51st International Congress of Meat Science and Technology
August 7-12, 2005 – Baltimore, Maryland USA

APPLICATION OF ANTIMICROBIAL TREATMENTS IN A COMMERCIAL SIMULATION TO REDUCE *ESCHERICHIA COLI O157:H7* AND *SALMONELLA SPP.* IN BEEF TRIM AND GROUND BEEF

D.D. Harris, M.M. Brashears, J.C. Brooks, and M.F. Miller

Department of Animal and Food Sciences, Box 42141 Texas Tech University, Lubbock TX 79409

Key Words: Acidified sodium chlorite, acetic acid, lactic acid, sterile water, pathogens, beef.

Introduction

Animals are natural reservoirs of food-borne pathogens including *Salmonella* spp. 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 from 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. Pathogens are of great concern for processors for both food safety issues and for economic reasons. While beef trimmings and ground beef are to be cooked by the consumer, the processor must recall the raw product if testing indicates the presence of *Escherichia coli* O157:H7. Processors have very few interventions for beef trimmings and ground beef.

A limited amount of research has been completed to determine the effectiveness of interventions under commercially simulated conditions on beef trim to reduce pathogens. Additionally, some results are conflicting and do not address “practical” issues that are encountered in a processing environment. It is essential that the long-term effects on the product quality be evaluated to determine if the product has a similar shelf-life and sensory qualities as non-treated products.

In previous studies, lactic acid and acidified sodium chlorite (ASC) have been effective in reducing pathogen loads on beef carcasses and to a limited extent, on beef trim. Castillo et al. (1999) reported that acidified sodium chlorite reduced populations of *Salmonella* Typhimurium and *Escherichia coli* O157:H7up to 3.9 log cycles when applied to beef carcass cuts after water wash. The Food and Drug Administration (FDA) has recently approved the use of ASC as a food additive to reduce pathogens loads on both pre-chill and post-chill meat and poultry products. However, the effectiveness of antimicrobials in a commercially simulated application on reduction of pathogens on beef trim has not been thoroughly reported in peer-reviewed journals. A limited amount of industry generated and non-peer reviewed data exist, but the effectiveness of the product needs to be studied under controlled conditions that mimic production conditions so processors can make informed, science-based decisions about the use of this product.

Studies related to the use of acetic and lactic acids as decontaminants for beef trim have been conflicting. Conner et al. (1997) reported that 2 and 4% organic acid sprays did not significantly reduce populations of *Escherichia coli* O157:H7or *Listeria monocytogenes* in beef trim or in the ground beef produced from that trim. On the other hand, Castillo et al (2001) reported that applying a 4% lactic acid spray to cold carcasses and then subsequently producing ground beef from the carcasses reduced

Escherichia coli O157:H7 and *Salmonella* spp. populations by 3.3-3.4 log cycles and additional reductions in the ground beef made from these carcasses. Kang et al. (2001) reported that multiple antimicrobial treatments were effective in reducing microbial populations on beef trim. They utilized hot water, hot air, and lactic acid to produce the antimicrobial effect on the trim and subsequently in the ground beef. They evaluated the effects of the treatments on naturally occurring microorganisms and did not report the effect on microbial pathogens. The numbers of aerobic organisms, psychotrophs, coliforms, and lactic acid bacteria were significantly lower in the treated samples.

Many currently published studies are conducted in a very controlled laboratory conditions and do not mimic the actual processing environment. For example, some studies report that trim was treated, held for 24 h, and then ground (Connor et al.). It is highly unlikely that this practice would be followed in industry. Additionally other studies report that the pieces of beef trim are treated evenly on all sides with the intervention. Again, it is unlikely that an on-line intervention process for beef trim will allow complete coverage of the pieces with the antimicrobial treatments. If the pieces of trim are dipped then they will likely contain too much of the antimicrobials and will adversely affect the product quality. If they are sprayed, then only a portion of the surface will be covered. Processors need validation in a setting that is similar to actual processing environments. At Texas Tech University we have a Pathogen Processing Area with processing equipment and facilities to produce both fresh and processed meat products that are pathogen inoculated. We are in a unique position to validate current interventions using “real world” conditions and generate data that are directly applicable to the meat processing industry. We recently completed a real world simulation at Texas Tech that showed the effectiveness of organic acids and acidified sodium chlorite in reducing the levels of both *Salmonella* spp. and *Escherichia coli* O157:H7. The results given in the final report to National Cattlemen’s Beef Association (NCBA) show promising results; however, an automated spray application system that flips the trim needs to be validated for their effectiveness in application of the pathogen reducing interventions.

