Validation of the Use of Organic Acids and Acidified Sodium Chlorite To Reduce *Escherichia coli* O157 and *Salmonella* Typhimurium in Beef Trim and Ground Beef in a Simulated Processing Environment

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ABSTRACT

A study was conducted to determine if acidified sodium chlorite (1,200 ppm) and acetic and lactic acids (2 and 4%) were effective in reducing foodborne pathogens in beef trim prior to grinding in a simulated processing environment. The reduction of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 at high (4.0 log CFU/g) and low (1.0 log CFU/g) inoculation doses was evaluated at various processing steps, including the following: (i) trim just after treatment application, (ii) in ground beef just after grinding, (iii) in ground beef 24 h after refrigerated storage, (iv) in ground beef 5 days after refrigerated storage, and (v) in ground beef 30 days after frozen storage. All antimicrobial treatments reduced the pathogens on the trim inoculated with the lower inoculation dose to nondetectable numbers in the trim and in the ground beef. There were significant reductions of both pathogens in the trim and in the ground beef inoculated with the high inoculation doses. On the trim itself, *E. coli* O157:H7 and *Salmonella* Typhimurium were reduced by 1.5 to 2.0 log cycles, with no differences among all treatments. In the ground beef, the organic acids were more effective in reducing both pathogens than the acidified sodium chlorite immediately after grinding, but after 1 day of storage, there were no differences among treatments. Overall, in the ground beef, there was a 2.5-log reduction of *E. coli* O157:H7 and a 1.5-log reduction of *Salmonella* Typhimurium that was sustained over time in refrigerated and frozen storage. Very few sensory differences between the control samples and the treated samples were detected by a consumer panel. Thus, antimicrobial treatments did not cause serious adverse sensory changes. Use of these antimicrobial treatments can be a promising intervention available to ground beef processors who currently have few interventions in their process.

Animals are natural reservoirs of foodborne pathogens, including *Salmonella* Typhimurium and *Escherichia coli* O157:H7. The muscle of a healthy animal is essentially sterile, but even under the most stringent conditions, it becomes contaminated during the slaughter process from the environment, hide, or direct contact with the intestinal tract contents. This contamination ultimately can cause consumer illness if the product is not appropriately handled by the processor or the consumer. Pathogen contamination is a great concern for processors, not only for human health issues but also for financial reasons. Processors must recall ground beef if it contains *E. coli* O157:H7 because *E. coli* O157:H7 is considered an adulterant by the U.S. Department of Agriculture, Food Safety Inspection Service (USDA-FSIS) (9). Many processors receive trim, grind it, and package it with very little control over the contamination coming in on the raw materials and with no validated interventions to be used to reduce the contamination. Most ground beef processors use cold temperatures, which will stop growth of pathogens but will not reduce them, to ensure the safety of the product.

The U.S. Food and Drug Administration has recently approved the use of acidified sodium chlorite (ASC) as a food additive to reduce pathogen loads on both prechill and postchill meat and poultry products, and the effectiveness in reducing pathogens on beef trim and in ground beef has not been previously reported. Organic acids are commonly used in the slaughter environment to reduce pathogen loads on carcasses but are not commonly used in ground beef facilities.

Previous studies on the use of interventions in ground beef or on trim have been conducted under controlled laboratory conditions and not under conditions that would typically be encountered in an industry environment. Additionally, data are conflicting due to application methods, holding times, pathogens of interest, and inoculation doses.

The objective of this study was to determine if application of ASC and organic acids to beef trim resulted in the reduction of foodborne pathogens under simulated industry conditions and if the treatments resulted in adverse effects on the sensory properties of the product during short-term and long-term storage.

MATERIALS AND METHODS

Experimental design. The antimicrobial effects of organic acids and ASC were evaluated by inoculating beef trim with a cocktail mixture of either *Salmonella* Typhimurium or *E. coli*
O157:H7 and then treating the trim with one of the interventions or with sterile, ambient temperature water (control). Samples were collected before treatment and at the following points during production: (i) immediately after treatment, (ii) immediately after grinding, (iii) 24 h after grinding, (iv) after 5 days of refrigerated storage, and (v) after 30 days of frozen storage. The pathogen experiments were conducted in the BL2 pathogen processing facility in the Food Technology Building at Texas Tech University under simulated industry conditions. The sensory experiments were conducted in the Texas Tech Meat Laboratory with samples not subjected to pathogen inoculation.

