The antimicrobial efficacies of novel organic acids as single antimicrobial intervention for the control of *Escherichia coli* O157:H7 in inoculated beef trimmings

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**Story in Brief**

The microbial safety of raw ground beef remains challenged by the potential for emergence of new pathogens and re-emergence of known pathogens. *E. coli* O157:H7 is known to have an inherent ability to evolve, mutate, and adapt to several stressors including antimicrobial treatments and allowing them to survive. The objective of this study was to determine the antimicrobial efficacies of peroxyacetic and other novel food grade organic acids on reduction of *E. coli* O157:H7 on beef trimmings. Beef trimmings inoculated with *E. coli* O157:H7 (10⁵ CFU/g) were dipped for 15 s in solutions of novel organic acids [fumaric acid, malic acid, citric acid, gluconic acid, levulinic acid, pyruvic acid, caproic acid, caprylic acid, and capric acid], and peroxyacetic acid. Following antimicrobial treatment, beef trimming samples were processed and spread plated on aerobic plate count, *E. coli* coliiform Petrifilm® plates. Among all treatments, caprylic acid was most effective in reducing more than 4.78, 4.73, and 2.48-logs of *E. coli*, coliiform, and of aerobic plate counts, respectively. Results of microbial assays revealed that 3% of pyruvic acid yielded 2.48, 0.31, and 0.2-log reduction of *E. coli*, levulinic acid, and caprylic acid respectively. Results of microbial assays revealed that 3% of pyruvic acid yielded 2.48, 0.31, and 0.2-log reduction of *E. coli*, levulinic acid, and caprylic acid respectively.

**Introduction**

Ground beef is characterized as a commodity of high potential risk of food-borne illness. Since ground beef is sourced from different cattle and locations, further grinding and mixing operations during ground beef production could potentially contaminate the uncontaminated product. In the past decades, *E. coli* O157:H7 has emerged as a high profile food-borne pathogen, and frequent ground beef product recalls due to *E. coli* O157:H7 contamination continue to be a serious concern to the U.S. meat industry and consumers. Direct antimicrobial application to meat as a decontamination technique has been shown to be effective in reducing pathogenic bacteria populations in the final product (Dorsa et al., 1998). Recent studies have suggested that decontamination of beef trimmings destined for ground beef production prior to grinding is effective in reducing bacterial counts in ground beef (Pohlman et al., 2002; Stivarius et al., 2002). This study was designed to evaluate the effectiveness of antimicrobial interventions on reducing *Escherichia coli* O157:H7 (EC), coliform (CO), and aerobic plate count (APC) of pre-inoculated beef trimmings. Therefore, our objective was to determine the antimicrobial efficacies of different concentrations of peroxyacetic acid (PAA) and other novel food grade organic acids [fumaric acid (FA), malic acid (MA), citric acid (CA), gluconic acid (GA), levulinic acid (LA), pyruvic acid (PY), caproic acid (CR), caprylic acid (CL); capric acid (CP)] on reduction of *E. coli* O157:H7 on beef trimmings.

**Material and Methods**

*Inoculation Preparation and Inoculation. Escherichia coli* O157:H7 (EC) inoculum was prepared from frozen (-80 °C) stock cultures. Frozen cultures of EC were thawed, and 0.1 ml of EC suspension was inoculated into 40 ml aliquots of Brain Heart Infusion broth (BHI). Following 18 h of incubation at 37 °C, bacteria were then harvested by centrifugation (3500 × g for 20 min at 25 °C) (Beckman GS-6 series, Fullerton, Ca.), and re-suspended with 40 ml of 0.1% buffered peptone water (BPW) (Difco Laboratories, Becton Dickinson and Company, Sparks, Md.). The bacterial suspension (log 10⁵CFU/ml EC) was cooled to 4 °C and then mixed. Beef trimmings were inoculated with the bacterial suspension and placed in a sterile bag and placed in a 4 °C cooler for 12 to 14 hr to allow for further microbial attachment.

*Antimicrobial Treatment Application and Sample Processing.* A model system was developed to mimic a commercial dip treatment system for beef trimmings. Beef trimmings inoculated with *E. coli* O157:H7 (10⁵ CFU/g; 25 g/treatment) were processed with antimicrobial treatments by dipping for 15 s in 100 mL solution of peroxyacetic acid (PAA; 0.02%) and 3% of novel organic acids [fumaric acid (FA); malic acid (MA); citric acid (CA); gluconic acid (GA); levulinic acid (LA); pyruvic acid (PY); caproic acid (CR), caprylic acid (CL), capric acid (CP), and peroxyacetic acid (PAA)]. Following antimicrobial treatment, beef trimming samples were processed and spread plated using serial 10-fold dilution in duplicates on aerobic plate count (APC), *E. coli* (EC) / coliform (CO) Petrifilm® plates. Plated samples were incubated at 37 °C for up to 48h in an aerobic incubation chamber. Inoculated beef trimmings treated with and without water (IN; IN+W) and un-inoculated (UN) samples served as controls.

