FINAL REPORT

Antimicrobial interventions/application methods for the reduction of *Escherichia coli* O157:H7 and *Salmonella* in beef trimming and/or ground beef.

August 15, 2012

SUBMITTED TO:
American Meat Institute Foundation

SUBMITTED BY:
University of Arkansas Division of Agriculture
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EXECUTIVE SUMMARY:

Project Title: Antimicrobial interventions/application methods for the reduction of *Escherichia coli* O157:H7 and *Salmonella* in beef trimming and/or ground beef.

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Objectives:

The overall goal of this research was to evaluate practical and cost-effective decontamination technologies for beef processors that can be rapidly implemented using antimicrobial properties of peroxyacetic acid, novel organic acids alone or in combination with a non-ionic surfactant (ethoxylated glyceride, EG) on beef trimmings against *E.coli* O157:H7, O26, O103, O111, O121, O45, and O145 and *Salmonella* Typhimurium DT 104, Newport MDR-AmpC to achieve maximum ground beef product safety without altering product quality through effective treatment application technologies.

Conclusions:

The results of this study indicated that conventional spray application of peroxyacetic acid (PA) followed by conventional or electrostatic spray application of octanoic acid (PO), pyruvic acid (PP), malic acid (PM) or fumaric (PF) on beef trimmings may effectively reduce *E.coli* O157:H7, non-O157:H7 and *Salmonella* populations on subsequent ground beef. Futhermore, the use of electrostatic spray application of antimicrobials was effective for reducing microbial numbers but used much less antimicrobials than conventional spray applications. Thid resulted in greater savings to achieve enhanced safety using electrostatic spray as compared to conventional spray application. In addition these treatments had little or no impact on ground beef instrumental color, sensory color and odor, and processing characteristics thereby leaving them similar in color and sensory characteristics as untreated ground beef.

Deliverables: The electrostatic atomization uses electrically charged droplets of antimicrobials that will be attracted to the target meat samples. The results indicate that ES application of some organic acids may have similar or greater efficiency in controlling ground beef microbial populations application compared to the CS application of the same acid. An added advantage was that the ES method provided uniform coverage of antimicrobial on exposed surfaces of meat allowing efficient antimicrobial usage with less wastage. Therefore, the ES system establishes a

more economical and waste manageable decontamination approach in controlling *E. coli* O157:H7 or non-O157H7 and *Salmonella* populations in ground beef without causing deleterious effects on color properties. This opens new avenues to utilize economically unfeasible yet efficient natural organic acids such as pyruvic, malic, octanoic and fumaric acids in a more cost effective manner in meat decontamination.

Technical Abstract

Recent large numbers of ground beef recalls due to presence of possible *Escherichia coli* or *Salmonella* contaminations warrants novel and efficient decontamination applications to enhance ground beef microbial safety and quality. The purpose of this study was to evaluate the effectiveness of antimicrobial interventions using peroxyacetic acetic acid (PAA) followed by novel organic acids with and without ethoxylated glycerol (EG) using electrostatic spray (ES) and conventional spray (CS) on beef trimmings prior to grinding on ground beef microbial populations. Beef trimmings (80/20; 50 lb) were inoculated with *E. coli* O157:H7 and non-STEC O157:H7 (EC) and *Salmonella* spp. (SA) cocktail mixture at 10^5 CFU/g. Inoculated trimmings (3 lb/treatment/replicate, 2 replicates) were treated through conventional spray application of 0.02% PAA alone or followed by CS or ES application of 3% octanoic acid (PO), 3% pyruvic acid (PP), 3% malic acid (PM), saturated solution of fumaric acid (PF) or deionized water (W) with or without incorporation of 1% ethoxylated glyceride (EG). Following treatment applications, trimmings were ground twice and were placed on plastic foam trays with absorbent pads and overwrapped with polyvinyl chloride film and sampled on days 0, 1, 2, 3 and 7 days for microbial counts and instrument color properties. An additional study using the same treatments was carried out on un-inoculated beef trimmings to evaluate ground beef visual color and odor characteristics. Findings from this study suggest that conventional spray application of PA as a single or multiple chemical hurdle approach with conventional or electrostatic spray application of malic, pyruvic, octonoic and fumaric acid on beef trimmings may be effective in reducing *E. coli* O157:H7 as well as non-STEC serotypes and *Salmonella* up to day 2 of display. The incorporation of surfactant (EG) in the treatment did not enhance the microbial safety by both application methods. The results also indicate that ES application of some organic acids may have similar or greater efficiency in controlling ground beef microbial populations application compared to the CS application of the same acid. Results of this study showed that instrumental color properties and sensory color odor and processing characteristics of ground beef treated with peroxyacetic acid followed by organic acids had little or no difference ($P > 0.05$) compared to the untreated un-inoculated control ground beef samples. The results also suggests that ES system establishes a more economical and waste manageable decontamination approach in controlling *E. coli* O157:H7 on non-O157H7 and *Salmonella* populations in ground beef without causing deleterious effects on color and odor quality properties.

Goals/Objectives

1. Evaluate antimicrobial properties and effectiveness of peroxyacetic acid alone, or in combination with novel organic acids (fumaric, malic, citric gluconic, levulinic, pyruvic, caprioc, caprylic, capric acids using electrostatic spray versus conventional spray for the inactivation of *E.coli* O157:H7, O26, O103, O111, O121, O45, and O145 and *Salmonella* Typhimurium DT 104, Newport MDR-AmpC on beef trimmings destined for ground beef.
2. Compare conventional spray treatment versus electrostatic spray method using peroxyacetic acid and novel organic acids from objective 1 alone or in combination with a non-ionic surfactant (ethoxylated glycerides, EG) as an effective decontamination method against *E.coli* O157:H7, O26, O103, O111, O121, O45, and O145 and *Salmonella* Typhimurium DT 104, Newport MDR-AmpC on beef trimmings before ground beef production.
3. Optimize quality attributes and retail display properties of un-inoculated ground beef produced from decontaminated beef trimmings from most effective antimicrobial treatments selected from objectives 1 & 2 during a simulated retail display for 0, 2, 5, and 7 days.

Material and Methods

Inoculation preparation.

Screening study: Frozen culture (-80°C) of *Escherichia coli* O157:H7 was thawed and 0.1 ml of bacterial suspension was dispensed into 10 ml aliquots of brain heart infusion (BHI; BD, BBL™, Becton Dickinson and Company, Sparks, MD) broth. Following 18 h of incubation at 37°C (Beckman GS-6 series, Fullerton, CA), bacteria were harvested by centrifugation (3500 *g* for 20 min at 25°C; Beckman GS-6 series, Fullerton, CA), and re-suspended in 0.1% buffered peptone water (BPW; Difco Laboratories, Becton Dickinson and Company, Sparks, MD). The bacterial suspension was further diluted with BPW to achieve 10^5 CFU/ml of *E. coli* O157:H7 suspension and stored at 4 °C until further use.

Objectives 1 and 2: A bacterial cocktail containing 10^5 log CFU *E.coli* O157:H7, O26, O103, O111, O121, O45 and O145, *Salmonella* Typhimurium DT 104, and *S. Newport* were prepared from frozen (-80°C) pure cultures. To make the cocktail, 0.1 ml of each strain were inoculated into 10 ml aliquots of Brain Heart Infusion solution (BHI; Difco Laboratories Becton Dickinson and Company, Sparks MD). The inoculated tubes were incubated at 37°C for 18 hours non-shaking. Following incubation, the tubes were centrifuged at 3500*g* for 20 minutes at 37°C (Beckman GS-6 series, Fullerton, CA). Then the liquid supernatant was discarded and the bacterial pellets were re-suspended with buffered peptone water (BPW; Difco Laboratories, Becton Dickinson and Company, Sparks MD) to achieve a 10^5 CFU/ml cocktail mixture of EC and SA.

Meat inoculation.

Screening study: Beef trimmings (80/20; 5 kg) obtained from Cargill Meat Solutions (Plainview, TX) were thawed and inoculated with the *E. coli* O157:H7 (EC) bacterial suspension (10^5 CFU/ml) in a sterile bag and placed at 4 °C for 12 to 14 hr for further microbial attachment.

Objective 1 and 2: Thawed beef trimmings (80/20; 27.3 kg) obtained from Cargill Meat Solutions (Plainview, TX) were submerged in a cocktail mixture of *E.coli* (EC) and *Salmonella* (SA) at10^5 CFU/g in a sterile bags. Then the inoculated trimmings were separated into 18 -20 batches and left overnight at 4°C for bacterial attachment.

Treatment application.

Screening study: Inoculated beef trimmings (25g/treatment/replicate) were dipped in 100 mL solutions of 0.02% peroxyacetic acid (PAA) and 3% of novel organic acids [fumaric acid (FA); malic acid (MA); citric acid (CA); gluconic acid (GA); levulinic acid (LA); pyruvic acid (PY); caprioc (decanoic) acid (CR), caprylic (octanoic) acid (CL), and capric (hexanoic) acid (CP)] for 15 s as single antimicrobial interventions. In a second screening study, inoculated beef trimmings (25 g/ treatment/replicate) were dipped in ethoxylated glyceride (EG) incorporated novel organic acids [fumaric acid (FA); malic acid (MA); citric acid (CA); gluconic acid (GA); levulinic acid (LA); pyruvic acid (PY); caprioc (decanoic) acid (CR), caprylic (octanoic) acid (CL), and capric (hexanoic) acid (CP)] for 15 s. In addition, inoculated beef trimmings treated with and without water (IN and IN+W) and un-inoculated samples without treatments (UN) were run as controls of the experiment.

Objective 1: The inoculated beef trimmings (1.36 kg/treatment/replicate) were arranged on stainless steel trays. Then each side of the beef trimmings were treated with conventional spray (~0.1ml/g) applications of 0.02% peroxyacetic acid (PA; Blitz®, FMC Corporation, Philadelphia, PA) alone or followed by conventional (CS) or electrostatic spray (ES; Electrostatic Spraying Systems, Inc. Watkinsville, GA) applications (~0.06ml/g) of deionized water (W), 3% Malic acid (PM; Sigma Aldrich St. Louis MO), 3% Pyruvic acid (PP; Sigma Aldrich St. Louis MO), 3% Octonoic acid (PO; Sigma Aldrich St. Louis MO), and saturated solution of Fumaric (PF; Sigma Aldrich St. Louis MO). As per manufacturer's instructions peroxyacetic acid treatment was applied only through conventional spray system. The PA- treated samples were allowed to drip for 3 min prior to and after assigned second antimicrobial applications (2 replicates / treatment). Inoculated beef trimmings were also treated with the conventional and electrostatic spray applications of de-ionized water (W) at the same rates used in antimicrobial applications and dripped for 3 min.

Objective 2: The same procedures explained in objective 1 were followed to inoculate beef trimmings (22.7 kg). Beef trimmings arranged in sterile stainless steel trays were spray treated using an electrostatic spray system (ES) and conventional sprayer (CS) with PA (0.02%) alone or followed by deionized water (W), 3% pyruvic acid (PP) or 3% octanoic acid (PO) with (PP+EG, PO+EG) or without (PP, PO) incorporation of 1% ethoxylatedd glyceride.

Objective 3: The same treatments described in objective 2 was applied on un-inoculated beef trimmings (80/20; 27.2 kg) through ES and CS spray methods and ground beef was evaluated for instrumental color and sensory characteristics.

Meat processing.

Objectives 1, 2, and 3: All treated and untreated inoculated (CON) beef trimmings were ground (American Eagle Model: AEG-12N, #14 (32 cm) chopper plate) twice and 200g of individual

samples were placed on plastic foam trays and over wrapped with polyvinyl chloride film (O_2 transmission rate = 14,000cc/mm2/24h/1atm; Koch Supplies, Inc. Kansas City, MO). The packages were stored under retail display condition (4°C; 1,630 lx of deluxe warm white fluorescent lighting; Phillips Inc., Somerset, NJ, USA) and sampled on day 0, 1, 2, 3, and 7 day of display for microbial analysis and CIE L*, a* and b* measurements.

