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Application of Multiple Antimicrobial Interventions for Microbial Decontamination of Commercial Beef Trim†

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ABSTRACT

Commercially produced, irregularly sized (range, 100 to 400 cm^2), uninoculated beef trim was treated by a previously optimized multihurdle antimicrobial process under spray system or hot air gun with set-up speed (1 cm/s): W (water wash at 65 psi for five passes) + HW (82°C water at 30 psi for three passes) + HA (510°C air for five passes) + L (2% [vol/vol] room temperature lactic acid wash at 30 psi for three passes). After treatment, the trim was finely ground, vacuum packaged, and stored at 4°C for up to 20 days. At regular intervals (0, 5, 10, 15, and 20 days of storage at 4°C), the ground beef was analyzed to measure mesophilic aerobic bacteria (APC), coliforms, psychrotrophic bacteria (PCT), and presumptive lactic acid bacteria (PLAB) and compared with the untreated control. The numbers of APC, coliforms, PCT, and PLAB were reduced to nearly nondetectable levels immediately after treatment, with significant differences compared with the control ($P < 0.05$), then started to increase after 5 to 10 days of storage at 4°C. After 20 days, microbial populations of treated ground beef were significantly lower than those of nontreated ground beef for the numbers of APC, coliforms, PCT, and PLAB ($P < 0.05$), with differences of 1.2, 2.4, 1.6, and 1.6 log CFU/g, respectively. Based on microbial reduction and quality aspects, the multihurdle antimicrobial process was identified as an effective intervention to reduce coliforms on beef trim.

Antimicrobial interventions have resulted in significant reduction of inoculated foodborne pathogens on animal carcasses *(2, 4, 6–9, 11, 17, 20, 21, 26)*. Microbial contamination of beef carcasses is an inevitable result of converting live animals to meat *(5, 10, 18)*. Internal muscles of the healthy animal are generally sterile at the time of slaughter *(3)*; however, under normal processing conditions, equipment and workers spread bacteria to newly exposed surfaces throughout processing from evisceration to packaging and storage *(14, 26)*. Ground beef accounts for 44% of the total beef consumed in the United States *(1)*. For a variety of reasons, such as raw material used (most of the carcass surface is used in ground beef production), number of steps required for its production, and reduction in particle size (increase in surface area), ground beef usually has a higher potential for bacterial contamination than steaks and roasts. Although the currently used antimicrobial interventions reduce microorganisms on beef carcasses, beef trim can be recontaminated during ground beef production. Treatments that cause denaturation of muscle proteins, such as steam, cannot be used as interventions for beef trim. Using more than one antimicrobial intervention, each having minimal effect on quality, should retain quality and achieve desirable microbial reductions. Several researchers have reported the processes for beef trim decontamination *(3, 5, 13–15)*. We recently reported the development of a multihurdle antimicrobial intervention process for uniform beef trim *(16)*. Briefly, we achieved a 2- to 3-log reduction of inoculated fecal coliforms using the process of multihurdle interventions. The efficiencies of microbial reductions might be different when the aforementioned process is applied to commercially produced beef trim. The objective of these experiments was to determine the effectiveness of the multihurdle intervention process on uninoculated commercial beef trim.

MATERIALS AND METHODS

Description of trim intervention table and chamber. Located at the Roman L. Hruska U.S. Meat Animal Research Center at Clay Center, Nebr., the trim intervention chamber is composed of an adjustable-speed, moving chain food processing table, two adjustable spray units, and one hot air cabinet with three heat guns *(16)*. This custom-built chamber is designed for testing trim antimicrobial interventions in a highly controlled environment with tightly controlled sprayers, pumps, heat sources, and exposure times as described previously *(16)*.

Trim samples. Beef trim was randomly obtained from a local beef processing company. Trim was fabricated from chilled beef carcasses. Beef trim was collected and held at 4°C overnight; the following day, half of the sampled beef trim was treated with the multihurdle antimicrobial process *(16)*. Half of the beef trim was reserved as control.

Application of combined antimicrobial treatments on beef trim. The multihurdle antimicrobial intervention was evaluated using the trim intervention chamber described above. The treatment conditions were as follows: W (water wash at 65 psi for five passes) + HW (82°C water at 30 psi for three passes) + HA

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TABLE 1. *The pH changes of control and ground beef treated by the multiple step antimicrobial process*[a]

	Before treatment[b]	After treatment[c]	Periods of storage at 4°C			
			5days	10 days	15 days	20 days
Control	A[d] 6.18 ± 0.03 A[e]	A 6.18 ± 0.03 A	A 6.08 ± 0.04 AB	A 6.06 ± 0.02 B	A 5.90 ± 0.05 C	A 6.00 ± 0.05 D
Treatment	A 6.18 ± 0.02 A	B 5.78 ± 0.04 B	B 5.77 ± 0.05 B	B 5.80 ± 0.03 B	B 5.73 ± 0.01 BC	B 5.71 ± 0.05 C

[a] Values in a column presented the mean of six replications ± standard deviation.
[b] Before multihurdle antimicrobial treatment.
[c] After multihurdle antimicrobial treatment: W (water wash at 65 psi for five passes) + HW (65°C water at 30 psi for three passes) + HA (510°C air for six passes) + L (2% [vol/vol] lactic acid wash at 30 psi for three passes).
[d] Means with the same letter within a column (preceding the values) are not significantly different ($P < 0.05$).
[e] Means with the same letter within a row (following the values) are not significantly different ($P < 0.05$).

