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Reduction of *Escherichia Coli* Surrogates for Shiga Toxin-Producing *Escherichia Coli* on Beef cuts by dipping in Lactic or Peroxyacetic acid for Short or Extended Times and the effect on Ground Beef Quality

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**REDUCTION OF *ESCHERICHIA COLI* SURROGATES FOR SHIGA TOXIN-
PRODUCING *ESCHERICHIA COLI* ON BEEF CUTS BY DIPPING IN LACTIC
OR PEROXYACETIC ACID FOR SHORT OR EXTENDED TIMES AND THE
EFFECT ON GROUND BEEF QUALITY**

By

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REDUCTION OF *ESCHERICHIA COLI* SURROGATES FOR SHIGA TOXIN-
PRODUCING *ESCHERICHIA COLI* ON BEEF CUTS BY DIPPING IN LACTIC OR
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University of Nebraska, 2017

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Shiga Toxin-producing *Escherichia coli* (STEC) are adulterants in ground beef. Antimicrobial interventions reduce STEC, but effectiveness may depend on application time and the impact on ground beef quality.

Objectives were to evaluate reductions in rifampicin-resistant *E. coli* (*E. coli*^{RIF}) on beef clod surfaces, and the effect on ground beef quality. Beef clods were sectioned and dipped in 4.5% lactic acid (LA) or 380 ppm peroxyacetic acid (PA) for 15 or 180 s. *E. coli*^{RIF} were inoculated ($\sim 5.3 \log \text{CFU/cm}^2$) onto fat or lean tissue surfaces. Non-inoculated treatments were ground into $\sim 454 \text{ g}$ portions. Lipid oxidation, meat pH, APC, L^* a^* b^* , and percentage of discoloration were all measured during retail display.

Greater reductions of inoculated *E. coli*^{RIF} counts occurred with increased exposure time. The PA180 treatment had greater ($P = 0.004$) reductions than the control. When differing beef surfaces were treated, LA180 had lower *E. coli*^{RIF} counts for APC (P

< 0.0001) and *E. coli* petrifilms ($P < 0.0001$) than control treatments. Fat surfaces had greater ($P = 0.024$) reductions than lean on *E. coli* petrifilms.

Ground beef from LA treatments were not different ($P > 0.05$) for APC within day of display. Treatment lipid oxidation was different on days 3, 5, and 7 ($P < 0.0001$, $P = 0.0001$, $P = 0.009$, respectively). Ground beef pH was different on days 0, 1, and 3 ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$, respectively). Visual percent discoloration was different on days 1, 2, and 3 ($P = 0.017$, $P = 0.013$, $P = 0.042$, respectively). The LA180 treatment increased lipid oxidation, decreased meat pH, and increased discoloration.

Dipping lean trim in organic acids for extended periods increases reduction of *E. coli*. However, excessive time reduced ground beef quality. Processors should consider quality impacts of organic acid antimicrobial interventions.

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INTRODUCTION

Escherichia coli (*E. coli*) contains a classification of bacteria known as Shiga Toxin-producing *E. coli* (STEC). This subgroup is comprised of two major types, *E. coli* O157:H7 and non-O157 STEC. Both major types are responsible for multiple food outbreaks and deaths every year (CDC, 2017). In 2015, there were 463 culture confirmed cases per 100,000 population leading to an incidence of 0.95, with 180 hospitalizations and 3 deaths for *E. coli* O157:H7. Likewise, there were 796 cases per 100,000 population with an incidence of 1.64 for non-O157 STEC. This lead to 126 hospitalizations and 1 death reported in 2015 (Huang et al., 2016).

Cattle are a natural reservoir for STEC, and beef can be a source of STEC that causes foodborne illness. While non-intact steaks have been implicated in some outbreaks, the major source of transmission is ground beef. This can occur through mixing of trim lots during grinding, where one infected carcass can contaminate large quantities of ground beef. The contaminated product can then be eaten unknowingly by consumers. STEC illnesses have also been attributed to other food commodities, such as leafy green vegetables, tomatoes, and other vegetable crops. The USDA estimates that ground beef represents 30 to 35% of STEC illnesses.

While *E. coli* O157:H7 can cause gastrointestinal illness such as bloody diarrhea, vomiting, and severe cramps, it can also cause Hemolytic Uremic Syndrome (HUS) in children, leading to acute renal failure and death. Hemolytic Uremic Syndrome affects 5-10% of people infected with *E. coli* O157:H7 (CDC, 2017). Therefore, it is important to

put safeguards in place to reduce the risk of foodborne illnesses, hospitalizations, and deaths.

The animal industry has already developed several antimicrobial interventions to reduce the risk of contracting STEC. Pre-harvest antimicrobial interventions are one way to reduce this risk, through the use of an *E. coli* O157:H7 secreted protein vaccine for cattle, or by using probiotic bacteria to inhibit *E. coli* O157:H7 through secreted metabolites. Both of these methods act to reduce the carriage and load of *E. coli* O157:H7 in the live bovine animal (Potter et al., 2004; Zhao et al., 1997). Once at slaughter, more antimicrobial interventions are utilized, including decontamination of contaminated areas after hide removal through steam vacuuming. Pre-evisceration antimicrobial interventions include carcass water washing and rinsing with organic acid solutions. These methods can then be followed by thermal pasteurizing and visual inspection of carcasses after splitting. Post-evisceration antimicrobial interventions include a final hot water carcass wash and application of an organic acid solution (Bacon et al., 2000). Carcasses are then fabricated, which produces beef trim available to grind for ground beef. This offers another point for an antimicrobial intervention application. Organic acids, such as acetic or lactic acid, can be used to reduce pathogens through spray or wash applications, as well as acidified sodium chlorite (Harris et al., 2006). Additionally, oxidizing agents such as peroxyacetic acid, can also act as antimicrobial interventions. These different organic acids can be used in various applications, including electrostatic sprays and dips (Pohlman et al., 2014).

While a variety of antimicrobial intervention applications exist, small business meat processors are limited in financial resources to invest in major facility renovations

required by some antimicrobial interventions strategies. Thus, applicable antimicrobial interventions must take into account the resources needed to produce safe, high quality products. To this end, research is needed to determine appropriate parameters for antimicrobial organic acid interventions on beef trim, specifically time spent in the solution. While concentrations of antimicrobial interventions have been studied, the time the beef trim spent in an organic acid based antimicrobial application has had little research conducted. Additionally, how time spent in solution has an effect on ground beef quality over a period of retail display has not been examined.

Therefore, the objective of the first study was to determine the effect of dipping cut pieces of beef shoulder clods for short or extended times in organic acids on the reduction of *E. coli* surrogates. The difference in meat surface type on reduction of *E. coli* surrogates was also evaluated for one organic acid antimicrobial in the second study. The objective of the third study was to evaluate the quality attributes of ground beef after pieces of beef shoulder clod were dipped in organic acids for short or extended times.

REVIEW OF LITERATURE

Escherichia coli

Escherichia coli (*E. coli*) is a type of bacteria commonly found in the digestive tracts of both humans and other warm-blooded animals. These bacteria survive mainly in the intestines and are usually harmless. However, some serotypes cause disease and illness to the population. These are known as the diarrheagenic *E. coli*, and can be separated into distinct groups based on certain characteristics such as virulence properties and symptoms (Montville et al., 2012). Additionally, each *E. coli* strain is designated with an O:H serotype based on major surface antigens. The “O” designates the somatic antigen, while “H” indicates the flagellar antigen. These antigens allow researchers to separate each strain into a distinct category. Categories include: enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffusely adhering *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), and enterohemorrhagic *E. coli* (EHEC). These EHEC serotypes that produce Shiga toxin (Stx) are known as Shiga toxin-producing *E. coli* (STEC). Within STEC, one serogroup is separated from the rest and known as O157. This serogroup contains the major serotype *E. coli* O157:H7. The other serogroups are classified together as non-O157 STEC (Montville et al., 2012). The U.S. Department of Agriculture Food Safety and Inspection Service (USDA FSIS, 2012) has identified six serogroups within the non-O157 STEC that cause about 70% of non-O157 STEC foodborne illnesses as O26, O45, O103, O111, O121, and O145.

History

The EHEC category remains the most prevalent pathogenic *E. coli* to the meat industry today. While considered rare when first discovered in 1982, today the principal EHEC found in the United States is *E. coli* O157:H7 (Montville et al., 2012). In 1982, this serotype was isolated from a single fast food chain of restaurants in two different states involving an undercooked hamburger. Prior to 1982, this serotype had only been detected from a California woman who had bloody diarrhea and severe abdominal cramps in 1975 (Riley et al., 1983). Little was known about the reservoir for this organism until 1986, when *E. coli* O157:H7 was isolated from fecal samples of dairy heifers in two different herds (Martin et al., 1986). In 1992, beef cattle carcasses were sampled and found positive for *E. coli* O157:H7 isolates, further confirming that bovines acted as a natural reservoir for this pathogen. Contamination through slaughter and processing of cattle had become a possible vector for transmission of this bacterium to humans (Chapman et al., 1993).

In 1993, a large multistate outbreak spanning Washington, Idaho, Nevada, and California occurred from consumption of undercooked hamburgers at a fast food restaurant chain. The fast food restaurant chain's hamburger patties were undercooked at less than 60°C, a temperature that was significantly lower than the required 68.3°C for instantaneous bacterial cell death. The insufficient cooking temperature led to a large number of hospitalizations (178) as well as development of Hemolytic Uremic Syndrome (HUS) by 56 people and the death of 4 children (Montville et al., 2012). Because of this outbreak, in 1994, the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) declared *E. coli* O157:H7 an adulterant.

In 1997, USDA FSIS announced a recall of frozen ground beef patties due to contamination of *E. coli* O157:H7. The initial 20,000-pound recall eventually reached 25 million pounds in 1999, prompting a shut down of the Columbus, Nebraska Hudson Foods facility in what would be one of the largest recalls recorded until 2008 (Brougher and Greene, 2011). The development of large recalls and outbreaks lead USDA FSIS and the meat industry to conduct an overhaul of the food safety system and a Federal Register final rule notice was published in 1996. The notice required all USDA inspected meat and poultry facilities to implement a program known as the Hazard Analysis Critical Control Points (HACCP) beginning in 1998 for large processors with 500 or more employees. The HACCP system is a science-based, preventative approach designed to reduce food safety hazards and is now required in all federally inspected meat and poultry facilities. A HACCP plan or system is used in conjunction with microbial testing to ensure plants are meeting food safety performance standards (USDA FSIS, 1996). Despite the preventative controls in place, in July 2002, 18 million pounds of beef trim and ground beef were recalled by a Colorado company after being linked to *E. coli* O157:H7 and causing 43 illnesses. Also in the fall of 2002, 2.8 million pounds of ground beef were recalled by a Wisconsin company after testing positive for *E. coli* O157:H7 (USDA FSIS, 2013).

Through 2003-2012, *E. coli* O157:H7 was responsible for 390 outbreaks with 33 deaths and 1,272 hospitalizations (Heiman et al., 2015). In 2014, the National Enteric Disease Surveillance: Shiga toxin-producing *Escherichia coli* (STEC) Annual Report generated by the CDC reported 4,437 cases of STEC infections that were culture-confirmed. This is 3% fewer than previously reported in 2013 (CDC, 2014) and an

overall reduction of 50% since 1996 (Healthy People, 2014). In 2011, USDA FSIS determined six other serogroups known as O26, O45, O103, O111, O121, and O145 to be adulterants as well. Collectively, this group is known as non-O157 STEC (USDA FSIS, 2012). Outbreaks and recalls show the need to continue control of *E. coli* O157:H7 and non-O157 *E. coli* strains.

Characteristics of E. coli

Growth/ Lethality Conditions

E. coli are gram negative rods included in the family Enterobacteriaceae. These bacteria are facultative aerobes that are oxidase-negative and catalase-positive (ICMSF, 1996). *E. coli* O157:H7 also tests negative for β -glucuronidase and sorbitol fermentation reactions, which is unlike other *E. coli* strains (Ratnam et al., 1988). The optimum growth range for *E. coli* is 35-40°C, but some strains can grow as low as 7°C while others can grow in temperatures as high as 46°C. While the optimum growth temperature for *E. coli* O157:H7 is 37°C, growth can occur in a slightly larger range than other *E. coli* between 8°C and 45°C (ICMSF, 1996). Non-O157 STEC grow in ranges from 6°C to above 47°C (Lin, 2014). In refrigerated temperatures, pathogenic *E. coli* can survive for over five weeks of storage with only a 0.5 to 1.5-log reduction. *E. coli* O157:H7 can survive for over nine months in ground beef held at -20°C. However, when non-pathogenic *E. coli* are held at -25.5°C for 38 weeks, a 10-fold reduction can occur (ICMSF, 1996).

Water activity can range between 0.95-0.995 for growth of *E. coli*, while the optimum growth is 0.995. Minimum pH for growth is 4.4. However, optimum growth uses a pH between 6-7 with a maximum of 9.0 (ICMSF, 1996). *E. coli* O157:H7 can

actually grow in a pH of 4.0 by depending on three metabolic systems to increase its acidic tolerance. These three metabolic systems include an acid-induced oxidative system, an acid-induced arginine-dependent system, and a glutamate-dependent system (Montville et al., 2012). To inactivate *E. coli* O157:H7, USDA Appendix A guidelines are used for cooking (USDA FSIS, 1999). These guidelines are based on inactivation of *Salmonella*, as it was shown by Doyle and Schoeni (1984) that more time is needed to inactivate *Salmonella* than *E. coli* O157:H7 based on *D* values. At 64.3°C, the *D* value for *E. coli* O157:H7 is 9.6 seconds (Doyle and Schoeni, 1984). When internal temperature of a product reaches 70°C (158°F), the inactivation time is considered instant and lethality achieved (USDA FSIS, 1999).

Virulence Factors

Both *E. coli* O157:H7 and non-O157 STEC use different virulence factors as a primary way of causing disease. One virulence factor found in both types is intimin, an adhesin, which helps colonize the intestine through adhesion to the epithelial cells (Serna and Boedeker, 2008) by inserting a receptor into the cell membrane. This causes lesions to form on the epithelial cells of the small and large intestines, and may ultimately lead to diarrhea (Muniesa et al., 2006). To form these adhesion lesions, a large chromosomal locus must be present. This is known as the locus of enterocyte effacement (LEE) pathogenicity island (McDaniel et al., 1995). The LEE island contains two important genes necessary for attachment and effacing. These are the *eae* gene, which encodes intimin, and the *tir* gene, which encodes the Tir protein. The Tir protein is inserted into the host cell, where it acts as the receptor for intimin (Montville et al., 2012). However,

little is known about the actual adhesion mechanism that helps colonize the intestine (Serna and Boedeker, 2008).

Other virulence factors also play a role in pathogenicity. One of these factors includes shiga toxins (Stx) that cause injury to epithelial cells after being spread through the bloodstream. Specific Stx genes are encoded on lamboid bacteriophages that are only transcribed when the phage is in the lytic stage (Serna and Boedeker, 2008). Shiga toxins are comprised of two different types, Stx1 and Stx2, with multiple genotypes associated with each type. The outcome of an infection may be determined by which type of shiga toxin is present. *E. coli* that produce Stx2 only are more virulent than those that generate Stx1 or a combination of both Stx1 and Stx2. Each Stx contains 2 different subunits, A and B. The A subunit has both a catalytic domain and an attachment zone for the B subunit (Serna and Boedeker, 2008). It acts to inhibit protein synthesis once inside the cell (O'Brien et al., 1992). This occurs by binding to the 28S RNA portion of the 60S ribosomal subunit, which prevents t-RNA binding and inhibits protein synthesis (Johnson et al., 2006). The B subunit attaches to a receptor for the Stx toxin that is located primarily in the renal tissue of humans. This receptor is known as glycosphingolipid globotriosyl ceramide (Gb₃) (Boyd and Lingwood, 1989) and is considered the primary receptor for shiga toxin (O'Brien et al., 1992).

