IN-PLANT VALIDATION OF TWO ANTIMICROBIAL AGENTS APPLIED DURING PRODUCTION OF FURTHER PROCESSED BEEF PRODUCTS

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Abstract - In 1999, the United States Department of Agriculture – Food Safety and Inspection Service declared *Escherichia coli* O157:H7 as an adulterant in non-intact beef products. As a result, all beef processors are required to address *E. coli* O157:H7 in their hazard analysis, and validate their systems to ensure identified pathogens of concern are adequately controlled. This project was designed to validate in-plant application of two antimicrobial interventions (lactic acid and Beefxide [lactic acid and citric acid mixture]) applied to multiple processing schemes (e.g. single pass or multiple pass tenderization, and marination). Surrogate microorganisms were applied to beef products prior to treatment with an antimicrobial (2.5% Beefxide or 2.9% lactic acid). Following inoculation and antimicrobial spray, products were subjected to a single or multiple pass tenderization and/or marination process. Beefxide and lactic acid treatments resulted in statistically significant ($P < 0.05$) log reductions of surrogate microorganisms on product surfaces for all beef subprimals. Surrogate microorganisms also were recovered from interior samples of all beef product types after mechanical tenderization. These data indicate that (1) Beefxide and lactic acid are similar ($P > 0.05$) in their efficacy as antimicrobial interventions in the production of non-intact beef products, and (2) non-intact processes can transfer microorganisms into the interior of whole-muscle cuts.

I. INTRODUCTION

In 1992-1993, a deadly foodborne outbreak occurred on the nation's west coast involving ground beef contaminated with *Escherichia coli* O157:H7, which caused consumers to question the safety of beef products. In 1994, the United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) responded to the outbreak by declaring *E. coli* O157:H7 an adulterant in ground beef under the Federal Meat Inspection Act. USDA also mandated new food safety measures including the development and implementation of a Hazard Analysis and Critical Control Point (HACCP) plan, implementation of sanitation standard operating procedures, and microbiological testing. As part of the HACCP plan, meat processing facilities must identify hazards that are likely to occur and implement critical control points designed to prevent, eliminate, or reduce to acceptable level the pathogen of concern. Various antimicrobial interventions, such as hot water, lactic acid, acetic acid, and other organic acid sprays are applied during harvest to reduce contamination on the carcass. With the declaration of *E. coli* O157:H7 as an adulterant in non-intact beef products, many further processors are also applying antimicrobial interventions. All processors are required to validate their systems to ensure that the pathogens of concern are adequately controlled. This project was designed to validate in-plant application of two different antimicrobial interventions (lactic acid and Beefxide [lactic acid and citric acid mixture]) applied to multiple processing schemes (e.g., single pass or multiple pass tenderization, and marination). These data will help establishments validate in-plant pathogen reduction processes.

II. MATERIALS AND METHODS

Texas A&M University worked with a commercial further-processing establishment in Texas to complete this project. The establishment is federally inspected, so the project was designed to comply with all USDA regulatory requirements related to process validation and use of surrogate microorganisms. According to FSIS [1], "an establishment that chooses to conduct a validation study may use a *surrogate* indicator organism to measure change, but it should do so only after giving careful consideration to specific precautions. These precautions include ensuring that a microbiologist trained in food science and in the design of inoculated-pack studies introduces the non-pathogenic cultures within the establishment. In addition, the establishment should ensure that the introduction of the non-pathogenic cultures does not create an insanitary condition in the facility or cause the food to become adulterated. Finally, establishments should ensure that the non-pathogenic cultures are necessary and proven to be effective for the intended purpose."