**Microbiological cultures.** Streptomycin-resistant (1,000 µg/ml) *Salmonella* Typhimurium (strains 1 and 2) (Texas Tech University Stock Culture Collection, originally isolated from ground beef) and *Escherichia coli* O157:H7 (strains 922, 925, 944, and 966) (Texas Tech University Stock Culture Collection) were used in this study. Frozen stock cultures were grown in Trypticase soy broth (TSB) containing 1,000 µg/ml of streptomycin (TSBS) at 37°C for 24 h. Cultures were passed three times prior to experimental use.

**Preparation of cocktail cultures.** A concentrated cocktail culture was prepared to facilitate inoculation of large quantities of beef trim. A 200-ml portion of TSBS was prepared for each individual strain of each pathogen. After 24 h, cells were harvested from the broth by centrifugation and resuspended in 20 ml of sterile water to create a concentrated culture. All *E. coli* O157:H7 strains were suspended together and all *Salmonella* Typhimurium were suspended together to make a concentrated cocktail mixture that was added to the beef trim as described below.

**Sample preparation.** From a commercial beef-packaging facility, beef trim was obtained with a 75% lean and 25% fat blend. For each replication (three for each pathogen), 54.4 kg of trim was processed. In the pathogen processing area, 49.8 kg of the trim was inoculated with the cocktail mixture of either *E. coli* O157:H7 or *Salmonella* Typhimurium by dipping each piece of trim into a container containing the pathogen mixture diluted with a buffer solution. Two separate lots of trim were prepared with two separate inoculation levels. The first portion of beef trim was dipped into an inoculation solution with the pathogen concentrations at a level of $1 \times 10^6$ CFU/ml, and the second set was inoculated at a level of approximately $1 \times 10^5$ CFU/ml. Concentrations in the dip solution were determined by direct plating as described below. The concentrations in the solution resulted in concentrations on the trim of approximately $1 \times 10^5$ CFU/g (high) and $<2.5 \times 10^1$ CFU/g (low), respectively. The actual concentration of the low-level inoculations was not determined because the numbers were below the enumeration detection limits. Prior to experimentation, the presence of the pathogen was determined by using the BAX system as described below. The total number of pathogen cells present in the high inoculation samples were enumerated throughout the study, whereas the pathogens were detected only in samples containing the low inoculation doses.

After the inoculation dip, the trim was held for 20 min on sanitized stainless steel mesh racks to allow for pathogen attachment. After attachment, trim for each inoculation dose was divided into equal portions for treatment. Trim was fed to the grinder on an Intralox conveyor belt system (series 800, Intralox, Inc., Harahan, La.) similar to those used in the meat industry. While traveling down the conveyor, the trim was treated by spraying one of the antimicrobial treatments onto the surface as it moved down a conveyor system toward the grinder. All treatments were applied at ambient temperature. Trim was only sprayed with the antimicrobial treatment on one side (to simulate conditions encountered in the industry) but was mixed together in the grinder, which exposed most surfaces to the treatment. Individual portions were sent down the conveyor and treated with one of the six treatments: (i) 2% acetic acid, (ii) 4% acetic acid, (iii) 2% lactic acid, (iv) 4% lactic acid (Fisher Scientific International Inc., Hampton, N.H.), (v) acidified sodium chloride (1,200 ppm) (Sanova, Alcide Corporation, Redmond, Wash.), and (vi) sterile ambient-temperature water. After the trim was processed, the conveyor system and grinder were cleaned with a commercial detergent and sanitized with a 0.05% chlorine solution between treatments within a replication. All processing equipment was cleaned with a detergent and sanitized with the application of a 0.05% chlorine solution and resanitized with Bi-Quat (Birkco Corp., Henderson, Colo.) between replications. Prior to experimentation, steam was applied to all surfaces for additional sanitization and to remove all sanitizer residue. A portion of uninoculated control samples was processed before the pathogen-inoculated samples to remove all residues. Samples from this grinding were also used to determine if any background flora were present prior to experimentation.

Samples of the trim were taken after the intervention application and immediately prior to grinding. The remaining trim was ground, and the ground beef was divided into four equal portions, packaged with a commercial vacuum-packaging unit (no vacuum was applied), and three portions were stored at 4°C in the processing lab for either 6 or 24 h or 5 days when samples were collected for microbiological analysis. The fourth portion of the ground beef was frozen and analyzed 30 days after processing. The frozen sample was thawed at 4°C for 24 h prior to sampling. All experiments were repeated three times. Both *E. coli* O157:H7 and *Salmonella* Typhimurium inhibition were evaluated separately.

