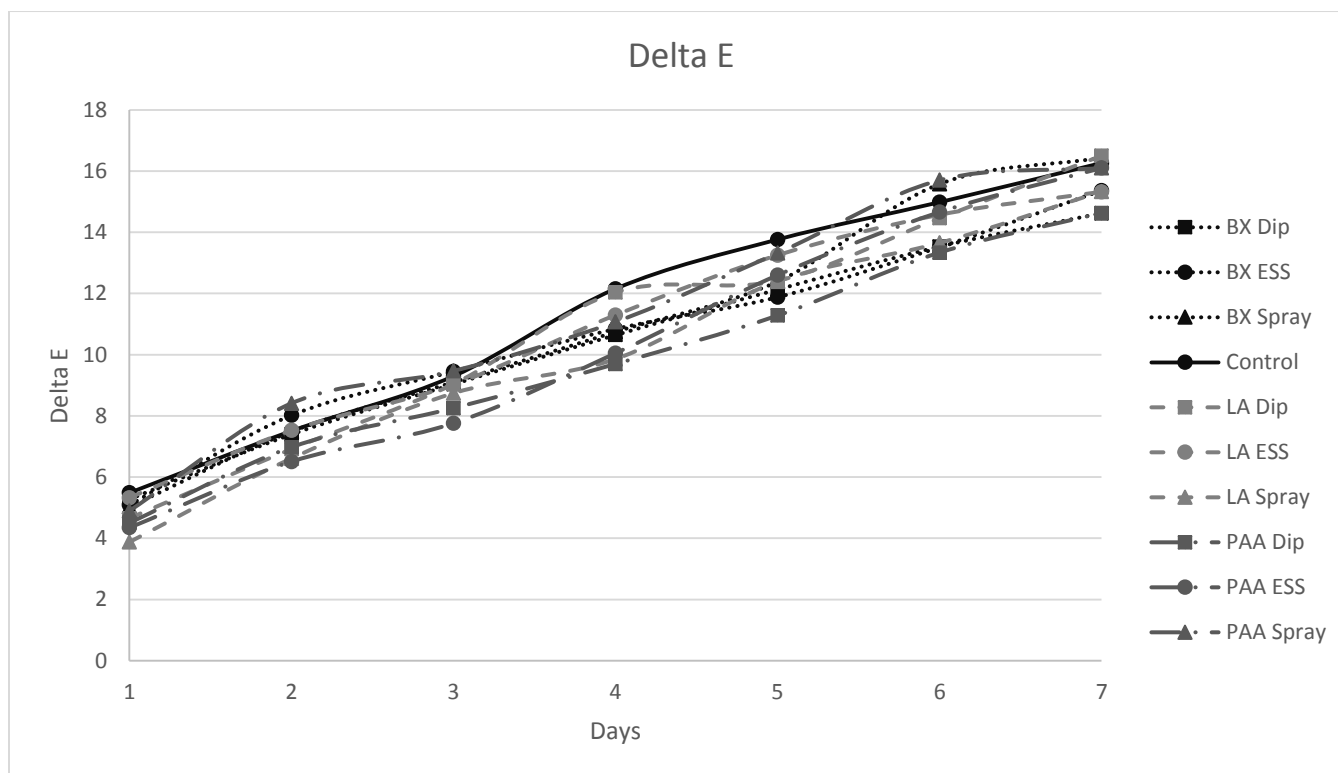
**Figure 8: Organic acid and application method treatment<sup>1</sup> differences for delta E values without the untreated control**



Acid\*app interaction ( $P = 0.0163$ ,  $SEM = 0.4895$ ); With Tukey's adjustment no means separation were found