Microbial enumeration.

Screening study: Following the antimicrobial treatment dipping applications, beef trim (25g/treatment/replicate) was placed in sterile whirl pack bags (Nasco, Ft Atkinson, WI) and homogenized for 2 minutes in a stomacher (Model 400 Lab Stomacher; Seward, London, UK) with 225 ml of 0.1% BPW. Subsequently, serial 10-fold dilutions were made and spread plating was performed in duplicates on aerobic plate counts (APC), and E. coli O157:H7 (EC) / coliform (CO) counts of Petrifilm® plates (3M Corporation, St. Paul, MN, USA) and incubated at 37 °C in an aerobic incubation chamber (VWR Model 5015 and Model 3015 incubators, VWR Scientific, Cornelius, OR). All counts were recorded as colony forming units per gram (CFU / g).

Objectives 1 and 2: Each ground beef sample (25g) was mixed with 225 ml of 0.1% buffered peptone water in sterile whirlpack bags (Nasco, Ft Atkinson, WI) separately and stomached for 2 minutes at normal speed (Model 400 Lab Stomacher; Seward, London, UK). Subsequently, serial 10-fold dilutions were made and spread plated (SA counts on *Salmonella* shigella agar (DIFCO Laboratories, Detroit, MI), aerobic plate count (APC), and *E. coli* (EC) / coliform (CO) counts on Petrifilm® (3M Corporation, St. Paul, MN) were done in duplicates. The EC, APC and ST counts were read after 48 h incubation at 37 °C, whereas coliform plates were read after 24 h (*2, 4*).

Instrumental color.

Objectives 1, and 3*:* Instrumental color was measured (n=3/treatment) using a Hunter-Lab MiniScan XE Spectrocolorimeter, Model 4500L (Hunter Associates Laboratory, Reston, West Virginia). The samples were evaluated for CIE L^* (Lightness), a^* (redness), and b^* (yellowness), hue angle (arctan (b^*/a^*), saturation index $((a^{*2}+ b^{*2}))^{0.5}$, and reflectance ratio (630/580 nm). All the values were determined from the mean of three measurements of each ground beef sample using Illuminant A/10° observer (*1, 3, 4*).

Sensory color and odor characteristics.

Objective 3: An eight member panel was selected and trained by an experienced panel leader according to the American Meat Science Association guidelines (AMSA, 1995). Packages were evaluated under simulated retail lighting conditions (deluxe warm white fluorescent lighting, 1630 lx) for overall color, worst point color and percentage discoloration. Packages were then opened, and evaluated by panelists for beef odor and off odor characteristics. Linear scales were used to evaluate overall color and worst point color (5=bright red, 4=dull red, 3= slightly brownish red, 2= moderately brownish red, 1= brown), percent discoloration (7=no discoloration 0%, 6=slight discoloration 1-20%, 5=small discoloration 20-39%, 4=modest discoloration 40-59%, 3=moderate discoloration 60-79%, 2=extensive discoloration 80-95%, 1=total discoloration 96-100%). Panelists also evaluated beef odor (8= extremely beef like, 7=very beef like, 6=moderately beef like, 5=slightly beef like, 4=slightly non beef like, 3=moderately non beef like, 2=very non beef

like, 1=extremely non beef like) at the same display intervals. Off odor were also evaluated (5= no off odor, 4=slight off odor, 3=small off odor, 2=moderate off odor, 1=extreme off odor) (*1, 3, 5*).

Processing properties.
Objective 3: A trained panel (8 members) evaluated smearing (6=extreme smearing, 5=moderate smearing, 4=slight smearing, 3=slight cut-grind, 2=moderate cut grind, 1=extreme cut grind) and patty forming ability (6=extremely fragile, 5=moderately fragile, 4=slightly fragile, 3=slightly fragile, 2=moderately cohesive, 1=extremely cohesive) of ground beef for each treatment on day 0 immediately after the grinding process (*3*).

Statistical analysis.
The experimental design included treatments applied using two methods of spraying and 5 display days (0, 1, 2, 3 and 7). Treatments were blocked by replicate and then analyzed for the main effects of antimicrobial treatment, day of display and treatment by day interactions. Sensory data was analyzed using Proc Mixed procedure of SAS to perform Type III test of fixed effects. Least square means for significant main effect were identified using the LSMEANS PDIFF option of SAS (version 9.2, SAS Institute Inc., Cary, NC).

Results

Effects of peroxyacetic acid and other novel organic acids on microbial properties of beef trimmings (Screening study I).

Coliform
Among all novel organic acids, 3% CL was most effective ($P < 0.05$) in reducing the counts of CO to non-detectable limits on inoculated beef trimming (Fig. 1). Antimicrobial treatment with 3% of FA, MA, PY, and CR reduced ($P < 0.05$) 3.33, 2.23, 2.48, and 0.82 logs of CF counts as compared to IN+W. Treatments of beef trimmings with 0.02% PAA and 3% of CA, LA, and CP reduced ($P < 0.05$) ~ 3.5 logs of CF as compared to IN. The decontamination treatment with GA was comparable to treatment with water (IN+W) in reducing in CF counts.

Escherichia coli
The CL treatment (Fig. 2) reduced ($P < 0.05$) EC counts to a non-detectable level on inoculated beef trimmings. Among all novel organic acids, CL had the most deleterious effect on EC survival followed by FA and MA treatments. Antimicrobial treatment of beef trimming with 3% of FA and MA reduced ($P < 0.05$) 3.07 and 2.26 logs of EC as compared to IN+W. Novel organic acids such as CA, PY, LA, CR, CP, and PAA had ~0.40 logs less ($P < 0.05$) EC counts compared to IN. Among all novel organic acids GA was least effective ($P < 0.05$) in reducing EC.

APC counts
Antimicrobial decontamination of beef trimmings using CL and FA resulted ($P < 0.05$) in 2.60 and 2.16 log CFU/g reduction of APC, respectively compared to IN (Fig.3). Un-inoculated beef trim not treated with antimicrobials showed 2.63 logs of aerobic bacterial loads. Treatment of inoculated beef trimming with other novel organic acids (MA, PY, CR, and CP) including PAA

reduced ($P < 0.05$) ~ 0.2 log of APC counts as compared with controls. Among organic acids, CA and GA exhibited least numeric reductions (≤ 0.1 logs) of APC counts.

Effects of non-ionic surfactant incorporated novel organic acids as antimicrobial interventions (Screening study II).

Beef trimmings microbial populations

Coliform

Incorporation of 0.5% ethoxylated glyceride surfactant in 3% novel organic acids and 0.02 % peroxyactic acid solutions did not result a significant coliform count reduction ($P > 0.05$) compared to the same treatment without surfactant except in the case of MA (Fig. 4). Among the most effective organic acids, CL reduced ($P < 0.05$) CO counts to non-detectable level when applied alone. However CO populations were reduced ($P < 0.05$) by 2.04-log CFU/g when beef trimmings were treated with CL in combination with 0.5% EG (CL+Surf). There was a 1.37 and 1.31 log CFU/g reduction ($P < 0.05$) in coliform counts when beef trimmings were treated with 3% PY alone and 3% PY in combination with 0.5% EG (PY+Surf), respectively (Fig. 4). Treatment of inoculated beef trimming with CR and CR+Surf reduced ($P < 0.05$) coliform populations by 1.04 log CFU/g and 0.82-log CFU/g, respectively. Among all antimicrobials used in this study, PAA exhibited a reduction ($P < 0.05$) of coliform populations by 0.64 when treated alone and 0.72-log CFU/g in combination with 0.5% EG (Surf) as compared with control. Among all novel organic acids, 3% MA exhibited a greater reduction of coliform population with 0.5% EG (Surf). When applied alone 3% MA was not effective in reducing coliform counts while combination of 0.5% EG (Surf) and 3% MA reduced ($P < 0.05$) coliform population by 0.61 log CFU/g. Among other novel organic acids, 3% LA reduced ($P < 0.05$) 0.3-log individually and 0.2 log CFU/g in combination with 0.5 % EG (LA+Surf) as compared with IN. Compared to the control (IN), 0.5 % EG (Surf) had 0.37 log CFU/g less ($P < 0.05$) coliform counts (Fig. 4).

Escherichia coli

Novel organic acids at 3% level when applied in combination with 0.5% EG (Surf) enhanced the reduction of *E. coli* O157:H7 (EC) on inoculated beef trimmings except CL and LA (Fig. 5). Among all treatments, 3 % CL without surfactant was most effective and reduced ($P < 0.05$) EC population to a non-detectable level (Fig. 5). A combination of 0.5% EG (Surf) and 3% CL however, was relatively less effective and reduced EC populations ($P < 0.05$) by 2.09 log CFU/g on inoculated beef trimming (Fig. 5). EC populations were decreased by 0.19 and 0.66-log CFU/g by 3% MA and 3% MA in combination with 0.5% EG (MA+Surf) respectively. Antimicrobial treatment of inoculated beef trimming with 3% of PY reduced ($P < 0.05$) EC populations by 1.14 log CFU/g. However (PY+Surf) enhanced microbial reduction of EC and reduced 1.55 log CFU/g of EC populations on inoculated beef trimming. Inoculated beef trimmings with EC when treated with CR alone reduced EC counts by 0.67 log while combination of 3% PY with 0.5% EG (Surf) resulted into 0.93-log CFU/g reduction. Application of 0.02% PAA on inoculated beef trimming was also effective against *E. coli* O157:H7 population (Fig. 5). When treated alone, 0.02% PAA reduced EC populations by 0.33 log CFU/g and the combination of 0.02% PAA with 0.5% EG (Surf) provided 0.89-log CFU/g reduction in microbial counts of EC. Among other novel organic acids, addition of 0.5% EG (Surf) with 3% LA did not enhance LA antimicrobial activity against *E. coli* O157:H7 population. Beef trimming when treated with 3% LA alone or in combination with 0.05% EG (Surf) resulted in ~ 0.3 log reduction of EC populations as compared to the control.

The 0.5 % EG (Surf) alone reduced ($P < 0.05$) EC counts by 0.73 log CFU/g on inoculated beef trimmings (Fig. 5).

Aerobic plate counts:

Effect of 0.5% surfactant (Surf) alone was not effective ($P > 0.05$) in reducing APC on inoculated beef trimmings (Fig. 6). However, 0.5% EG (Surf) when applied in combination with novel organic acids such as 3% MA was effective ($P < 0.05$) in reducing APC counts by 1.13 log CFU/g on inoculated beef trim. Without surfactant (0.5% EG (Surf)), 3% MA alone reduced ($P < 0.05$) 0.79 log CFU/g of APC as compared to the control IN (Fig. 6). Among all novel organic acids, CL was the most effective in decreasing APC; when treated with 3% of CL on inoculated beef trimming, CL reduced ($P < 0.05$) APC counts by 3.38 log CFU/g, however when beef trimmings were treated with 3% of CL in combination with 0.5% EG (Surf), APC reduced ($P < 0.05$) by 2.0 log CFU/g. Antimicrobial treatment of inoculated beef trimming with 3% PY in combination with and without 0.5% EG (Surf) reduced ($P < 0.05$) APC by 1.46 log CFU/g and 1.86 log CFU/g, respectively (Fig. 6). Treatment with 3% of CR of inoculated beef trimming reduced ($P < 0.05$) APC by 1.29 log CFU/g while 3% CR in combination with 0.5% EG (Surf) reduced ($P < 0.05$) APC by 1.49 log CFU/g. Among all novel organic acids, LA seemed to be the least effective when applied with 0.5% EG (Surf) against APC on inoculated beef trimmings. The LA treatment reduced ($P < 0.05$) 0.96 log CFU/g of APC whereas (LA+Surf) reduced ($P < 0.05$) 0.5 log CFU/g of APC on inoculated beef trimmings. Among all antimicrobials tested in this study for the reduction of APC on inoculated beef trimmings, 0.02% PAA exhibited 0.64 log CFU/g reduction, PAA+Surf reduced ($P < 0.05$) 0.41 log CFU/g as compared with control (Fig. 6).

