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Microbiological and organoleptic characteristics of beef trim and ground beef treated with acetic acid, lactic acid, acidified sodium chlorite, or sterile water in a simulated commercial processing environment to reduce *Escherichia coli* O157:H7 and *Salmonella*

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ABSTRACT

The objective of this study was to validate the effectiveness of acetic and lactic acids (2% and 5%), acidified sodium chlorite (1000 ppm), and sterile water in reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in inoculated beef trim in a simulated processing environment. Samples were collected to assess microbial characteristics at three processing points. Results from this study indicate that all treatments, including sterile water, reduced pathogen concentrations ($P<0.05$) of both *E. coli* O157:H7 and *Salmonella* Typhimurium in ground beef up to 0.5 and 0.6 log by 24 h, respectively. In some cases, there were no significant differences between the antimicrobial treatments and the sterile water using this application method. Triangle sensory test results of non-inoculated beef indicated there were no differences ($P<0.05$) in the means of correct responses between controls or antimicrobial treatments at 6 or 24 h. While interventions are important for beef trim, use of the interventions must be validated under industry conditions to ensure proper effectiveness.

1. Introduction

The muscle of a healthy animal is essentially sterile, but even under the most stringent conditions, muscle can become contaminated during the harvest process from the environment, hide, or from direct contact with the intestinal tract contents. Contamination ultimately can cause consumer illness if the product is not appropriately handled by the processor or the consumer. Pathogens are of great concern for processors for both food safety and for economic reasons. While beef trimmings and ground beef are to be cooked by the consumer, processors should recall raw product if testing indicates the presence of *Eschericia coli* O157:H7. Processors have very few interventions for beef trimmings and ground beef.

In previous studies, organic acids and acidified sodium chlorite (ASC) have effectively reduced pathogen loads on beef carcasses or cuts (Castillo et al., 2001; Kang, Koohmaraie, & Siragusa, 2001); however, organic acids and ASC as decontaminants of beef trim have produced conflicting results. Connor, Kotrola, Mikel, and Tamblyn (1997) reported that 2 and 4% organic acid sprays did not reduce populations of *E. coli* O157:H7 or *Listeria monocytogenes* in beef trim or ground beef. Even so, Castillo et al. (2001) reported that ground beef from carcasses sprayed with 4% lactic acid had significantly reduced populations of *Salmonella* spp. and *E. coli* O157:H7, and Harris, Miller, Loneragan, and Brashears (2006) showed a 2.5 log reduction for *E. coli* O157:H7 and 1.5 log reduction for *Salmonella* from the spray application of organic acids. However, it is highly unlikely that the coverage system as well as the dwell time observed in a laboratory setting would be followed in industry. For example, Connor et al. (1997) held trim 24 h after treatment application before grinding. In an attempt to replicate a commercial processing environment, Harris et al. (2006) used a belt system to transport beef trim to a grinder, but manually sprayed the trim on only one side.

A limited amount of research has been completed to determine the effectiveness of interventions under commercially simulated conditions on beef trim to reduce pathogens. Therefore, the objective of this study was to validate the effectiveness of ASC (1000 ppm), acetic and lactic acids (2% and 5%), and sterile water in reducing *E. coli* O157:H7 and *Salmonella* Typhimurium on beef trim prior to and after grinding in a simulated processing environment utilizing an

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automated spray application with a belt system that rotates and flips the trim for more even distribution. Also, the treatment effects on sensory quality and organoleptic properties will be determined.

2. Materials and methods

2.1. Experimental design

The antimicrobial effects of organic acids used at 2 and 5% and ASC (1000 ppm) were evaluated by inoculating beef trim with either *E. coli* O157:H7 or *Salmonella* Typhimurium and allowing for pathogen attachment. Trim was treated with one of the interventions, and samples of trim were collected in triplicate form before treatment (control) and at the following points during production: (i) immediately after treatment (20 min); (ii) immediately after grinding (6 h); and (iii) 24 h after grinding. The experiment was conducted at the Texas Tech University Pathogen Processing Laboratory under simulated industry conditions using a belt and spray system similar to those used in the industry.

2.2. Preparation of pathogen cultures

In order to test the efficacy against multiple strains, three strains of streptomycin-resistant (1000 μg/ml) *E. coli* O157:H7 (922, 944, and 966), and two strains of *Salmonella* Typhimurium (strains 1 and 2), all isolated from beef animals or products, were selected for use in the study. Streptomycin resistant strains were used to facilitate recovery on non-selective media in the presence of background flora. These cultures were obtained from the stock culture collection at Texas Tech University. Stock cultures were maintained in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) at −80 °C. Strains were grown separately and subcultured three times in TSB containing 1000 μg/ml of streptomycin sulfate salt (TSBS; Sigma, S9137-100G, Sigma-Aldrich, Inc., St. Louis, MO) for 24 h at 37 °C. A concentrated cocktail culture was prepared to facilitate inoculation of large quantities of beef trim. A frozen stock culture of each strain was thawed to room temperature (25–27 °C), placed into 200 ml of TSBS, and incubated at 37 °C for 24 h. Cells were harvested from the broth by centrifugation (4000 rpm × 3 g for 20 min at 18 °C) and re-suspended in 20 ml of sterile water to create a concentrated culture. Re-suspended solutions of each stain were mixed in equal volumetric parts to obtain a cocktail for *E. coli* O157:H7 and a cocktail for *Salmonella* Typhimurium.