Food Sources

Pathogenic *E. coli* is found in a wide variety of food sources. While mainly attributed to beef, there have been recent outbreaks in several other foods. For instance, in 2017 an outbreak occurred in soy nut butter (CDC, 2017b). This was a multi state outbreak, with 32 people infected and 12 hospitalized. In 2016, the CDC informed the

public of outbreaks in flour and alfalfa sprouts (CDC, 2017b). These outbreaks included 63 and 11 infected persons, respectively. In addition, raw clover sprouts were reported as an outbreak in both 2012 and 2014. In-shell hazelnuts were implicated in an outbreak in 2011, while in 2009 prepackaged cookie dough was contaminated with *E. coli* O157:H7 and infected 72 people of whom 10 people developed HUS. Spinach, romaine lettuce, and pizza have also all been implicated in various outbreaks (CDC, 2017b). Beef was also implicated in several recalls over this time period; however, *E. coli* is now being found in multiple products across the food industry. From 1998-2015, *E. coli* was implicated in 554 outbreaks resulting in 12,250 illnesses, 2,005 hospitalizations, and 35 deaths (CDC, 2016).

E. coli O157 Human and Animal Disease

From 2003-2012, there were 390 outbreaks associated with *E. coli* O157. Within those 390 outbreaks, there were 33 deaths and 4,928 people reported illnesses. Food remained the most common route of transmission with 65% (255 outbreaks total) of the outbreaks associated with food products or ingestion of *E. coli* O157:H7. Of the 255 food related outbreaks, 78 (55%) were associated with beef. Within the 55%, ground beef was responsible for 69%, or 54 outbreaks (Heiman et al., 2015). In 2003, the annual cost of illness from *E. coli* O157 was estimated annually at \$405 million. This includes \$5 million in lost productivity and \$30 million for medical care. However, the major economic loss came from premature death, which accounted for 93%, or \$370 million, of the total estimated cost of illness (Frenzen et al., 2005).

Symptoms of illness can include severe abdominal cramps and non-bloody diarrhea for a few days, followed by bloody diarrhea. Nausea and vomiting, as well as no

or low fever are also symptoms of this infection. These broad signs of infection normally subside in less than a week with little complication. However, there are cases where HUS develops after diarrhea, particularly in children. Elderly populations are also very susceptible to this infection (Boyce et al., 1995). There is no single treatment available for *E. coli* O157:H7. Both antimicrobial and antimotility drugs are not recommended because the chance of developing HUS increases (Boyce et al., 1995). Thus, preventing the primary infection of *E. coli* O157:H7 remains the best way to prevent HUS (Chandler et al., 2002).

Non-O157 Human and Animal Disease

In 2011, the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) classified six other serogroups of *E. coli*, besides O157:H7, as adulterants in raw, non-intact beef products. These include serogroups O26, O45, O103, O111, O121, and O145 (USDA FSIS, 2012). These are typically grouped together as non-O157 Shiga toxin-producing *E. coli* (STEC). However, other serogroups have caused illnesses and the term non-O157 can also refer to these serogroups as well. Non-O157 STEC strains can also cause disease and infections through different virulence factors. A full range of symptoms can include mild diarrhea to hemorrhagic colitis and HUS. However, certain serotypes of non-O157 are more virulent than other non-O157 serotypes in regards to HUS occurrence. Indeed, just like O157:H7, those non-O157 strains containing Stx2 are more likely to result in the onset of HUS (Johnson et al., 2006).

There were over 2000 cases of non-O157 STEC reported over a 10-year period from 2000-2010. Due to improvements in detection methods and monitoring, the

incidences of non-O157 STEC have increased. However, those persons with an O157 infection were two-thirds more likely to be connected to an outbreak than those with a non-O157 infection (Gould et al., 2013). In 2014, the CDC estimated a rise of infection due to non-O157 serotypes, with approximately 0.79 cases occurring per 100,000 people (CDC, 2014). Current estimates continue to rise, as the 2015 report from FoodNet indicated an incidence rate of 1.65 (CDC, 2017a).

Goals for STEC Reduction of Infection

The United States Department of Health and Human Services has set baseline goals for 2020 concerning the reduction of *E. coli* O157:H7 transmitted through food known as Healthy People 2020 (Healthy People, 2014). Baseline levels given from 2006-2008 show an infection rate of 1.2 cases per 100,000 individuals. The 2020 Healthy People target is 0.6 cases per 100,000 in the population. As of 2015, a reduction to 0.9 cases per 100,000 people was reached (Healthy People, 2014). This is a major decrease, but appears to be holding steady as both 2014 and 2015 had similar infection rates. Another goal set by the committee of Healthy People 2020 is to decrease the number of outbreak-associated infections in beef from the baseline given in 2006-2008 from 200 cases to 180 cases, which is a 10 percent improvement. However, this is intended for multiple bacterial species including *E. coli* O157:H7, *Campylobacter*, *Listeria*, and *Salmonella* (Healthy People, 2014). Additionally, there is a goal to reduce the number of cases of postdiarrheal HUS in children under 5. The baseline, set from 2006-2008, averaged 2.0 cases per 100,000. A 50% reduction with a target of 1 case per 100,000 is the goal for 2020. In 2014, 1.1 cases per 100,000 were reported (Healthy People, 2014). This is a significant improvement, and shows promise for reaching the

2020 goal. These goals help producers measure improvements in food safety and show the commitment to a food safety culture across the United States food industry.

Prevalence of E. coli

Cattle act as a natural reservoir for *E. coli* and the bacteria can be found both internally and externally, in the gastrointestinal tract and environment. During slaughter, the main source of carcass contamination is the hide. In a study by Arthur et al. (2006), cattle hides sampled for *E. coli* O157:H7 before transport to the slaughter facility had a prevalence of 50.3%. After harvest, hides sampled during processing had an increased prevalence of 94.4%. In addition, pre-evisceration samples detected *E. coli* O157:H7 on 28 of 286 carcasses, or 9.8% (Arthur et al., 2006). However, a study by Bosilevac et al. (2009) measured the prevalence of *E. coli* O157:H7 on 1,995 hide samples at 33.2%, while 2.1% of pre-evisceration beef carcasses had enumerable levels (> 0.5 CFU/100 cm²) of *E. coli* O157:H7 in small commercial abattoirs processing under 1,000 head of cattle per day.

Hide contamination rate is directly related to carcass contamination levels. However, hide prevalence varied between days and facilities, with daily prevalence of *E. coli* O157:H7 ranging from 18.9% to 100% (Bosilevac et al., 2009). Pre-evisceration carcasses sampled in 2 large commercial processing operations showed a prevalence of 3.1% and 10.9% for *E. coli* O157:H7. Post-intervention carcasses had decreased *E. coli* O157:H7 prevalence of 0% and 1%, respectively for those 2 large commercial processing operations (Rivera-Betancourt et al., 2003). Another post-intervention carcass sampling recovered 1.2% (15) of *E. coli* O157:H7 from 1,232 samples from 3 Midwestern beef processing facilities (Barkocy-Gallagher et al., 2003). When Most Probable Number

(MPN) assays were conducted, all 15 positive carcasses were shown to have <3.0 cells per 100 cm² (Barkocy-Gallagher et al., 2003). This indicates the effectiveness of interventions in controlling microbial populations throughout the slaughter process.

In the process of carcass cooling and fabrication, there are multiple opportunities for contamination and spread of pathogens. One such opportunity comes from subprimals. While considered independent, microbiological contamination is thought to vary from one subprimal cut to another. Prior to further processing, intact beef subprimal surfaces have a very low contamination rate of *E. coli* O157:H7 (<0.083%) when sampled at four different commercial processing plants. However, both coliforms and non-O157 *E. coli* were present at low numbers, indicating that there was still potential for pathogen growth (Kennedy et al., 2006). In a study conducted in a commercial Irish abattoir, beef trimmings had a 2.4% prevalence of *E. coli* O157:H7 with concentrations in a range of 0.70 to 1.61 log₁₀ cfu/g⁻¹ (Carney et al., 2006). Small or very-small beef-processing facilities tested 118 samples of ground beef with 18.6% (22) positive for one or more O-groups, but none of the samples had either *Stx1* and/or *Stx2* present (Svoboda et al., 2013). This suggests that STEC can be present regardless of facility size.

The USDA estimated the national prevalence in beef trim of *E. coli* O157:H7 as 0.39%, with a 95% confidence interval ranging from 0.05% to 0.73%. The data were obtained from December 2005 through January 2007, with approximately 250 federally inspected facilities participating (USDA FSIS, 2011). In another study, 4,133 commercial ground beef samples were screened for non-O157 STEC serotypes and overall prevalence was determined to be as high as 24.3% (1,006 positive samples) for the presence of either *Stx1* or *Stx2* (Bosilevac and Koohmaraie, 2011). STEC isolates

were then confirmed in 7.3% (300 samples) of the 4,133 samples. However, prevalence varies highly between location and facility, with several factors to consider such as location and the facility itself (Bosilevac and Koohmaraie, 2011). Regardless of facility size, processors need to implement interventions and processes that can be used to decrease the prevalence of these organisms from pre-harvest to further processing, covering all facets of the beef supply chain.

Surrogate Usage

Non-pathogenic indicator organisms that share most of the same characteristics of pathogenic strains are ideally suited for learning more about pathogens without compromising the biological integrity of a facility. For this reason, non-pathogenic *E. coli* strains that share many of the same characteristics as *E. coli* O157:H7 were adapted into surrogate (indicator) organisms for use in studies conducted in processing facilities. Indicator organisms have several criteria that must be met before being considered suitable. These include: being detectable both rapidly and easily, being able to be separated from microorganisms commonly found in the food, and must be found in the same foods as the pathogen they represent. Additionally, indicator organisms should ideally have the same growth requirements, growth rate, and death curve as the pathogen (Montville et al., 2012). One of the biggest criteria for surrogate organisms is thermal resistance, measured in *D*-values. *D*-values are usually expressed in minutes, and represent the time it takes to kill 90% of a bacterial population at a given temperature (Marshall et al., 2005).

Overall, combinations of non-pathogenic *E. coli* isolates appear to be most successful at mimicking *E. coli* O157:H7 behavior (Cabrera-Diaz et al., 2009; Marshall et

al., 2005). A specific combination of five *E. coli* biotype I isolates was designated as surrogates with strains deposited with the American Type Culture Collection (ATCC) with accession numbers of BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431. In different combinations, these surrogate organisms provide additional degrees of safety depending on the critical control points of the process being considered. This means that the reduction in *E. coli* O157:H7 would be higher than the surrogate strains, leading to increased degrees of safety (Keeling et al., 2009).

Surrogate strains can also be modified for identification. This can be done by a few different methods, including transforming bacteria to fluoresce using plasmid vectors, or inserting rifampicin resistant genes (Cabrera-Diaz et al., 2009). To ensure rifampicin resistance, the surrogate strain is grown in a sterile solution containing rifampicin. After incubation, colonies are streaked onto selective media plates to ensure resistance to rifampicin (Kaspar and Tamplin, 1993). The strains can then be stored until needed, depending on the culturing procedure.

Strains of *E. coli* O157:H7 can also be genetically modified to serve as positive controls. One way to do this is to use modified green fluorescent proteins (GFP) to fluoresce in inoculated positive samples. To modify the protein, both a plasmid and transposon containing the *gfp* gene are transferred to the bacterial chromosome (Noah et al., 2005). Fluorescence can then be used to identify positive colonies instead of selective media, which can limit the growth of injured cells. This is done by fluorescence microscopy using UV light to detect colonies through visual examination (Noah et al., 2005).

Antimicrobial Interventions

Lactic Acid

Lactic acid has many uses in meat products, including use as an antimicrobial in fresh meats, and was recognized by the FDA in 1978 as a Generally Recognized As Safe (GRAS) substance (U.S. National Archives and Records Administration, 2006). There are no current regulatory limits of this acid besides those established with current good manufacturing practices (U.S. National Archives and Records Administration, 2006). Lactic acid is also known as 2-hydroxyproionic acid. In a pure dry form, this acid is a white powder. In aqueous solutions, it appears clear to slightly yellow, depending on concentration and purity. At 22°C, the pKa value is reported as 3.857. One way to produce lactic acid is to use a strain of lactic acid bacteria to ferment sucrose or dextrose to calcium lactate. Heat is applied to the solution and the impurities are separated off before sulphuric acid is added to release the lactic acid. The precipitant is then separated and the supernatant purified before being concentrated into specific amounts. To further purify the lactic acid, the processes of esterification, distillation, and hydrolysis are all utilized (Smulders et al., 1986).

Lactic acid inhibits gram-negative bacteria such as *E. coli* through reducing the pH (Gill and Newton, 1981). Lowering the pH prevents metabolic activities such as proteolysis, and reduces bacterial growth. This depends on both the acid and conditions of use. The effect of pH is also related to the amounts of dissociated and undissociated acids present in the solution. A lower pH leads to a greater concentration of undissociated acid, and more inhibition of bacterial growth is possible. However, the conditions of the system in which the acid is used must be considered. Different

organisms require different amounts of acid for neutralization, depending on where and how the acid attacks the cells, as well as how easily it can pass through the outer membrane (Ingram et al., 1956).

Permeabilization of the outer cell membrane of gram negative bacteria by lactic acid occurs by releasing the lipopolysaccharide (LPS) layer present on the outside of the membrane, allowing detergents and lysozymes to permeate through the outer barrier membrane into the cell (Alakomi et al., 1999). This also allows lipophilic acids such as lactic acid to enter the cell (Ray and Sandine, 1992). The cytoplasm is acidified causing a disruption to the proton motive force that pumps protons out of the cell through the membrane (Ray and Sandine, 1992). Additionally, once the electron transport system becomes disrupted, the processes of oxidative phosphorylation and substrate transport become uncoupled (Baird-Parker, 1980) leading to a loss of active transportation of nutrients across the membrane (Ray and Sandine, 1992).

Peroxyacetic Acid

Peroxyacetic acid is applied to meat surfaces through a blended processing water spray as an antimicrobial intervention. Normally known as peroxyacetic acid, it is actually comprised of an aqueous solution of known concentrations of peroxyacetic acid (PAA), hydrogen peroxide (HP), acetic acid, and 1-hydroxyethylidene-1, and 1-diphosphonic acid (HEDP). Thus, regulatory limits for this mixture are found in the Food Contact Substance Notification No. FCN 1132, where meat applications are not to exceed 400 parts per million (ppm) PAA, 155 ppm HP, and 20 ppm HEDP, when used primarily as a spray application (USDA FSIS, 2017). While peroxyacetic acid is considered the primary active ingredient, hydrogen peroxide and acetic acid act as

fortifiers and HEDP as a stabilizer (Block, 1991). A strong acetic acid odor is associated with this clear acid (Organic Materials Review Institute, 2000). Peroxyacetic acid is thermodynamically unstable and will decompose quickly (Block, 1991). When decomposed, peroxyacetic acid forms acetic acid, water, and oxygen. These products are considered virtually nontoxic when diluted (Cords et al., 1993). However, decomposition will lead to reduction in effectiveness as an antimicrobial intervention.

Peroxyacetic acid works primarily through oxidation of the outer cell membrane. In a study by Clapp et al. (1994), the hydroxyl radical was reported as the major lethal species in the oxidation reaction. This radical is highly reactive and can oxidize many components of the cell, including membrane lipids and DNA, with reactions cleaving double bonds (Block, 1991). Interestingly, peroxyacetic acid also helps inactivate discoloration and degradation enzymes, particularly in potatoes, as proved by Greenspan and Margulies (1950).