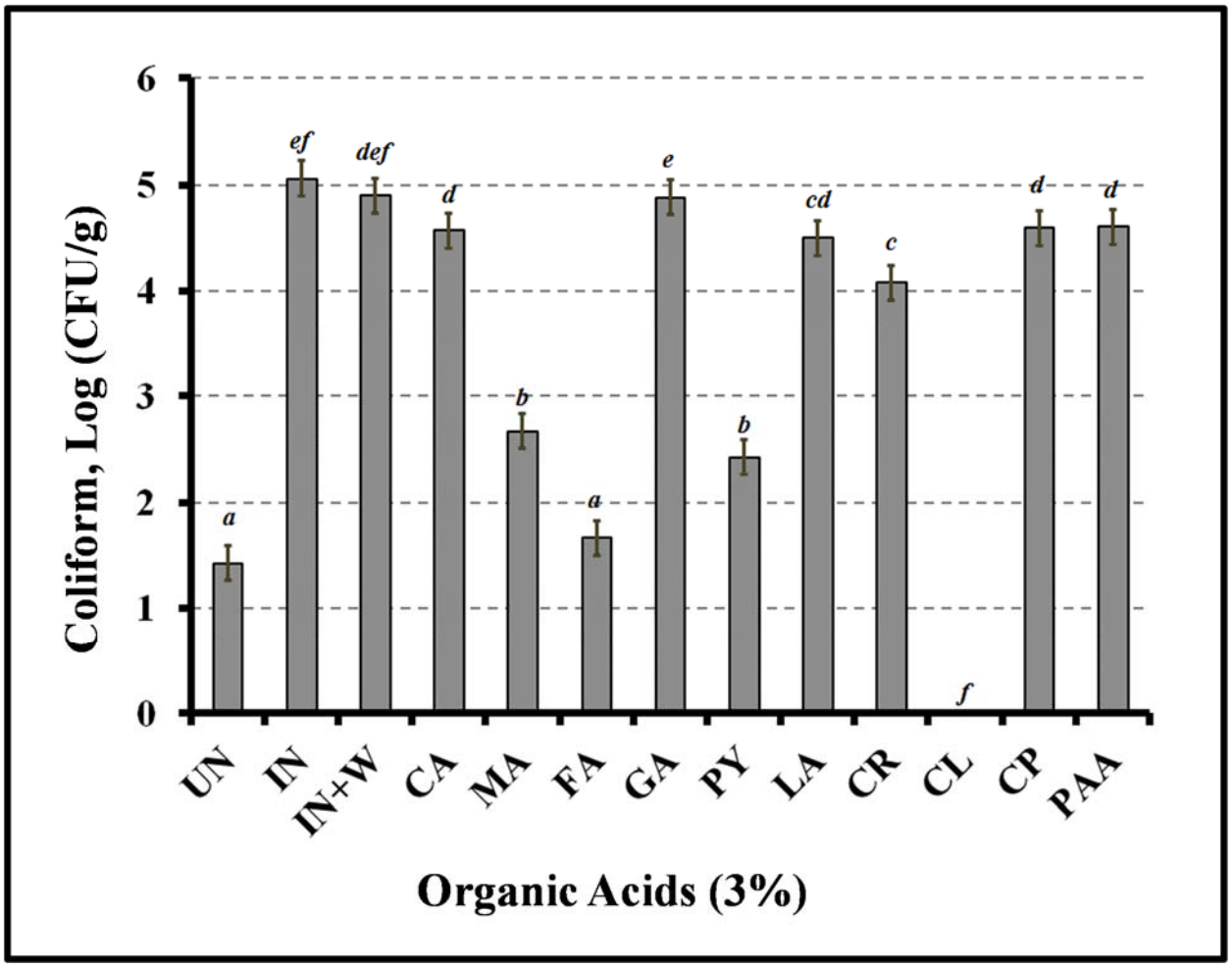

FIGURE 1. Effects of 3% novel organic acids on reduction of coliform counts on inoculated beef trimmings. Standard error = 0.16; UN = un-inoculated and untreated, IN = inoculated and untreated IN+W = inoculated and treated with water, CA = citric acid (3%), MA = malic acid (3%), FA = fumaric acid (3%), GA = gluconic acid (3%), PY = pyruvic acid (3%), LA = levulinic acid (3%), CR = caproic acid (3%), CL = caprylic acid (3%), CP = capric acid (3%), PAA = peroxyacetic acid (0.02%). $^{a\text{-}f}$*Bars with different superscripts differ (P < 0.05).*

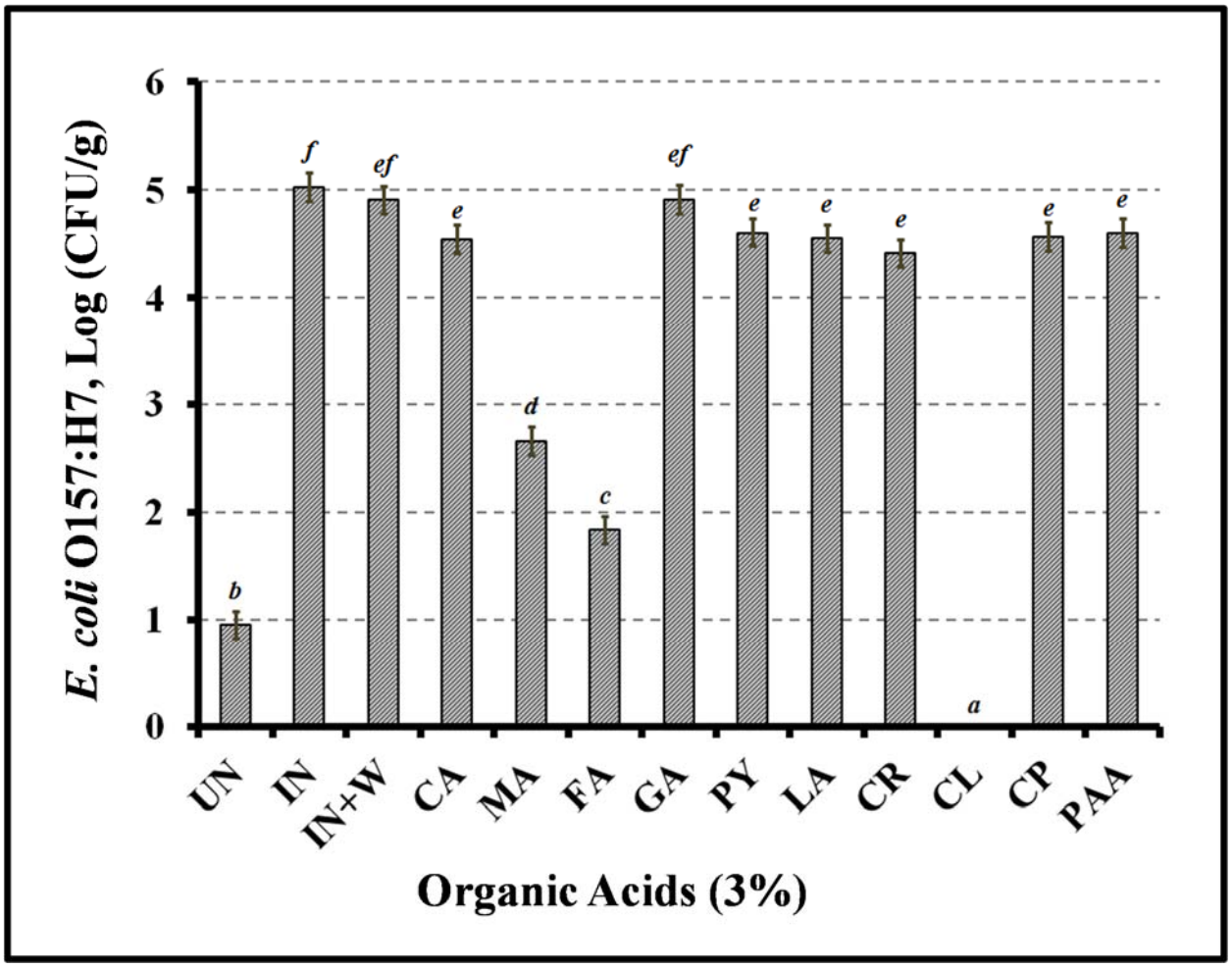

FIGURE 2. Effects of 3% novel organic acids on reduction of E.coli O157:H7 counts on inoculated beef trimmings. Standard error = 0.13; UN = un-inoculated and untreated, IN = inoculated and untreated IN+W = inoculated and treated with water, CA = citric acid (3%), MA = malic acid (3%), FA = fumaric acid (3%), GA = gluconic acid (3%), PY = pyruvic acid (3%), LA = levulinic acid (3%), CR = caproic acid (3%), CL = caprylic acid (3%), CP = capric acid (3%), PAA = peroxyacetic acid (0.02%). $^{a\text{-}f}$*Bars with different superscripts differ (P < 0.05).*

