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Antimicrobial Efficacy of Acidified Peroxyacetic Acid Treatments Against Surrogates for Enteric Pathogens on Prerigor Beef

Brianna C. Britton[1], Ifigenia Geornaras[1]*, James O. Reagan[2], Stephen Mixon[2], Dale R. Woerner[1†], and Keith E. Belk[1]

[1]Center for Meat Safety & Quality, Department of Animal Sciences, Colorado State University, 1171 Campus Delivery, Fort Collins, CO 80523, USA
[2]Zoetis, Inc., 10 Sylvan Way, Parsippany, NJ 07054, USA
*Corresponding author. Email: gina.geornaras@colostate.edu (Ifigenia Geornaras)
[†]Present address: Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX 79409, USA

Abstract: Two studies were conducted to evaluate the antimicrobial effects of pH-adjusted solutions of peroxyacetic acid (PAA) against nonpathogenic *Escherichia coli* surrogates for Shiga toxin–producing *E. coli* and *Salmonella*, inoculated on beef. In both studies, prerigor beef carcass surface tissue (10 × 10 cm pieces) was inoculated (6–7 log colony-forming units [CFU]/cm^2) on the adipose side with a 5-strain mixture of *E. coli* biotype I. In the first study, samples were left untreated (control) or were immersed (10 s) in solutions of PAA (300 parts per million [ppm]) acidified with a sulfuric acid and sodium sulfate blend (SSS) (pH 1.2) or PAA (400 ppm) acidified with acetic acid (2%), citric acid (1%), lactic acid (3.5%), or SSS (pH 1.2 or pH 1.8). In the second study, samples were left untreated or were spray treated (10 s) using a spray cabinet, with water, PAA (350 ppm or 400 ppm), PAA (350 ppm or 400 ppm) acidified with SSS (pH 1.2), or PAA (400 ppm) acidified with acetic acid (2%). All immersion treatments effectively ($P < 0.05$) reduced inoculated *E. coli* populations (6.2 log CFU/cm^2) by 2.3 to 2.8 log CFU/cm^2. When the test solutions were applied by spraying, the water and all PAA-containing treatments lowered inoculated populations (6.4 log CFU/cm^2) by 0.4 ($P \geq 0.05$) and 1.7–1.9 ($P < 0.05$) log CFU/cm^2, respectively. No ($P \geq 0.05$) differences in decontamination efficacy were observed between the 5 PAA-containing spray treatments. Overall, the results showed that PAA and the pH-adjusted PAA treatments were effective in reducing levels of the surrogates for Shiga toxin–producing *E. coli* and *Salmonella*. Although no differences in antimicrobial efficacy were noted between the nonacidified and acidified PAA treatments immediately after treatment application, further studies are needed to evaluate how the acidified PAA treatments perform as part of a sequential multi-hurdle decontamination strategy to reduce pathogen contamination on beef carcasses.

Key words: prerigor beef, antimicrobial interventions, peroxyacetic acid, surrogates, Shiga toxin–producing *E. coli, Salmonella*
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Introduction

An estimated 9.4 million cases of human illness occur annually in the United States as a result of consumption of undercooked or improperly handled foods contaminated with 31 known pathogens (Scallan et al., 2011). Nontyphoidal *Salmonella* spp. are estimated to be the leading cause of foodborne illness from a bacterial agent and are responsible for about 35% of hospitalizations and 28% of deaths related to foodborne illness (Scallan et al., 2011). Additionally, *Escherichia coli* O157:H7 and non-O157 Shiga toxin–producing *E. coli* (STEC) are estimated to cause approximately 175,000 illnesses and 20 deaths each year (Scallan et al., 2011). In the US, *E. coli* O157:H7 and 6 non-O157 STEC serogroups, including O26, O45, O103, O111, O121, and O145, are considered adulterants in raw nonintact beef products (USDA-FSIS, 2012; Wheeler et al., 2014).

Cattle are known reservoirs for STEC and *Salmonella*, and it has been reported that fecal contamination of the hide is likely the primary source of contamination of beef carcasses with these pathogens (Barkocy-Gallagher et al., 2002; Yang et al., 2017). In an effort to ultimately provide consumers with microbiologically safe products, the beef industry utilizes a number of sequential decontamination interventions during processing to reduce pathogen contamination levels on carcasses and beef products (Pohlman et al., 2002; Geornaras et al., 2012a; Buncic et al., 2014; Schmidt et al., 2014; Wheeler et al., 2014; Scott et al., 2015; Yang et al., 2017). These decontamination processes are physical and chemical in nature and include, among others, knife trimming, steam vacuuming, water washes, and application of antimicrobial treatments (Pohlman et al., 2002; Wheeler et al., 2014). The efficacy of various chemical interventions—such as organic acids (e.g., lactic acid) and peroxyacetic acid (PAA)—for reducing STEC and *Salmonella* have been extensively evaluated (Bacon et al., 2000; Berry and Cutter, 2000; Ransom et al., 2003; Gill and Badoni, 2004; Koohmaraie et al., 2005; Mohan and Pohlman, 2016; Kocharunchitt et al., 2020). Still, the beef industry continues to seek new chemical interventions for use in a multiple hurdle system to reduce pathogens on beef carcasses.