Other Antimicrobials for Meat and Poultry

In addition, other antimicrobial interventions are also used for various reasons in different facilities. These can include acetic acid (up to 4%), acidified sodium chlorite in ranges from 500 to 1200 ppm depending on pH, and anhydrous ammonia followed by carbon dioxide on ground beef. Additionally, aqueous mixtures of various organic compound solutions are available (USDA FSIS, 2017). Mixtures can include different combinations of citric acid, sorbic acid, hydrochloric acid, phosphoric acid, sulfuric acid, and sodium sulfate as well as many others depending on regulations found in USDA FSIS Directive No. 7120.1. Calcium hypochlorite can be used on beef primals (20 ppm free available chlorine), as well as chlorine dioxide (3 ppm residual chlorine dioxide).

Lactoferrin can also be used on beef carcasses up to 2%. Ozone is approved for use on meat and poultry products using current industry standards (USDA FSIS, 2017). A combination of sodium diacetate, sodium propionate, and sodium benzoate is also approved with maximum levels at 0.25%, 0.5% and 0.1% for each ingredient in ready to eat products. Many more antimicrobial interventions are approved for use in meat products. Each antimicrobial intervention used depends on the type of product, ex. poultry, red meat, carcasses, ready to eat products, or fresh products. Regulatory limits are also placed on the antimicrobial interventions according to various USDA FSIS directives regarding safe usage (USDA FSIS, 2017).

Application Factors of Antimicrobial Interventions

Methods

Organic acids can be used in a variety of applications to meat products. Some of these include the use of cabinet washes on carcasses, dip solutions, sprays, and washes on subprimals and beef trim. Beef carcasses sprayed twice (after rail inspection and after an 8-hr spray-chill cycle) with lactic acid showed greater reduction than carcasses sprayed with only water or chlorine (Kenney et al., 1995). Using a multi-step system of trimming or water washing, and then rinsing hot boned beef carcasses with hot water (95°C) before spraying with 2% lactic acid (55°C) proved more effective, especially with low levels of contamination, than just rinsing with hot water after trimming or water washing (Castillo et al., 1998). A simulated spray chilling study on beef carcass tissue revealed reductions of approximately 1.5 log of *E. coli* O157:H7 when sprayed by 0.02% peroxyacetic acid every 30 minutes for 10 hours at -3°C. That same study also showed greater reductions when 2% lactic acid was sprayed with the same application method (Stopforth et al.,

2004). A 2% lactic acid wash was shown to be slightly more effective than just water washing alone, with less than 1 log reduction on inoculated beef plates; however, the no wash control was not statistically different than any of the organic acid washes or water wash alone (Carpenter et al., 2011). When treating beef trimmings, combining a 3-second hot water treatment with an 11 second 2% lactic acid dip reduced *E. coli* O157:H7 by 1.1 log CFU/g (Ellebracht et al., 1999). A 4.4% lactic acid dip was shown to be more effective than a 4.4% lactic acid spray when applied to inoculated beef trim before grinding, leading to reductions of 0.91 to 1.41 log CFU/g of *E. coli* O157:H7, as well as reducing non-O157 STEC by 0.48 to 0.82 log CFU/g. The lactic acid spray reduced *E. coli* O157:H7 by 0.5 log CFU/g, but was not significantly different from untreated control counts (Wolf et al., 2012).

Additional Factors

When deciding which application to use, factors such as type of food, temperature, time, and concentration should be considered when choosing an organic acid application method. Time and concentration appear to have a direct proportional effect on inhibition of microbial growth, with longer times and higher concentrations showing the most effect (Greer and Dilts, 1992; Kalchayanand et al., 2016; Kotula and Thelappurath, 1994). At shorter exposure times (15 s), 200 ppm peroxyacetic acid was less effective when high levels of organic material were present. However, 200 ppm peroxyacetic acid and 4% lactic acid were reported to have similar effects after 60 s of exposure time (Kalchalyanand et al., 2016).

Organic acid concentration has also been reported to be a factor impacting effectiveness (Anderson and Marshall, 1990). As concentration of lactic acid increased,

greater reductions were measured with concentrations ranging from 0 to 3% (Anderson and Marshall, 1990). However, this conclusion was refuted by Harris et al. (2006), with evidence that beef trim and ground beef sprayed with higher concentrations of organic acids had no additional reduction of pathogen contamination when compared to lower concentrations. Additionally, increasing concentrations of peroxyacetic acid up to 1000 ppm applied to individual pieces of fresh beef trim showed no increase in reduction of *E. coli* O157:H7 (Ellebracht et al., 2005). This was also shown by Krug et al. (2017), who found no reductions in STEC when beef subprimals were sprayed with concentrations of 3.5-10% lactic acid. Temperature of solution can also be a factor when applying organic acids. A study by Anderson and Marshall (1990) found greater reductions in microbial populations when 2 or 3% lactic acid was applied at 55 or 70°C compared to 25 or 40°C.

Contamination levels, pre-exposure to acidic conditions, and serotype also have an effect on reducing microbial loads. In a study by Youssef et al. (2012), lightly contaminated beef cuts (~1 log cfu/cm²) sprayed with 5% lactic acid showed a reduced effectiveness when compared to heavily contaminated beef (~4 log cfu/cm²). Bacteria that have been exposed to sub lethal acidic conditions may also be more acid resistant than those not exposed, particularly to lactic acid (Stopforth et al., 2004). Certain serotypes of pathogenic STEC also appear to have highly variable reduction levels. When 3% lactic acid was applied for 2 minutes to pure cultures of *E. coli* O26:H11 and O121:H2, reductions measured 2.1 and 0.3 log CFU/mL for each pathogen, respectively (Zhao et al., 2014). Meat surface type may also have a varying effect on reductions, with a study by Gill and Badoni (2003) suggesting that distal surfaces of beef brisket had a

reduction greater than 2 log units, but medial surfaces less than 2 log units when treated with 4% lactic acid on chilled beef carcass quarters. When trimming was applied to chilled subprimals, fat surfaces had greater reductions than lean surfaces. Even before trimming, lean surfaces had fewer inoculated surrogate *E. coli* present, implying that there would be less initial bacteria present on lean surfaces than fat surfaces on subprimals (Laster et al., 2012). Lactic acid treated lean beef tissue showed maximum reductions of approximately 0.5 log for generic *E. coli* in a study by Greer and Dilts (1992). This is consistent with a study by Marshall et al. (2005), which reported greater reductions on fat tissue versus lean tissue. Meat temperature at the time of antimicrobial treatment can impact efficiency. When applied to chilled carcasses, reductions of 2.0 to 2.4 logs have been reported (Castillo et al., 2001). However, a conflicting study by Acuff et al. (1987) showed that little effect on microbial populations was found during the shelf life of PVC-overwrapped steaks from vacuum-packaged subprimal beef cuts sprayed with 1% lactic acid and kept in cold storage up to 84 days. When inoculated beef trim was treated with 3 or 5% lactic acid, reduction of STEC was greatest for trim with surface temperatures $>8^{\circ}\text{C}$ (Zhao et al., 2014).

Meat Characteristics

Bacterial Attachment

Bacteria survive and grow by attaching to a meat surface. One way this is done is through attracting and repulsing forces acting on both the bacteria and meat surface structures (Li and McLandsborough, 1999). Bacteria have 2 types of adhesion to surfaces, reversible (loosely attached) and non-reversible (strongly attached). Reversible

adhesion is considered instantaneous, where bacteria are weakly held to the surface.

Non-reversible attachment occurs when bacteria are firmly held to the surface (Marshall et al., 1971). Bacterial strain can also play a role in attachment, with both surface charge and hydrophobicity determined to be strain-specific. When the surface charges of *E. coli* O157:H7 (pathogenic strain) and *E. coli* JM109 (laboratory strain) were compared, *E. coli* O157:H7 was not as affected by changes in environment such as pH, ionic strength, or the addition of a surfactant (Li and McLandsborough, 1999). Initial cell concentration can be a factor for attachment with an increase in attachment rates occurring as initial cell concentration increases (Chung et al., 1989; Li and McLandsborough, 1999). Loosely attached cells also appear to have higher cell concentrations than strongly attached cells (Rivas et al., 2006). Most attachment of *E. coli* occurs between 1 and 20 minutes, with little increase occurring after 20 minutes. Additionally, temperature of attaching medium appears to have little effect, with *E. coli* attachment occurring over a wide (2.5 to 37°C) temperature range (Butler et al., 1979).

Meat surface type does not seem to affect bacterial attachment rate, despite the surface quality differences between fat and lean tissue (Chung et al., 1989). Negative bacterial cell surface charges correlate with initial attachment to both lean and fat tissues (Dickson and Koohmaraie, 1989), suggesting that cell surface charge plays a larger role in initial bacterial attachment than meat surface type. The hydrophobicity of the cell surface may also be a factor in attachment with both negative charge and hydrophobicity increasing with an increase in attachment to fat surfaces (Dickson and Koohmaraie, 1989). These results agree with Benito et al. (1997), who found that hydrophobicity of the bacterial cell surface correlated ($r=0.80$) with the strength of attachment to beef

muscle. Strongly attached bacterial concentrations of planktonic and sessile cultures are higher on fat tissue surfaces than muscle tissue (Rivas et al., 2006).

Motile gram-negative species such as *E. coli* also appear to have an increased attachment rate as compared to non-motile gram-positive species of bacteria (Butler et al., 1979). Attachment rate also appears affected by sessile or planktonic culture growth, with sessile culture grown strains of STEC having a greater attachment rate (Rivas et al., 2006). The attachment mechanism of bacteria to meat surfaces appears poorly understood due to the complexity of the system. Many factors appear to affect bacterial attachment to meat surfaces, with multiple areas of variability between bacteria, and even within bacterial strain.

Buffering Capacity

While organic acids have been used for many years in a variety of applications, many inconsistencies in reduction have been reported when applied to lean tissue surfaces. One of the reasons this may occur is due to the apparent buffering capacity of lean muscle tissue. Consisting of 75% moisture, lean muscle tissue may dehydrate on the surface depending on storage conditions, leading to bacterial reductions (Dickson and Siragusa, 1994). A high amount of moisture may also impact lean muscle tissue by diluting the organic acid or using the cellular components to solubilize the acid. Additionally, lean muscle cells may have taken up the acid more quickly than the bacterial cells (Podolak et al., 1996). Muscle fiber type is also speculated to have an impact on buffering capacity. In the beef chuck, a variety of fiber types are present including red, white, and intermediate fibers. Each type has different compositions of adipose and lean tissue amounts, leading to different processing characteristics and

ultimately, a difference in meat quality (Kirchofer et al., 2002). However, ultimate pH appears to have a greater impact on buffering capacity than fiber type due to the differences in non-protein nitrogen and lactic acid content. As pH declines, buffering capacities differ in muscles due to the change in lactic acid content. It is also speculated that larger amounts of creatine increases buffering power when muscle is at or below a pH of 3 (Rao and Gault, 1989). Muscle buffering will also differ depending on physiological state with living muscle much more complex than post mortem muscle. At rigor, buffering capacity is not dependent on the amount of bicarbonate and esterified phosphate is hydrolyzed to orthophosphate (Smith, 1938). The buffering capacity in post mortem muscle is reported between 40-60 mmol $H^+/(pH \cdot kg)$, although values outside the range have also been reported (Pösö and Puolanne, 2005). Organic acids, such as lactic acid, lower the pH on the meat surface. However, a study by Rodríguez-Melcón et al. (2017) reported similar pH values for homogenized beef samples using several concentrations of lactic acid (2%, 3%, 4%, 5%) after 24 hours with pH ranging from 5.61 to 6.02. By 120 hours, there was still no significant difference in beef samples between any concentration and the untreated control for pH (Rodríguez-Melcón et al., 2017). Moisture, storage conditions, muscle fiber type, and ultimate pH all have an impact on the potential buffering capacity of lean muscle tissue.

Quality Characteristics of Fresh Meat

Shelf Life

Shelf life is dependent on bacterial presence and growth. It is normally a measurement of the amount of time before a meat product is considered unacceptable in organoleptic properties, such as taste and smell (Borch et al., 1996). Meat is

considered spoiled when bacterial cell counts exceed $10^6/\text{cm}^2$ and amino acid degradation begins. When cell density exceeds $10^8/\text{cm}^2$, bacterial growth slows dramatically due to the limitation of substrates, regardless of surface type (Gill and Newton, 1980).

Large numbers of background microflora can also help inhibit pathogenic bacteria, such as *E. coli* O157:H7, in ground beef. This is especially true under anaerobic conditions. In particular, *Lactobacillus* spp. that consist of homofermentative lactic acid bacteria appear to dominate growth during storage, especially strains of *L. sakei* (Vold et al., 2000). Using this strain as an inhibitor may provide an alternative method to help reduce pathogenic *E. coli* in ground beef. The shelf life for ground beef is considered to be 5 to 7 days in an oxygen permeable overwrap. Reduced oxygen packages have a shelf life of 14 to 18 days. However, primal cuts that have been vacuum packaged and stored at less than 3°C may have up to 45 days of shelf life (Aberle et al., 2012).

Color

Color is one of the most important characteristics of meat. Consumers use color as an indicator of quality more than any other fresh meat property. Several factors influence color in a meat product, but pigments in meat are predominantly considered as meat color changes. Myoglobin is the major pigment in meat, but hemoglobin may contribute to a lesser extent. Myoglobin has a major impact on color due to the structure of the protein. With a porphyrin heme ring attached to an iron atom, the oxidation state has the primary influence on color changes and reactions to other compounds (Aberle et al., 2012).

When organic acids are applied to the surfaces of muscle tissue, various results for color change have been reported. Steaks placed in storage for 84 days after treatment with 1% lactic acid, 1% acetic acid, untreated control, or mixture of 1% lactic acid, 2% acetic acid, 0.25% citric acid, and 0.1% ascorbic acid showed only minor differences in appearance, with no acid treatment consistently different than the others (Acuff et al., 1987). When beef steaks were dipped in lactic acid, L* values for 1.2% lactic acid samples were higher than the treated and untreated controls (Kotula and Thelappurate, 1994).

Ground beef appears to have inconsistent color effects with acid treatment. Ground beef was lighter in color with less oxymyoglobin content when 5% lactic acid was applied compared to control and hot water treatments (Stivarius et al., 2002). These findings are consistent with Jimenez-Villarreal et al. (2003b), who reported that ground beef treated with 2% lactic acid had less oxymyoglobin content and were a lighter color when compared to an untreated control. Additionally, lactic acid treated ground beef patties had lower a* values and were less red than an untreated control (Jimenez-Villarreal et al., 2003a). This agrees with a study by Rodríguez-Melcón et al. (2017) in which beef sprayed with 5% lactic acid was slightly lighter and less red than the control. However, a study by Harris et al. (2012) observed no differences in color by trained visual color panelists over a 3-day retail display of ground beef patties sprayed with 2% lactic acid, 5% lactic acid, or acidified sodium chlorite at 1000 ppm. In agreement, 0.02% peroxyacetic acid sprayed on beef trim before grinding showed no differences in L* a* or b* values when compared to an untreated control and displayed in retail

conditions (Pohlman et al., 2014). This could be due to the low application rate (~0.1 ml/g) of the conventional spray method (Pohlman et al., 2014).