**Microbiological analysis: high inoculation samples.** Samples were removed from the cooler, and 10 g of the sample was aseptically weighed and placed into a sterile stomacher bag (model 400 bags 6041, Stomacher Lab System, Seward Medical, London, UK). Buffered peptone water (90 ml) was added with the sample in the sampling bag for mixing to make a 1:10 dilution of the sample. The bag and contents were placed in a laboratory blend stomacher (model 400, Seward Medical) and processed for 2 min. After stomaching, 3 ml of the sample was extracted and placed into a spiral plater sampling cup. Samples were automatically serially diluted and plated with the Spiral Biotech Autoplate system (Spiral Biotech, Norwood, Mass.). The samples inoculated at high doses were exponentially plated onto two Trypticase soy agar plates with the addition of 1,000 µg of streptomycin antibiotic (TSAST). The streptomycin antibiotic inhibited growth of the background flora while allowing for pathogen growth. Total numbers of the pathogen present were determined with the Spiral Biotech Q Count (version 2.0, Spiral Biotech). Uninoculated control samples were also subjected to plating in the TSAST agar to validate that there were no background flora resistant to the streptomycin.

**Microbiological analysis: low inoculation samples.** A 10-g sample of the trim and/or ground beef was subjected to preenrichment in either buffered peptone water (for *Salmonella* Typhimurium) or TSB (for *E. coli* O157:H7). Preenrichment broth was incubated at 37°C for 18 h, and the preenriched cultures were streaked onto either XLT4 agar (Salmonella Typhimurium) or onto sorbitol MacConkey agar (*E. coli* O157:H7) to observe typical colony formation. Typical colonies were confirmed with commercially available agglutination kits. Additionally, the preenriched cultures were subjected to the BAX system (DuPont Qualicon,
Sensory evaluation. The sensory portion of this project was performed in the same manner as the microbiological portion, except none of the trim was inoculated with a pathogen. Samples were processed in the Texas Tech Meat Laboratory (a separate building from the pathogen laboratory). Likewise, ground beef pattie samples were collected at the following production points: (i) immediately after pattie production, (ii) 24 h after pattie production, (iii) 5 days after pattie production, (iv) 30 days after pattie production, and (v) 90 days after pattie production.

From a commercial beef-packing facility, 240 lb of beef trim were obtained. One hundred eighty pounds of beef trim was used in each replication \( (n = 3) \). Upon arrival at the Texas Tech Meat Laboratory, the trim was divided into a 60-lb portion to serve as the control and five 24-lb portions to serve as trim for the antimicrobial treatments. The control portion was treated with a sterile water spray instead of an intervention.

The remaining portions of trim were treated with one of the following six treatments: (i) 2% acetic acid, (ii) 4% acetic acid, (iii) 2% lactic acid, (iv) 4% lactic acid, (v) acidified sodium chloride (1,200 ppm), and (vi) sterile ambient temperature water. The trim was sprayed with one of the five treatments, and only one side of the trim was treated.

After being sprayed, the trim was coarsely ground through a 1.3-cm plate on a three-phase Biro meat grinder (model 346, Biro, Ft. Smith, Ark.). The coarse-ground samples were then ground through a 0.3-cm fine-grind plate on the same Biro meat grinder. Ground beef was formed into 16-lb patties by a Super Model 54 Hollymatic pattie machine (Hollymatic, Park Forrest, Ill.). A total of 72 patties was formed for each of the five treatments and 180 control patties for each replication. Ground beef patties were labeled, divided, and packaged according to treatment groups.

Samples to be analyzed at 6 and 24 h were overwrapped with Reynolds 914 oxygen-permeable overwrap film (Reynolds, Richmond, Va.) and refrigerated at 4°C until sampling. Samples for 5, 30, and 90 days were vacuum packaged by a Koch vacuum-packaging machine (model 88045, Koch Equipment, Kansas City, Mo.) in Cryovac 9 × 14, 250-ml, plain tapered, two-sided, straight-seal vacuum bags (no. 9C93, Cryovac, Saddle Brook, N.J.) and then refrigerated at 4°C (5 days) or frozen at −15°C (30 and 90 days) for further analysis.

Sensory evaluation. Patties were thawed at a temperature between 2 and 5°C for 24 h (30- and 90-day samples). A three-digit number was randomly assigned to each pattie, which was identified by this number throughout the cooking process and sensory panel evaluation. Precooking and postcooking weights and temperatures were recorded to determine cooking loss and the final internal temperature. All patties were cooked on the Belt Grill (model TBG-60 Magigrill, MagiKitch’n, Inc., Quakertown, Pa.) to an internal temperature of 71°C. Each pattie was cut into eight identical pieces and placed on prenumbered and trisectioned Styrofoam plates.