Chemical interventions should be inexpensive and easily implemented into existing systems while meeting regulatory standards (Scott et al., 2015). Use of PAA has been shown to effectively reduce STEC and *Salmonella* contamination on various beef products (Kitis, 2004; King et al., 2005; Geornaras et al., 2012a, b; Mohan and Pohlman, 2016), utilizing an oxidative mechanism for killing bacteria (Kitis, 2004). However, to our knowledge, there are no published studies reporting on the efficacy of PAA solutions that are pH adjusted with organic or inorganic acids (hereafter referred to as "acidified PAA") for reducing bacterial contamination on beef. Therefore, the objective of two studies that were conducted was to evaluate the antimicrobial effects of PAA acidified with different acids against inoculated surrogates for STEC and *Salmonella* on prerigor beef carcass surface tissue.

Materials and Methods

Bacterial strains and inoculum preparation

A 5-strain mixture of *E. coli* surrogates for STEC and *Salmonella* (Cabrera-Diaz et al., 2009; USDA-FSIS, 2020) was used in both studies to inoculate the prerigor beef carcass surface tissue pieces. The 5 *E. coli* biotype I strains (ATCC-BAA 1427, ATCC-BAA 1428, ATCC-BAA 1429, ATCC-BAA 1430, and ATCC-BAA 1431) were individually cultured and subcultured (35°C, 22 h) in 10 mL of tryptic soy broth (Difco, Becton Dickinson and Co. [BD], Sparks, MD). Following subculturing, broth cultures of all 5 strains were combined, and cells were harvested via centrifugation (6,000 × *g*, 15 min, 4°C; Sorvall Legend X1R, Thermo Scientific, Waltham, MA). Resulting cell pellets were washed in 10 mL of phosphate-buffered saline (pH 7.4; Sigma-Aldrich, St. Louis, MO), re-centrifuged as previously described, and resuspended in 50 mL of phosphate-buffered saline. The concentration of the inoculum mixture was approximately 8 to 9 log colony-forming units (CFU)/mL.

Inoculation of prerigor beef carcass surface tissue

Two replicate trials were performed on separate days for each of the 2 studies. For each trial, 40 sections of prerigor beef carcass surface tissue were collected from carcasses on the harvest floor of a commercial beef processing facility in northern Colorado. The beef tissue sections were obtained from the brisket area of carcasses after they had been subjected to electrical stimulation but before application of the final antimicrobial intervention treatment. Tissue samples were placed in insulated containers and were immediately transported to the Center for Meat Safety & Quality (Department of Animal Sciences, Colorado State University, Fort Collins, CO).

Beef surface tissue sections were cut into 10 × 10 cm portions, and each piece was randomly assigned to an untreated control group or one of the immersion (first study) or spray (second study) treatment groups. For each treatment, 5 beef tissue samples were placed onto trays lined with ethanol-sterilized foil, with the adipose side facing up, and were inoculated under a biosafety cabinet. A 0.2 mL aliquot of the *E. coli* inoculum was deposited, using a micropipette, on each tissue surface. The inoculum was then spread over the entire 100-cm^2 area with a sterile disposable spreader. The target inoculation level was 6 to 7 log CFU/cm^2. Inoculated samples were allowed 15 min for bacterial cell attachment before application of the antimicrobial treatments or analysis of the untreated (control) samples for determination of initial inoculated bacterial populations.

Application of antimicrobial treatments

In the first study, an immersion treatment method was used to screen 6 acidified PAA treatments for

antimicrobial effects against the inoculated surrogate populations, while in the second study, a spray application method was used to compare the antimicrobial efficacy of 3 acidified PAA treatments to that of PAA (i.e., PAA that was not pH adjusted) and a water treatment.