When percent discoloration was measured, by day 2 of retail display, lactic acid treated beef trimmings were more discolored than a hot water treatment or non-treated control (Stivarius et al., 2002). This was confirmed again by Jimenez-Villarreal et al. (2003b), when they reported that lactic acid treated ground beef showed the most discoloration on days 2 and 3 of retail display amongst all treatments. Unlike lactic acid, peroxyacetic acid showed less discoloration than an untreated control on days 0 through 3 of retail display (Quilo et al., 2008). However, when beef clods were treated with 4.5% lactic acid or 380 ppm peroxyacetic acid, there were no differences between either organic acid when compared to an untreated control in ground beef over a 7 day retail display period (McCarty, 2016).

pH

The pH of meat decreases from 7.4 in living muscle to an ultimate pH of around 5.3 within 24 hours of slaughter. This occurs through the accumulation of H^+ ions through glycolysis and hydrolyzing ATP to ADP (Aberle et al., 2012). However, the use of organic acids tends to lower pH in meat even further. Lactic acid appears to particularly decrease the pH depending on concentration. Not surprisingly, higher concentrations tend to decrease pH values, with 5% lactic acid having lower pH values than samples treated with 2% lactic acid (Rodríguez-Melcón et al., 2017). When surface pH of lean beef trim was measured, a significant drop in pH was observed when compared to non-acid-treated lean beef trim. In the same study, fat beef trim also had decreased pH values when treated with lactic acid and hot water. However, it was

reported that lean beef trim surface pH values returned to above 5.0 faster than fat beef trim samples (Kang et al., 2001). Ground beef from beef shoulder clods dipped in lactic acid (4.5%) or peroxyacetic acid (380 ppm) was not different from an untreated control throughout a 7-day retail display period. However, throughout the retail display life, pH decreased for all treatments (McCarty, 2016).

Color and pH are related in meat. A low pH increases L* values (McCarty, 2016). Using organic acids, with their tendency to lower pH to kill bacteria, seems to increase L* values, particularly in ground meat. Ground beef patties treated with lactic acid had a lower pH and higher L* value than controls (Jimenez-Villarreal et al., 2003a). When a culture of lactic acid bacteria was added to ground beef, there were no differences in pH values for treated or untreated samples held over a 12 day storage period (Smith et al., 2005). This shows that organic acids appear to have an effect on meat pH, but cultures of lactic acid bacteria don't cause the same effect. Therefore, producers should select the antimicrobial intervention that is best suited for the product's intended use.

Lipid oxidation

Lipid oxidation is a chemical reaction that occurs through two pathways during the shelf life of a product and leads to rancidity. This can occur through either an enzymatic reaction or autooxidation free radical process (Aberle et al., 2012). To slow lipid oxidation, free radical scavengers can be added or oxygen eliminated (Aberle et al., 2012). This can be difficult with ground product, as oxygen is introduced during the grinding process.

Organic acids applied as an antimicrobial intervention appear to have a positive effect on lipid oxidation in ground product. In a study by Quilo et al. (2009), beef trimmings treated with organic acids (4% sodium metasilicate, 0.02% peroxyacetic acid) and then ground into patties had less lipid oxidation, particularly on day 3 of retail display. Peroxyacetic acid, while similar to other treatments, was lower than the untreated control in TBARS value (mg malonaldehyde per kg of meat; Quilo et al., 2009). However, lactic acid has been shown to actually increase lipid oxidation, especially when compared to untreated controls (Jimenez-Villarreal et al. 2003b). As a general trend, by day seven lipid oxidation increased significantly regardless of treatment (Jimenez-Villarreal et al. 2003b). When lactic acid (4.5%) and peroxyacetic acid (380 ppm) were applied to beef shoulder clods, ground beef from lactic acid treated clods had greater lipid oxidation than treatment with peroxyacetic acid (McCarty, 2016).

Sensory Characteristics

Organic acid applications can affect the sensory properties of meat products. For instance, 5% lactic acid treated beef had a slight acidulous odor when compared to untreated, 2%, 3%, and 4% lactic acid samples (Rodríguez-Melcón et al., 2017). Additionally, Stivarius et al. (2002) reported that lactic acid treated beef trimmings had a more non-beef like odor than either the control or hot water treatment by day 3. In contrast, peroxyacetic acid had a similar beef odor when compared to control ground beef patties (Quilo et al., 2009). Harris et al. (2012), reported untrained panelists were not able to find differences between treated and non-treated control ground beef patties when triangle sensory tests were used.

When shear values of beef rib steaks were evaluated after organic acid application, they showed no significant differences between controls, 0.6% acetic acid, 1.2% acetic acid, 0.6% lactic acid, and 1.2% lactic acid. However, the mean shear-value for 0.6% lactic acid dipped beef was 5.9 kg/g, whereas the mean shear-value for 1.2% lactic acid was 4.9 kg/g. In the same study, 1.2% lactic acid samples were significantly less juicy than the water treated control. Still, either concentration of lactic acid was not different than the water treated control in other sensory aspects such as flavor, color, and overall acceptability (Kotula and Thelappurath, 1994). This is also true for peroxyacetic acid, with no significant differences between 0.02% peroxyacetic acid and untreated control ground beef patties when beef flavor, off flavor, and juiciness were evaluated. However, peroxyacetic acid ground beef patties showed a lower shear force value than the untreated control (Quilo et al., 2009). While organic acids appear to slightly affect several palatability factors, overall, there was little difference when compared to untreated controls. However, many of these studies used lower concentrations (~1-2%) of organic acids. This may not be an accurate representation of current meat industry usage of organic acid antimicrobials.

Summary

Although historically associated with ground beef, STEC have been found in a variety of food sources, owing to their wide range of possible growth conditions. However, these organisms are heat labile and can be destroyed through proper heating. If not heated correctly, these organisms can invade the epithelial cells of the gastrointestinal tract of humans and cause disease, specifically Hemolytic Uremic Syndrome, which can

lead to death. With only a low infectious dose needed to cause illness, goals have been developed to reduce these risks by 2020. These goals have drawn attention to other pathogenic STEC besides *E. coli* O157:H7, leading to prevalence studies to determine baseline levels in each sector of beef, from live cattle to ground trim. After finding other non-pathogenic strains of *E. coli* that existed in the same conditions, surrogate cocktails of these organisms were developed to further the understanding and control of pathogenic *E. coli* without exposing the facility to unnecessary risk.

To help reduce STEC, various antimicrobial interventions, including organic compound solutions, can be used. Both lactic acid and peroxyacetic acid are approved for use on meat and are currently listed as processing aids. Each organic compound has their own unique way of inhibiting bacteria. Lactic acid drops the pH and causes permeabilization of the outer cell membrane, while peroxyacetic acid acts as an oxidizer. There are many ways of applying antimicrobial interventions, from carcass applications such as cabinet washes to hand held sprays of pieces of beef trim. While each method has a designated use, other factors can also cause variations in reductions of bacteria. These factors include temperatures of the organic acid solution and meat, meat surface type, and solution concentration. Lactic acid appears to be more effective with increasing concentrations, unlike peroxyacetic acid. Still, each organic acid is effective at reducing the number of bacteria. However, when too concentrated, lactic acid appears to have detrimental affects on sensory properties, especially in ground beef.

Organic acids can be useful for reducing the number of *E.coli* present in meat. However, how successful these antimicrobial treatments are will also be dependent on the degree of bacterial attachment to the meat surface. Meat quality characteristics such as

color, pH, and lipid oxidation can be affected by the use of antimicrobial organic acids. Key sensory characteristics appear to be unaffected by small concentrations of organic acids. However, there is a need for research at higher concentrations to discover if sensory properties remain unaffected. Additionally, these higher concentrations will provide more knowledge about bacterial reductions. Although organic acid usage has been well documented for the first 24 hours after application, there have been few studies to determine if time from organic acid application has an effect on both reduction of bacteria and quality properties over retail shelf life. Therefore, the objective of these studies was to determine the effect of dipping beef trim in lactic and peroxyacetic acid solutions on *E. coli* reduction and on quality characteristics of ground beef during a simulated retail shelf life period.

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**REDUCTION OF *E. COLI* SURROGATES ON
DIFFERENT SURFACES OF BEEF CLOD SECTIONS
AFTER DIPPING IN ORGANIC ACIDS FOR SHORT OR
EXTENDED TIMES**

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Abstract

Small business meat processors can use antimicrobial interventions to reduce Shiga Toxin-Producing *Escherichia coli* (STEC) in beef prior to grinding. Dipping subprimals in organic acids can be used but the effectiveness of these antimicrobials may depend on application parameters such as exposure time and subprimal tissue type. The objective of these studies was to evaluate reductions in *E. coli* on beef clods using short or extended times of application, and the impact of fat or freshly cut lean tissue surfaces. In study 1, sections of beef clod were dipped in 4.5% lactic acid (LA) or 380 ppm peroxyacetic acid (PA) for 15 or 180 seconds. The meat surface was inoculated with rifampicin-resistant *E. coli* (*E. coli*^{RIF}) at ~5.3 log CFU/cm² and *E. coli*^{RIF} counts were taken before and after antimicrobial treatment, and after ground beef production. In study 2, fat or freshly cut lean tissue sections of beef clod were inoculated with *E. coli*^{RIF} at ~5.3 log CFU/cm² and dipped in 4.5% LA for 15 or 180 seconds. *E. coli*^{RIF} counts were taken before and after antimicrobial treatment and reductions were calculated.

Study 1 *E. coli*^{RIF} counts before treatment plated on aerobic count plates (ACP) were not different ($P = 0.948$). After treatment, PA 180s had lower ($P = 0.001$) *E. coli*^{RIF} counts than the control. When reductions were calculated, PA 180s had greater ($P = 0.004, 0.022$) reductions in *E. coli*^{RIF} counts than the control and LA 15s treatment. *E. coli*^{RIF} counts in ground beef samples were different ($P = 0.013$) for the control when compared to PA 15s, PA 180s, and LA 15s treatments.

In study 2, *E. coli*^{RIF} counts before treatment had no treatment and surface interaction ($P = 0.718$). There were no differences for treatment ($P = 0.991$) or surface type ($P = 0.200$) in *E. coli*^{RIF} counts plated on ACP petrifilm. *E. coli*^{RIF} counts post

treatment had no treatment by surface interaction ($P = 0.760$). Treatment was different in *E. coli*^{RIF} counts ($P = 0.0003$) with LA 180s as the lowest treatment compared to LA 15s and control treatments ($P = 0.037$, $P < 0.0001$, respectively). Calculated reductions for *E. coli*^{RIF} plated on APC petrifilm had no treatment by surface interaction ($P = 0.694$). Both LA treatments reduced ($P = 0.005$) *E. coli*^{RIF} counts when compared to the control, while meat surface type was not different ($P = 0.374$).

When plated on *E. coli* petrifilm, before treatment there was no treatment by surface interaction ($P = 0.815$). No differences between treatment ($P = 0.772$) or meat surface ($P = 0.281$) were observed. Post treatment no treatment by surface interaction ($P = 0.055$) was observed. Post treatment *E. coli*^{RIF} counts were lower ($P = 0.001$, $P < 0.0001$) for LA 180s than either LA 15s or control treatments. Surface type was different ($P = 0.041$) post treatment. Fat surfaces had lower ($P = 0.041$) *E. coli*^{RIF} counts than lean surfaces. Calculated reductions had no interaction between treatment and surface type ($P = 0.141$). Both treatment ($P = 0.0001$) and surface type ($P = 0.024$) were different. Reductions for LA 180s were greater ($P = 0.004$, $P < 0.0001$) than both LA 15s and control treatments. Fat surface treatments had greater ($P = 0.024$) reductions of *E. coli*^{RIF} counts than lean surface treatments. Overall, these studies demonstrated that organic acids could reduce surrogate *E. coli*^{RIF}. Additionally, fat or lean surfaces have an effect on potential reductions obtained from use of an antimicrobial intervention. When using an organic acid dip as an antimicrobial intervention, processors should consider application time, as well as the meat surface type being treated.

Introduction

The U.S. Department of Agriculture Food Safety and Inspection Service considers *E. coli* O157:H7 and six non-O157 Shiga Toxin-producing *E. coli* (STEC) serogroups O26, O45, O103, O111, O121, and O145 to be adulterants in non-intact beef due to the risk of disease from contamination of these pathogens (17). Ground beef has traditionally been a major source for illnesses from STEC and has been implicated in several outbreaks (1). To decrease the risk of *E. coli* O157:H7 and non-O157 STEC, small business meat processors are using a variety of antimicrobial intervention measures. One of these includes using organic acids as an antimicrobial intervention. Research has reported reductions in pathogenic bacteria for carcasses and subprimal cuts (3, 5, 8, 12). For chilled beef carcass quarters, a 4% lactic acid spray with a holding time of 60 minutes has been found to have substantial reductions in *E. coli*, with up to 2 log unit reductions on carcass quarters treated with lactic acid (5). Similar results were stated by Castillo et al. (3) who sprayed chilled carcass surfaces with 4% lactic acid at 55°C and reported significant bacterial reductions. While carcass surfaces can have significant reductions (>2 logs), spraying pieces of meat with lactic acid yielded reductions in a range between 0.57 to 0.95 log units (12). This agrees with work by McCarty (8) who found reductions between 0.39 and 1.13 log CFU/cm² on beef shoulder clods using various acids. These smaller reductions could be due to a variety of factors, including application temperature, meat storage time, and meat surface type.

Low levels of bacterial populations appear to be protected and may not be as affected by antimicrobial treatments such as 5% lactic acid (18). The temperature of the organic acid solution and meat surface can influence efficacy as well.

Ground beef is a major vehicle of transmission for these organisms. *E. coli* are naturally present in cattle populations and can be transferred onto carcasses during slaughter. Additionally, a small number of contaminated animals can cause contamination in a large amount of ground beef due to the mixing that occurs through the grinding process (2). Thus, small business meat processors that grind subprimals for ground beef should be using an antimicrobial intervention to decrease the risk of *E. coli* O157:H7 and non-O157 STEC.

Antimicrobial interventions offer an effective way to control or reduce pathogens present in meat, particularly ground beef. Various factors, such as length of time of application and meat surface available may affect the efficiency of the antimicrobial intervention and the product quality. The objective of this work was to evaluate the reduction of *E. coli* on beef shoulder clod surfaces using either a short or extended dip application time of an organic acid.

Materials and Methods

Experimental design

This study evaluated the effectiveness of dipping in organic acid solutions for short or extended times on the reduction of rifampicin resistant *E. coli* (*E. coli*^{RIF}) surrogates on pieced beef clods. An inoculated, non-dipped treatment was used as a positive control. Pieces of beef clod were randomly assigned to treatments with different organic acids. The first study utilized fresh cut lean inner surfaces while the second used both freshly cut lean inner surfaces and medial fat surfaces of pieces with only one acid. Each study had 4 independent replicate and was designed as a randomized complete design. Study 1

was arranged as a 2 x 2 + 1 factorial, while study 2 utilized a 3 x 2 arrangement of treatments.

Raw meat materials

To imitate small business meat processors receiving meat, a local distributor transported beef shoulder clods (IMPS 114; 16) to the University of Nebraska Loeffel Meat Laboratory in Lincoln, Nebraska for sample preparation and used within 30 days of fabrication. Boxes of vacuum packaged beef clods were kept refrigerated from 1.5-3°C until use. Beef shoulder clods (4) were divided by separating the top blade from the arm portion of the clod. The separated portions were then cut into approximately 12 cm² sections before being randomly assigned to a treatment. Within a replication, at least 5 sections were assigned to each treatment, and 2 cores excised from each section selected.

Culture preparation

The inoculum was prepared by using a five-strain cocktail of rifampicin resistant *E. coli* including USDA-FSIS 011-82, ATCC 43888, ATCC 43889, ATCC 43890, and USDA-FSIS 45756. Each strain was grown separately by adding 10 ml of tryptic soy broth (Bectin, Dickson, and Company; Sparks, MD) to 15 ml tubes and adding a small amount of each frozen culture strain to individual tubes. Tubes were then incubated for 48 hours at 37°C. After incubation, 1 ml of culture was pipetted into a different 15 ml tube and 10 mL of tryptic soy broth was added, along with 5 µL of rifampicin (100 µg/ml deionized double distilled water; Sigma-Aldrich Company; St. Louis, MO). Each tube was incubated again for 24 hours at 37°C. After 24 hours, a 1:1 ratio of each strain was combined and buffered peptone water (45 ml; BBL Buffered Peptone water; Bectin, Dickson, and Company, Sparks, MD) was added to create a 9:1 ratio of buffer to culture.