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

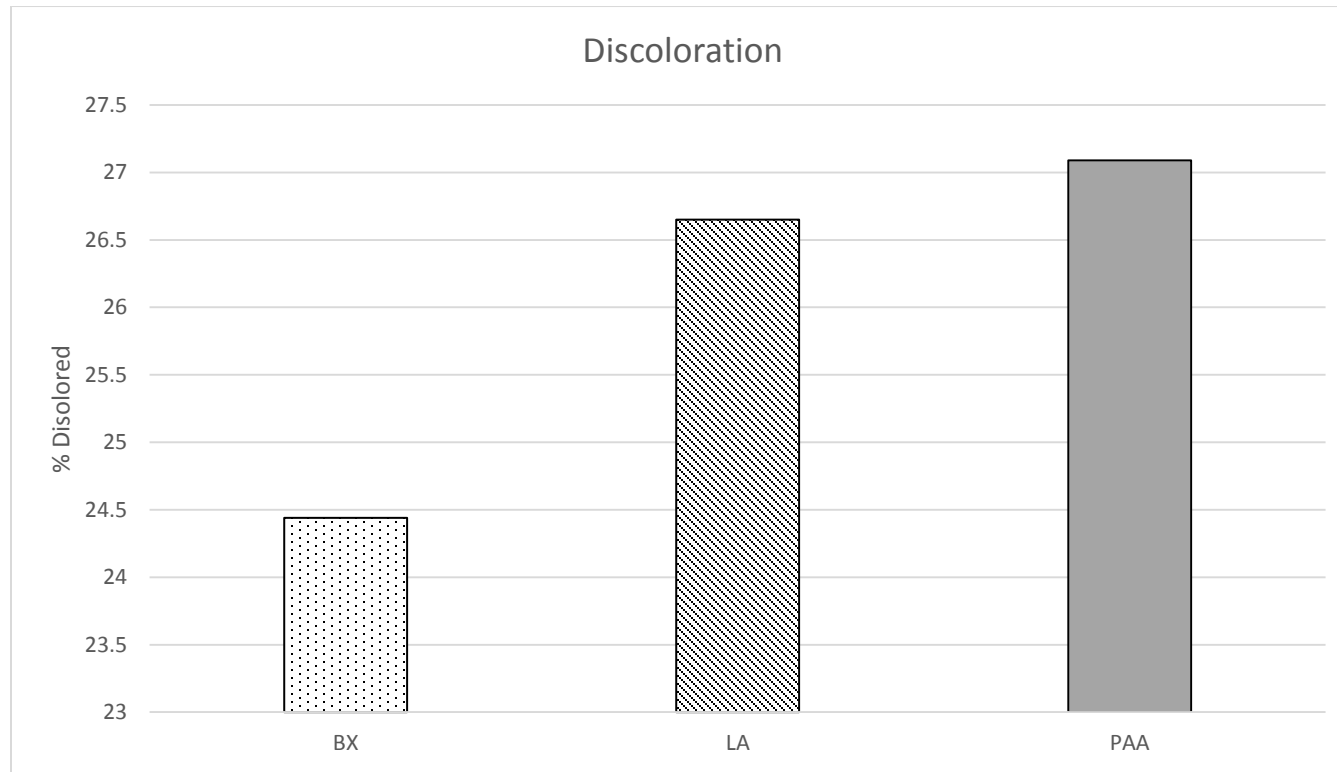
**Figure 9: Day effect for delta E values with organic acid and application method treatments<sup>1</sup> and the untreated control**



Day Effect ( $P < 0.0001$ , SEM = 0.2868)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; ESS= Electrostatic spray, 10 sec/side

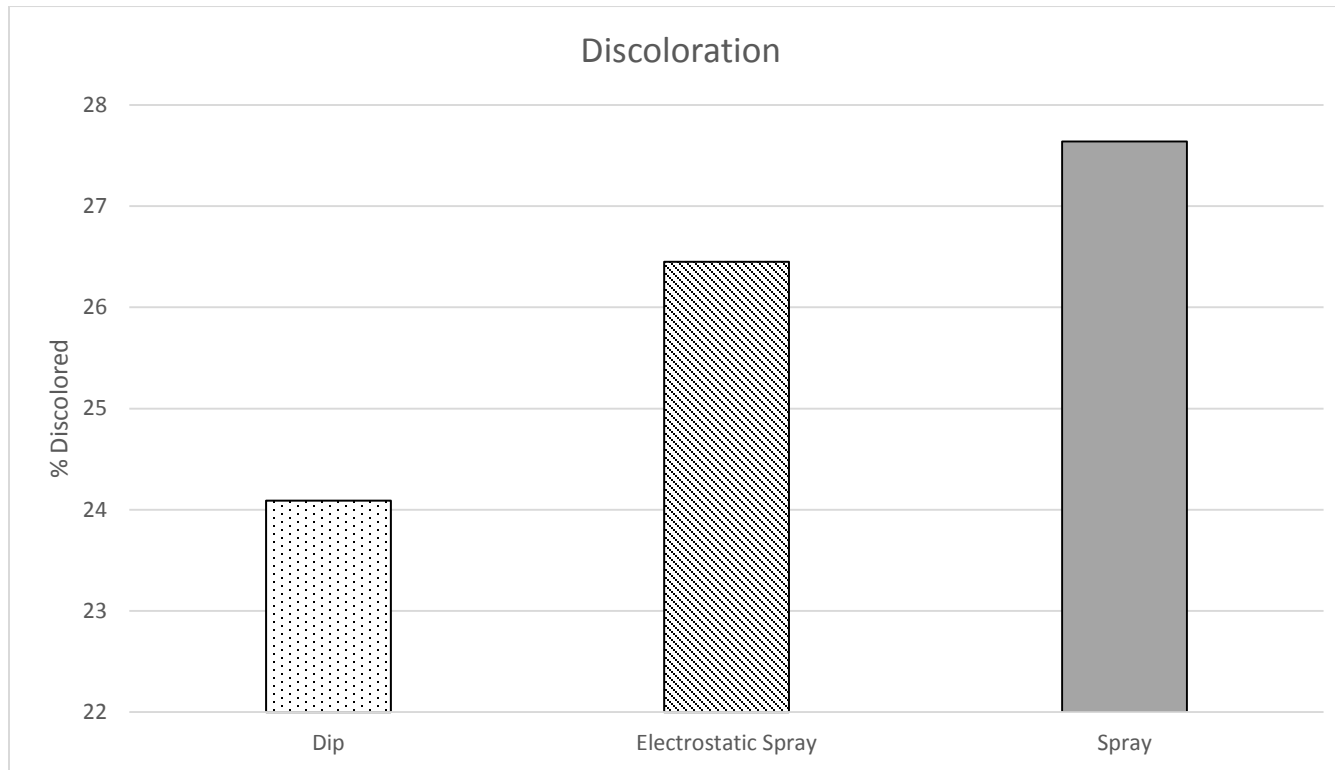
**Figure 10: Organic acid<sup>1</sup> effects for percent discoloration without the untreated control**