(510°C air for six passes) + L (2% [vol/vol] room temperature lactic acid wash at 30 psi for three passes). Both sides of the beef trim pieces were treated with this antimicrobial intervention and held at 4°C for up to 60 min. Within 1 h following treatment, the beef trim was ground to 1-cm-diameter portions with a commercial grinder (Davpol Enterprises Inc., New York, N.Y.). After separately grinding, 50 g of each treated or untreated ground beef portion was sampled and analyzed as described below. Each sample (approximately 100) was separately vacuum packaged in plastic film (0% oxygen permeability, Advantage Food Equipment Systems Co., Omaha, Nebr.) with a vacuum packager (Hollymatic, New Age Industrial Co., Norton, Kans.).

The pH of untreated and treated ground beef was examined at regular intervals (0, 5, 10, 15, and 20 days of storage at 4°C) at a depth of 2 cm, with pH flat bulb electrode (Cole Parmer Instrument Co., Vernon Hills, Ill.) and a pH meter (Corning Scientific Products, Corning, N.Y.).

Sampling and bacterial enumeration. Samples were taken by weighing 50 g of ground beef using alcohol-flamed forceps *(12)*. Samples were placed in filtered stomacher bags (Spiral Biotech, Inc., Bethesda, Md.) with 100 ml of buffered peptone water (Difco Laboratories, Detroit, Mich.) with 0.1% (vol/vol) Tween 20 (Sigma Chemical Co., St. Louis, Mo.), then pummeled for 2 min in a model 400 stomacher (Tekmar, Inc., Cincinnati, Ohio). Appropriate sample dilutions were made in buffered peptone water, and the numbers of microorganisms were enumerated. Populations of mesophilic aerobic bacteria (APC), total coliforms, psychrotrophic bacteria (PCT), and presumptive lactic acid bacteria (PLAB) were enumerated and monitored before and after treatment and at regular intervals (0, 5, 10, 15, and 20 days of storage at 4°C). APC and PCT were enumerated using 3M Petrifilm aerobic count plates (3M, Inc., St. Paul, Minn.) incubated at 37°C for 48 h and at 15°C for 7 days, respectively. Coliforms were enumerated using 3M Petrifilm *Escherichia coli* count plates (3M) for 24 h. For the enumeration of PLAB, lactobacilli MRS (Difco) agar containing 0.02% (wt/vol) sodium azide (Sigma) was used and anaerobically incubated in a Brewer anaerobic jar (BBL, Cockeysville, Md.) with AnaeroGen (anaerobic gas-generating envelopes, Oxoid, Hampshire, England) for 48 h at 30°C.

Statistical analysis. Bacterial numbers for each treatment were converted to log10 CFU/g and analyzed statistically by analysis of variance using the SAS General Linear Models procedure *(24)*. Means were separated using the least significant difference test (PROC MIXED) at 0.05 probability level.

RESULTS

The resulting microbial reductions from the treatment were calculated, and posttreatment oxymyoglobin formation was evaluated. Mean pH for control and treated ground beef is summarized in Table 1. During incubation at 4°C for 20 days, the pH values remained unchanged. In the case of the treated ground beef, the pH initially decreased to approximately 5.78 ± 0.04, a statistically significant difference compared with the control ($P < 0.05$), and it remained less than 5.8 through 20 days of incubation at 4°C. The pH of control ground beef was 6.2 ± 0.03 and changed to 6.00 ± 0.05 after 20 days of incubation. According to our preliminary experiments *(16)*, the surface pH of beef trim treated by the multihurdle antimicrobial intervention continuously increased from 3.8 to 5.0. However, in the case of subsequent grinding of treated trim, the pH was not significantly changed. The color of ground beef muscle from trim treated by the multihurdle antimicrobial intervention deteriorated rapidly and was unacceptable soon after treatment. However, on further storage for 1 to 2 h at 4°C, the color of the ground beef reverted to an acceptable red.