The inoculum was mixed thoroughly before 15 ml was placed into each 15 ml tube for inoculation and stored at $<2.5^{\circ}\text{C}$ until use. Just before inoculation, tubes were inverted several times to ensure inoculum was thoroughly mixed.

Inoculation

Before inoculation, surface temperature of the meat was measured with a calibrated thermometer. The pre-made inoculate (15ml) was applied to the surface of the meat pieces using a hand-held sprayer and allowed to attach for 20 minutes at room temperature ($12\text{-}16^{\circ}\text{C}$). Meat surface temperature was measured again before 5 cores (Individual core: 3.5 cm diameter; Total: 47 cm^2) were excised from the meat surface for pre-treatment inoculation counts and placed in Whirl-Pak™ bags (Item #01-812-6C; Fisher Scientific; Pittsburgh, PA) for microbiological analysis. Sections for sampling were selected to have an area large enough to excise 2 core sites (pretreatment and post treatment).

Organic acid preparation and titration

To prepare the lactic acid solution, 776 ml of concentrated lactic acid (88%; Birko; Henderson, CO) was mixed with 15.1 liters of water to create a 4.5% lactic acid solution. The peroxyacetic acid solution was prepared using 34 ml of peroxyacetic acid (BirkoSide MP-2; Birko; Henderson, CO) mixed with 15.1 liters of water for a final concentration of 380 parts per million. Titration kits (Peracetic Acid Test Kit; ChemWorld; Kennesaw, GA and Lactic Acid Test Kit; Birko; Henderson, CO) were used to verify that final concentrations of solutions were within an acceptable range (4.0-4.9% LA, 300-340 ppm, PA). In the second study, only lactic acid was used to determine if there was a difference between lean and medial fat surfaces. Immersion times remained the same.

Organic acid application

To apply the organic acid treatments, 15.1 liters of each organic acid solution were prepared in a standard meat lug (ToteAll 2000 Item No. 319507071; Bunzl Processor Division; Riverside, MO) and an ANOVA Precision™ Cooker (ANOVA Applied Electronics, Inc; San Francisco, CA) was placed in the solution to ensure the temperature stayed consistent (22.2°C) and mixing occurred throughout dipping treatment. At least 5 beef clod sections were submerged into the solution using a metal wire basket for 15 seconds before being placed on racks and surface temperature taken again. The temperature of the solution was recorded both immediately before and after dipping. This process was then duplicated, with sections submerged for 180 seconds instead of 15 seconds before being placed on racks. Total treatments included dipping in lactic acid for 15 seconds (LA15), lactic acid for 180 seconds (LA180), peroxyacetic acid for 15 seconds (PA15), peroxyacetic acid for 180 seconds (PA180), and an inoculated, untreated control (control).

After treatment, clods were allowed to drip for ~2 minutes before 5 core samples (Individual core diameter: 3.5 cm; Total: 47 cm²) were excised from the meat surface and stored in Whirl-Pak™ bags for microbial analysis. Core samples and treated pieces were held in a refrigerated cooler (<2.5°C) immediately after the second sampling for microbiological analysis. Study 1 treated pieces were ground for further sampling.

Temperature protocol

The first temperature measurement of the meat surface was taken before inoculation with a calibrated thermometer (Thermocouple Thermometer (Type T); Omega Engineering Inc, Norwalk, CT) within 0.75cm of the surface. After the

attachment period, temperature of the surface was measured again with a calibrated thermometer (Thermocouple Thermometer (Type T)). The final temperature measurement of the meat surface was obtained within 5 minutes after the dipping treatment. The solution temperature was taken before and after dipping any treatment by a calibrated thermometer (Thermocouple Thermometer (Type T)).

Grinding protocol Study 1

Beef pieces from each treatment were ground twice, first with a plate with 1.27 cm holes and then again with a plate with 0.63 cm holes. A 25 gram sample for each treatment was randomly collected throughout the 0.63 cm plate grind by choosing 3-5 smaller portions and combining them before being placed in a Whirl-Pak™ bag for microbiological analysis. The grinder was disassembled and cleaned between each treatment using chlorine dioxide to sanitize and avoid cross-contamination of samples.

Microbiological analysis

Core samples (5) from each treatment were combined with 25 ml peptone water containing rifampicin (100 µg/ml deionized double distilled water) before undergoing a 3 minute stomaching (AES Laboratoire Stomacher; AES Laboratoire; Bruz, France). Ground beef samples were transferred to sterile lateral filter bags (Item # F-78860; interscience; St. Nom) for stomaching and combined with 25 ml rifampicin containing buffered peptone water. After stomaching, procedures remained the same for both core and ground samples, with 1 ml of fluid pipetted into a culture tube and combined with 9 ml of peptone water containing rifampicin. Samples were then serially diluted to the appropriate range. One milliliter serially diluted samples were plated onto ACP

Petrifilms™ (3M; St. Paul, MN) in duplicate and incubated for 48 hours at 37°C before colonies were manually counted.

Statistical analysis

In the first study, a completely randomized design was used, with treatments arranged in a 2 x 2 + 1 design, with 2 different organic acids dipped at 2 different times (15 or 180 seconds) with an untreated, inoculated control. A treatment effect was determined using SAS 9.2 PROC GLIMMIX procedures (SAS Institute; Cary, NC) with $P < 0.05$. The least squared means were separated using Tukey's adjustment. Direct treatment comparisons use Tukey's adjusted P values. The second study was designed as a completely randomized design as well, but only used lactic acid. Two different time points (15 or 180 seconds) with two different surface types, fat or lean tissue were used. Untreated controls for both meat surface types were also included for a 3 x 2 design. An interaction of treatment x location was used in the model statement for SAS 9.2 PROC GLIMMIX procedures with $P < 0.05$. Least squared means were separated using PDIF lines.

Results

Study 1

E. coli^{RIF} counts after inoculation and prior to treatment plated on Aerobic Plate Count (APC) petrifilms were not different ($P = 0.948$; Table 1). LSMeans for inoculation counts of *E. coli*^{RIF} ranged from 5.32 log CFU/cm² to 5.42 log CFU/cm². After treatment, *E. coli*^{RIF} counts (CFU/cm²) were different ($P = 0.002$). The *E. coli*^{RIF} counts for PA 180s and LA 180s were lower ($P \leq 0.028$) than *E. coli*^{RIF} counts for the

untreated control (Table 1). However, both PA 15s and LA 15s were similar to the control, regardless of acid type.

The reduction in the *E. coli*^{RIF} counts from inoculation to post treatment was different ($P = 0.007$) among the treatments and control (Table 1). The reduction in *E. coli*^{RIF} counts for the PA 180s treatment was greater ($P \leq 0.022$) than the control and LA 15s treatment. However, reduction in the *E. coli*^{RIF} counts was similar for the control when compared to PA 15s, LA 15s, and LA 180s treatments. Ground beef samples were different ($P = 0.013$) between treatment (Table 1) with PA 15s, PA 180s, and LA 15s lower ($P < 0.05$) than the control. The LA 180s treatment and control were similar in *E. coli*^{RIF} counts.

Temperatures of beef sections were not different ($P = 0.714$) for treatment before inoculation (Table 2). Additionally, meat temperature after inoculation before treatment was not different ($P = 0.968$) between treatments. After treatment, meat temperature was not different ($P = 0.078$) between treatments. Differences in meat temperature from before inoculation to after treatment were calculated, and were not different ($P = 0.050$). Solution temperature before treatment was not different ($P = 0.453$) between treatments. Post treatment, solution temperature was not different ($P = 0.999$) between treatments.

Study 2

No interaction between treatment and surface type ($P = 0.718$) was found before treatment. *E. coli*^{RIF} counts plated on APC petrifilms after inoculation prior to treatment were not different ($P = 0.991$) among the treatments and untreated control (Table 3). Additionally, there was no difference ($P = 0.200$) in fat or lean surface type. Post treatment *E. coli*^{RIF} counts plated on APC petrifilms had no significant treatment by

surface interaction ($P = 0.760$) or difference in surface type ($P = 0.879$); however there was a difference ($P = 0.0003$) in treatment (Table 3). The LA 180s treatment had lower ($P \leq 0.037$) CFU of *E. coli*^{RIF} than either LA 15s or the control.

The reduction for *E. coli*^{RIF} counts plated on APC petrifilms from inoculation to post treatment had no significant treatment by surface interaction ($P = 0.695$). There was a difference in treatment ($P = 0.005$). The LA 180s treatment was lower ($P = 0.001$) than the control, but similar to the LA 15s treatment. Fat or lean surface of meat tissue had no difference ($P = 0.374$).

No interaction ($P = 0.815$) was detected between treatment and surface type before treatment when *E. coli*^{RIF} counts were plated on *E. coli* petrifilms. Pre treatment, no differences ($P = 0.773$) were observed between treatment (Table 4). Additionally, no differences ($P = 0.281$) were observed between surface type (Table 4). After treatment, no treatment and surface interaction was observed ($P = 0.055$). Both treatment ($P < 0.0001$) and surface ($P = 0.041$) were different after treatment (Table 4). The LA 180s treatment had the lowest ($P \leq 0.001$) *E. coli*^{RIF} counts when compared to LA 15s and the control treatments. Fat meat surface had lower ($P = 0.041$) *E. coli*^{RIF} counts post treatment compared to lean meat surface.

No interaction between treatment and surface was observed ($P = 0.141$) when reductions of *E. coli*^{RIF} counts plated on *E. coli* petrifilms were calculated. However, reduction of *E. coli*^{RIF} counts for both treatment and surface type were different ($P = 0.0001$, $P = 0.024$, respectively; Table 4). When treatments were compared, LA 180s had the highest reduction of *E. coli*^{RIF} counts ($P = 0.0001$). Additionally, fat surface also had a greater ($P = 0.024$) reduction of *E. coli*^{RIF} counts than lean surface (Table 4).

No treatment and surface interaction ($P = 0.212$) for meat temperature was detected before inoculation. Temperature of beef section was not different ($P = 0.836$) before inoculation for treatment (Table 5). However, surface type was different ($P = 0.002$), with fat surface treatments higher ($P = 0.002$) than lean surface treatments (Table 5). After inoculation prior to treatment, there was no interaction of treatment and surface type ($P = 0.063$) for beef section temperature. Additionally, beef surface temperature was not different ($P = 0.145$) between treatments. Fat surface had an increased ($P = 0.004$) temperature when compared to lean surface after inoculation.

After treatment, no interaction between treatment and surface type ($P = 0.637$) was observed. However, differences ($P < 0.0001$) between treatments were observed after treatment (Table 5). The LA 180s treatment was higher ($P \leq 0.0002$) than LA 15s and control treatments. Surface type was not different ($P = 0.736$) post treatment (Table 5). The difference in meat temperature was calculated from before inoculation to after treatment. No treatment and surface type interaction was observed ($P = 0.918$). However, treatment was different ($P < 0.0001$) when meat temperature difference was calculated. The LA 180s treatment temperature increase was greater ($P \leq 0.0002$) than LA 15s and control treatments. Meat surface type was not different ($P = 0.865$) when temperature difference between pre inoculation and after treatment was calculated.

No interaction of treatment and meat surface ($P = 0.495$) was observed for pre treatment solution temperature. Treatment was different ($P = 0.019$) for pre treatment solution temperatures (Table 5). The LA 180s solution temperature was higher ($P = 0.019$) when compared to LA 15s before treatment. Surface type was not different ($P = 0.607$) for pre treatment solution temperature. After treatment, no interaction between

treatment and meat surface type ($P = 0.582$) was found for solution temperature.

Additionally, treatment was not different ($P = 0.241$) after treatment for solution temperature. Surface type was also not different ($P = 0.136$) after treatment for solution temperature.

Discussion

Study 1

In study 1, PA 180s and LA 180s were both lower ($P \leq 0.028$) than the untreated control after treatment in *E. coli*^{RIF} counts. This agrees with Pohlman et al. (10) who found reductions over 1 log in *E. coli* after treatment with peroxyacetic acid. Time of antimicrobial intervention application appears to affect *E. coli*^{RIF} counts. Both 15 s treatments were similar to the inoculated control in *E. coli*^{RIF} counts after treatment. An increased application time appears to increase reduction of bacteria (7). However, increasing immersion time of beef cheek meat in 2.5 or 5% lactic acid solution did not improve efficacy (13).

Calculated reductions were below 1 log CFU/g for all treatments. This is in agreement with Stivarius et al. (14), who tumbled 5.5% lactic acid with beef trimmings for 180 s and reported a reduction of 0.64 in APC and 0.66 in *E. coli*. Wolf et al. (15) reported that dipped pieces of beef trim in 4.4% lactic acid for 5 s reduced *E. coli* O157:H7 by 0.91 log CFU/g. The smaller reductions in *E. coli*^{RIF} CFU observed in this study could be due to the differing surface types of the pieces (5). Additionally, smaller reductions could be due to use of APC petrifilms, which allows for recovery of injured cells (9). Ground beef samples were different between treatment, with the inoculated control higher than LA 15s, PA 15s, and PA 180s treatments. However, lactic acid

180 s was similar to all treatments. The reason for this is unclear and therefore, the second study was chosen to focus on only on lactic acid and how differing meat surface type could affect reductions of *E. coli*^{RIF}.

Study 2

In study 2, when *E. coli*^{RIF} was plated on APC petrifilm, there was less *E. coli*^{RIF} present post treatment when beef clod sections were dipped in lactic acid for 180s (Table 3). In agreement with McCarty (8), LA 15s was lower than the inoculated control in *E. coli*^{RIF} counts post treatment. When reductions were calculated, lactic acid treated beef sections were greater than the control (Table 3). This agrees with McCarty (8). Results reported by Harris et al. (6), found no measureable reductions for *E. coli* O157:H7 immediately after spraying beef trim with 2% or 5% lactic acid. However, by 6 hours post treatment, 5% lactic acid had reduced *E. coli* O157:H7 by 0.8 log CFU/g (6). A contrasting report from Kalchayanand et al. (7), found 4% lactic acid to be most effective with 2-3 log reductions when exposed to beef purge for 15 seconds.

When using *E. coli* petrifilms, *E. coli*^{RIF} counts post-treatment were greater for the control than either lactic acid treatment (Table 4). Additionally, fat surfaces had less *E. coli*^{RIF} counts than lean surfaces. However, work by Rivas et al. (11) found STEC attachment to be stronger to fat tissue than lean muscle tissue. This was not found in the present study, where greater reductions of *E. coli*^{RIF} counts were found on fat surfaces, which is in agreement with Cutter and Siragusa (4). When reductions were calculated for treatment, lactic acid 180 s reduced *E. coli*^{RIF} counts by over 1 log CFU/cm². This is in agreement with Kalchayanand et al. (7), who reported that increasing exposure time in lactic acid resulted in greater reductions of STEC. Reductions calculated from *E. coli*

petrifilms appear to be greater than those observed from APC petrifilms. This is not surprising, as *E. coli* media can prevent injured cells from growing (9).

Fat surface temperature was higher than lean surface temperature before and after inoculation (Table 5). However, this difference is less than 1.5° C and thus may not be of practical importance in industry application. Post treatment meat surface temperatures were higher for LA 180s than all other treatments. This could be due to the length of exposure in the higher temperature of organic acid. When temperature differences were calculated from pre-inoculation to post treatment, LA 180s was higher than all other treatments. This could also be due to the length of time spent in the organic acid, which had a higher temperature than the meat surface. Solution temperature pre-treatment between the lactic acid treatments was higher for LA 180s. However, as this was less than one degree Celsius different it may not be of practical importance.