( $P = 0.4075$ , SEM = 2.1165)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid

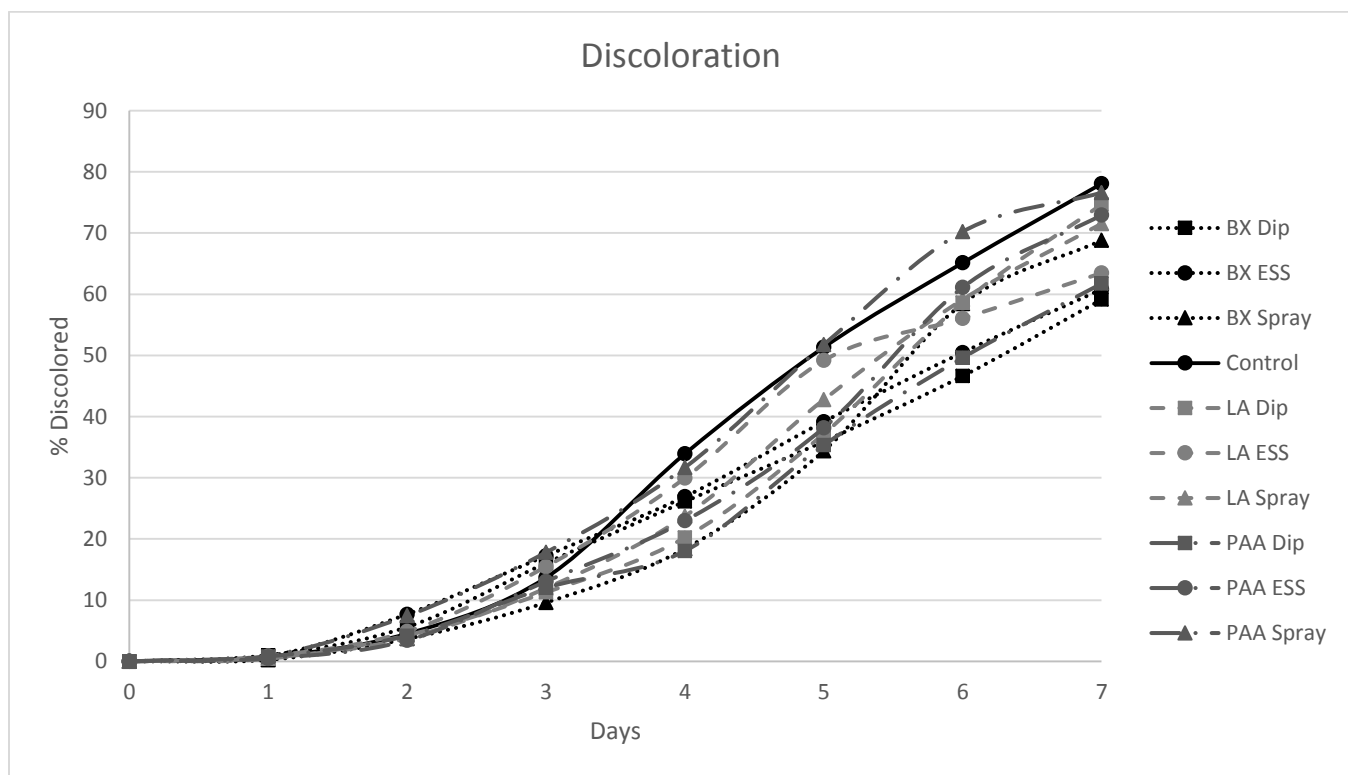
**Figure 11: Application method<sup>1</sup> effects for percent discoloration without the untreated control**



( $P = 0.2328$ , SEM = 2.1162)