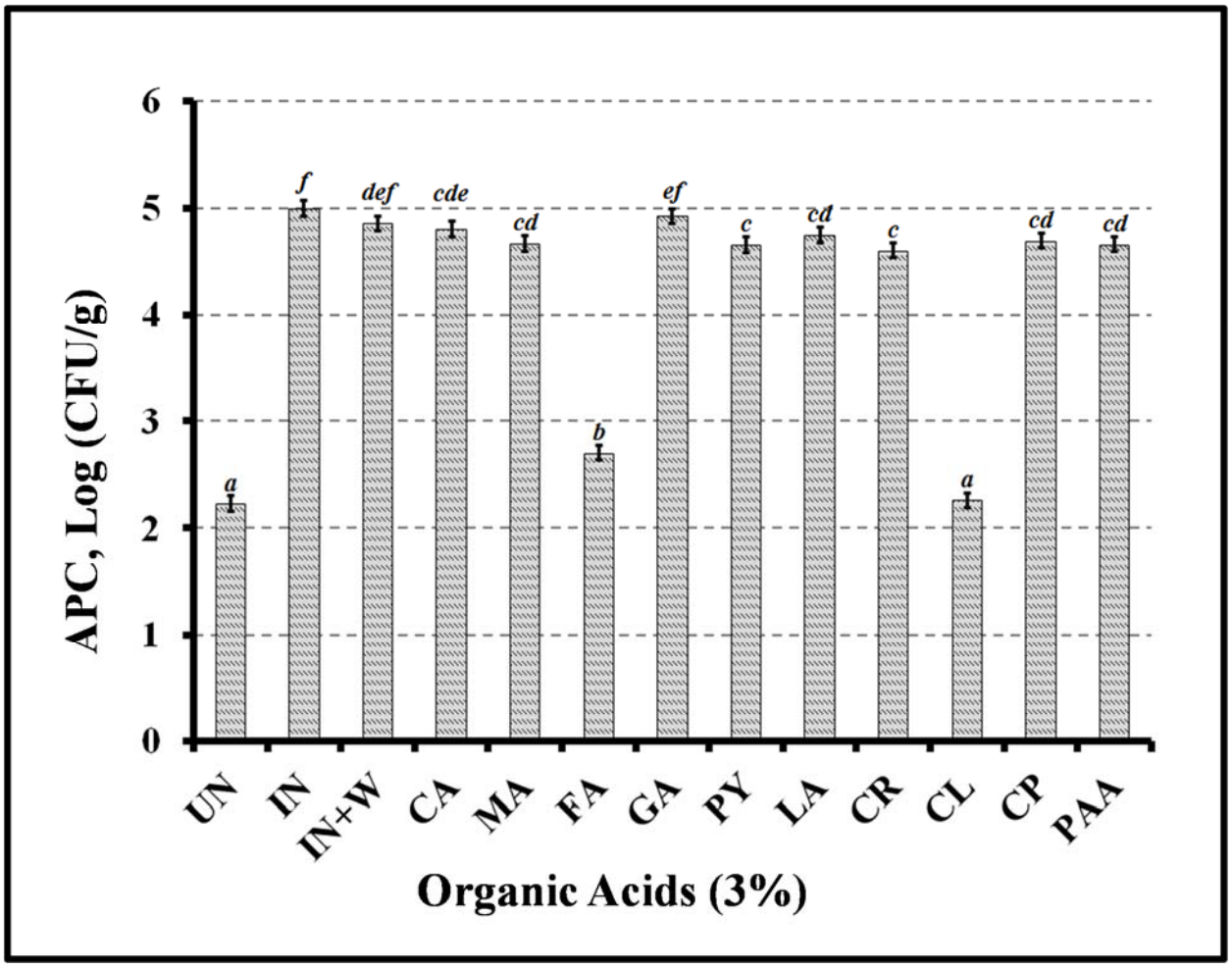

FIGURE 3. Effects of 3% novel organic acids on reduction of aerobic plate counts (APC) on inoculated beef trimmings. Standard error = 0.07; UN = un-inoculated and untreated, IN = inoculated and untreated IN+W = inoculated and treated with water, CA = citric acid (3%), MA = malic acid (3%), FA = fumaric acid (3%), GA = gluconic acid (3%), PY = pyruvic acid (3%), LA = levulinic acid (3%), CR = caproic acid (3%), CL = caprylic acid (3%), CP = capric acid (3%), PAA = peroxyacetic acid (0.02%). $^{a\text{-}f}$Bars with different superscripts differ ($P < 0.05$).

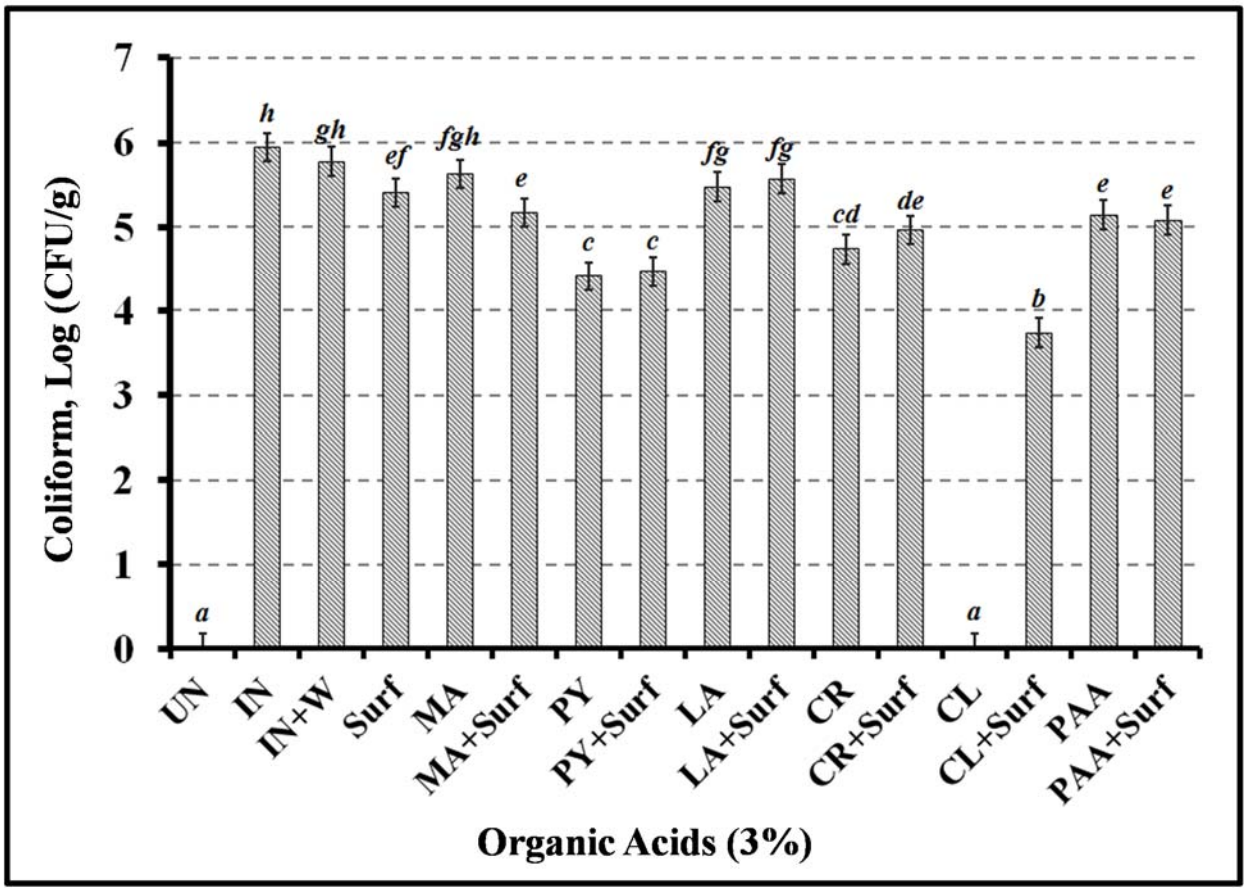

FIGURE 4. Effects of 3% novel organic acids with and without 0.5% ethoxylated glycerol (Surf; a non-ionic surfactant) on reduction of coliform counts on inoculated beef trimmings. Standard error = 0.17; UN = un-inoculated and untreated, IN = inoculated and untreated IN+W = inoculated and treated with water, CA = citric acid (3%), MA = malic acid (3%), FA = fumaric acid (3%), GA = gluconic acid (3%), PY = pyruvic acid (3%), LA = levulinic acid (3%), CR = caproic acid (3%), CL = caprylic acid (3%), CP = capric acid (3%), PAA = peroxyacetic acid (0.02%). $^{a\text{-}h}$*Bars with different superscripts differ (P < 0.05).*

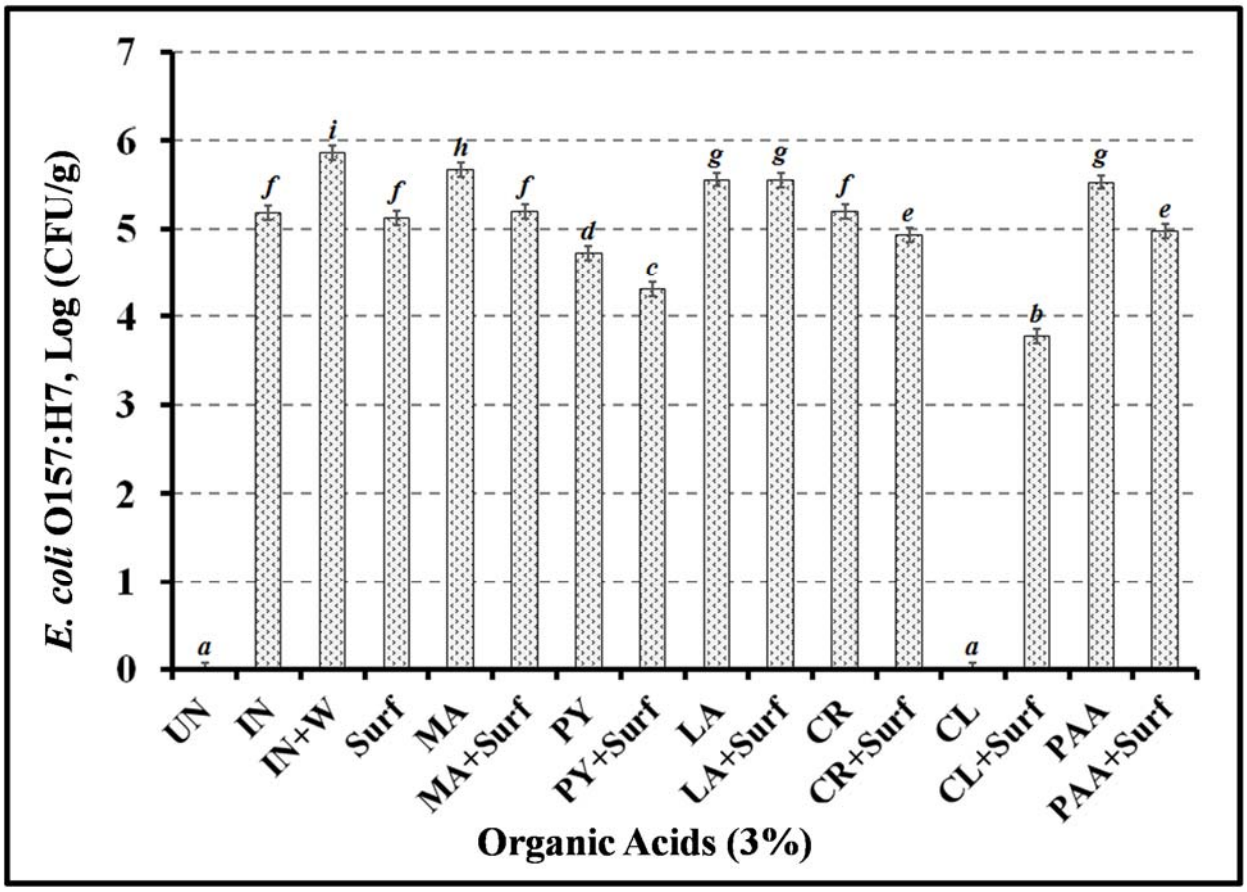

FIGURE 5. Effects of 3% novel organic acids with and without 0.5% ethoxylated glycerol (Surf; a non-ionic surfactant) on reduction of E.coli O157:H7 counts on inoculated beef trimmings. Standard error = 0.08; UN = un-inoculated and untreated, IN = inoculated and untreated IN+W = inoculated and treated with water, CA = citric acid (3%), MA = malic acid (3%), FA = fumaric acid (3%), GA = gluconic acid (3%), PY = pyruvic acid (3%), LA = levulinic acid (3%), CR = caproic acid (3%), CL = caprylic acid (3%), CP = capric acid (3%), PAA = peroxyacetic acid (0.02%). $^{a\text{-}i}$*Bars with different superscripts differ (P < 0.05).*