The treatments evaluated in the first study included (i) an untreated control, (ii) PAA (300 parts per million [ppm]; Kroff Food Services, Inc., Pittsburgh, PA) acidified with a proprietary blend of sulfuric acid and sodium sulfate (SSS) (pH 1.2; Zoetis, Parsippany, NJ), (iii) PAA (400 ppm) acidified with acetic acid (2%; pH 2.6; Fisher Scientific, Fair Lawn, NJ), (iv) PAA (400 ppm) acidified with citric acid (1%; pH 2.2; Fisher Scientific), (v) PAA (400 ppm) acidified with lactic acid (3.5%; pH 2.0; Corbion-Purac, Lenexa, KS), (vi) PAA (400 ppm) acidified with SSS (pH 1.2), and (vii) PAA (400 ppm) acidified with SSS (pH 1.8). Before mixing with the acidulant, PAA solutions (300 ppm and 400 ppm) had an average pH of 3.5. Target concentrations of PAA (300 ppm and 400 ppm) were verified with a hydrogen peroxide and peracetic acid test kit (LaMotte Company, Chestertown, MD). The antimicrobial immersion treatments were applied by placing individual inoculated beef tissue samples into sterile Whirl-Pak bags (55-oz; Nasco, Modesto, CA) containing 350 mL of the test solution. A separate Whirl-Pak bag with fresh, unused solution was used for treatment of each piece. Samples were completely immersed in the chemical solution for 10 s, after which they were removed from the bag with sterile forceps and placed on a sterile wire rack to drain (5 min) before analysis for surviving populations.

Seven treatments were tested in the second study and included (i) an untreated control, (ii) water, (iii) 350 ppm PAA (pH 3.5), (iv) 400 ppm PAA (pH 3.5), (v) PAA (350 ppm) acidified with SSS (pH 1.2), (vi) PAA (400 ppm) acidified with acetic acid (2%; pH 2.6), and (vii) PAA (400 ppm) acidified with SSS (pH 1.2). The water and PAA-containing treatments were applied using a custom-built spray cabinet (Birko-Chad Equipment, Olathe, KS) that was fitted with 18 FloodJet spray nozzles (0.1 gallons per minute; Spraying Systems Co., Glendale Heights, IL), with 10 nozzles above the product belt and 8 nozzles below. Solutions were applied at a pressure of 15 lb/in^2 with a product contact time of 10 s. After treatment, samples were placed onto sanitized wire racks and were left to drain for 5 min before being processed for microbial analysis.

Microbiological analysis

Untreated and treated beef tissue samples were placed into a Whirl-Pak filter bag (55-oz; Nasco) containing 175 mL of Dey/Engley neutralizing broth (Difco, BD). Samples were mechanically pummeled for 2 min (Masticator, IUL Instruments, Barcelona, Spain) and then serially diluted in 0.1% buffered peptone water (Difco, BD). Appropriate dilutions were plated, in duplicate, onto Petrifilm *Enterobacteriaceae* Count plates (3M, St. Paul, MN), and colonies were counted after incubation of plates at 35°C for 24 ± 2 h. On each of the experiment days, 3 uninoculated and untreated prerigor beef tissue samples were also analyzed to determine levels of naturally occurring *Enterobacteriaceae* populations. The detection limit of the microbial analysis was 0.2 log CFU/cm^2.

Statistical analysis

Two repetitions were conducted for each of the studies with 5 samples analyzed per treatment on each day (i.e., a total of 10 samples per treatment). Both studies were designed as a randomized complete block with experiment day serving as the block effect. Recovered *Enterobacteriaceae* populations were expressed as least-squares means for log CFU/cm^2 of prerigor beef surface tissue under the assumption of a lognormal distribution for plate counts. Data were analyzed using the lsmeans package in R (Rstudio, 2015) with antimicrobial treatment serving as the independent variable. Least-squares means were separated using a significance level of $\alpha = 0.05$.

Results

Levels of naturally occurring *Enterobacteriaceae* populations on the uninoculated beef tissue samples analyzed in the first study were ≤1.9 log CFU/cm^2, while in the second study, *Enterobacteriaceae* populations were not detected (<0.2 log CFU/cm^2) in any of the samples analyzed. Therefore, *Enterobacteriaceae* levels naturally associated with the prerigor beef samples used in the studies were lower than the *Enterobacteriaceae* populations recovered from any of the inoculated samples (Tables 1–2). As such, it can be concluded that the bacterial populations recovered from inoculated control (untreated) and immersion- or spray-treated beef samples were those of the *E. coli* inoculum.