<sup>1</sup> Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

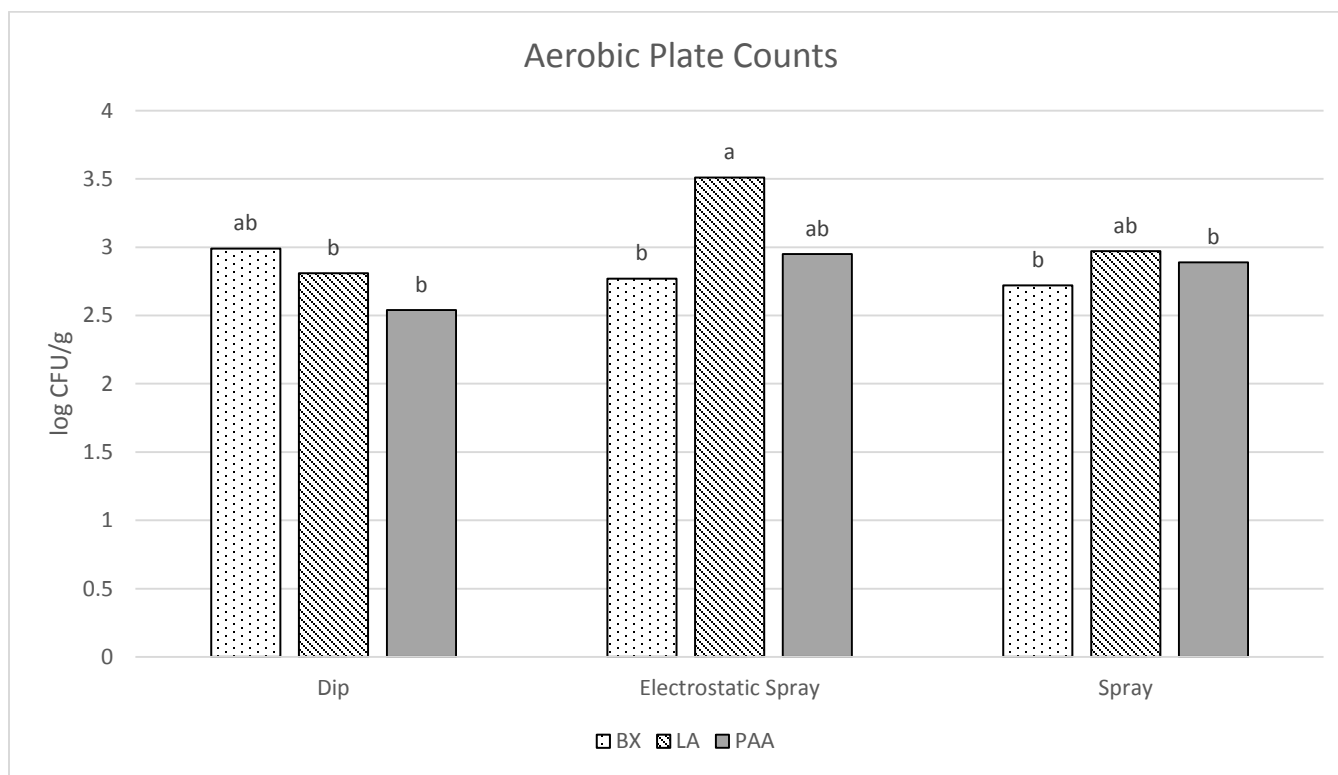
**Figure 12: Day effect for percent discoloration with organic acid and application method treatments<sup>1</sup> and the untreated control**



Day Effect ( $P < 0.0001$ , SEM = 2.28)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; ESS= Electrostatic spray, 10 sec/side

**Figure 13: Organic acid and application method treatment<sup>1</sup> differences for aerobic plate counts (log CFU/g) without the untreated control**