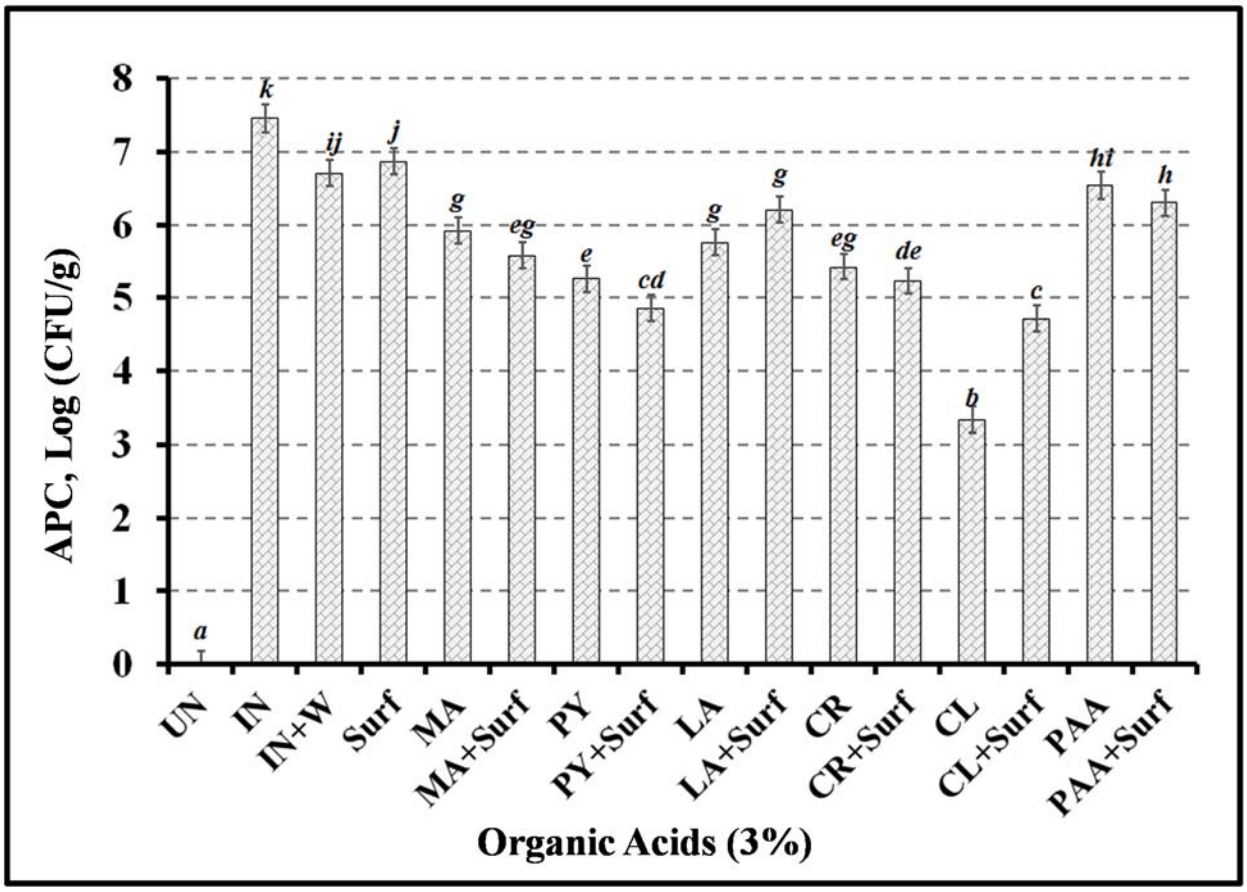

Figure 6. Effects of 3% novel organic acids with and without 0.5% ethoxylated glycerol (Surf; a non-ionic surfactant) on reduction of aerobic plate counts (APC) on inoculated beef trimmings. Standard error = 0.18; UN = un-inoculated and untreated, IN = inoculated and untreated IN+W = inoculated and treated with water, CA = citric acid (3%), MA = malic acid (3%), FA = fumaric acid (3%), GA = gluconic acid (3%), PY = pyruvic acid (3%), LA = levulinic acid (3%), CR = caproic acid (3%), CL = caprylic acid (3%), CP = capric acid (3%), PAA = peroxyacetic acid (0.02%). $^{a\text{-}k}$*Bars with different superscripts differ (P < 0.05).*

Microbial and Instrumental color characteristics of ground beef processed from beef trimmings decontaminated with electrostatic and conventional spray applications of peroxyacetic acid alone or followed by novel organic acids. (Objective 1)

Ground beef microbial populations

Coliform:

All the treatments showed a significant reduction ($P < 0.05$) in ground beef coliform counts compared to the inoculated control on day 0 (Table 1.1). However, the PA, W and PM treatments by CS application, PM, PP, and PF treatments by ES application showed more than 1 log reduction ($P < 0.05$) of ground beef coliforms (CO) compared to the other treatments by both CS and ES methods. Considering all the treatments and application methods, PM (1.8 log) and PP (1.75 log) by CS method and PP (1.11 log), and PO (1.02 log) by ES methods were most efficient in CO log reduction ($P < 0.05$) for day 1 of display. Conversely, PA, W by ES, PP by CS and PF by ES treatments exceeded ($P < 0.05$) the other treatments in controlling CO counts with > than 1 log reduction on day 2 of display except for PF by ES. None of the treatments showed significant CO reductions ($P > 0.05$) on day 7 of display. The PP treatment applied through ES system outperformed the CS application in controlling ground beef CO population on day 0 of display. On the other hand, there was no significant difference ($P > 0.05$) between CS vs. ES methods of W, PM, PO and PF treatments in ground beef CO reduction on day 0 of display. Therefore, ES application of these antimicrobials was able to achieve similar CO reduction as CS, but using much less antimicrobial.

Escherichia coli:

Ground beef processed from PA, W, and PP, treatments applied through CS method achieved ≥ 1 log reduction *Escherichia coli* (EC) on day 0 of display (Table 1.2). The PM, PP treatments applied through CS and ES methods respectively, showed significantly lower ($P < 0.05$) EC count compared to the control on day 1 of display with up to > 1.9 log reduction. However, PO and PF treatments by both application methods together with PM and PP applied through ES system also possessed significantly lower ($P < 0.05$) EC counts compared to the control with 1 or more log reduction on day 1 of display. While PA along with ES application of PP, PO, and PF showed > 1 log reduction, CS application of PM and PP treatments accounted for > 2 log reduction in EC counts on day 2 of display. The CS application of PM treatment had the highest EC log reduction (0.81) on day 3 of display. Also CS and ES were equally effective for reducing EC when used to apply PP, PO or PF treatments on day 3 of display. In contrast, PP and PM treatments were more efficient ($P < 0.05$) in CS application method compared to the ES method in reducing EC populations in ground beef on day 1 and 2 of display, respectively.

Aerobic plate counts:

The PA and PO-treated ground beef through CS application lead by CS application of PP, showed the lowest ($P < 0.05$) aerobic plate count (APC) on day 0 of display (Table 1.3). On day 1 of display PM and PP treatments applied by CS, ES application of PP treatment and PF treatment by both application methods obtained > 1 log reduction of ground beef APC. Treatment with PA, PO, and PF resulted in APC reductions ($P < 0.05$) compared to the control, regardless of application method. The PP- treated ground beef through CS application reported the lowest ($P < 0.05$) APC with 2.05 log reduction on day 2 of display. Although ground beef from PP treated ground beef by ES had less performance ($P < 0.05$) compared to CS, it accounted for > 1 log reduction of APC on day 2 of display. Further, the PP by ES treatment was able to maintain a 1.02 log reduction of APC on day 3 of display. Both ES and CS treatment application methods of PO and PF treatments showed a similar ($P > 0.05$) efficiency in controlling ground beef APC on day 1 of display. All treatments regardless of application method were effective for reducing ($P < 0.05$) APC by day 7 of display.

Salmonella:

Beef trimmings treated with PA, ES application of PM, PP, and PF along with CS application of PP and PO reduced ($P < 0.05$) *Salmonella* (ST) population with > 1 log reduction on day 0 of display (Table 1.4). These treatments along with W and PO treatments through ES and CS application of PM and PF had significantly lower ($P < 0.05$) *Salmonella* populations compared to CON, and CS application of W on days 1 and 2 of display. The CS application of PP indicated the lowest ($P < 0.05$) ground beef *Salmonella* count on day 7. The ES applications of PM and PP had a greater ($P < 0.05$) ST count reductions compared to CS applications of same organic acids on day 0 of display. However, PM, PP, and PF treatments applied by both methods showed similar ($P > 0.05$) ST reduction on days 1 and 2 of display. By day 1 of display, all treatments and application methods were effective ($P < 0.05$) for reducing *Salmonella* counts.

TABLE 1.1. Effects of antimicrobial treatment, application method and day of display against coliform population in ground beef during simulated retail display storage at 4ºC.

*Treatment	**Application Method	Coliform count (log CFU/g)				
		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	5.17^{a}	5.51^{a}	5.74^{a}	6.02^{b}	7.09
PA	CS	3.74^{d}	4.62^{bc}	4.56^{d}	5.98^{b}	6.79
W	CS	4.12^{bc}	5.67^{a}	5.73^{a}	6.27^{a}	7.09
W	ES	4.39^{b}	5.47^{a}	4.56^{d}	5.98^{b}	6.86
PM	CS	3.94^{cd}	3.71^{d}	4.91^{cd}	5.26^{f}	6.89
PM	ES	4.16^{bc}	4.97^{b}	5.42^{ab}	6.00^{b}	6.60
PP	CS	4.22^{cb}	3.76^{d}	4.03^{e}	5.79^{d}	6.68
PP	ES	3.73^{d}	4.40^{c}	4.84^{cd}	5.45^{e}	6.60
PO	CS	4.30^{b}	4.53^{bc}	5.61^{a}	5.56^{e}	6.78
PO	ES	4.33^{b}	4.49^{c}	5.08^{bc}	5.54^{e}	6.51
PF	CS	4.30^{b}	4.55^{bc}	5.41^{ab}	5.45^{e}	6.74
PF	ES	4.15^{bc}	4.65^{bc}	4.87^{cd}	5.48^{e}	6.95
	Standard error	±0.06	±0.09	±0.07	±0.02	±0.12

Coliform growth (log Colony Forming Units/g) reported as least squares means along with ± standard error.

$^{a-f}$ Least squares means within a column with different superscripts differed significantly ($P < 0.05$).

*Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

**Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 1.2. Effects of antimicrobial treatment, application method and day of display against Escherichia coli in ground beef during simulated retail display storage at 4ºC.

		Escherichia coli count (Log CFU/g)				
*Treatment	**Application Method	Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	5.22[a]	5.77[ab]	6.29[a]	6.30[a]	7.33[a]
PA	CS	3.77[f]	4.88[c]	4.96[f]	6.19[ab]	7.19[b]
W	CS	4.22[d]	5.90[a]	6.02[b]	6.31[a]	7.28[a]
W	ES	4.43[c]	5.71[b]	5.73[c]	6.28[ab]	7.13[c]
PM	CS	4.35[cd]	3.82[e]	4.09[g]	5.49[d]	7.13[c]
PM	ES	4.39[cd]	4.68[d]	5.44[d]	6.27[ab]	6.96[d]
PP	CS	3.99[e]	3.84[e]	4.03[g]	5.60[cd]	7.01[d]
PP	ES	4.81[b]	4.62[d]	4.95[f]	5.77[cd]	7.21[b]
PO	CS	4.47[c]	4.72[d]	5.95[b]	5.80[cd]	6.97[d]
PO	ES	4.45[c]	4.67[d]	5.14[e]	5.70[cd]	7.12[c]
PF	CS	4.32[cd]	4.62[d]	5.81[c]	5.92[bc]	7.12[c]
PF	ES	4.29[cd]	4.77[cd]	5.06[e]	5.76[cd]	7.11[c]
	Standard error	±0.04	±0.03	±0.02	±0.08	±0.01

Escherichia coli growth (log Colony Forming Units/g) reported as least squares means along with ± standard error.

[a-g] Least squares means within a column with different superscripts differed significantly ($P < 0.05$).

*Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

**Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 1.3. Effects of antimicrobial treatment, application method and day of display against total aerobic plate bacteria in ground beef during simulated retail display storage at 4ºC.

*Treatment	**Application Method	Aerobic plate count (Log CFU/g)				
		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	5.52^{b}	6.27^{a}	6.33^{a}	6.59^{abc}	7.89^{a}
PA	CS	4.57^{f}	4.95^{d}	6.26^{ab}	6.34^{bcd}	7.56^{b}
W	CS	5.04^{d}	6.19^{a}	6.08^{abc}	6.80^{a}	7.52^{bc}
W	ES	5.64^{a}	5.66^{b}	6.21^{ab}	6.68^{ab}	7.24^{d}
PM	CS	5.48^{b}	4.09^{ef}	5.89^{bcde}	5.67^{hg}	7.32^{cd}
PM	ES	5.47^{b}	4.92^{d}	5.68^{de}	6.33^{bcd}	7.36^{bcd}
PP	CS	4.36^{g}	4.00^{f}	4.28^{g}	5.94^{efg}	7.54^{b}
PP	ES	5.11^{d}	4.14^{e}	5.14^{f}	6.12^{def}	7.51^{bc}
PO	CS	4.60^{f}	5.04^{c}	5.98^{abcd}	6.28^{cde}	7.38^{bcd}
PO	ES	4.77^{e}	5.05^{c}	5.59^{e}	5.94^{efg}	7.30^{d}
PF	CS	5.30^{c}	4.11^{e}	5.98^{abcd}	5.86^{fgh}	7.50^{bc}
PF	ES	4.79^{e}	4.06^{ef}	5.74^{cde}	5.57^{h}	7.37^{bcd}
	Standard error	±0.02	±0.02	±0.07	±0.07	±0.04

Total aerobic bacterial growth (log Colony Forming Units/g) reported as least squares means along with ± standard error.