Enterobacteriaceae populations recovered from inoculated untreated and immersion-treated beef tissue samples are shown in Table 1. Least-squares means are presented for the main effect of antimicrobial treatment. When compared to the untreated control, all

Table 1. Adjusted least-squares mean ($n = 10$) *Enterobacteriaceae* populations (log CFU/cm^2 ± SD) for inoculated (*Escherichia coli* biotype I; 5-strain mixture) prerigor beef carcass surface tissue samples that were left untreated (control) or were treated by immersion (10 s) in solutions of PAA acidified with various acidulants

Treatment	Bacterial Populations ± SD
Control (untreated)	6.2 ± 0.0^{a}
PAA (300 ppm) acidified with SSS (pH 1.2)	3.9 ± 0.1^{b}
PAA (400 ppm) acidified with acetic acid (2%)	3.7 ± 0.1^{bc}
PAA (400 ppm) acidified with citric acid (1%)	3.9 ± 0.1^{b}
PAA (400 ppm) acidified with lactic acid (3.5%)	3.4 ± 0.1^{c}
PAA (400 ppm) acidified with SSS (pH 1.2)	3.4 ± 0.1^{c}
PAA (400 ppm) acidified with SSS (pH 1.8)	3.9 ± 0.1^{b}

$^{a–c}$Least-squares means without a common superscript letter differ ($P < 0.05$).

CFU, colony-forming units; PAA, peroxyacetic acid; ppm, parts per million; SD, standard deviation; SSS, sulfuric acid and sodium sulfate blend.

Table 2. Adjusted least-squares mean ($n = 10$) *Enterobacteriaceae* populations (log CFU/cm^2 ± SD) for inoculated (*Escherichia coli* biotype I; 5-strain mixture) prerigor beef carcass surface tissue samples that were left untreated (control) or were spray-treated (10 s, 15 lb/in^2) with water, PAA, or PAA acidified with acetic acid or SSS

Treatment	Bacterial Populations ± SD
Control (untreated)	6.4 ± 0.3^{a}
Water	6.0 ± 0.1^{a}
PAA (350 ppm)	4.7 ± 0.2^{b}
PAA (400 ppm)	4.5 ± 0.2^{b}
PAA (350 ppm) acidified with SSS (pH 1.2)	4.6 ± 0.3^{b}
PAA (400 ppm) acidified with acetic acid (2%)	4.7 ± 0.2^{b}
PAA (400 ppm) acidified with SSS (pH 1.2)	4.5 ± 0.3^{b}

$^{a–b}$Least-squares means without a common superscript letter differ ($P < 0.05$).

CFU, colony-forming units; PAA, peroxyacetic acid; ppm, parts per million; SD, standard deviation; SSS, sulfuric acid and sodium sulfate blend.

treatments were effective ($P < 0.05$) in reducing inoculated *E. coli* populations (Table 1). Specifically, all 6 immersion treatments reduced ($P < 0.05$) initial inoculated bacterial populations (6.2 log CFU/cm^2) by 2.3 to 2.8 log CFU/cm^2. No ($P \geq 0.05$) differences in antimicrobial effects against the surrogates were observed between the 400-ppm PAA acidified with acetic acid, lactic acid, or pH 1.2 SSS treatments. Furthermore, antimicrobial effects against the *E. coli* populations were similar ($P \geq 0.05$) for samples immersion-treated with 400-ppm PAA acidified with acetic acid, citric acid, or pH 1.8 SSS, as well as 300-ppm PAA acidified with pH 1.2 SSS. Overall, although statistical differences ($P < 0.05$) were noted between some of the treatments, the difference in log reductions between these treatments was small (0.5 log CFU/cm^2).

Results obtained for the untreated and spray-treated beef samples are shown in Table 2. Least-squares means are presented for the main effect of antimicrobial treatment. Application of the water treatment was not effective ($P \geq 0.05$) in reducing inoculated bacterial populations. However, all of the tested acid treatments effectively ($P < 0.05$) lowered the inoculated *E. coli* populations, when compared to the untreated control and water treatments. Specifically, the PAA and acidified PAA treatments reduced ($P < 0.05$) initial surrogate populations (6.4 log CFU/cm^2) by 1.7 to 1.9 log CFU/cm^2. Under the experimental conditions of the study, no differences ($P \geq 0.05$) in decontamination efficacy were obtained between the acidified and nonacidified PAA treatments.