Conclusions

These studies showed that time of application can be a critical factor when applying an antimicrobial intervention to beef tissue surfaces. In general, longer exposure time in the acid solution showed a greater reduction. Both peroxyacetic acid and lactic acid appear to be more effective at longer application time. Additionally, surface tissue type showed differences, with fat tissue more susceptible to the organic acid treatment than lean tissue. Therefore, producers should consider both what type of organic acid to use, as well as the composition of the product, before implementing an antimicrobial intervention.

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Tables

Table 1. LSMeans of *E. coli*^{RIF} count on Aerobic Plate Count petrifilm of organic acid treatment of beef for Study 1.¹

Treatment ²	Pre Treatment (log CFU/cm ²)	Post Treatment (log CFU/cm ²)	Reduction (log CFU/cm ²)	Ground Beef (log CFU/g)
PA 15	5.36	5.08 ^{abc}	0.28 ^{ab}	4.44 ^b
PA 180	5.42	4.70 ^c	0.72 ^a	4.32 ^b
LA 15	5.33	5.16 ^{ab}	0.16 ^b	4.45 ^b
LA 180	5.32	4.94 ^{bc}	0.38 ^{ab}	4.47 ^{ab}
Control	5.39	5.38 ^a	0.02 ^b	4.94 ^a
SEM	0.10	0.12	0.15	0.15
<i>P</i> value	0.948	0.002	0.007	0.013

¹LSmeans with different superscripts (a-c) in a column are significantly different ($P < 0.05$). For each treatment, n = 4.

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip; LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

Table 2. Temperatures of meat surfaces and solutions of each organic acid treatment and control for study 1.¹

Treatment²	Meat Temperature (°C)				Solution Temperature (°C)	
	Pre Inoculation	Pre Treatment	Post Treatment	Difference in Temp.³	Pre Treatment	Post Treatment
PA 15	3.2	4.7	7.4	4.2	21.7	21.0
PA 180	3.2	5.3	9.4	6.3	22.5	21.1
LA 15	3.2	5.1	8.2	5.0	21.0	21.1
LA 180	2.8	5.0	9.4	6.7	21.5	21.0
Control	3.5	5.5	6.0	2.5	.	.
SEM	0.48	0.96, 1.22	1.25	1.31	0.85, 0.76	1.12
<i>P</i> value	0.714	0.968	0.078	0.050	0.453	0.999

¹LSmeans with different superscripts (a-c) in a column are significantly different ($P < 0.05$). For each treatment, $n = 4$.

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip; LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

³Difference in temperature= pre inoculation to post treatment values.

Table 3. LSMeans of *E. coli*^{RIF} count on Aerobic Plate Count petrifilm of each organic acid treatment for Study 2.¹

Treatment²	Pre Treatment (log CFU/cm²)	Post Treatment (log CFU/cm²)	Reduction (log CFU/g)
LA 15	5.37	4.90 ^b	0.47 ^a
LA 180	5.38	4.60 ^c	0.78 ^a
Control	5.38	5.31 ^a	0.08 ^b
SEM	0.14	0.13	0.18
<i>P</i> value	0.991	0.0003	0.005
Surface			
Fat	5.45	4.94	0.51
Lean	5.30	4.93	0.37
SEM	0.11	0.11	0.14
<i>P</i> value	0.200	0.879	0.374

¹LSmeans with different superscripts (a-c) in a column are significantly different ($P < 0.05$). For each treatment, n = 4.

²LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment; Fat = fat tissue surface; Lean= lean tissue surface.

Table 4. LSMeans of *E. coli*^{RIF} Count on *E. Coli* Petrifilm of each organic acid treatment for Study 2.¹

Treatment²	Pre Treatment (log CFU/cm²)	Post Treatment (log CFU/cm²)	Reduction (log CFU/cm²)
LA 15	5.18	4.53 ^b	0.65 ^b
LA 180	5.23	3.88 ^c	1.35 ^a
Control	5.28	5.16 ^a	0.12 ^c
SEM	0.13	0.16	0.21
<i>P</i> value	0.773	<0.0001	0.0001
Surface			
Fat	5.29	4.37 ^b	0.92 ^a
Lean	5.17	4.67 ^a	0.50 ^b
SEM	0.11	0.13	0.17
<i>P</i> value	0.281	0.041	0.024

¹LSmeans with different superscripts (a-c) in a column are significantly different ($P < 0.05$). For each treatment, n = 4.

²LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment; Fat = fat tissue surface; Lean= lean tissue surface.

Table 5. Temperatures of beef surfaces and solutions of each organic acid treatment and control for study 2.¹

Treatment ²	Meat Temperature (°C)				Solution Temperature (°C)	
	Pre Inoculation	Pre Treatment	Post Treatment	Difference of Temp. ³	Pre Treatment	Post Treatment
LA 15	2.4	5.8	8.3 ^b	2.8 ^b	22.0 ^b	21.9
LA 180	2.4	6.0	12.3 ^a	7.4 ^a	22.7 ^a	22.1
Control	2.2	5.0	5.2 ^c	-0.3 ^c	.	.
SEM	0.41, 0.39	0.35	0.76, 0.73	0.83, 0.79	0.23	0.18
<i>P</i> value	0.836	0.145	<0.0001	<0.0001	0.019	0.241
Surface						
Fat	2.9 ^a	6.3 ^a	8.5	3.3	22.4	21.9
Lean	1.7 ^b	4.9 ^b	8.7	3.4	22.2	22.2
SEM	0.32	0.40	0.61	0.70	0.23	0.18
<i>P</i> value	0.002	0.004	0.736	0.865	0.607	0.136

¹LSmeans with different superscripts (a-c) in a column are significantly different ($P < 0.05$). For each treatment, $n = 4$.

²LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment, Fat = fat tissue surface; Lean = lean tissue surface.

³Difference in temperature= pre inoculation to post treatment values.

**EFFECT OF SHORT OR EXTENDED TIME
APPLICATION OF ORGANIC ACIDS ON SELECTED
GROUND BEEF QUALITY CHARACTERISTICS AND
AEROBIC PLATE COUNT**

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Prepared in the style of **Meat and Muscle Biology**

Abstract

Shiga Toxin-producing *E. coli* (STEC) are considered adulterants in ground beef and must be controlled. Small business meat processors can use various antimicrobial interventions to achieve control, but the effect on ground beef quality characteristics of dipping beef subprimals in organic acids may be dependent on time of exposure. Therefore, the objective of this study was to determine the effect of dipping sections of beef clods into lactic or peroxyacetic acid for short or extended times and the effect on ground beef quality characteristics. Beef clod sections (approximately 5.44 kg) were dipped in either 4.5% lactic acid (LA) or 380 ppm peroxyacetic acid (PA) for 15 or 180 s or left untreated as a control. Treatments were ground after acid application, pressed into approximately 454 g portions, and placed in retail display for 7 days. During simulated retail display, Total Aerobic Plate Counts, lipid oxidation, meat pH, objective color, and percentage of discoloration measurements were taken.

Total Aerobic Plate Count was not different ($P > 0.05$) within day for any treatment. Lipid oxidation had no differences ($P > 0.05$) on days 0 and 1. On days 3, 5, and 7 differences ($P < 0.0001$, $P = 0.0001$, $P = 0.009$, respectively) were observed between treatments. On day 3, the LA180 treatment had the greatest ($P < 0.05$) TBA values of all treatments. Ground beef pH was different on days 0 ($P < 0.0001$), 1 ($P < 0.0001$), and 3 ($P < 0.0001$) among treatments. On days 0 and 3, the pH of the LA180 treatment was less ($P < 0.05$) than the pH for all other treatments. No differences ($P > 0.05$) were detected between treatments on days 5 and 7. Percentage of discoloration was not different ($P > 0.05$) among treatment for day 0. Days 1, 2, and 3 had differences ($P = 0.017$, $P = 0.013$, $P = 0.042$, respectively) among treatments. On day 1, the LA180

treatment had a greater ($P \leq 0.049$) percentage of discoloration than PA15, PA180, and control treatments. Percentage of discoloration was not different ($P > 0.05$) among treatments for the remainder of the retail display period. No differences ($P > 0.05$) were observed until day 5 for L^* values. No differences ($P < 0.05$) for either a^* or b^* values among any treatments within day were observed.

Ground beef treated with lactic acid for extended times had detrimental effects on pH, TBA, and percentage of discoloration during days 0 to 5 of retail display.

Peroxyacetic acid treatments had less impact for many quality characteristics, regardless of time of exposure. Small business meat processors should carefully consider the time of application and the antimicrobial used to maintain quality in ground beef.

Introduction

Escherichia coli O157:H7 is a known pathogenic shiga toxigenic strain (STEC) that causes gastrointestinal illness, and in severe cases, can lead to death due to renal failure. Therefore, the U.S. Department of Agriculture Food Safety and Inspection Service (USDA FSIS) has deemed both *E. coli* O157:H7 and six other non-O157 Shiga Toxin-producing *E. coli* (STEC) serogroups (O26, O45, O103, O111, O121, and O145) to be adulterants (USDA FSIS, 2015). Ground beef is considered a major food borne transmission route for foodborne illness from its natural reservoir in cattle. STEC have been recovered from approximately 1.2% of chilled post intervention carcasses in a commercial beef processing plant (Barkocy-Gallagher et al., 2003). Therefore, it is important for meat processors to use antimicrobial interventions to control these organisms during processing to ground beef.

Using organic acids as antimicrobial interventions offers a method to reduce STEC in beef. However, treatment with organic acid may have an effect on ground beef quality. Ground beef patties tumbled with 2% lactic acid were lighter and less red than untreated control patties (Jimenez-Villarreal et al., 2003). This agrees with Kotula and Thelapurate (1994) who found that treatment with lactic acid produced paler beef rib steaks; in particular treatment with 1.2% lactic acid produced the lightest color when compared to both an untreated control and 1.2% acetic acid treatments. Stivarius et al. (2002) also reported lighter color and less oxymyoglobin content for ground beef from trimmings tumbled with 5% lactic acid. However, when 0.02% peroxyacetic acid was sprayed on beef trim, no color differences in L* values were found when compared to untreated controls during a seven day simulated retail display period (Pohlman et al.,

2014). In other quality characteristics, when the pH of lactic acid treated ground beef patties was measured, 2% lactic acid was lower than the untreated control (Jimenez-Villarreal et al., 2003). Rodríguez-Melcón et al. (2017) found that higher concentrations of sprayed lactic acid had lower pH values when compared to lower concentrations of lactic acid and a control. In addition, samples treated with 5% lactic acid had a slight sour odor and turned slightly whiter when evaluated by sensory panelists (Rodríguez-Melcón et al., 2017).

These results differ from those of Harris et al. (2012) who reported no differences in percent discoloration and beef color in ground beef patties manufactured from lactic acid treated beef trim in retail display for 3 days. Additionally, ground beef patties from beef trimmings treated with 0.02% peroxyacetic acid had no differences when compared to control patties in beef odor or flavor (Quilo et al., 2009). Ground beef from lactic acid treated beef trimmings was not different from the control in percentage discoloration on day 0 and 1 of retail display (Stivarius et al., 2002).

Reducing bacteria is the main purpose of antimicrobial intervention, but processors must be cognizant of their effect on quality. When organic acids are applied, a variety of effects can occur depending on concentration and type of acid chosen. The objective of this study was to determine the effect of dipping pieces of beef clod into lactic or peroxyacetic acid for short or extended time intervals on the quality characteristics of ground beef during a simulated retail display period.

Materials and Methods

Experimental Design

Beef clod sections were treated with 2 different organic acids, lactic acid or peroxyacetic acid, dipped for 2 different times, 15 s or 180 s, or were not dipped and treated as a control for a 2 x 2 +1 arrangement of a completely randomized design. Beef clod sections were randomly assigned to treatments, with a total of 6 independent replicates occurring. Treatments consisted of lactic acid 15 s (LA15), lactic acid 180 s (LA180), peroxyacetic acid 15 s (PA15), peroxyacetic acid 180 s (PA180), and control (control).

Raw meat materials

For each replication, the University of Nebraska Loeffel Meat Laboratory in Lincoln, Nebraska received beef shoulder clods (IMPS 114; USDA Agricultural Marketing Service, 2014) from a distributor that normally distributes to small business meat processors to represent usual industry distribution methods and the number of days since fabrication. Boxes of vacuum packaged beef clods were stored in a cooler from 1.5- 3°C until use and were used within 25 days of fabrication. Individual beef clods were unboxed and separated by splitting the arm portion of the clod from the top blade section into two sections into approximately 12 cm² pieces. The divided sections were randomly assigned to a treatment. Sections were then randomly combined until each treatment weighed approximately 5.44 kg.

Organic acid preparation/ titration

A 4.5% lactic acid solution was prepared by mixing 776 ml of concentrated lactic acid (88%; Birko; Henderson, CO) with 15.1 liters of 22.2°C water. Peroxyacetic acid

with a concentration of 380 parts per million was prepared by pipetting 34 ml of concentrated peroxyacetic acid (BirkoSide MP-2; Birko; Henderson, CO) into 15.1 liters of 22.2°C water. The solutions were then titrated with commercial titration kits (Lactic Acid Test Kit; Birko; Henderson, CO and Peracetic Acid Test Kit; ChemWorld; Kennesaw, GA) to determine the final concentrations of solutions.

Organic acid application

Each organic acid solution was prepared in a standard meat lug (ToteAll 2000 Item No. 319507071; Bunzl Processor Division; Riverside, MO) and an ANOVA Precision™ Cooker (ANOVA Applied Electronics, Inc; San Francisco, CA) was inserted into the lug to ensure thorough mixing of the solution occurred, as well as maintain a consistent target temperature of 22.2°C. A new organic acid solution was prepared for each treatment. The beef clod pieces were immersed into each solution using a large wire basket for the designated treatment of 15 or 180 seconds before the sections were placed on a rack to drip for approximately 2 minutes. Temperature of both the meat and solution was taken immediately before and after dipping in solution with a calibrated thermometer. Treated samples were then put into a cooler (1.5-3°C) for storage until grinding later that day.

Meat Surface and Solution Temperature Procedures

Temperature measurements of meat and solutions were taken for each treatment. The first temperature reading was measured within 0.75cm of the meat surface before treatment (Thermocouple Thermometer (Type T); Omega Engineering Inc, Norwalk, CT). Within 5 minutes of antimicrobial treatment, temperature was measured within 0.75cm of the meat surface. The organic acid solution was measured within 5 minutes

both before and after treatment by inserting the probe of the thermometer (Thermocouple Thermometer (Type T)) into the middle of the organic acid solution.

Grinding and retail display protocols

Treatments were ground twice, first with a plate with 1.27 cm holes and then again with a plate with 0.63 cm holes on the same day as organic acid application. The ground beef from each treatment was then formed into approximately 454 gram (1 lb) portions with a Colosimo press attached to a piston stuffer for each day. Portions were placed onto Styrofoam Retail Display trays (Genpak; Glens Falls, NY) and overwrapped in an oxygen permeable film (Bunzl; Kansas City, MO) before being placed on a table in a cooler under simulated retail display conditions. Samples were rotated daily to minimize any effect of lighting or temperature location effect. Display temperatures were maintained at 0 to 2°C with samples 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). On days 0, 1, 3, 5, and 7, ground beef samples were taken after portions were overwrapped in an oxygen permeable film by randomly selecting 125 g for analysis. Twenty-five g were collected for microbiological analysis and 100 g collected for pH and lipid oxidation analysis from an individual package for each treatment on days 0, 1, 3, 5, and 7. Samples for microbiological analysis were placed in Whirl-Pak™ bags and kept refrigerated at <2.5°C until use. The other 100 g sample was vacuum packaged and stored at -80°C until needed for further analysis.