$^{a-h}$ Least squares means within a column with different superscripts differed significantly ($P < 0.05$).

*Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

**Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 1.4. Effects of antimicrobial treatment, application method and day of display against Salmonella Typhimurium in ground beef during simulated retail display storage at 4ºC.

	Salmonella count (Log CFU/g)

*Treatment	**Application Method		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-		5.06^{a}	5.58^{a}	6.24^{a}	6.30^{a}	7.54^{a}
PA	CS		3.30^{f}	3.88^{ed}	5.01^{d}	6.19^{ab}	6.63^{d}
W	CS		4.11^{bc}	5.46^{a}	6.27^{a}	6.31^{a}	6.59^{d}
W	ES		4.24^{bc}	4.31^{cd}	5.45^{b}	6.28^{ab}	6.84^{cd}
PM	CS		4.14^{bc}	3.52^{fg}	3.86^{i}	5.49^{d}	6.80^{cd}
PM	ES		3.39^{ef}	3.67^{fg}	4.82^{e}	6.27^{ab}	7.27^{ab}
PP	CS		3.88^{cd}	3.44^{g}	3.72^{j}	5.60^{cd}	6.12^{e}
PP	ES		3.15^{f}	3.56^{fg}	4.17^{g}	5.77^{cd}	7.14^{bc}
PO	CS		3.71^{de}	3.71^{fg}	4.22^{g}	5.80^{cd}	7.02^{bc}
PO	ES		4.27^{b}	4.08^{ed}	4.05^{h}	5.70^{cd}	7.07^{bc}
PF	CS		4.09^{bc}	4.68^{b}	5.26^{c}	5.92^{bc}	7.01^{bc}
PF	ES		3.91^{bcd}	4.62^{bc}	4.64^{f}	5.76^{cd}	7.14^{bc}
		Standard error	±0.08	±0.07	±0.02	±0.07	±0.07

Total Salmonella species (log Colony Forming Units/g) reported as least squares means along with ± standard error.

$^{a-h}$ Least squares means within a column with different superscripts differed significantly ($P < 0.05$).

*Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

**Application methods: CS = conventional spray application, ES = electrostatic spray application.

Ground beef instrumental color properties:

The ground beef samples processed from PA, W, PM, and PF had similar lightness (L*) ($P > 0.05$) compared to CON on days 0, 1, 3 and 7 of display regardless of the treatment application

method (Table 1.5). Ground beef treated with PP by both application methods had similar lightness to the CON on days 0, 3 and 7 of display. The ground beef treated with PA, W, PM, and PO, despite of application method, had similar ($P > 0.05$) redness (a*) to CON on day 0 of display (Table 1.6). However, ground beef from PP and PF applied with ES were redder in color on day 0 of display. All the treatments applied through CS and ES methods, except ES application of malic acid, maintained a similar redness to control at day 7 of display. The treatment and application method did not show an interaction effect on ground beef yellowness on days 0 through 7 of display with all treatments being similar in yellowness to the control and each other (Table 1.7). The ground beef from all treatments maintained a similar hue angle ($P > 0.05$) or hue color on days 0 and 7 of display (Table 1.8). All treated samples possessed a saturation index similar ($P > 0.05$) to control throughout the display time except ES applications of PF on day 0of display (Table 1.9). Therefore, with the exception of PF applied by ES on day 0 of display, all treatments were as vivid in color throughout display as the control. Additionally, all treatments had similar reflectance ratio (estimated oxymyoglobin content) compared to CON on days 0, 3 and 7 of display (Table 1.10). Therefore, antimicrobial or application method had little impact on myoglobin state, keeping similar oxymyoglobin content as the control.

TABLE 1.5. Effects of antimicrobial treatment, application method and day of display on ground beef lightness (L*) during simulated retail display storage at 4ºC.

	Lightness (L*)

*Treatment	**Application Method		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-		51.08^{c}	50.45^{c}	48.59^{c}	46.98^{b}	46.42^{b}
PA	CS		54.45^{abc}	54.84^{abc}	53.74^{abc}	51.11^{ab}	50.25^{ab}
W	CS		53.61^{abc}	53.83^{bc}	51.92^{abc}	51.31^{ab}	49.86^{ab}
W	ES		53.53^{abc}	52.48^{bc}	49.46^{bc}	48.03^{b}	47.05^{ab}
PM	CS		54.55^{abc}	55.86^{abc}	55.23^{ab}	52.49^{ab}	50.43^{ab}
PM	ES		54.78^{abc}	55.14^{abc}	50.91^{abc}	50.44^{ab}	51.13^{ab}
PP	CS		55.59^{abc}	56.36^{ab}	56.38^{a}	53.84^{ab}	51.24^{ab}
PP	ES		53.79^{abc}	54.56^{abc}	51.05^{abc}	49.65^{ab}	48.30^{ab}
PO	CS		56.73^{ab}	56.53^{ab}	54.11^{abc}	53.07^{ab}	51.49^{ab}
PO	ES		58.45^{a}	59.56^{a}	56.19^{a}	56.34^{a}	54.28^{a}
PF	CS		52.78^{bc}	53.48^{c}	50.95^{abc}	48.78^{ab}	47.60^{ab}
PF	ES		52.68^{bc}	54.35^{abc}	51.85^{abc}	48.94^{ab}	48.06^{ab}
		Standard error	±0.97	±0.98	±1.17	±1.43	±1.39

Lightness (L*) reported as least squares means along with ± standard error, $^{a-c}$ Least squares means within a column with different superscripts differed significantly ($P < 0.05$).

*Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

**Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 1.6. Effects of antimicrobial treatment, application method and day of display on ground beef redness (a*) during simulated retail display storage at 4ºC.

			Redness (a*)

*Treatment	**Application Method		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-		13.97^{c}	13.09	18.40^{abc}	20.30	20.21^{a}
PA	CS		15.59^{abc}	9.33	12.36^{abc}	17.78	18.16^{a}
W	CS		15.75^{abc}	11.17	18.68^{ab}	19.93	17.92^{ab}
W	ES		17.18^{abc}	12.16	19.97^{a}	19.60	17.81^{ab}
PM	CS		16.09^{abc}	8.95	10.12^{bc}	17.02	18.04^{ab}
PM	ES		17.19^{abc}	8.71	15.90^{abc}	16.12	14.62^{b}
PP	CS		14.56^{bc}	9.85	9.37^{c}	14.29	17.99^{ab}
PP	ES		18.06^{ab}	8.97	15.04^{abc}	19.21	18.02^{ab}
PO	CS		16.13^{abc}	9.45	16.93^{abc}	19.49	19.80^{ab}
PO	ES		15.12^{abc}	10.08	17.43^{abc}	18.80	18.65^{ab}
PF	CS		17.89^{abc}	9.59	15.82^{abc}	20.04	19.97^{ab}
PF	ES		18.98^{a}	9.56	14.10^{abc}	17.93	18.85^{ab}
		Standard error	±0.70	±0.86	±1.63	±1.43	±0.97

Redness (a*) reported as least squares means along with ± standard error.

[a-c] Least squares means within a column with different superscripts differed significantly ($P < 0.05$).

*Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

**Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 1.7. Effects of antimicrobial treatment, application method and day of display on ground beef yellowness (b*) during simulated retail display storage at 4ºC.

	yellowness (b*)

*Treatment	**Application Method		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-		16.34	15.36	15.03	15.37	14.27
PA	CS		16.88	16.40	15.06	16.19	14.25
W	CS		16.87	16.08	17.36	16.57	13.64
W	ES		16.76	15.60	16.55	15.17	12.51
PM	CS		17.25	15.89	16.73	17.55	14.89
PM	ES		17.31	15.50	14.97	15.09	12.41
PP	CS		17.39	17.24	16.77	17.42	15.21
PP	ES		17.31	15.97	16.42	16.80	13.88
PO	CS		18.00	16.55	17.31	17.62	15.73
PO	ES		16.52	15.76	16.56	17.23	15.15
PF	CS		18.11	16.77	16.99	17.59	14.95
PF	ES		18.55	16.14	16.41	15.89	14.35
		Standard error	0.50	0.49	0.87	0.45	0.66

Yellowness (b*) reported as least squares means along with ± standard error, Least squares means within a column with different superscripts differed significantly ($P < 0.05$).

*Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

**Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 1.8. Effects of antimicrobial treatment, application method and day of display on ground beef hue angle during simulated retail display storage at 4ºC.

			Hue angle

*Treatment	**Application Method		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-		49.48	49.56^{b}	39.32^{c}	37.19^{b}	35.27
PA	CS		47.27	60.33^{a}	50.71^{abc}	42.33^{ab}	37.98
W	CS		46.97	55.45^{ab}	42.95^{c}	39.73^{b}	37.33
W	ES		44.26	52.36^{ab}	39.72^{c}	37.84^{b}	35.11
PM	CS		47.07	60.65^{a}	58.81^{abc}	45.99^{ab}	39.57
PM	ES		45.18	60.65^{a}	43.24^{c}	43.17^{ab}	40.35
PP	CS		50.09	60.23^{a}	60.79^{a}	51.39^{a}	40.14
PP	ES		43.81	60.67^{a}	47.63^{bc}	41.17^{ab}	37.61
PO	CS		48.14	60.25^{a}	46.07^{c}	42.09^{ab}	38.45
PO	ES		47.50	57.47^{ab}	43.52^{c}	42.51^{ab}	39.08
PF	CS		45.37	60.26^{a}	47.50^{bc}	41.29^{ab}	36.79
PF	ES		44.35	59.33^{a}	49.44^{abc}	41.55^{ab}	37.30
		Standard error	1.17	1.62	2.09	1.99	1.12

Hue angle [$\tan^{-1}$(b*/a*)] reported as least squares means along with ± standard error.
$^{a-c}$Least squares means within a column with different superscripts differed significantly ($P < 0.05$).
*Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.
**Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 1.9. Effects of antimicrobial treatment, application method and day of display on ground beef saturation index during simulated retail display storage at 4ºC.