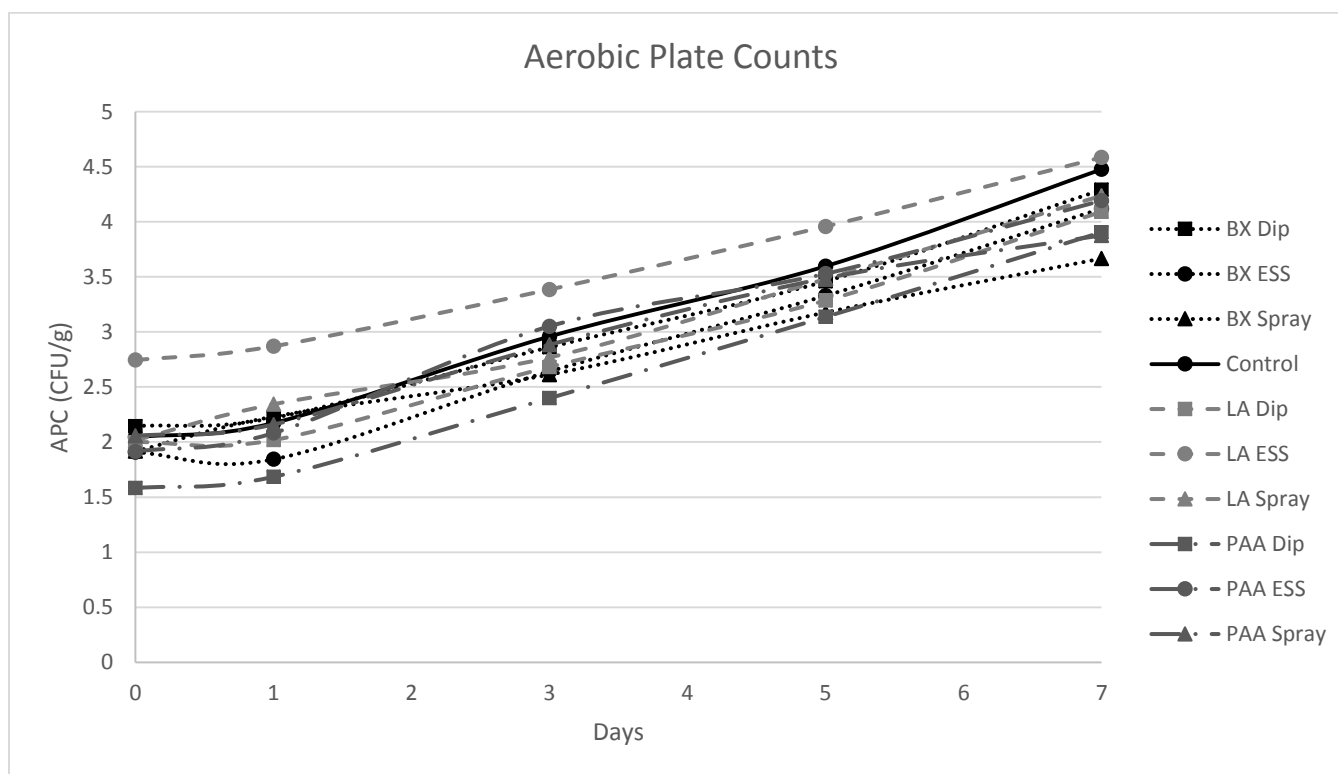


Acid\*app interaction ( $P = 0.0052$ , SEM = 0.1880)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>ab</sup> Treatments with different superscripts indicate significant differences.

**Figure 14: Day effect for aerobic plate counts (log CFU/g) with organic acid and application method treatments<sup>1</sup> and the untreated control**

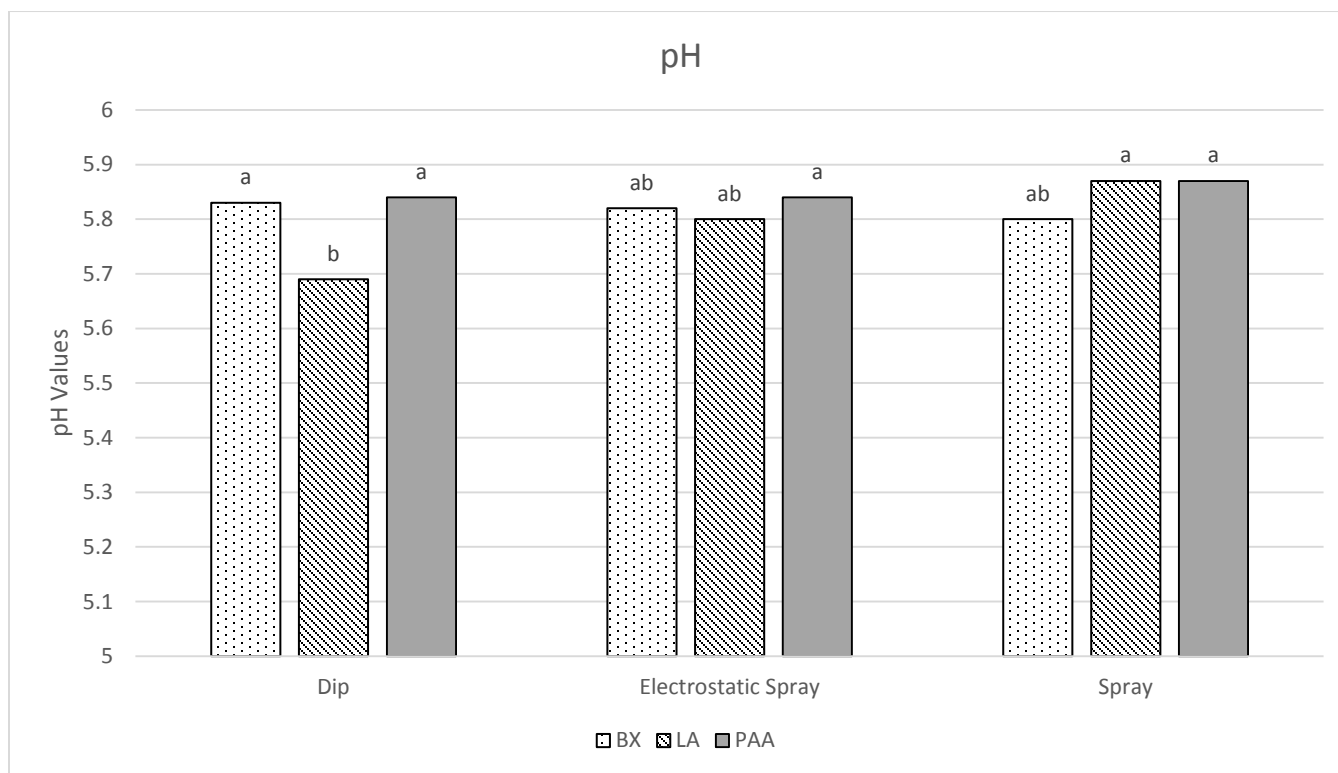


Day Effect ( $P < 0.0001$ , SEM = 0.1366)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; ESS= Electrostatic spray, 10 sec/side