Objective

The objective of this study was to validate the effectiveness and application of acidified sodium chlorite (1000 ppm), acetic and lactic acids (2% and 5%) and sterile water in reducing *Escherichia coli* O157:H7 and *Salmonella* spp. levels on beef trim prior to and after grinding in a simulated processing environment utilizing a belt turning and spray application.

Methodology

The antimicrobial effects of organic acids and acidified sodium chlorite (ASC) were evaluated by inoculating beef trim *Escherichia coli* O157:H7 or *Salmonella* spp. allowing for pathogen attachment, treating the trim with one of the interventions and collecting samples of trim before treatment (control) and at the following points during production: (1) immediately after treatment (20 min); (2) immediately after grinding (6 h); (3) 24 h after grinding. The experiment was conducted in the pathogen processing facility in the Food Technology Building at Texas Tech University under simulated industry conditions.

From a commercial beef-packing facility, 163.29 kg. of beef trim were obtained with an 80% lean and 20% fat blend. For each replication of *Escherichia coli* O157:H7 inoculated samples (n=3) and *Salmonella* spp. inoculated samples (n=3), 81.65 kg. of trim was processed. In the pathogen processing area, 81.65 kg. of the trim was inoculated with a cocktail mixture of a streptomycin-resistant strains of either *Salmonella* spp. (strains 1 and 2) (University of Nebraska, Lincoln, NE) or *Escherichia coli* O157:H7 by dipping each piece of trim into a sanitized container containing the pathogen mixture along with a buffer solution. The target population on the beef trim was1 x 10^5 cfu/g, on the surface of the trim.

Prior to inoculation a sample of trim was taken to ensure no pathogens were present on the trim prior to inoculation. 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 (4.54 kg.). Trim was fed to the grinder in simulated processing environment utilizing a belt turning and spray application. The trim was treated by spraying one of the antimicrobial treatments onto its surface as it moved down a conveyor system towards the grinder. Trim was fed to the grinder using a conveyor belt similar to those used in the industry. Trim was treated by spraying with an automatic premixed spray system, onto the surface of one side of the trim as it moves down a conveyor. The trim was flipped on to the same conveyor and the other side was sprayed prior to grinding. The individual portions were sent down the conveyor and treated with one of the six treatments: (1) 2% acetic acid; (2) 5% acetic acid; (3) 2% lactic acid; (4) 5% lactic acid (Fisher Scientific International Inc., Hampton, NH); (5) acidified sodium chlorite (1000 ppm) (Sanova®, Alcide Corporation, Redmond, WA); and (6) sterile water. The conveyor system and grinder were cleaned and sanitized with a 0.05% chlorine solution in between treatments within a replication. All processing equipment was cleaned with the application of a 0.05% chlorine solution and sanitized with Bi-Quat (Birko Corp., Henderson, CO) between replications.

Samples of trim were taken after the intervention application and immediately prior to grinding. The remaining trim was ground and ground samples were collected immediately after grinding for microbiological analysis. The remaining ground beef was divided into two equal portions, vacuum packaged and the two portions were stored at 4^{o}C in the processing lab for either 6 or 24 h. Ground beef samples were processed at 6 and 24 h after processing. Both *Escherichia coli* O157:H7 and *Salmonella* spp. inhibition were evaluated separately.

Microbiological Analysis

During the sampling process 10 g of the sample were collected and placed in a sampling bag (Model 400 Bags 6041, Stomacher Lab System Seward Limited, London, UK). Buffered Peptone Water (99 ml) was added with the sample in the sampling bag. The bag and contents were placed in a laboratory blend stomacher (Model 400, Seward Medical, London UK) and processed at a normal speed for 60 s. Afterwards, 3 ml of the sample was collected and placed into a spiral-platter sampling cup. Samples were automatically plated using the Spiral Biotech Autoplate® (Spiral Biotech, Norwood, MA). The 10^5 samples were exponentially plated with 50 ul of the sample on two Trypicase Soy Agar (TSA) plates and two TSA (TSAST) plates with the addition of 50 ug of streptomycin antibiotic.

The streptomycin antibiotic inhibited growth of the background flora while allowing for pathogen growth. Total aerobic plate counts and coliform counts were determined using Trypicase Soy Agar with the absence of the streptomycin antibiotic. Plates were incubated for 48 h at 37^{o}C. 10^{5} plates were counted using the Spiral Biotech Q Count™ (Version 2.0, Spiral Biotech, Norwood, MA).

Results

Trim Samples

The antimicrobial treatments on the surface of beef trim showed no effects ($P < 0.05$) for *Escherichia coli* O157:H7.

The antimicrobial treatments of 5% acetic acid and acidified sodium chlorite (1000 ppm) on the surface of beef trim showed significant reductions ($P < 0.05$) for *Salmonella* spp. (Figure 1).