			Saturation index

*Treatment	**Application Method		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-		21.51[b]	20.21	23.77	25.48	24.76[ab]
PA	CS		23.00[ab]	18.88	19.51	24.06	23.14[ab]
W	CS		23.09[ab]	19.61	25.52	25.92	22.53[ab]
W	ES		24.02[ab]	19.82	25.94	24.79	21.77[ab]
PM	CS		23.60[ab]	18.24	19.56	24.48	23.40[ab]
PM	ES		24.40[ab]	17.78	21.84	22.09	19.18[b]
PP	CS		22.68[ab]	19.86	19.21	22.65	23.56[ab]
PP	ES		25.04[ab]	18.32	22.28	25.56	22.75[ab]
PO	CS		24.18[ab]	19.06	24.27	26.29	25.29[a]
PO	ES		22.41[b]	18.72	24.05	25.51	24.03[ab]
PF	CS		25.46[ab]	19.31	23.30	26.68	24.95[ab]
PF	ES		26.55[a]	18.76	21.65	23.97	23.70[ab]
		Standard error	0.72	0.82	1.64	1.01	1.08

Saturation index ($[(a^{*2}+b^{*})^{0.5}]$ reported as least squares means along with ± standard error.
[a-b]Least squares means within a column with different superscripts differed significantly ($P < 0.05$).
*Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid. ** **Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 1.10. Effects of antimicrobial treatment, application method and day of display on ground beef reflectance ratio during simulated retail display storage at 4 ºC

	Reflectance ratio

*Treatment	**Application Method		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-		2.82^{abc}	1.38^{a}	2.20^{ab}	2.53	2.37
PA	CS		2.83^{abc}	1.04^{b}	1.45^{bcd}	2.21	2.59
W	CS		2.86^{ab}	1.21^{ab}	2.12^{ab}	2.46	2.36
W	ES		3.18^{a}	1.29^{ab}	2.41^{a}	2.55	2.68
PM	CS		2.84^{abc}	1.05^{ab}	1.11^{d}	2.08	2.38
PM	ES		2.92^{ab}	1.05^{ab}	1.89^{abc}	2.19	2.13
PP	CS		2.58^{bc}	1.09^{ab}	1.29^{cd}	1.81	2.37
PP	ES		3.14^{ab}	1.07^{ab}	1.86^{abcd}	2.44	2.48
PO	CS		2.78^{abc}	1.07^{ab}	1.97^{abc}	2.33	2.35
PO	ES		2.28^{c}	1.34^{ab}	1.96^{abc}	2.33	2.68
PF	CS		3.17^{a}	1.02^{b}	1.77^{abcd}	2.33	2.42
PF	ES		3.34^{a}	1.06^{ab}	1.65^{bcd}	2.26	2.21
		Standard error	0.10	0.06	1.33	0.18	0.22

Reflectance ratio (580/630 nm) reported as least squares means along with ± standard error, [a-c]Least squares means within a column with different superscripts differed significantly ($P < 0.05$).
Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.
**Application methods: CS = conventional spray application, ES = electrostatic spray application.

Evaluation of conventional vs. electrostatic spray applications of pyruvic and octanoic acids alone or in combination with ethoxylated glyceride surfactant on beef trimmings on ground beef microbial properties. (Objective 2).

Effect of treatment on ground beef microbial populations:

Table 2.1 summarizes the effect of treatment pooled across the days of display on ground beef APC, coliform, E. coli (EC) and Salmonella (SA) populations. The Electrostatic spraying of water on beef trimming resulted significantly less ($P < 0.05$) ground beef APC, coliform and EC counts compared to conventional application of water. Incorporation of 1% EG in pyruvic acid (PP+EG) did not show a (P>0.05) beneficial influence on ground beef APC, coliform, EC and SA counts demonstrating a slightly increased ($P < 0.05$) or similar ($P > 0.05$) values compared to the pyruvic acid with no added EG treatment (PP), despite the application method. The CS application of pyruvic acid treatment with or without added EG had slightly lower ($P < 0.05$) ground beef APC and coliform counts compared to the ES application of the same treatments. However, there was no difference ($P > 0.05$) between spray application methods of pyruvic acid treatment, irrespective of the EG incorporation, on ground beef E. coli and Salmonella populations.

Octanoic acid with added EG (PO+EG) application through CS exhibited no difference ($P > 0.05$) in ground beef coliform and EC counts however had higher ($P < 0.05$) APC and lower ($P > 0.05$) Salmonella counts compared to the octanoic acid treatment with no added EG applied through the same method. Similarly, the ES application of PO+EG treatment showed no significant differences ($P > 0.05$) in APC, coliform, and Salmonella counts yet resulted 0.42 less ($P < 0.05$) EC counts compared to its CS counterpart. In addition, the ES application of PO+EG showed better efficiency with significantly less ($P < 0.05$) ground beef APC, coliform, EC, and SA counts compared to that of beef trimmings treated with PO+EG treatment through CS application method. However, PO treatment showed no difference ($P > 0.05$) between CS and ES methods for ground beef APC, EC and SA counts.

Effect of duration of display on microbial growth

The effect of duration of display on microbial populations is summarized in Table 2.2. The ground beef microbial populations showed an increasing trend across the 7 days of display ($P < 0.05$). As expected APC, coliform, E. coli and Salmonella counts all increased from 0 to 7 days of display.

TABLE 2.1. Effect of antimicrobial treatments on least squares means (± SE) log CFU[b]/g aerobic plate count (APC), Coliform, E. coli and Salmonella.

		Log $_{10}$ counts (***CFU/g)			
*Treatment	** Application Method	APC	Coliform	E. coli	Salmonella

CON	None	5.40^{de}	4.86^{d}	5.30^{e}	6.00^{b}
PA	CS	5.78^{b}	5.67^{a}	5.63^{bc}	5.86^{cde}
W	CS	5.68^{b}	5.16^{c}	5.74^{ab}	5.82^{de}
W	ES	5.44^{cde}	4.83^{d}	5.51^{cd}	5.81^{e}
PP	CS	5.50^{cd}	5.19^{c}	5.38^{de}	5.81^{e}
PP+EG	CS	5.54^{c}	5.29^{c}	5.68^{abc}	6.05^{ab}
PP	ES	5.80^{b}	5.24^{c}	5.31^{e}	6.05^{ab}
PA+EG	ES	6.00^{a}	5.46^{b}	5.81^{a}	5.95^{bcd}
PO	CS	5.40^{de}	5.29^{c}	5.45^{de}	6.18^{a}
PO+EG	CS	5.78^{b}	5.27^{c}	5.42^{de}	5.97^{bc}
PO	ES	5.46^{cde}	4.94^{d}	5.39^{de}	6.08^{ab}
PO+EG	ES	5.34^{e}	4.79^{d}	4.97^{f}	5.95^{bcd}
SE		0.019	0.02	0.02	0.02

Total APC, coliform, E.coli and Salmonella (log Colony Forming Units/g) reported as least squares means along with ± standard error (SE).

[a-e] Least squares means within a column with different superscripts differed significantly ($P < 0.05$).

[*]Treatments: CON = Inoculated control with no antimicrobial treatment; W = deionized water; PA= 0.02%Peroxyacetic acid; PP = 0.02% PA followed by pyruvic acid (3%); PP+EG = 0.02 % PA followed by 3% pyruvic acid with added 1% ethoxylated glycerides, PO = 0.02 % PA followed by octanoic acid (3 %); PO+EG = 0.02% PA followed by 3% octanoic acid with added 1 % ethoxylated glyceride.

**Application methods: CS = conventional spray application; ES = electrostatic spray application.

[***] Colony forming units.

TABLE 2.2. Effect of duration of display pooled across antimicrobial treatments, on least square mean log *CFU/g aerobic plate count (APC), Coliform, E. coli and Salmonella.

Microorganism	Day of Display				
	0	1	2	3	7
APC	3.94^{e}	4.13^{d}	5.27^{c}	6.10^{b}	8.52^{a}
Coliform	3.66^{e}	3.70^{d}	4.96^{c}	5.95^{b}	7.56^{a}
E. coli	3.80^{e}	3.95^{d}	5.13^{c}	6.10^{b}	8.34^{a}

Salmonella	4.23[e]	4.74[d]	5.94[c]	6.75[b]	8.16[a]

*Colony forming units.

Least- squares means within a microorganism within a row bearing different letters (a-d) are different ($P < 0.05$).

Effect of peroxyacetic acid followed by organic acids decontamination interventions on ground beef instrumental color and sensory color, odor and processing characteristics. (Objective 3).

Table 3.1 to 3.6 summarizes the effect of treatment, application method and day of display interaction effects on instrumental color properties. All the treatment showed a similar ($P > 0.05$) lightness (L*) to control on days 0 through 7 of display (Table 3.1). In addition, no significant

differences ($P > 0.05$) were found between CON and any of the treatments on days 0 through 7 of display for ground beef redness (a*) (Table 3.2) and yellowness (Table 3.3). The application method did not differ ($P > 0.05$) in ground beef L*, a* and b* values for any given treatment on any given day of display. The hue angle (Table 3.4), saturation index (Table 3.5) and reflectance ration (Table 3.6) of all the treatments were significantly not different ($P > 0.05$) from that of the control ground beef irrespective of the application method on day 0 through 3 of display. However, PP applied by ES and PO applied by ES or CS were slightly less ($P < 0.05$) red as indicated by hue angle from the control (Table 3.4). The effect of treatments on ground beef sensory color odor and processing attributes is reported on Table 3.7. With the exception of PP applied by CS, sensory panelists indicated a superior red worst point color, overall color and less percent discoloration in all treatments compared with CON. However, PP treatment applied through CS system surpassed other treatments in maintaining a similar ground beef overall color and percent discoloration to CON. All the treatments had a similar ($P > 0.05$) beef odor to CON. Likewise PA, W, PM, PF and PP treatments by CS method and W, PM, PF by ES method had a similar ($P > 0.05$) off odor to CON. The panelist indicated all treatments had a similar ($P > 0.05$) smearing and patty foaming ability characteristics to the CON ground beef except ES application of W treatment.

TABLE 3.1. Effects of antimicrobial treatment, application method and day of display on ground beef lightness (L*) during simulated retail display storage at 4ºC.

		Lightness (L*)				
*Treatment	**Application Method	Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	41.05	41.19	40.99	39.98	37.20

PA	CS	43.78	45.24	45.80	45.15	40.85
W	CS	43.45	45.19	46.52	42.40	41.64
W	ES	46.52	49.19	45.28	46.21	41.84
PM	CS	46.25	47.02	45.07	45.47	43.50
PM	ES	46.82	47.27	45.10	45.83	43.45
PP	CS	44.57	44.72	43.97	44.48	40.63
PP	ES	45.47	46.51	44.64	45.67	38.51
PO	CS	42.74	42.67	42.25	42.15	37.94
PO	ES	45.69	48.54	46.65	42.31	40.66
PF	CS	44.58	44.30	45.29	45.78	40.88
PF	ES	45.95	47.18	45.77	42.63	43.21

Lightness (L*) reported as least squares means.
Least squares means within a column did not differ ($P > 0.05$).
*Treatments: CON = untreated un-inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.
**Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 3.2. Effects of antimicrobial treatment, application method and day of display on ground beef redness (a*) during simulated retail display storage at 4ºC.

		Redness (a*)				
*Treatment	**Application Method	Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	17.48	13.14	11.01	10.22	13.45
PA	CS	21.73	14.56	10.62	10.09	17.70
W	CS	20.81	14.02	10.11	12.74	19.74

W	ES	20.35	16.33	12.23	11.43	19.80
PM	CS	24.43	14.78	12.07	10.42	18.00
PM	ES	21.75	15.32	11.79	10.25	18.25
PP	CS	18.36	13.57	11.36	9.93	15.11
PP	ES	22.46	15.68	13.40	10.71	21.00
PO	CS	20.39	15.65	13.02	10.62	19.95
PO	ES	16.28	15.40	10.81	16.72	22.11
PF	CS	22.52	14.44	13.23	11.27	14.52
PF	ES	21.31	14.57	10.80	18.88	19.52

Redness (a*) reported as least squares means.
Least squares means within a column did not differ ($P > 0.05$).
*Treatments: CON = untreated un-inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.
**Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 3.3. Effects of antimicrobial treatment, application method and day of display on ground beef yellowness (b*) during simulated retail display storage at 4ºC.

		yellowness (b*)				
*Treatment	**Application Method	Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	15.69	15.94	15.16	15.11	12.88
PA	CS	18.68	16.25	16.10	16.27	12.85
W	CS	17.91	16.02	16.10	16.40	13.70
W	ES	18.06	17.33	16.48	16.59	15.39

PM	CS	20.46	16.22	16.16	15.83	13.78
PM	ES	18.66	15.99	16.11	16.14	13.77
PP	CS	17.01	16.05	15.84	15.98	15.33
PP	ES	19.53	16.25	15.93	15.70	13.30
PO	CS	16.98	14.68	14.69	14.83	12.85
PO	ES	18.23	17.09	17.32	15.11	14.53
PF	CS	19.41	16.40	16.49	16.62	15.48
PF	ES	19.86	16.66	16.64	16.01	14.78

Yellowness (b*) reported as least squares means.