**Figure 15: Organic acid and application method treatment<sup>1</sup> differences for pH values without the untreated control**

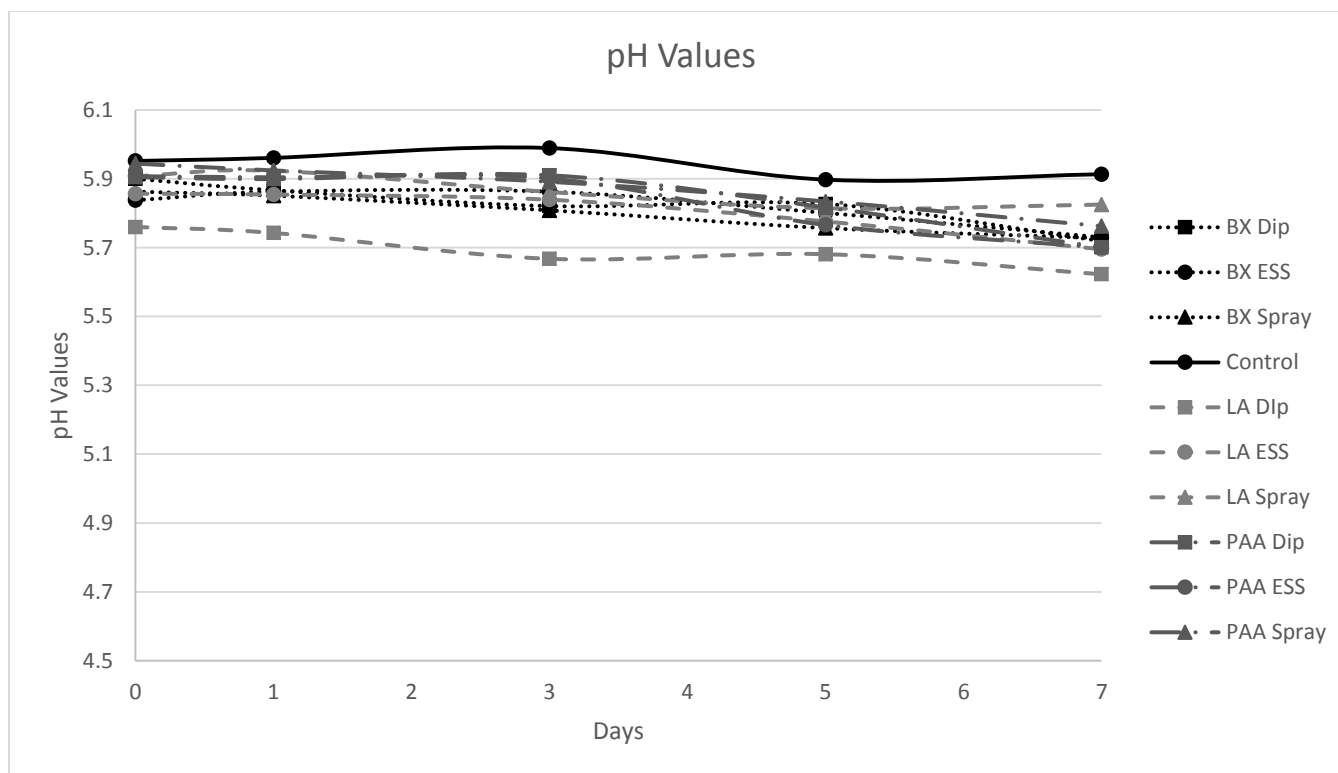


Acid\*app interaction ( $P = 0.0100$ , SEM = 0.0406)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

<sup>ab</sup> Treatments with different superscripts indicate significant differences.