Compared with initial levels, the multihurdle antimicrobial intervention process used in this study inhibited the tested microbial groups for 5 and 10 days of cold storage (Fig. 1). Compared with the control, the treatment suppressed microbial growth level for the 20-day duration of the experiments. The initial APC count of approximately 2.73 log CFU/g was reduced ($P < 0.05$) to 0.26 log CFU/g by the treatment (Fig. 1a). The APC count on ground beef treated with the multihurdle antimicrobial process remained less than 1.0 log CFU/g for 5 days and then increased to 6.9 log CFU/g by 20 days of storage at 4°C (Fig. 1a), whereas the APC count of the control samples increased from 2.73 log to 8.19 log CFU/g after 20 days of incubation at 4°C. After 20 days of incubation, the difference in numbers of total APC was about 1.2 log units between treated and control ground beef.

Throughout the entire storage period, the population of coliform bacteria also significantly increased in ground beef (Fig. 1b). Following treatment, the numbers of coliforms were reduced to below a detectable (<3 CFU/g) level, then increased to 4.88 log CFU/g after 20 days of storage at 4°C. For the 10 days of storage, the numbers of coliforms in the treated ground beef remained less than 1.00 log CFU/g, whereas the levels of coliforms in control ground beef increased from 1.65 log to 7.24 log CFU/g after 20 days of incubation at 4°C (Fig. 1b). After 10 days, there was

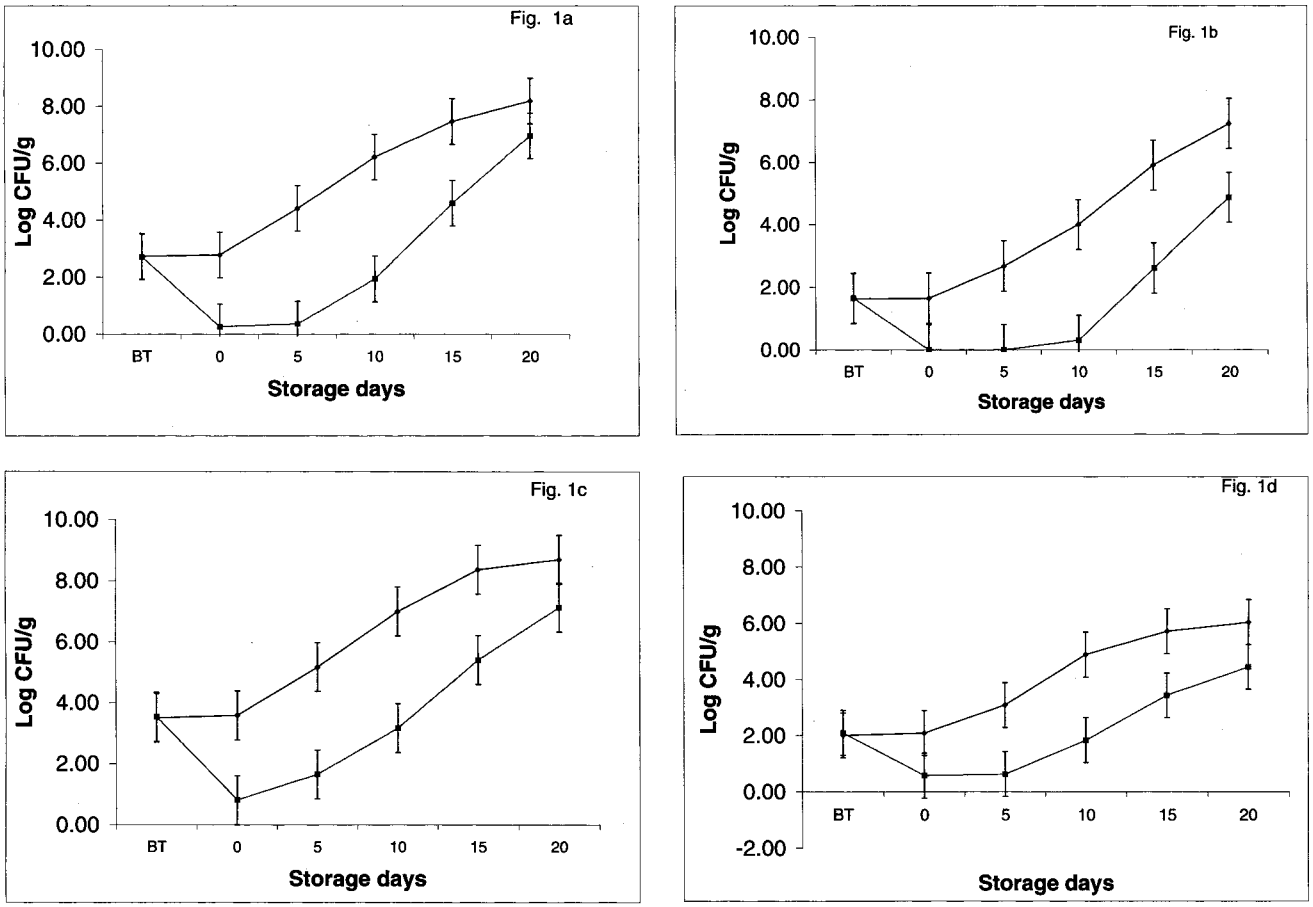

FIGURE 1. *Effect of beef trim multihurdle antimicrobial intervention treatment on populations of (a) APC, (b) coliforms, (c) PCT, and (d) PLAB immediately before treatment (BT) after treatment (day 0), and during storage at 4°C for 20 days.* ◆, *control;* ■, *multihurdle antimicrobial treated.*