Discussion

Various chemical interventions used within the beef industry have been reported to have differing efficacies against bacterial populations on different beef tissues (Podolak et al., 1996; King et al., 2005; Geornaras et al., 2012a, 2012b; Schmidt et al., 2014; Yang et al., 2017; Cap et al., 2019; Thomas et al., 2020). In the US, PAA is commonly applied as a carcass wash intervention in beef processing facilities at a maximum concentration of 400 ppm (USDA-FSIS, 2019). In the current study, PAA (300 ppm or 400 ppm) solutions that were pH adjusted with one of 3 organic acids or SSS were initially screened, using an immersion application method, for their decontamination efficacy against the surrogate populations. In the second study, a spray application method was used, which is more representative of how the industry would apply antimicrobial interventions to beef carcasses, to compare the antimicrobial effects of 3 acidified PAA treatments to those of PAA solutions (350 ppm and 400 ppm) that were not acidified. Overall, the results showed that all acidified PAA immersion and

spray treatments were effective ($P < 0.05$) in reducing bacterial contamination on prerigor beef carcass surface tissue. Initial surrogate populations were lowered by 2.3 to 2.8 log CFU/cm^2 when samples were immersion treated and by 1.7 to 1.9 log CFU/cm^2 when spray treated (Tables 1–2). However, as seen, antimicrobial effects of the acidified PAA spray treatments were not ($P \geq 0.05$) different from those of PAA that was not acidified (Table 2). It should be noted that since a selective-differential plating medium, namely *Enterobacteriaceae* Petrifilm, was used for recovery of surviving populations, selective ingredients present in the culture medium could have hindered resuscitation of bacterial cells sublethally injured from exposure to the treatments (Bosilevac et al., 2004). As such, it is possible that surviving bacterial populations could actually be greater than those reported here.

To our knowledge, this is the first report on the antimicrobial effects of pH-adjusted solutions of PAA against inoculated bacterial populations on prerigor beef carcass surface tissue. In comparison, there are numerous published reports on the decontamination efficacy of the individual chemicals used in the current studies, including PAA (nonacidified), lactic acid (LA), and SSS, against pathogen populations on various beef tissue types (Berry and Cutter, 2000; King et al., 2005; Geornaras et al., 2012b; Schmidt et al., 2014; Scott-Bullard et al., 2017; Yang et al., 2017; Signorini et al., 2018). Due to differences in experimental parameters (e.g., antimicrobial concentrations tested, treatment application parameters, target populations, beef tissue type), direct comparisons of the results of these studies with those obtained in the current work cannot be made; however, the findings of these studies can be discussed as alternative intervention methods. In one study (Schmidt et al., 2014), researchers evaluated the efficacy of LA (2.5% and 5%), SSS (1%), and PAA (220 ppm), applied as immersion treatments, for reducing levels of pathogen populations inoculated on the adipose side of beef cheek meat. Cheek meat samples were inoculated with either *E. coli* O157:H7 (3.9 log CFU/cm^2), non-O157 STEC (4.0 log CFU/cm^2), or *Salmonella* (4.1 log CFU/cm^2) and were immersed in the treatment solutions for 1 min, 2.5 min, or 5 min. Schmidt et al. (2014) reported that, regardless of pathogen type and immersion treatment time, LA (2.5% and 5%), PAA, and SSS reduced inoculated populations by 1.4 to 2.1, 0.8 to 1.3, and 1.1 to 1.5 log CFU/cm^2, respectively. Geornaras et al. (2012b) evaluated 30-s immersion treatments of PAA (200 ppm) or SSS (pH 1.2) for their antimicrobial effects against inoculated populations of *E. coli* O157:H7 and non-O157 STEC on beef trimmings. The authors reported that treatment with 200 ppm PAA reduced inoculated populations (3.4–3.9 log CFU/cm^2) by 0.6 to 1.0 log CFU/cm^2 and treatment with SSS (pH 1.2) reduced pathogen populations by 0.3 to 0.4 log CFU/cm^2 (Geornaras et al., 2012b). In another study (King et al., 2005), PAA (200 ppm, 43°C)—applied as a 15-s spray treatment to prerigor tissue obtained from the outside round, plate, clod, and brisket regions of beef carcasses—reduced inoculated STEC and *Salmonella* populations by 0.7 log CFU/cm^2.

Overall, under the experimental conditions of the studies conducted, PAA and pH-adjusted PAA treatments effectively reduced populations of the *E. coli* surrogates for STEC and *Salmonella* on prerigor beef carcass surface tissue. However, our results showed that acidification of PAA (350 ppm or 400 ppm) with 2% acetic acid or pH 1.2 SSS did not enhance the antimicrobial effects of PAA. Chemical spray washes are commonly applied to beef carcasses at one or more points throughout the harvesting process (e.g., after hide removal, after evisceration, during carcass chilling) as part of a multiple-hurdle approach to reduce pathogen levels during slaughter. To better elucidate the decontamination efficacy, future studies should consider testing the pH-adjusted PAA in such a multihurdle system.

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