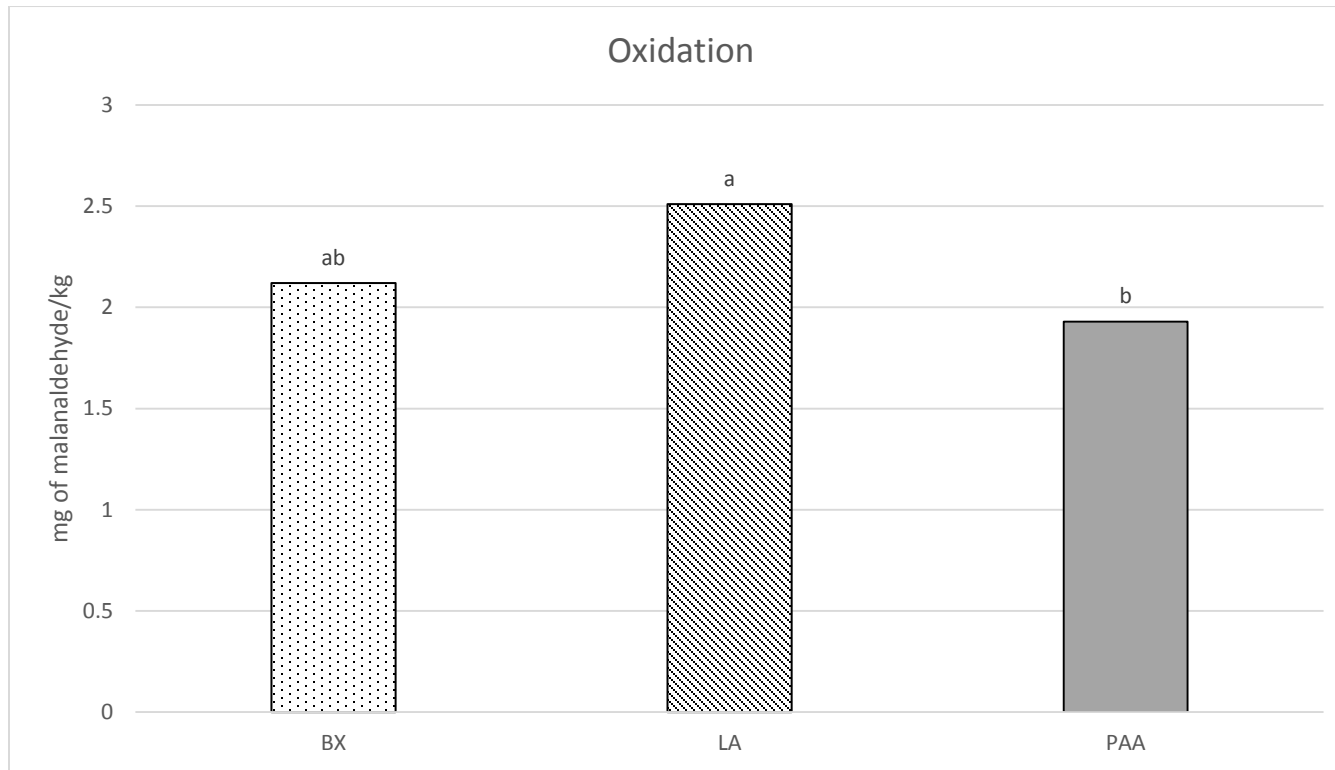
**Figure 16: Day effect for pH values with organic acid and application method treatments<sup>1</sup> and the untreated control**



Day Effect ( $P < 0.0001$ , SEM = 0.0301)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; ESS= Electrostatic spray, 10 sec/side

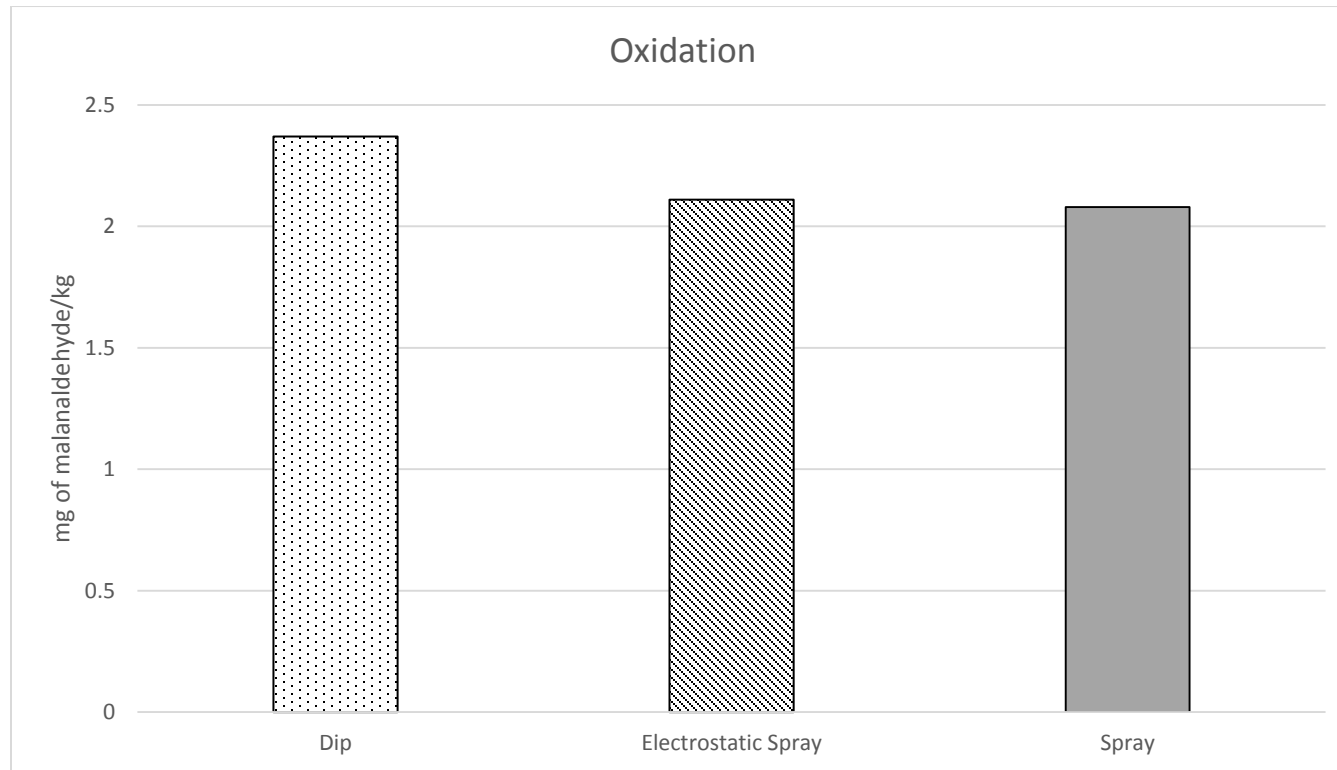
**Figure 17: Organic acid<sup>1</sup> effects for oxidation values (mg of malanaldehyde/kg) without the untreated control**