Microbiological analysis

Ground beef treatment samples (25 g) were removed from Whirl-Pak™ bags and placed in sterile lateral filter bags (Item # F-78860; interscience; St. Nom) with 25 ml peptone water (BBL Buffered Peptone Water; Bectin, Dickson, and Company, Sparks, MD). Treatments were then stomached (AES Laboratoire Stomacher; AES Laboratoire; Bruz, France) for 180 seconds, after which at least 5 ml of sample was placed in a sterile culture tube. The sample was then serially diluted using 1 ml of sample and 9 ml of buffered peptone water to the appropriate dilution. Serially diluted samples were then plated onto ACP Petrifilms™ (3M, St. Paul, MN) in duplicate and incubated for 48 hours at 37°C. Colonies were then manually counted after 48 hours.

Objective color analysis

Objective color was measured daily using a Konica Minolta Colorimeter (Chroma Meter CR-400; Konica Minolta Sensing Americas, Inc.; Ramsey, NJ) with a 2° observer, 8 mm aperture, and a D65 illuminate to calculate L* a* b* values. The colorimeter was calibrated using a blank white tile, which was covered with the same oxygen permeable film as the samples. One sample portion (454 g) was measured in 6 different locations, which were then averaged together as the sample value. Objective color was measured on all days (0-7) and recorded. Samples were rotated daily to minimize any effect of lighting or temperature location effect.

Subjective color analysis

Percentage of discoloration was measured subjectively by a trained 7-9 member panel of University of Nebraska-Lincoln graduate students in the Department of Animal Science. The panel was asked to evaluate the amount of discoloration of ground beef samples as a percentage (0-100%) daily through the 7 days of simulated retail display.

Panelist measurements were averaged together within each treatment during a single day period to produce the final value for that treatment within each day.

pH analysis

Ground beef samples were powdered using liquid nitrogen before usage in pH analysis. Ten grams of powdered sample were weighed out in duplicate into small beakers and placed on a stir plate. Ninety milliliters of distilled, deionized water was then added to the beaker along with a stir bar to keep the samples mixing continuously throughout the measurement process. The sample pH was then measured as the sample was stirred using a pH meter (Orion 410Aplus; ThermoFisher Scientific; Waltham, MA) that had been calibrated using 4.0 and 7.0 standards. Each sample measurement was conducted in duplicate, with values averaged together for final pH measurements. Treatment pH analysis was measured on days 0, 1, 3, 5, and 7 of simulated retail display.

Lipid oxidation analysis

Lipid oxidation procedures were performed from the Thiobarbituric Acid Assay developed by Buege and Aust (1978) and modified by Ahn et al. (1998). Ground beef samples that had been powdered with liquid nitrogen were used in the lipid oxidation analysis. Five grams of powdered ground beef sample was placed in a 50 ml conical tube with 14 ml of double distilled water (ddH₂O) and 1 ml of Butylated hydroxyanisole stock solution (BHA). The mixture was then homogenized for 15 seconds using a polytron before being centrifuged for 5 minutes at 2000xg. One milliliter of the homogenate was then transferred to a 15 ml conical tube and 2 ml of TBA/TCA solution (2-Thiobarbituric Acid/ Trichloroacetic acid) was added. Samples were placed in 70°C water bath for 30 minutes to develop color before cooled in a cold water bath for 10 minutes. Tubes were

then centrifuged for 15 minutes at 2000xg before duplicate aliquots of 200µl were inserted into wells of a 96 well plate. The absorbance was read at 540 nm on a plate reader (Biotek; Biotek Instruments, Inc.; Winooski, VT) and analyzed. Final results were calculated as mg malonaldehyde/ kg of tissue.

Statistical analysis

In this study, 2 different organic compounds at 2 different application times were utilized with an untreated control for a 2 x 2 + 1 arrangement of treatments using a completely randomized design. Data were analyzed for treatment effect within day of retail display using SAS 9.2 GLIMMIX procedures (SAS Institute; Cary, NC) with $P < 0.05$. Least squared means for treatment were then separated and adjusted using Tukey's adjustment. Direct treatment comparisons are noted in the text with Tukey's adjusted P values.

Results

The Total Aerobic Plate count was not different ($P > 0.05$) when treatments were compared within day on days 0, 1, 3, 5, and 7 (Table 1).

Lipid oxidation had no differences ($P > 0.05$) among treatments until day 3 (Table 2). On day 3, 5, and 7 differences ($P < 0.0001$, $P = 0.0001$, $P = 0.009$, respectively) between treatments were observed. The LA180 treatment had the greatest ($P < 0.05$) TBA values of all treatments on day 3. On day 5, the LA180 treatment TBA values were greater ($P \leq 0.004$) than the TBA values for PA180 and control treatments. On day 7, TBA values for the PA180 treatment were less ($P \leq 0.047$) than either LA15 or LA180 treatments. As a general trend, LA180 had greater ($P < 0.05$) TBA values than the control and both PA treatments on days 3 and 5 while LA15 TBA values were greater

($P < 0.05$) than the control and both PA treatments on day 3. However, TBA values for both PA treatments were similar to the control TBA values regardless of day of display.

When pH was measured, on days 0, 1, and 3, differences ($P < 0.0001$, ($P < 0.0001$, $P < 0.0001$, respectively) were observed between treatments (Table 3). The pH of the LA180 treatment was less ($P < 0.05$) than the pH for all other treatments on days 0 and 3. On day 1, the pH of the LA180 treatment was less ($P \leq 0.0003$) than the pH for PA15, PA180, and control treatments, but similar to LA15 pH. As an overall trend, pH for LA180 was less ($P < 0.05$) than pH for both PA treatments and the control. The pH of both PA treatments remained similar to the control, regardless of display day.

Percentage of discoloration was not different ($P = 0.307$) on day 0 between treatments. However, on days 1, 2, and 3, differences ($P = 0.017$, $P = 0.013$, $P = 0.042$, respectively) among treatments were observed. On day 1, the LA180 treatment had a greater ($P \leq 0.049$) percentage of discoloration than PA15, PA180, and control treatments. On day 2, the percentage of discoloration for the LA180 treatment was greater ($P \leq 0.041$) than the percentage of discoloration for PA180 and LA15 treatments. On day 3, the LA180 treatment percentage discoloration was greater ($P = 0.039$) than the PA180 percentage discoloration. For the remainder of the retail display period, there were no differences ($P > 0.05$) between any treatments for percentage of discoloration. Overall, LA180 had a greater ($P < 0.05$) percent discoloration than PA180 throughout retail display. Additionally, the percent discoloration of both PA treatments remained similar to the control regardless of display day.

When L^* values were measured, there were no differences ($P > 0.05$) found between any treatments until day 5 (Table 5). On day 5, LA180 had higher ($P \leq 0.022$) L^* values than the control and PA15 treatments. No differences ($P > 0.05$) in L^* values between treatments were observed for the remainder of the retail display period. There were no differences ($P > 0.05$) observed in a^* or b^* values when treatments were compared within day (Table 6 and 7, respectively).

Discussion

Within day of retail display, there were no differences in ground beef aerobic plate counts (APC) between treatments. It appears the microflora from the meat surface of the clod sections were not changed by organic acid treatment. This could be due to the length of storage before use of the beef clods. Additionally, as the beef clods were cut into pieces, treated, and then ground, more meat surface would be exposed. This agrees with work by Rodríguez-Melcón et al. (2017), who reported no differences between lactic acid treated beef samples and the untreated control on day 0. However, this contrasts with research by Kotula and Thelappurate (1994) who found that lactic acid treated rib eye steaks had lower total CFU than control samples. This could be due to a residual acid effect on the native microflora present.

Lipid oxidation had no differences between treatments until day 3 of retail display. Lactic acid appears to increase lipid oxidation values, regardless of exposure time. The LA treatments may also have a residual acid effect that would help increase the rate of lipid oxidation during the later days of retail display. This is in agreement with work by McCarty (2016) that showed greater lipid oxidation TBA values of ground beef from 4.5% lactic acid treated beef shoulder clods as compared to 380 ppm

peroxyacetic acid treated ground beef samples. The present study found no differences between peroxyacetic acid and control treatments. This differs from results of Quilo et al. (2009), who showed lower oxidation values on day 3 for peroxyacetic acid treated (0.02%) ground beef patties than the untreated control.

The low pH of LA180 throughout the retail display period could be due to the extended time application leading to a residual acid effect with an increase in TBA values during the latter half of retail display. This agrees with research by Jimenez-Villarreal et al. (2003) and Rodríguez-Melcón et al. (2017) who found lactic acid treated beef had a lower pH when compared to untreated controls. Indeed, McCarty (2016) found lactic acid treated ground beef to have the lowest pH and highest TBA values among all treatments.

Percentage of discoloration was greater for lactic acid treated ground beef, particularly the LA180 treatment. The increased time of application in lactic acid could be the reason for the increase in percentage of discoloration. Both peroxyacetic acid treatments were similar to the control. This differs from Quilo et al. (2009), who found less discoloration for peroxyacetic acid treated ground beef patties compared to an untreated control. However, McCarty (2016) found no differences in ground beef percentage discoloration through a seven day retail shelf life when beef shoulder clods were treated with 4.5% lactic acid or 380 ppm peroxyacetic acid. Additionally, Harris et al. (2012) found no differences in percentage of discoloration when beef trim was sprayed with 2% acetic acid, 5% acetic acid, water, 2% lactic acid, or 5% lactic acid.

Little difference was observed between all treatments throughout the shelf life period in L* values. This is in agreement with Rodríguez-Melcón et al. (2017) who also

found little variation in L^* values when lactic acid was applied. However, Kotula and Thelappurath (1994) reported paler meat after application of lactic acid. Peroxyacetic acid treated ground beef was also reported to be similar to an untreated control (Pohlman et al., 2014). No differences were reported in this study between a^* or b^* values in all treatments, in agreement with work by Kotula and Thelappurath (1994). However, Rodríguez-Melcón et al. (2017) described lower a^* values in meat treated with 5% lactic acid, as well as an increase in b^* values for lactic acid treatments.

This study demonstrated little variation in objective color measurements (L^* a^* b^*) or Aerobic Plate Count. However, lipid oxidation, pH, and percentage of discoloration were all impacted by treatment with a LA antimicrobial intervention with an extended exposure time. In particular, using a LA180 treatment will decrease ground beef quality due to extended length of exposure. Thus, small business meat processors should carefully consider the quality aspects in deciding what antimicrobial intervention to implement.

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Tables

Table 1. LSMeans of Aerobic Plate Counts (CFU/g) of all treatments and days of retail display.¹

Treatment ²	Day of Display				
	0	1	3	5	7
PA15	3.02	3.24	3.98	4.13	4.27
PA180	3.10	3.21	3.86	4.22	4.39
LA15	3.14	3.19	3.85	4.00	3.95
LA180	3.30	3.07	3.43	3.56	3.43
Control	3.10	3.38	4.17	4.17	4.26
SEM	0.37	0.36	0.39	0.37	0.36
<i>P</i> value	0.959	0.941	0.441	0.398	0.088

¹ LSmeans with different superscripts are significantly different ($P < 0.05$) within a column. For each treatment $n = 6$.

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip; LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

Table 2. LSMeans of lipid oxidation values (mg malonaldehyde/kg tissue) for all treatments and days of retail display.¹

Treatment ²	Day of Display				
	0	1	3	5	7
PA15	0.96	1.47	1.80 ^c	2.46 ^{bc}	2.86 ^{ab}
PA180	1.82	1.06	1.52 ^c	2.15 ^c	2.62 ^b
LA15	1.16	1.78	2.69 ^b	3.87 ^{ab}	4.65 ^a
LA180	1.34	1.72	3.62 ^a	4.75 ^a	4.58 ^a
Control	0.83	1.17	2.00 ^c	2.67 ^{bc}	3.22 ^{ab}
SEM	0.52	0.31	0.22	0.52	0.66
<i>P</i> value	0.376	0.102	<0.0001	0.0001	0.009

¹ LSmeans with different superscripts in a column (a-c) are significantly different ($P < 0.05$) within a column. For each treatment $n = 6$.

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip; LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

Table 3. LSMeans of pH values for all treatments and days of retail display.¹

Treatment ²	Day of Display				
	0	1	3	5	7
PA15	5.76 ^{ab}	5.75 ^a	5.64 ^a	5.41	5.33
PA180	5.62 ^{ab}	5.73 ^a	5.69 ^a	5.35	5.28
LA15	5.54 ^b	5.55 ^{ab}	5.50 ^a	5.29	5.16
LA180	5.25 ^c	5.32 ^b	5.21 ^b	5.18	5.18
Control	5.81 ^a	5.76 ^a	5.58 ^a	5.31	5.35
SEM	0.08	0.08	0.07	0.08	0.10
<i>P</i> value	<0.0001	<0.0001	<0.0001	0.114	0.242

¹LSmeans with different superscripts (a-c) are significantly different ($P < 0.05$) within a column.
For each treatment n = 6.

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip;
LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

Table 4. LSMeans for percentage of discoloration for all treatments and days of retail display.¹

Treatment ²	Day of Display							
	0	1	2	3	4	5	6	7
PA15	0.0	0.2 ^b	2.9 ^{ab}	13.0 ^{ab}	45.2	77.0	93.5	99.9
PA180	0.0	0.2 ^b	1.5 ^b	9.1 ^b	37.7	68.1	91.9	99.8
LA15	0.4	0.7 ^{ab}	2.7 ^b	12.1 ^{ab}	43.1	67.9	94.1	99.9
LA180	0.3	1.9 ^a	8.8 ^a	32.9 ^a	67.0	90.8	98.0	100.0
Control	0.0	0.3 ^b	4.2 ^{ab}	16.5 ^{ab}	61.5	89.2	98.3	100.0
SEM	0.24	0.52	2.02	7.81	13.00	9.46	4.42	0.13
<i>P</i> value	0.307	0.017	0.013	0.042	0.144	0.052	0.527	0.661

¹LSmeans with different superscripts (a-c) are significantly different ($P < 0.05$) within a column.

For each treatment $n = 6$.

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip;
LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

Table 5. LSMeans of L* values for all treatments and days of retail display.¹

Treatment ²	Day of Display							
	0	1	2	3	4	5	6	7
PA15	50.24	49.99	50.70	49.25	49.36	48.57 ^b	49.07	49.23
PA180	51.24	50.88	51.13	50.65	50.84	50.52 ^{ab}	50.01	50.25
LA15	51.45	50.82	50.52	49.95	50.35	49.79 ^{ab}	49.68	49.66
LA180	50.73	51.30	51.72	51.20	51.35	51.91 ^a	51.32	51.52
Control	51.07	50.28	50.38	49.51	49.51	48.81 ^b	49.04	48.67
SEM	0.96	1.04	0.95	1.04	0.87	0.94	1.16	0.96
<i>P</i> value	0.739	0.734	0.630	0.336	0.143	0.010	0.299	0.061

¹LSmeans with different superscripts (a-b) are significantly different ($P < 0.05$) within a column. For each treatment n = 6.

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip; LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

Table 6. LSMeans of a* values for all treatments and days of retail display.¹

Treatment ²	Day of Display							
	0	1	2	3	4	5	6	7
PA15	24.57	19.96	17.41	15.16	12.85	10.59	8.81	7.90
PA180	25.04	20.39	17.64	15.96	13.55	10.71	9.30	8.01
LA15	25.10	20.45	17.66	15.03	12.25	10.17	8.59	7.68
LA180	24.03	19.55	16.16	13.28	10.96	8.95	7.90	7.34
Control	25.13	20.75	17.01	14.02	11.05	9.14	7.99	7.56
SEM	1.00	0.55	0.63	1.02	1.23	1.16	1.03	0.42
<i>P</i> value	0.770	0.238	0.132	0.111	0.184	0.427	0.639	0.524

¹LSmeans with different superscripts are significantly different ($P < 0.05$) within a column. For each treatment $n = 6$.