3.70-log difference ($P < 0.05$) between treated and nontreated ground beef for coliform counts, which decreased to a 2.40-log difference at 20 days of storage ($P < 0.05$).

The initial numbers of PCT were also reduced by the multihurdle antimicrobial treatment ($P < 0.05$). The numbers of PCT on meat treated by the multihurdle antimicrobial intervention increased to higher than initial numbers after 5 days of storage at 4°C (Fig. 1c). The numbers of PCT in treated ground beef increased to 7.13 log CFU/g after 20 days of incubation (Fig. 1c). The numbers of PCT in control ground beef increased from 3.50 log to 8.70 log CFU/g after 20 days of incubation ($P < 0.05$).

The numbers of PLAB were monitored through 20 days of storage at 4°C. The numbers were significantly reduced by the multihurdle antimicrobial treatment compared with control values ($P < 0.05$). Immediately following multihurdle antimicrobial treatment, the reduction was about 1.50 log CFU/g. By 20 days of storage at 4°C, the numbers of PLAB in treated ground beef increased to 4.45 log CFU/g versus 6.04 log CFU/g in control ground beef (Fig. 1d).

DISCUSSION

To date, antimicrobial intervention processes have focused either on the carcass stage of production or on ground beef *(6, 7, 17)*. The most obvious difference between applying decontaminating interventions to carcasses versus trim is that carcasses are still covered with an intact fascia tissue, which is somewhat protective to the underlying muscle, whereas trim surfaces are cut-exposed muscle tissues that are highly sensitive to heat and other denaturants *(13–16)*. Therefore, for trim, interventions such as prolonged high-temperature exposure cannot be used because of their adverse effects on color and, possibly, protein functionality. Using several less severe interventions should retain quality and achieve desirable microbial reductions *(15, 16)*. For developing the multihurdle antimicrobial process, uniformly sized and inoculated beef trim was used *(16)*; however, for validation, commercial trim obtained from a local processing plant was used. The natural microflora of the beef trim and its highly irregular shape and composition are more realistic conditions for evaluation of the multihurdle antimicrobial treatment. After multihurdle antimicrobial treatment, the surface of beef trim was discolored to a depth of about 2 to 3 mm (data not shown). On grinding, the discolored surface meat of the trim is further "diluted" by the overwhelming mass of unaffected interior tissues. After grinding, the color of treated beef trim was slightly different from the control but appeared to recover to control color levels during 4°C storage for 1 to 2 h (data not shown). The impact of this apparent color effect on customer acceptance of the final product is unknown. Our observations

of effect on color are empirical, and the effects of these treatments on color warrant further study.

The tested process achieved about a 4.00-log difference of naturally occurring coliforms after 10 days of storage at 4°C. We interpret a reduction in coliform counts as a broad indication of inhibition or reduction in numbers of enteric pathogens, including enterohemorrhagic *E. coli.*

The multihurdle approach to inhibiting microbial contaminants has been studied previously for other foods *(19).* Research has also shown that sublethally injured bacteria are more susceptible to antimicrobial food processes and microbial inhibitors *(23)* than are unstressed bacteria. Therefore, by taking advantage of a series of sublethal injuries, an overall greater microbial reduction may be achieved than when simple intervention is applied alone.

This research is the application of multihurdle antimicrobial interventions for commercial beef trim. Previous workers *(6, 11, 25)* have studied the effects of interventions applied to carcass tissues and the subsequent changes in microbial populations of ground beef made from the treated carcass tissues. Treatments included water, lactic and acetic acids, trisodium phosphate, hot water, and steam vacuuming, all as single rather than combined interventions *(4, 6, 10, 22).* In brief, no major changes in microbial progression of the resulting ground beef were observed, nor was there unchecked growth of inoculated pathogens, including *E. coli* O157 and salmonellae, under the controlled conditions of the respective studies.

Collectively, our data indicate that, for commercial beef trim, a multihurdle antimicrobial process can reduce the natural level of coliform bacteria in ground beef, offering an immediate reduction with a residual inhibitory effect for at least 20 days of 4°C refrigeration after processing. The currently reported process retains favorable color quality and does not greatly alter the normal microbial population of the trim.

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