2.3. Sample preparation and inoculation

Beef trim (163.6 kg) was obtained from a commercial beef packing facility with an 80% lean and 20% fat blend and stored at 2 °C until inoculation. During each of the three replications for each pathogen, 81.8 kg of trim was processed. Beef trim was transported to the Texas Tech University Pathogen Processing Laboratory, which is a BioSafety Level 2 facility. Prior to inoculation a sample of trim was taken to ensure no pathogens were present on the trim prior to inoculation. The product was tested using the standard protocols on the BAX system (Dupont Qualicon, Wilmington, DE) that is capable of detecting very low levels of pathogens. Trim (81.8 kg) was inoculated with either the *E. coli* O157:H7 or *Salmonella* Typhimurium cocktail mixture. The frozen concentrated culture cells were washed prior to freezing in buffered peptone water (EMD Chemicals, Inc., Gibbstown, NJ) and were resuspened in 2 l of buffered peptone water at a pH of 7.2 after thawing. Trim was inoculated by dipping each piece of trim (average size 15.2 × 25.4 cm) for 2 min into a sanitized container containing the pathogen mixture mixed with buffered peptone water, which was held at ambient temperature (25 °C) for 30 min. The target populations on the beef trim were 1×10^5 CFU/g on the surface of the trim. Samples of trim were plated onto two trypicase soy agar (TSA; EMD Chemicals, Inc., Gibbstown, NJ) plates with the addition of 1000 μg/ml of streptomycin antibiotic to ensure the target population was obtained. After the inoculation dip, the trim was held for 20 min on sanitized stainless steel mesh racks to allow for pathogen attachment. Control samples of trim were taken before intervention application.

After attachment, trim for each study was divided into equal portions (9.1 kg). The individual portions were transported via conveyor belt and treated with one of the six treatments: (i) 2% acetic acid; (ii) 5% acetic acid; (iii) 2% lactic acid; (iv) 5% lactic acid (Fisher Scientific International Inc., Hampton, NH); (v) ASC (1000 ppm; pH 2.5–2.9) (Sanova, Alcide Corporation, Redmond, WA); and (vi) sterile water. All treatments were applied to the trim at ambient temperature (25 °C). The trim was treated by spraying one of the antimicrobial treatments onto its surface of the trim as it moved through a six-nozzle, trim-sanitizing spray cabinet (Chad Co., Olathe, KS) via a conveyor belt system (Series 800, Intralox, Inc., Harahan, LA) towards the grinder. The conveyor belt was set to expose the trim to the intervention for 10 s throughout the entire study. Trim was treated by spraying an automatic premixed spray onto both surfaces of the trim with nozzles located 15.2 cm above the trim and 5.1 cm below the trim at both the beginning and end of the conveyor belt. The flow rate for each nozzle was 0.42 l/min with a pressure of 138 kPa. As the trim entered the chamber, the intervention was sprayed onto the top and bottom surfaces. Trim was then flipped on to the same conveyor, and sprayed a second time again on both the top and bottom surfaces prior to grinding. The conveyor system, grinder, and processing equipment were cleaned with a chlorinated alkaline cleaner and sanitized with Bi-Quat (Birko Corp., Henderson, CO) between replications. Steam was applied to remove all cleaning residues.

Samples of trim from each treatment were taken in triplicate form after the intervention application and immediately before grinding. The remaining trim was ground and samples were immediately collected in triplicate form for microbiological analysis. The remaining ground beef was divided into two equal portions and vacuum packaged by a Koch vacuum-packaging machine (Ultravac 250, Koch Equipment, Kansas City, MO) in non-permeable Cryovac vacuum bags (Cryovac, Saddle Brook, NJ). Those portions were stored at 4 °C in the processing lab for either 6 or 24 h. Ground beef samples were collected in triplicate form for each treatment and tested at 6 and 24 h after processing. Both *E. coli* O157:H7 and *Salmonella* Typhimurium inhibition were evaluated separately.

2.4. Microbiological analysis

During the sampling process, 10 g of the sample were collected and placed in a stomacher bag (Model 400 Bags 6041, Stomacher Lab System Seward Limited, London, UK). Buffered Peptone Water (99 ml) was added to the sample in the stomacher bag. The bag and contents were placed in a laboratory blend stomacher (Model 400, Seward Medical, London UK) and processed at 230 rpm for 60 s. Afterwards, 3 ml of the sample was collected and placed into a spiral-plater sampling cup. Samples were automatically plated using the Spiral Biotech Autoplate® (Spiral Biotech, Norwood, MA). The samples were exponentially plated in duplicate with 50 μl of the sample on both TSA plates and TSA plates with the addition of 1000 μg/mL of streptomycin antibiotic. The two plates for each media type were averaged prior to statistical analysis. The streptomycin antibiotic inhibited growth of the background flora while allowing for pathogen growth. Total aerobic plate counts (APC) were determined using TSA with the absence of the streptomycin antibiotic. Plates were incubated for 48 h at 37 °C. After incubation, plates were counted using the Spiral Biotech Q Count™ (Version 2.0, Spiral Biotech, Norwood, MA).

2.5. Sensory and retail display preparation

Beef trim (217.7 kg) was obtained from a commercial beef-packing facility with an 80% lean and 20% fat blend. Trim was divided