Ground Beef Samples

The antimicrobial treatments of: sterile water, 5% lactic acid, 2% acetic acid, 5% acetic acid, and acidified sodium chlorite significantly reduced *Escherichia coli* O157:H7 ($P < 0.05$) in ground beef when compared to the control at 6 h. 5% lactic acid showed the most effectiveness 6 h after processing. However, 2% acetic acid and acidified sodium chlorite (1000 ppm) showed the most effectiveness in reducing *Escherichia coli* O157:H7 24 h after processing (Figure 2).

The antimicrobial treatments of: sterile water, 2% lactic acid, 5% lactic acid, 2% acetic acid, 5% acetic acid, and acidified sodium chlorite significantly reduced ($P < 0.0001$) pathogen loads of *Salmonella* spp. on the ground beef after both 6 and 24 h after processing (Figure 3).

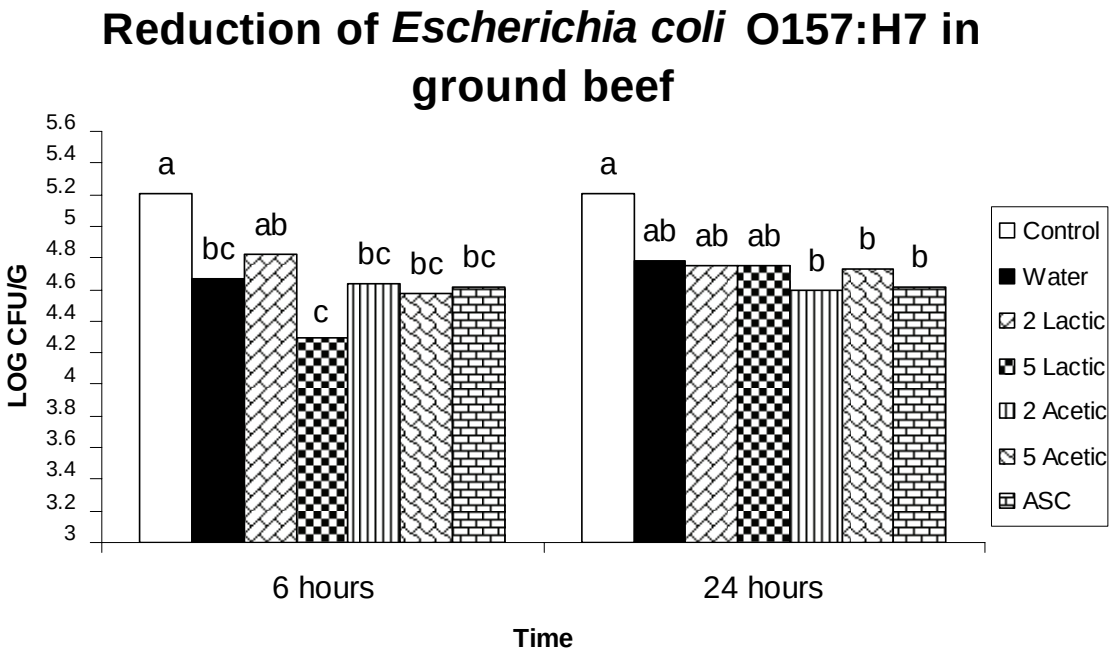

Figure 1. Effects of sterile water, acetic acid, lactic acid, and acidified sodium chlorite in reducing *Escherichia coli* O157:H7 in ground beef stored at refrigerated temperatures.