A. Product and process parameters

This experiment was conducted in two phases. The first phase utilized the establishment's normal production practices. Three beef products: Boneless Strip Loin (n = 12), Top Sirloin Butt-Cap Off (n = 12), and Bottom Sirloin Flap (n = 12) were selected by the establishment based on availability of product, number of passes through the blade tenderizer, and the marination process. Antimicrobial interventions were applied to each product using a commercial spray cabinet (with conveyor belt) prior to blade tenderization. Bottom sirloin flaps were passed through the tenderizer one (1) time, split, and then marinated (proprietary commercial marinade) using a vacuum-tumbler. The strip loins were passed through the tenderizer two (2) times, and the top sirloin butts were passed through the tenderizer three (3) times. The second experimental phase involved strip loins (n = *6)* that were assigned to one of three non-intact treatments: single tenderization, double tenderization, and triple tenderization. The second phase of this study was designed to help researchers better control the variables that may influence internalization of surrogate microorganisms (i.e. varying subprimal thicknesses and/or non-intact processes).

Phase 1 products were randomly assigned to either Beefxide (lactic acid and citric acid mixture) or lactic acid treatment. Phase 2 product was only exposed to lactic acid spray since the focus of phase two was on microorganism internalization, not antimicrobial effectiveness. The average operating parameters for the antimicrobials were as follows: Beefxide: pH 2.18, temperature 24.4 °C, concentration 2.4%; lactic acid: pH 1.97, temperature 27 °C, concentration 2.9%. To ensure proper coverage with the antimicrobial, all products were placed in a single layer with no overlap. For both study phases, products that were passed through the tenderizer two or three times, did not receive additional antimicrobial treatment, just additional tenderization.

B. Inoculum preparation

Three non-pathogenic *E. coli* Biotype I strains (1427, 1428 and 1430) were obtained from the American Type Culture Collection (ATCC - ww.ATCC.org) for use in this study. These strains then were selected in the Food Microbiology Laboratory at Texas A&M University for their inherent ability to naturally resist rifampicin. Through previous scientific research, these surrogate organisms have demonstrated identical thermal and acid resistance to the human pathogen *E. coli* O157:H7. These marker organisms were designed for use in a "cocktail" to represent possible contamination with enteric pathogens of fecal origin such as *Salmonella* or *E. coli* O157:H7. In previous research [2], these marker organisms demonstrated identical thermal and acid resistance to *E. coli* O157:H7. At 48 h before each collection day, the Rif^R cultures of *E. coli* organisms (1427, 1428, 1430) were propagated by transferring a loop of each stored microorganism from a tryptic soy agar (TSA, Becton, Dickinson and Co., Sparks, MD) slant to a fresh 10 ml tryptic soy broth (TSB, Becton, Dickinson and Co., Sparks, MD) tube and incubated aerobically at 37 °C for 18 to 24 h. Each

culture then was transferred individually by pipetting 0.1 ml into Falcon™ (Thermo-Fisher Scientific, Waltham, MA) conical centrifuge tubes containing 10 ml TSB before incubating for 18 h at 37 °C. After incubation at 37°C for 18 h, cells from each culture were harvested by centrifugation at 1,620 × g for 15 min. The supernatant was discarded and the pellet suspended in 10 ml of phosphate buffered saline (PBS). Each cell suspension was centrifuged again (1,620 × g for 15 min) and the procedure was repeated once. The final pellets were suspended in 10 ml of PBS each. Following the final suspension, cell suspensions from each culture were combined to form a cocktail of Rif^R *E. coli* organisms. All beef products were experimentally contaminated with surrogate microorganisms.

C. Product preparation and sampling

Background microbiological samples were taken randomly from six strip loins, top butts, and flaps to show that no naturally occurring rifampicin-resistant microorganisms were present prior to inoculation. All samples for this study were collected by excising two pieces of product (10cm^2 x 2 mm deep) using sterile stainless-steel borer, scalpel and forceps, and compositing them for a total of 20cm^2 sample area. Both sides of each cut were inoculated with: 2 ml (per side) for strip loins and flaps and 1 ml (per side) top butts. Amounts of inoculum used were based on the surface area of the product. Following inoculation of the cuts, 30 minutes was allowed for attachment of microorganisms to the product surfaces. After attachment was achieved, microbiological samples then were collected from both sides of the product, both before and after the intervention/tenderization process. The bottom sirloin flaps also were sampled after 2 additional steps as their production process included splitting and 20-minute marination/vacuum tumbling steps. Each sample was placed in a sterile stomacher bag, inside an insulated container, and transported to the Food Microbiology Laboratory at Texas A&M University. Along with surface samples, the subprimals were transported in insulated containers to the laboratory to accommodate internal microbiological sample collection under aseptic conditions. Once in the laboratory, 99 ml of sterile 0.1% peptone water was added to each sample. The samples then were pummeled for 1 minute at 260 rpm using a Stomacher-400 (Tekmar Company, Cincinnati, OH). For each sample, counts of the surrogate microorganisms were determined by plating the appropriate serial dilutions on pre-poured and dried rifampicin-tryptic soy agar (rif-TSA, Difco, Sparks, MD) plates. The plates were incubated for 24 h at 37 °C. Colonies then were counted, recorded, and reported as log CFU/cm^2.