Styrofoam plates were divided into three equal sections with a black permanent marker. Each section was labeled from left to right with a 1, 2, or 3. Identifying the order from 1 to 3, the panelist would evaluate the samples. Styrofoam plates were also labeled with the panelist number \( (n = 24) \) and panel number \( (n = 5) \) for identification purposes. Additionally, each of the three sections was assigned the pattie’s random three-digit number corresponding to the panelist’s answer sheet. Panelists were provided a prenumbered answer sheet corresponding to the panel number, panelist number, and sample numbers.

The sensory tests were conducted based on the work of Meilgaard et al. (8). A nontrained panel \( (n = 24) \) was asked to determine the odd sample between two like samples and one different sample. If panelists could not determine a difference among the samples, they were instructed to guess. Panelists were asked to identify the odd sample by the sample’s randomly assigned three-digit number on the worksheet space provided. Samples were served under red lights to mask color differences. The panelists received water and apple juice to rinse their palates between samples.

Statistical analysis. Data were analyzed as a completely randomized design using the mixed models procedures (PROC MIXED) of SAS (SAS/STAT Users Guide, version 6, 4th ed., SAS Institute, Cary, N.C.). Where appropriate, repeated measures methodologies were employed and first-order autoregression matrices were used to model within observation dependency over time. Pathogen counts (log transformed), media type, and form were dependent variables of interest, and treatment and sampling times were independent variables.

Replication and its interactions were forced into the model as random effects. Full second-order models were used and terms were systematically removed from the model if their \( P \)-values were >0.05 while maintaining hierarchy within terms. When a significant \( F \) statistic was detected, multiple comparisons of means (least significant difference) was used to separate effects.

RESULTS

Microbiological evaluation. For all samples inoculated with the low dose of *Salmonella* Typhimurium and *E. coli* O157:H7, there were no detectable pathogens on the trim after all of the five treatments were applied, whereas all of the control samples contained detectable amounts of both the pathogens (data not shown). Treated trim samples were positive before application of the treatment. The same was observed in the ground beef, with all treatments resulting in nondetectable numbers of *E. coli* O157:H7 and *Salmonella* Typhimurium at all sampling intervals and all of the control samples having detectable pathogens at all sampling intervals. The low numbers of pathogens would be more indicative of what would be encountered in the industry. A second study was necessary to determine quantitative amounts of reductions at a higher initial inoculation dose because quantitative reductions could not be measured in the first study.

The initial populations of the pathogens on both the *Salmonella* Typhimurium and *E. coli* O157:H7 inoculated at high doses were not significantly different before treatment (Figs. 1 and 3). The total numbers ranged from 5.0 log CFU/g to 5.3 log CFU/g in the *E. coli* O157:H7–inoculated samples to 4.2 log CFU/g to 4.4 log CFU/g in the *Salmonella* Typhimurium–inoculated trim. The antimicrobial treatments effectively reduced pathogen loads on the trim prior to grinding (Figs. 1 and 3) compared with the trim treated with an ambient room–temperature water rinse. The water rinse did not significantly reduce pathogen loads compared with the initial numbers found on the trim prior to treatment for both *E. coli* O157:H7 and *Salmonella* Typhimurium. For both *E. coli* O157:H7 and *Salmonella* Typhimurium, there was an approximate 1.5-log reduction of the number of pathogens, with no significant differences
among the treatments. In both trim and ground beef samples, there were no streptomycin-resistant organisms detected in the background control samples that were not inoculated.

The total numbers of E. coli O157:H7 and Salmonella Typhimurium in the ground beef were significantly lower ($P < 0.05$) than in the numbers of pathogens detected in the control ground beef at all sampling intervals during both refrigerated and frozen storage (Figs. 2 and 4).

E. coli O157:H7 was reduced in the ground beef by 2.0 to 2.5 log cycles in the samples treated with organic acids (Fig. 2). Generally, the ground beef made from trim treated with ASC had significantly higher ($P < 0.05$) numbers of E. coli O157 detected at all sampling intervals compared with the organic acid–treated samples, but the numbers were still significantly less than the control samples. After 5 days of refrigerated storage, the number of E. coli O157:H7 detected in the ground beef treated with 4% lactic acid had total numbers similar to those in the ASC-treated sample and was significantly higher ($P < 0.05$) than the numbers found in the other organic acid–treated samples. An important observation was that treatment with a 4% organic acid did not have additional benefits over treatments with 2% organic acid treatments for both Salmonella Typhimurium and E. coli O157:H7.