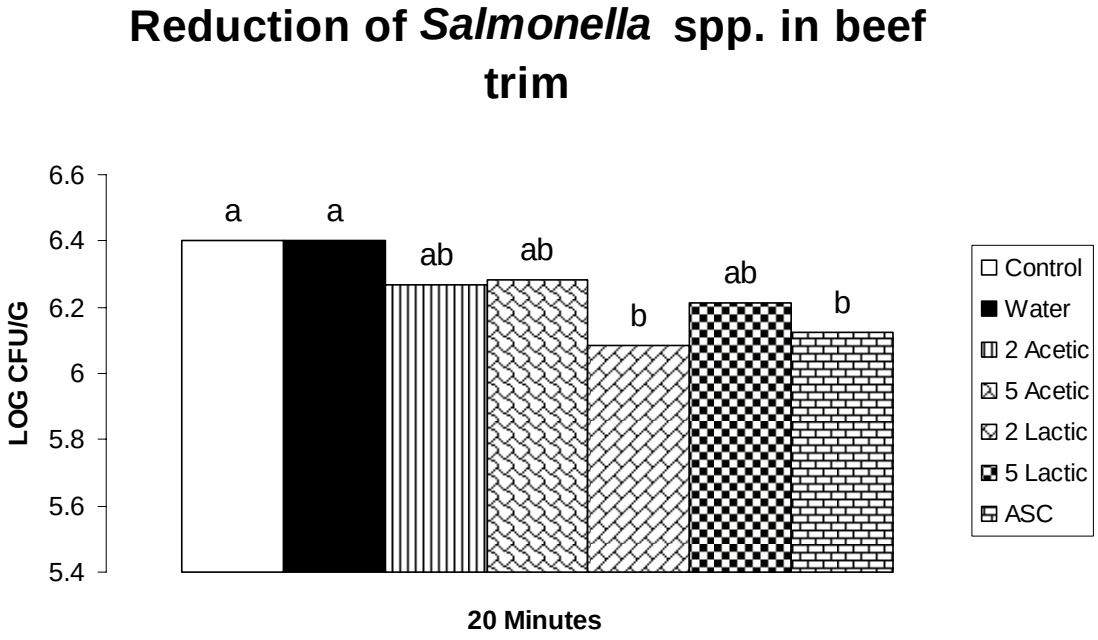

Figure 2. Reduction of *Salmonella* spp. in beef trim after treatment with sterile water, acetic acid, lactic acid and acidified sodium chlorite.

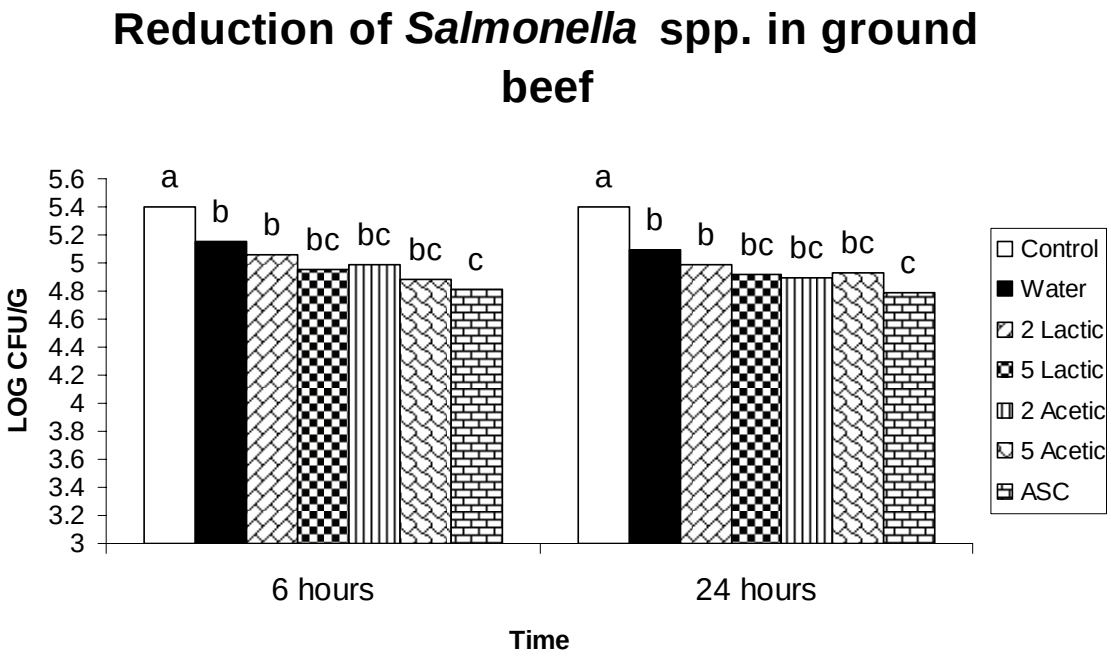

Figure 3. Effects of sterile water, acetic acid, lactic acid, and acidified sodium chlorite in reducing *Salmonella* spp. in ground beef stored at refrigerated temperatures.

Conclusion

In conclusion, research from this present study indicates that industries can utilize antimicrobial interventions under commercially simulated conditions on beef trimmings to reduce pathogens in ground beef.

References

Castillo, A., L. M. Lucia, D. B. Roberson, T. H. Stevenson, I. Mercado, and G.R. Acuff. 2001. Lactic acid sprays reduce bacterial pathogens on cold beef carcass surfaces and in subsequently produced ground beef. J. Food Prot. 64:58–62.

Conner, D.E., J.S. Kotrola, W. B. Mikel, and K. C. Tamblyn. 1997. Effects of acetic-lactic acid treatments applied to beef trim on populations of Escherichia coli O157:H7 and Listeria monocytogenes in ground beef. J. Food Prot., 12:1560–1563.

Kang, D.H., M. Koohmaraie, and G.R. Siragusa. 2001. Application of multiple antimicrobial interventions for microbial decontamination of commercial beef trim. J. Food Prot. 64:168–171.