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip; LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

Table 7. LSMeans of b* values for all treatments and days of retail display.¹

Treatment ²	Day of Display							
	0	1	2	3	4	5	6	7
PA15	12.17	10.58	10.10	9.70	10.32	10.35	10.98	11.70
PA180	12.81	11.02	10.13	10.10	10.13	10.40	10.96	11.56
LA15	12.85	11.38	10.47	10.27	10.29	10.63	11.52	11.91
LA180	12.39	11.25	10.59	10.39	10.98	11.32	11.62	12.37
Control	12.58	11.14	10.04	9.84	10.18	10.70	11.07	11.35
SEM	0.61	0.38	0.33	0.36	0.39	0.55	0.47	0.47
<i>P</i> value	0.771	0.315	0.363	0.289	0.221	0.419	0.477	0.270

¹LSmeans with different superscripts are significantly different ($P < 0.05$) within a column. For each treatment n = 6.

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip; LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

Future Research Recommendations

These studies showed the effects of dipping sections of beef clods in organic acids for short or extended times. Each application time was applied using a new solution of organic acid. To further understanding of how effective dipping beef clods into organic acids can be, more research needs to be completed on the efficiency of one organic acid solution when used multiple times. Using one organic acid solution multiple times for different meat sections as an antimicrobial intervention may cause a loss of efficiency at some point. However, little research has been completed to show where this reduction of antimicrobial intervention effectiveness occurs. Small business meat processors implementing this antimicrobial intervention method should have an understanding of when their organic acid solutions will lose effectiveness and should be replaced.

Additionally, treating meat using a multiple-use organic acid dip solution also requires more knowledge of how time application affects bacterial growth. In these studies, beef sections were dipped for 180 s, but this extended application time would not be applicable to large meat processors. Instead, more research needs to be conducted at other exposure times to determine the effectiveness. Once the other exposure times have been studied, a curve of effectiveness can be determined for dipping organic acids. Producers could then use this curve to determine the best usage for their facility.

After multiple treatment effectiveness is determined, more research is also needed on the difference of meat surface type. These studies showed more microbiological reductions for fat meat surfaces when compared to freshly cut lean surfaces. More research is now needed to determine why these reductions are occurring

on fat surfaces instead of lean surfaces. The buffering capacity of a freshly cut lean surface could be responsible for the lesser reduction observed. However, additional research should be conducted to further the understanding of how bacteria are attaching and other additional factors that could be different among meat surface types.

Additionally, conducting further research on the sensory aspects of antimicrobial interventions is needed. Lactic acid dipped for 180 s had detrimental effects on percentage of discoloration and increased lipid oxidation TBA values in these studies. There is now a need to conduct research on how other sensory properties, like smell and taste, affect meat over a period of time when treated with antimicrobial interventions. This could have a significant impact on consumer purchasing decisions. Both consumer and trained sensory panels should be used for this research to determine how organoleptic properties can change over time for meat treated with antimicrobial interventions.

Lastly, the majority of research for antimicrobial interventions has been focused on beef due to the risk of foodborne illness from STEC. More research is now needed on using antimicrobial interventions on other species, such as pork, to reduce pathogens and the effect these antimicrobial interventions have on meat quality. Little research is available for the efficacy of antimicrobial interventions of other red meat species. Small business meat processors could use these antimicrobial interventions for other species besides beef, and thus must be assured of the effectiveness. Therefore, critical research is needed to determine antimicrobial intervention parameters for other species. Effectiveness of multiple applications of organic acids, determining the effect of organic acid solutions on differing meat surface types, as well as other application lengths are all needed areas of research. Additionally, more research on sensory aspects, and

establishing parameters for other species are all needed to determine an effective antimicrobial intervention using an organic acid dip solution.

APPENDICES

Appendix I

Culture Preparation of rifampicin-resistant *E. coli*

1. Pipet 10 mL Tryptic Soy Broth (TSB) into individually labeled 15 mL tubes.
2. Scrape frozen *E. coli* from -80°C (labeled 1427-1431) and combine (step 1).
3. Incubate 15 mL tubes for 2 days at 37°C.
4. Pipet 10 mL TSB into individually labeled 15 mL tubes (1427-1431).
5. Pipet 5 µL Rifampicin into each 15 mL tube.
6. Vortex tubes, then pipet 1 mL of each culture into individually labeled 15 mL tubes (1427-1431).
7. Vortex cultures, then incubate (step 4-6) for 24 hours at 37°C.
8. Mix a 1:1 ratio of culture from each 15 mL tube (1427-1431) together.
9. Combine (step 8) with peptone buffer to create a 9:1 ratio (buffer: culture).
Vortex.
10. Pipet 15 mL of mixture (step 9) into 15 mL tubes for inoculation.
11. Store in <4.4°C before use.
12. When inoculating, invert tubes several times for thorough mixing before application.

Appendix II

Meat Preparation and Inoculation

1. Unwrap beef shoulder clod (IMPS 114) from vacuum package.
2. Separate the top blade muscle from the arm portion by making a straight cut to the blocktop.
3. Cut each portion (top blade and arm) into approximately 2 x 2 inch pieces (12 cm²).
4. Inoculate meat by hand spraying 15 mL of prepared inoculum onto surface of pieces.

Appendix III

pH Measurement

1. Weigh 10 g powdered sample in small beakers in duplicate.
2. Add 90 mL of distilled, deionized water to each small beaker.
3. Homogenize the solution by placing a stir bar into the solution and placing the solution onto a stir plate.
4. Read the pH using a pH meter calibrated using 7.0 and 4.0 Buffer.

Appendix IV

Lipid Oxidation TBA Assay

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 μ l TEP (97%) bring volume to 100 mL ddH₂O

Working Solution: Dilute stock solution to 1:3 (TEP Solution:ddH₂O) (1×10^{-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 ddH₂O.

Dissolve 2.88 g TBA in warm ddH₂O first, then add TCA (150g) and ddH₂O to 1L

BHA (ButylatedHydroxyAnisole) Stock Solution:

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

10g BHA dissolved in 90 mL ethanol (90%) + 5mL ddH₂O

Standards: In duplicate

		<u>Moles of TEP</u>
Blank:	1 ml ddH ₂ O	
Standard 5:	100 μ l working TEP + 1.90 mL ddH ₂ O	(5×10^{-5} M)
Standard 4:	1 mL Std. 5 + 1 mL ddH ₂ O	(2.5×10^{-5} M)
Standard 3:	1 mL Std. 4 + 1 mL ddH ₂ O	(1.25×10^{-5} M)
Standard 2:	1 mL Std. 3 + 1 mL ddH ₂ O	($.625 \times 10^{-5}$ M)
Standard 1:	1 mL Std. 2 + 1 mL ddH ₂ 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 ddH₂O 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 μ 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 (1×10^{-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: $.012 \times \text{concentration} \times 72.063 \times 10^6 = \text{mgs Malonaldehyde/kg of tissue}$

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

Appendix V

Additional Tables And Figures For Study 3

A different statistical analysis was considered with the following results. The following results were found by comparing treatments across all days of retail display. Model included response to an interaction between treatment and day. LSmeans were adjusted using Tukey's adjustment.

Table 1. LSmeans for Total Colony Forming Units (CFU/g) of all treatments and days of retail display.¹

Treatment ²	Day of Display				
	0	1	3	5	7
PA15 ³	3.02 ^g	3.24 ^{fg}	3.98 ^{abc}	4.13 ^{ab}	4.27 ^{ab}
PA180	3.02 ^{fg}	3.21 ^{fg}	3.86 ^{abcde}	4.22 ^{ab}	4.39 ^a
LA15	3.14 ^{fg}	3.19 ^{fg}	3.85 ^{bcde}	4.00 ^{abc}	3.95 ^{abcd}
LA180	3.30 ^{fg}	3.06 ^{fg}	3.43 ^{defg}	3.56 ^{cdef}	3.43 ^{defg}
Control	3.10 ^{fg}	3.38 ^{efg}	4.17 ^{ab}	4.17 ^{ab}	4.26 ^{ab}

¹ LSmeans with different superscripts (a-g) are significantly different ($P < 0.0001$) regardless of row or column.

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip;

LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

³Standard error for LA15, PA15, LA180, and control is 0.14 while PA180 standard error is 0.15.

Table 2. LSmeans for L* a* b* values of all treatments through days of retail display.¹

Treatment²	L*	a*	b*
PA15	49.55 ^c	14.66 ^a	10.74 ^c
PA180	50.69 ^{ab}	15.08 ^a	10.89 ^{bc}
LA15	50.28 ^{bc}	14.62 ^{ab}	11.16 ^{ab}
LA180	51.38 ^a	13.52 ^c	11.36 ^a
Control	49.66 ^c	14.08 ^{bc}	10.86 ^{bc}
SEM	0.2885	0.2046	0.1286
<i>P</i> value	<0.0001	<0.0001	<0.0001

¹ LSmeans with different superscripts (a-c) are significantly different ($P < 0.05$) within a column. No treatment x day interaction for L* ($P = 0.9207$), a* ($P = 0.2333$), b* ($P = 0.8782$).

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip; LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

Table 3. LSmeans for pH for all treatments and days of retail display.¹

Treatment ²	Day of Display				
	0	1	3	5	7
PA15 ³	5.76 ^{ab}	5.75 ^{ab}	5.64 ^{abcd}	5.41 ^{defgh}	5.33 ^{fghi}
PA180	5.62 ^{abcd}	5.73 ^{abc}	5.69 ^{abc}	5.35 ^{efghi}	5.28 ^{ghi}
LA15	5.54 ^{bcdef}	5.55 ^{bcdef}	5.50 ^{cdefg}	5.29 ^{ghi}	5.16 ⁱ
LA180	5.24 ^{hi}	5.32 ^{fghi}	5.21 ^{hi}	5.18 ^{hi}	5.18 ^{hi}
Control	5.81 ^a	5.76 ^{ab}	5.58 ^{abcde}	5.31 ^{fghi}	5.35 ^{efghi}

¹ LSmeans with different superscripts (a-i) are significantly different ($P < 0.0001$) regardless of row or column.

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip; LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

³Standard error for all treatments is 0.06.

Table 4. LSmeans for Lipid Oxidation (mg Malonaldehyde/kg Tissue) for all treatments and days of retail display.¹

Treatment ²	Day of Display				
	0	1	3	5	7
PA15 ³	0.96 ^{gh}	1.47 ^{efgh}	1.80 ^{defgh}	2.46 ^{bcdefgh}	2.86 ^{bcde}
PA180	1.82 ^{defgh}	1.06 ^{fgh}	1.52 ^{defgh}	2.15 ^{cdefgh}	2.62 ^{bcdefg}
LA15	1.15 ^{efgh}	1.78 ^{defgh}	2.69 ^{bcdef}	3.87 ^{ab}	4.65 ^a
LA180	1.34 ^{efgh}	1.71 ^{defgh}	3.62 ^{abc}	4.75 ^b	4.58 ^a
Control	0.82 ^h	1.17 ^{efgh}	2.00 ^{cdefgh}	2.68 ^{bcdefg}	3.22 ^{abcd}

¹ LSmeans with different superscripts (a-h) are significantly different ($P = 0.0009$).

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip; LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

³Standard error for all treatments is 0.46.

Table 5. LSmeans for Percentage of Discoloration for all treatments and days of retail display. ¹

Treatment ²	Day of Display							
	0	1	2	3	4	5	6	7
PA15 ³	0.00 ^j	0.21 ^j	2.88 ^j	12.95 ^{ij}	45.23 ^{efg}	76.96 ^{abcd}	93.50 ^a	99.94 ^a
PA180	0.00 ^j	0.17 ^j	1.46 ^j	9.11 ^j	37.73 ^{gh}	68.10 ^{bcde}	91.94 ^a	99.83 ^a
LA15	0.38 ^j	0.66 ^j	2.67 ^j	12.08 ^{ij}	43.09 ^{fg}	67.87 ^{bcde}	94.14 ^a	99.89 ^a
LA180	0.30 ^j	1.87 ^j	8.77 ^j	32.89 ^{ghi}	67.00 ^{cde}	90.78 ^{ab}	97.97 ^a	100.00 ^a
Control	0.00 ^j	0.34 ^j	4.24 ^j	16.49 ^{hij}	61.52 ^{def}	89.20 ^{abc}	98.31 ^a	100.00 ^a

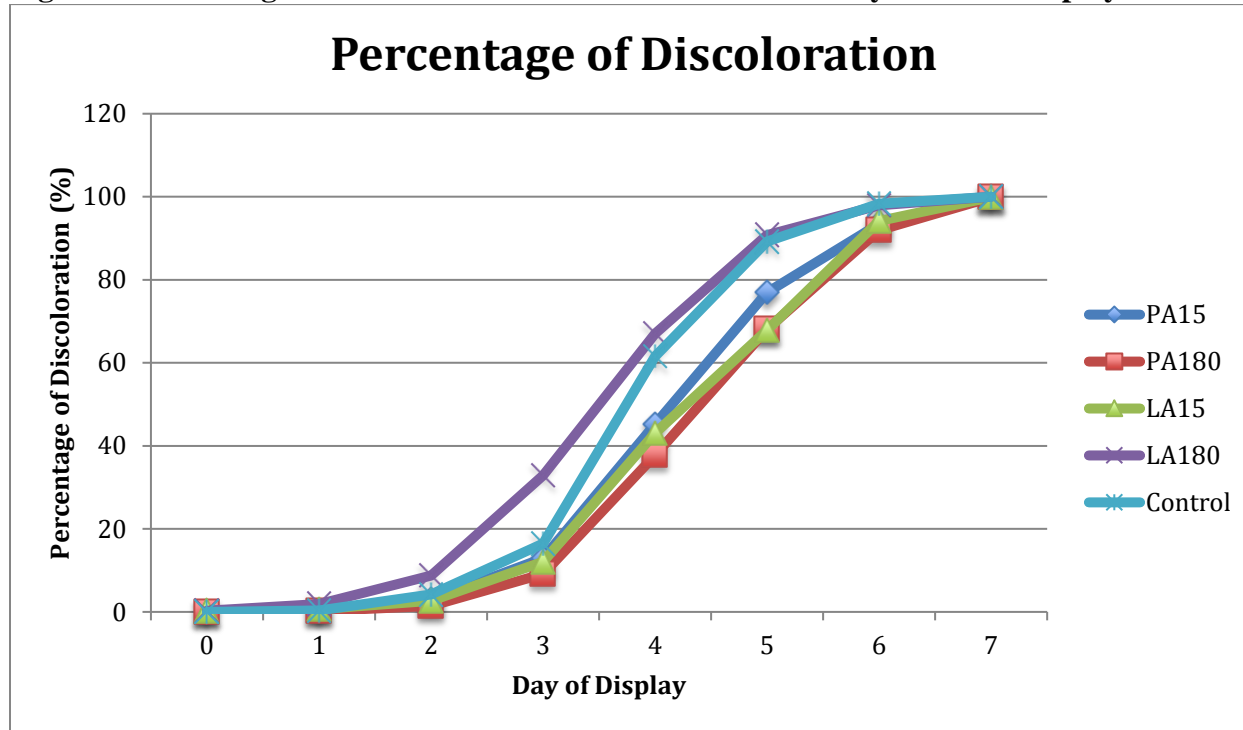
¹ LSmeans with different superscripts (a-i) are significantly different ($P = 0.016$) regardless of row or column.

²PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip;

LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

³Standard error for all treatments is 5.85.

Figure 1. Percentage of discoloration for all treatments¹ and days of retail display.



¹PA15=peroxyacetic acid 380ppm, 15 s dip; PA180=peroxyacetic acid 380ppm, 180 s dip; LA15=lactic acid 4.5%, 15 s dip; LA180=lactic acid 4.5%, 180 s dip; Control = no organic acid treatment.

²Standard error for all treatments is 5.85.