The reduction of Salmonella Typhimurium was also sustained during refrigerated and frozen storage. Treatments with all organic acids resulted in significant ($P < 0.05$) 1 to 1.5 log reductions on samples taken just after grinding with ASC resulting in no significant reductions ($P > 0.05$) in the ground beef compared with the control. After 1- and 5-day storage periods at refrigerated temperatures and after 30 days of frozen storage, there were no significant ($P > 0.05$) differences among treatments (Fig. 4).

**Sensory evaluation.** A significance level determined to be $P < 0.05$ was used to evaluate the correct responses in the sensory study. With a panelist size of 24, the minimum number of correct responses was established at 13, to
TABLE 1. Results of triangle tests conducted on ground beef treated with organic acids and acidified sodium chlorite

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*a A total of 13 responses are needed to detect significance at the P < 0.05 level of significance.

show a significant difference at P < 0.05 (6). A correct response was characterized as correctly identifying the odd or different sample. Thus, there was not a significant difference in the mean correct responses between controls, 2% acetic acid, 4% acetic acid, 2% lactic acid, or ASC within treatments or days of storage (Table 1). Four percent lactic acid did not show a significant difference in the mean correct responses for 6- or 24-h and 5-day samples. However, at day 30, 4% lactic acid did show a significant difference at P < 0.05.

DISCUSSION

A limited number of studies have been conducted to determine the antimicrobial effects of interventions on beef trim. In previous studies, lactic acid and ASC have been effective in reducing pathogen loads on beef carcasses and, to a limited extent, on beef trim.

Studies related to the use of acetic and lactic acids as decontaminants for beef trim have been conflicting. Applications of 2 and 4% organic acids did not significantly reduce populations of E. coli O157:H7 or Listeria monocytogenes in beef trim or in the ground beef produced from that trim in one study (3). However, other studies (2, 6) reported that applying various antimicrobial treatments, including organic acids, hot water, and hot air, to cold carcasses or trim significantly reduced Salmonella and E. coli O157:H7 populations and naturally occurring microorganisms on trim and in ground beef.

The treatment of trim with antimicrobial interventions including 2 and 4% acetic acid and 2 and 4% lactic acid significantly reduced E. coli O157:H7 and Salmonella Typhimurium on beef trim and ground beef. Reductions were 1.5 to 2.5 log cycles depending on the pathogen and the treatment method. The higher inoculation reductions were sustained during short-term refrigerated and long-term frozen storage for most antimicrobial intervention treatments. The higher concentrations of organic acids did not have additional reductions of the pathogen loads on the trim or ground beef samples. The lower concentrations should be used based on economic aspects and to minimize sensory changes in the product.

Studies on the use of ASC have also been conflicting. Bosilevac et al. (1) evaluated the effects of 600 ppm and 300 ppm on the reductions of aerobic plate counts and total Enterobacteriaceae counts in beef trim. They reported significant reductions of both groups of bacteria, with the 600 ppm giving the most reductions and the 300 ppm giving the best sensory results. In another study, Lim and Mustapha (7) reported that a 1,000-ppm solution of ASC alone or in combination with other chemicals reduced L. monocytogenes, Staphylococcus aureus, and E. coli O157 on beef cubes. However, there was a loss of redness associated with the beef surfaces treated with the ASC. Gill and Badoni (4) examined the effects of ASC on beef carcasses in a commercial processing plant. They reported that a 1,600-ppm solution had little effect on total aerobic organisms, generic E. coli, and coliforms on the carcasses. Hajmeer et al. (5) reported that a 1,000-ppm solution of ASC applied to beef briskets significantly reduced E. coli O157 and S. aureus.

In this study, a low-level inoculation study was also conducted to mimic what would actually be encountered in the industry. All treatments reduced the lower inoculation level of the pathogens to nondetectable levels, further illustrating that any of these treatments could be possible interventions for ground beef processors.

The triangle tests indicate that panelists could only detect a difference between the controls and 4% lactic acid–treated patties frozen for 30 days. Panelists could not detect any differences between the short- or long-term storage of the controls or treated patties at the postproduction sampling periods. Because 2% lactic acid is as effective as 4%, and no differences were detected in the 2% treatment, lactic acid is still a viable option for ground beef processors.

In conclusion, all antimicrobial interventions reduced the lower inoculation level of pathogens to a nondetectable level, with significant reductions of the higher inoculation levels of pathogens on trim and in ground beef indicating that the organic acids applied at the 2% level or ASC applied at 1,200 ppm could be effective interventions for ground beef processors. There were not any visual differences assessed by a trained visual panel for patties undergoing short-term refrigerated storage, which is more indicative of consumer perceptions in the retail case, so the use of these interventions should not result in adverse quality changes in the product. Use of interventions in ground beef production is a viable option to further ensure the safety of the ground beef supply.

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REFERENCES