*Microbial Sampling.* The microbial enumeration was carried out by homogenizing 25 g beef trim in a sterile whirl pack bag (Nasco, Ft Atkinson, Wis.) in 225 ml of 0.1% buffered peptone for 2 minutes in a stomacher (Model 400 Lab Stomacher; Seward, London, UK)

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Subsequently, serial 10-fold dilutions were made and spread plating was done in duplicates on aerobic plate counts (APC), and *E. coli* (EC)/coliform (CO) counts of Petrifilm® (3M Corporation, St. Paul, Minn.). Plates were then incubated at 37 °C in an aerobic incubation chamber (VWR Model 5015 and Model 3015 incubators, VWR Scientific, Cornelius, Ore.). The EC and APC counts were read after 48 h, whereas CO plates were read at 24 h. All counts were recorded as colony forming units per gram (CFU/g).

**Statistical Analysis.** The bacterial values were transformed to log values and then analyzed for the main effects of antimicrobial treatment using the PROC Mixed procedure of SAS (SAS Inst., Inc., Cary, N.C.). Least-squares means for protected F-tests ($P < 0.05$) were separated by using least significant differences (LSD; $P < 0.05$). The experiment was replicated two times.

**Results and Discussion**

The effect of antimicrobial treatment with PAA and other novel organic acids [FA, MA, CA, GA, LA, PY, CR, CL, CP, and PAA] on *E. coli* O157:H7 population of beef trimmings is shown in Figs. 1, 2, and 3. Treatment of beef trimmings prior to grinding with MA, FA, PY, and CL reduced ($P < 0.05$) survival of CO (Fig. 1). Among all antimicrobials MA, FA, and CL were most effective in reducing ($P < 0.05$) EC counts (Fig. 2) while PY yielded only 0.3, and 0.2-log reduction ($P<0.05$) of EC, and APC counts as compared to the control (IN+W) (Figs. 2 and 3). Among all antimicrobial treatments, CL and FA were the most effective treatments in reducing CO and EC populations within 15 s (Figs. 1 and 2). Caprylic acid reduced ($P < 0.05$) 4.78, 4.73, and 2.48-logs and FA reduced 3.23, 3.07, and 2.16-logs of EC, CO, and APC respectively (Figs. 1, 2, and 3). Among other treatments, 3% MA resulted in 2.23, 2.25, and 0.2-log reduction ($P < 0.05$) of EC, CO, and APC counts as compared to the control (IN+W). Dorsa et al., (1998) reported similar findings that antimicrobial treatment with 2% lactic acid on beef trimmings was not effective on reducing mesophilic aerobic bacteria through 7 days of storage.

Treatment of beef trimmings with PAA, CA, LA, and CP resulted in ~0.3 to 0.5 log reduction of EC and CO and APC. The highest concentration (3%) of novel organic acid (GA) tested in this study yielded no reduction in EC, CO, and APC counts compared to the control. Results suggest that the deleterious effects exhibited by the organic acids and PAA were consistent with other studies (King et al., 2005; Pohlman et al., 2002ab). These data suggest that CL negatively affects EC and CO enumeration efficacy. The results of this study indicated that CL, PA, and MA were the most effective antimicrobial treatments in reducing *E.coli* O157:H7 population on beef trimmings. The results of the antimicrobial application on beef trimmings were as expected for potent antimicrobial agents: PAA and other novel organic acids exhibited deleterious effects on survival of EC, CO, and APC.

**Implications**

This research provides a practical and cost-effective, novel decontamination technology for beef processors that can be immediately implemented for commercial application of antimicrobial interventions during ground beef production. The results from this research validate the antimicrobial efficacies of peroxoacetic acid and/or novel organic acids and their application on beef trimmings and/or ground beef to substantially reduce *E.coli* O157:H7 contamination.

**Literature Cited**


Fig. 1. Effects of 3% of novel organic acids [fumaric acid (FA); malic acid (MA); citric acid (CA); gluconic acid (GA); levulinic acid (LA); pyruvic acid (PY); caprioc acid (CO), caprylic acid (CL), and capric acid (CP)] and peroxyacetic acid (200 ppm) on log CFU/g of coliform (CO) counts of inoculated beef trimming.

Fig. 2. Effects of 3% of novel organic acids [fumaric acid (FA); malic acid (MA); citric acid (CA); gluconic acid (GA); levulinic acid (LA); pyruvic acid (PY); caprioc acid (CO), caprylic acid (CL), and capric acid (CP)] and peroxyacetic acid (200 ppm) on log CFU/g of E. coli O157:H7 (EC) counts of inoculated beef trimming.
Fig. 3. Effects of 3% of novel organic acids [fumaric acid (FA); malic acid (MA); citric acid (CA); gluconic acid (GA); levulinic acid (LA); pyruvic acid (PY); caprioc acid (CO), caprylic acid (CL), and capric acid (CP)] and peroxyacetic acid (200 ppm) on log CFU/g of Aerobic Plate counts (APC) of inoculated beef trimming.