F. Statistical analysis

Microbiological counts were transformed into logarithms before obtaining means and performing statistical analyses. When counts were found to be below the detection limit for the counting method used, a number between 0 and the lowest detection limit was used to facilitate data analysis. All data were analyzed using JMP Software (JMP Pro, Version 10.0, SAS Institute Inc., Cary, NC). The Fit Model function was used for analysis of variance, determining interactions from the full model, and least squares means comparisons were performed using a Student's t-test.

III. RESULTS AND DISCUSSION

Initial inoculum level was an important consideration for this project, to ensure that the level would be sufficient to measure a reduction; mean initial inoculum level of 7.8 and 8.7 log CFU/cm^2 were used for study phases 1 and 2, respectively. In phase 1, the reduction of surrogate microorganisms from the pre-intervention to post-intervention steps for Beefxide and lactic acid were statistically equal across all subprimal types. Following a 30-minute inoculum attachment period, pre-intervention product surface counts were found to be 5.6 log CFU/cm^2 across subprimals treated with either antimicrobial. Again displaying no difference ($P > 0.05$) in antimicrobial efficacy, post-intervention counts were 4.1 and 4.3 log CFU/cm^2 for Beefxide and lactic acid, respectively. The ability to compare the effectiveness of these antimicrobials was one of the main objectives of this project.

Internalization data from phase 1 of this study are in line with the findings of previous research describing the internalization of *E. coli* O157:H7 in mechanically tenderized meat [3]. While microbiological counts were recovered from the internal surfaces of all subprimals, bottom sirloin flap samples presented significantly higher ($P < 0.05$) counts than the other two subprimal types. It should be noted that the flaps were subjected to marination and vacuum tumbling, whereas the other subprimals were not. This finding as well as findings from other scientific research tell us that microorganisms are pushed further inside the product during further processing steps, such as marination and vacuum-tumbling [4]. To better understand the internalization of the surrogate microorganisms, data from phase 2 of this experiment is presented in Table 1 below.

Table 1. Least squares means for log CFU/cm^2 of Biotype I *Escherichia coli* surrogates for Phase 2 beef strip loins subjected to various blade tenderization applications following lactic acid treatment.

Sampling Interval	Fat surface	Lean surface	Internal
Pre-intervention[1]	6.1 A[2]	5.6 A	NC[3]
Post-intervention			
Single tenderization	5.1 B	5.3 A	2.3 A
Double tenderization	4.8 B	5.1 A	2.2 A
Triple tenderization	4.8 B	4.9 A	1.7 A

[1] Pre-intervention samples were collected following a 30-minute attachment of inoculum.
[2] Means within a column lacking a common letter differ ($P < 0.05$).
[3] NC, not collected.

These data show no differences ($P > 0.05$) in microbiological counts among tenderization treatments (single, double or triple tenderization passes).

IV. CONCLUSIONS

These data indicate that tenderization and marination processes can transfer microorganisms into the interior of whole-muscle cuts, and suggest that Beefxide and lactic acid may be similar in their efficacy as antimicrobial interventions applied during the production of non-intact beef products. Additionally, this project demonstrates the usefulness of surrogate microorganisms in validating an establishment's food safety/HACCP system and provides information that can be used by other further processors to validate their in-plant processes.

ACKNOWLEDGEMENT

This project was funded, in part, by The Beef Checkoff.

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