( $P = 0.0079$ , SEM = 0.1873)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid

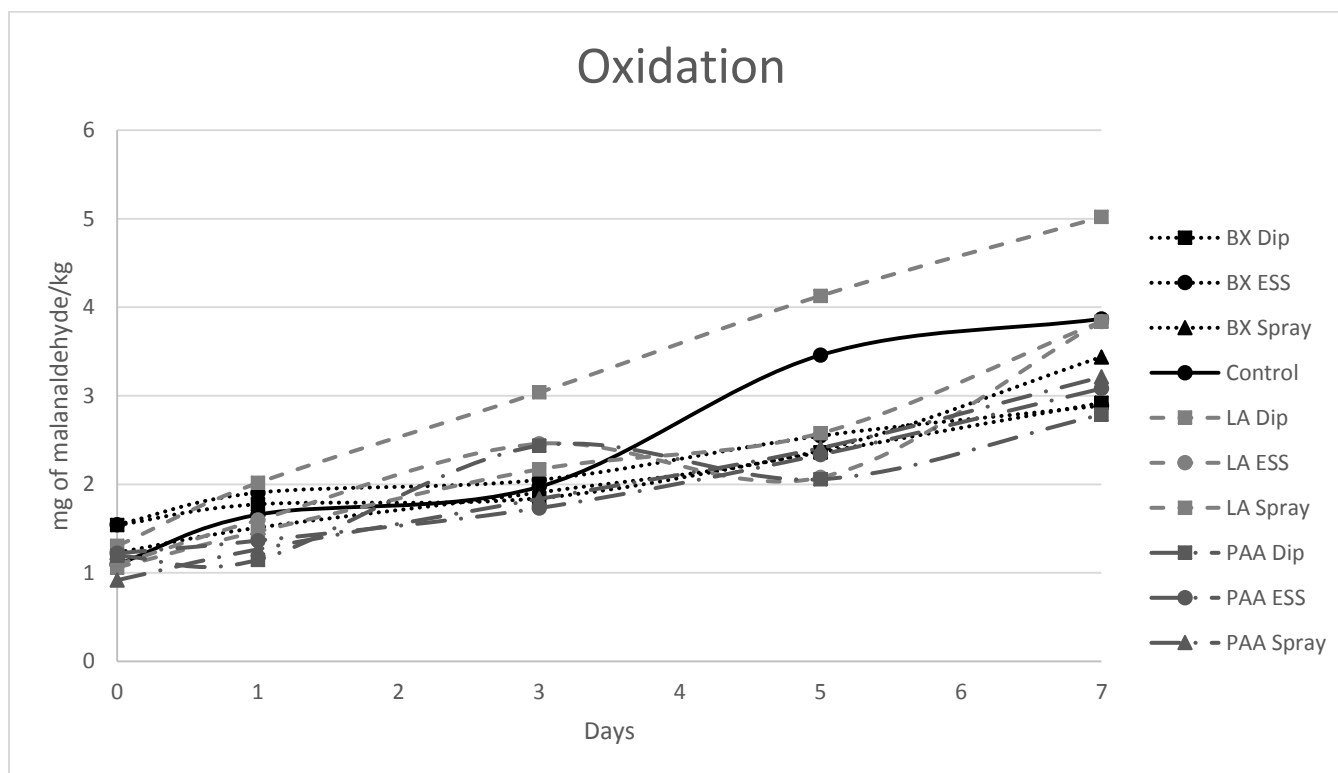
**Figure 18: Application method<sup>1</sup> effects for oxidation values (mg of malanaldehyde/kg) without the untreated control**



( $P = 0.2443$ , SEM = 0.1873)

<sup>1</sup> Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; Electrostatic spray = 10 sec/side

**Figure 19: Day effect for oxidation values (mg of malanaldehyde/kg) with organic acid and application method treatments<sup>1</sup> and the untreated control**



Day Effect ( $P < 0.0001$ , SEM = 0.2288)

<sup>1</sup> BX = 2.5% Beefxide™; LA = 4.5% lactic acid; PAA = 380 ppm peroxyacetic acid; Dip = 15 sec; Spray = 11 sec/side, 137,895 Pa; ESS= Electrostatic spray, 10 sec/side