Least squares means within a column did not differ ($P > 0.05$).

*Treatments: CON = untreated un-inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

**Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 3.4. Effects of antimicrobial treatment, application method and day of display on ground beef hue angle during simulated retail display storage at 4ºC.

Treatment	Application Method	Hue angle				
		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	41.93	50.52	54.01^{abc}	54.51^{abc}	43.99^{ab}
PA	CS	40.69	48.15	56.58^{ab}	58.22^{a}	35.97^{bc}
W	CS	40.74	48.81	57.87^{a}	52.17^{abc}	34.73^{cd}
W	ES	41.62	46.71	53.38^{abc}	55.34^{abc}	37.84^{ab}
PM	CS	39.96	47.67	53.26^{abc}	56.59^{ab}	37.41^{ab}

PM	ES	40.67	46.21	53.79^{abc}	57.59^{a}	37.04^{abc}
PP	CS	42.81	49.78	54.36^{abc}	58.16^{a}	45.46^{a}
PP	ES	41.02	46.02	49.94^{c}	55.67^{abc}	32.35^{c}
PO	CS	39.78	43.12	48.30^{c}	54.35^{abc}	32.80^{c}
PO	ES	47.30	47.98	55.50^{ab}	42.42^{c}	33.28^{c}
PF	CS	40.96	48.70	51.29^{bc}	55.91^{abc}	47.21^{a}
PF	ES	43.00	48.82	56.99^{ab}	40.34^{c}	37.11^{ab}

Hue angle [$\tan^{-1}$(b*/a*)] reported as least squares means.

$^{a-c}$Least squares means within a column with different superscripts differed significantly ($P < 0.05$).

*Treatments: CON = untreated un-inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

**Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 3.5. Effects of antimicrobial treatment, application method and day on ground beef saturation index during simulated retail display storage at 4ºC.

		Saturation index				
*Treatment	**Application Method	Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	23.49^{ab}	20.66	18.74^{b}	19.59^{ab}	18.65^{b}
PA	CS	28.66^{ab}	21.83	19.30^{ab}	19.15^{ab}	21.87^{ab}
W	CS	27.46^{ab}	21.29	19.01^{b}	20.76^{ab}	24.04^{ab}
W	ES	27.22^{ab}	23.83	20.53^{ab}	20.17^{ab}	25.10^{ab}
PM	CS	31.87^{a}	21.95	20.18^{ab}	18.96^{b}	22.68^{ab}
PM	ES	28.66^{ab}	22.14	19.97^{ab}	19.12^{b}	22.87^{ab}
PP	CS	25.04^{ab}	21.01	19.49^{ab}	18.81^{b}	21.53^{ab}
PP	ES	29.77^{ab}	22.58	20.81^{ab}	19.00^{b}	24.86^{ab}

PO	CS	26.54^{ab}	21.46	19.66^{ab}	18.24^{b}	23.73^{ab}
PO	ES	24.82^{ab}	23.00	20.55^{ab}	22.69^{ab}	26.48^{ab}
PF	CS	29.74^{ab}	21.86	21.14^{ab}	20.09^{ab}	21.32^{ab}
PF	ES	29.13^{ab}	22.13	19.85^{ab}	24.76^{ab}	24.49^{ab}

Saturation index ([(a*^{2}+b*)$^{0.5}$] reported as least squares means.

$^{a-c}$Least squares means within a column with different superscripts differed significantly ($P < 0.05$).

*Treatments: CON = untreated un-inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

**Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 3.6. Effects of antimicrobial treatment, application method and day of display on ground beef reflectance ratio during simulated retail display storage at 4ºC.

		Reflectance ratio				
*Treatment	**Application Method	Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	2.34	2.34	1.20	1.08	1.76
PA	CS	3.09	3.09	1.10	1.02	2.55
W	CS	3.45	3.45	1.03	1.28	2.89
W	ES	2.56	2.56	1.29	1.15	2.53
PM	CS	3.80	3.80	1.30	1.08	2.45
PM	ES	2.80	2.80	1.24	1.04	2.41
PP	CS	2.36	2.36	1.26	1.07	2.11
PP	ES	3.21	3.21	1.50	1.13	3.31
PO	CS	2.83	2.83	1.50	1.10	3.07
PO	ES	2.87	2.87	1.05	2.14	3.41

PF	CS	3.39	3.39	1.49	1.21	1.85
PF	ES	2.81	2.81	1.09	2.47	2.42

Reflectance ratio (580/630 nm) reported as least squares means.
Least squares means within a column did not differ ($P > 0.05$).
Treatments: CON = untreated un-inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.
**Application methods: CS = conventional spray application, ES = electrostatic spray application.

TABLE 3.7. Effects of antimicrobial treatment and application method ON ground beef sensory color, odor and processing characteristics during simulated retail display storage at 4ºC.

**** Treatment	Application Method	Worst Point Color	Overall color	Percent Discoloration	Beef odor	Off odor	Smearing	Patty forming ability
CON	-	1.78^{b}	1.97^{c}	2.56^{c}	4.97	3.42^{cde}	2.87^{bc}	2.56^{ab}
PA	CS	2.46^{a}	2.74^{ab}	3.61^{ab}	4.64	3.18^{ef}	3.06^{abc}	2.93^{ab}
W	CS	2.54^{a}	3.05^{ab}	3.89^{ab}	4.46	3.15^{ef}	3.06^{abc}	2.43^{b}
W	ES	2.44^{a}	2.91^{ab}	3.67^{ab}	4.56	3.35^{de}	3.50^{a}	3.12^{ab}
PM	CS	2.53^{a}	2.94^{ab}	3.87^{ab}	4.97	3.64^{abc}	3.25^{ab}	2.43^{b}
PM	ES	2.41^{a}	2.93^{ab}	3.80^{ab}	4.97	3.52^{bcd}	2.62^{c}	3.12^{ab}
PF	CS	2.46^{a}	2.85^{ab}	3.60^{ab}	5.01	3.59^{abcd}	3.12^{abc}	3.00^{ab}
PF	ES	2.71^{a}	3.19^{a}	4.26^{a}	4.38	3.25^{e}	2.56^{c}	2.50^{b}
PP	CS	2.11^{ab}	2.47^{bc}	3.11^{bc}	5.34	3.68^{abc}	2.87^{bc}	2.56^{ab}
PP	ES	2.51^{a}	2.92^{ab}	3.79^{ab}	5.00	3.81^{a}	2.93^{abc}	3.31^{a}
PO	CS	2.73^{a}	3.16^{a}	4.08^{a}	5.12	3.73^{ab}	3.12^{abc}	2.81^{ab}
PO	ES	2.58^{a}	3.01^{ab}	3.96^{ab}	4.43	2.93^{f}	3.06^{abc}	3.00^{ab}

$^{a-c}$Least squares means within a column with different superscripts differed significantly ($P < 0.05$).

****Treatments: CON = untreated un-inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

**Application methods: CS = conventional spray application, ES = electrostatic spray application. Linear scales for overall color and worst point color (5=bright red, 4=dull red, 3= slightly brownish red, 2= moderately brownish red, 1= brown), for percent discoloration (7=no discoloration 0%, 6=slight discoloration 1-20%, 5=small discoloration 20-39%, 4=modest discoloration 40-59%, 3=moderate discoloration 60-79%, 2=extensive discoloration 80-95%, 1=total discoloration 96-100%). Beef odor (8= extremely beef like, 7=very beef like, 6=moderately beef like, 5=slightly beef like, 4=slightly non beef like, 3=moderately non beef like, 2=very non beef like, 1=extremely non beef like) at the same display intervals. Off odor were also evaluated (5= no off odor, 4=slight off odor, 3=small off odor, 2=moderate off odor, 1=extreme off odor). Smearing (6=extreme smearing, 5=moderate smearing, 4=slight smearing, 3=slight cut-grind, 2=moderate cut grind, 1=extreme cut grind) and patty forming ability (6=extremely fragile, 5=moderately fragile, 4=slightly fragile, 3=slightly fragile, 2=moderately cohesive, 1=extremely cohesive).

Cost Comparison of electrostatic versus conventional spray application methods.

Table 4 provides a cost comparison of antimicrobial usage with conventional and electrostatic spray. One advantage in electrostatic spray application of antimicrobials is reduced antimicrobial cost. In general, at the rates used in these research trials, electrostatic spray of antimicrobials produced similar reductions in microorganisms as conventional spray but did it using approximately 55% less antimicrobial which would result in a 55% antimicrobial savings.

TABLE 4. Cost comparison of electrostatic versus conventional spray application systems.

Organic acid and concentration	Organic acid listed price/kg	Cost for CS Application @ 0.1ml/g $/100kg ($/100lb)	Cost for ES Application @ 0.06ml/g $/100kg ($/100lb)
Pyruvic acid ≥97%	10.64	3.29 (1.50)	1.97 (0.90)
Numeric acid ≥99%	3.62	1.10 (0.50)	0.66 (0.30)

Malic acid ≥99%	1.45	0.44	(0.20)	0.26	(0.12)
Octanoic acid ≥98%	3.08	0.94	(0.26)	0.57	(0.26)

Conclusions

Antimicrobial treatments of 3% novel organic acids were effective against CO, EC, and APC on inoculated beef trimmings and combination of 3% of some novel organic acids with 0.5% EG (Surf) further enhanced the bactericidal activity of organic acids on inoculated beef trimmings in most cases when inoculated beef trimmings were dipped in treatments for 15 s. Therefore, decontamination treatments using novel organic acids as an intervention strategy may lead to increased microbial safety and quality of the ground beef prior to grinding and processing. CL, FA, and PY at the 3% level will be more effective than CA, GA, LA, MA for improving the microbial safety of the ground beef applied before grinding and processing. Our data clearly show that CL, MA, FA, and PY alone and in combinations with 0.5% EG (Surf) will effectively inhibit the growth of CO, EC, and APC on inoculated beef trimmings intended for the production of ground beef.

Peroxyacetic acid alone or followed by conventional or electrostatic spray application of malic, pyruvic, octonoic or fumaric acid on beef trimmings may be effective in reducing *E. coli* O157:H7 as well as Non-STEC serotypes and *Salmonella* through 2 days of display. ES application of some organic acids may have similar or greater efficiency in controlling ground beef microbial populations compared to the CS application of the same acid. Incorporation of surfactant (ethoxylatedd glyceride) did not show beneficial impact on enhancing microbial properties of ground beef when treatment was applied through conventional or electrostatic spray. These interventions had no or little interference on quality attributes of ground beef such as ground beef color, odor and processing characteristics. The ES application of organic acid established a cost-conscious treatment application with less antimicrobial usage as well as less waste management. Therefore, the outcome of this study opens new avenues for cost-effective utilization of natural organic acids in more efficient decontamination interventions in ground beef production lines to reduce pathogens of recent concern.

Recommendations for Future Research

Evaluation of a multiple hurdle (chemical and physical) approach of these organic acids for inactivation of *E. coli* O15:H7, Non-O157:H7 STEC and *Salmonella* is required. In addition, further investigation of these organic acids would be prudent especially with octanoic, fumaric, malic and pyruvic acids in other carriers that will increase concentration given that these acids had very good reduction at very low levels.

Presentations and publications

The outcome of this project is submitted to American Meat Institute Foundation as the final report. Two technical abstracts were submitted to Institute of Food Technologists in 2011 and 2012. One manuscript was submitted to Arkansas Animal Science Report. Dr. Fred Pohlman delivered a presentation on "Non- O157:H7 shiga -toxin producing E. coli in meat systems, their incidence and control measures" at American Meat Institute Conference in Dallas, TX in May 2012.

Literature Citation

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