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Development of a Multiple-Step Process for the Microbial Decontamination of Beef Trim†

DONG-HYUN KANG, MOHAMMAD KOOHMARAIE,* WARREN J. DORSA,‡ AND GREGORY R. SIRAGUSA§

U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, Nebraska 68933, USA

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ABSTRACT

A multiple-hurdle antimicrobial process for beef trim was developed. The microbial profiles of inoculated lean beef trim tissue (BTL) and fat-covered lean beef trim (BTF) were monitored during prolonged refrigerated storage following the application of successive multiple antimicrobial treatments applied to inoculated beef trim on a processing conveyor belt set at a belt speed of 1 cm/s. Beef trim (meat size approximately 15 by 15 cm) was preinoculated with bovine feces before all treatments that included the following: control, no treatment; water wash at 65 psi for five passes; water plus lactic acid (2% [vol/vol] room temperature lactic acid wash at 30 psi for three passes); combination treatment 1 (water plus 65°C hot water at 30 psi for one pass plus hot air at 510°C for four passes plus lactic acid), combination treatment 2 (water plus hot water at 82°C for one pass plus hot air at 510°C for five passes plus lactic acid), and combination treatment 3 (water plus hot water at 82°C for three passes plus hot air at 510°C for six passes plus lactic acid). The effects of treatments on bacterial populations were monitored by enumerating mesophilic aerobic bacteria (APC), presumptive lactic acid bacteria (PLAB), psychrotrophic bacteria (PCT), coliforms, and *Escherichia coli* biotype 1 on product stored for up to 7 days at 4°C. In the case of BTL, the numbers of APC, PCT, and PLAB increased during storage at 5°C, whereas the numbers of coliform and *E. coli* decreased on average by 1.8 log CFU/cm^2, then remained constant following the initial reduction. Negligible effects on color quality were observed from multihurdle treatment combination 1. In the case of the BTF, the microbial reductions by treatments were much greater than the reduction on BTL. The pH of treated BTF increased more slowly than the pH of treated BTL, resulting in further reduction of the microflora on BTF. Except for control and water treatments, all sample treatments involving lactic acid resulted in continuously decreasing microbial populations. Based on microbial reduction and quality aspects, it was concluded that successively applied combination antimicrobial treatments for meat trim could offer potential food safety benefits.

Red meat animal processors are actively exploring possible interventions for minimizing the risk of introducing bacterial pathogens to processed meats from contaminated raw carcasses. Significant reductions of inoculated foodborne pathogens on carcasses have been demonstrated with various antimicrobial interventions *(2, 4, 6–10, 14, 19, 22, 23, 27, 29)*. To date, limited research has been reported for antimicrobial interventions to reduce microorganisms on beef trim stocks used to make ground beef *(7, 18, 19)*. Microbial contamination of beef carcasses is an inevitable result of converting live animals to meat *(5, 11)*. Muscles within the healthy animal are sterile at the time of slaughter *(3)*; however, under normal processing conditions, equipment and workers spread bacteria to newly exposed meat surfaces, throughout processing, from evisceration to packaging and storage *(17, 20, 24)*. Ground beef, which currently accounts for 44% of the total beef consumed *(1)*, requires extensive handling during production; therefore, there is a higher degree of bacterial contamination in ground beef than in whole-muscle products. Although the currently used antimicrobial interventions reduce microorganisms on beef carcasses, beef trim can be recontaminated by worker hands and tools. For trim, exceedingly harsh interventions, such as heating the surface with steam, cannot be used because of their adverse impact on trim quality. However, Gill and Badoni *(16)* reported that ground beef prepared from hot water–pasteurized beef trim appeared to be acceptable for at least hamburger patty manufacture.

Sequential use of less severe or minimal processing interventions might better retain quality and achieve desirable microbial reduction. The goal of this research was to develop a multihurdle antimicrobial intervention for reducing fecal microflora in beef trim destined for ground beef production with a minimal effect on resulting color quality.

MATERIALS AND METHODS

Trim intervention table and chamber. The trim intervention chamber is composed of a food processing grade, adjustable

* Author for correspondence. Tel: 706-546-3596; Fax: 706-546-3771; E-mail: siragusa@saa.ars.usda.gov.

† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

‡ Present address: John Morrell & Co., 805 East Kemper Road, Cincinnati, OH 45246–2515, USA.

§ Present address: USDA, ARS, Russell Research Center, P.O. Box 5677, Athens, GA 30604, USA.

speed, moving chain table; two adjustable spray units (each spray unit had four nozzles [catalog no. QJJ, Spraying Systems Co., Kansas City, Mo.] with ⅛-in. orifice diameter); and one hot air cabinet with three heat guns (Varytemp Heat Gun, Master Appliance Co., Racine, Wis.). This custom-built chamber is designed for testing various antimicrobial interventions for trim in a controlled environment and with controlled sprays, pumps, heat sources, and exposure times. The system is identical in scale to an industrial fabrication table. Two individual spray bar units, each with four spray nozzles aligned along a single bar horizontal to the conveyor belt path (Spraying Systems Co., Wheaton, Ill.), are used to deliver cold water, hot water, or lactic acid solutions onto the product. These treatments are manually controlled from one control panel located at the front of the chamber. The lines contain a total of four elliptical orifice spray nozzles. In-line water pressure is monitored using dial-type pressure gauges (Marshalltown, Inc., Hastings, Neb.) placed within 50 cm of each nozzle orifice, which reach 65 psi for water spray and 30 psi for hot water and lactic acid. The distance from each nozzle to the beef trim is 20 cm. Water temperature in the feed line delivered to the cabinet is adjustable and monitored by a dial-type thermometer (Marshall Instruments, Inc., Anaheim, Calif.) located within 120 cm of the orifice. The temperature of water delivered to the surface of beef trim from the nozzles is monitored using a portable thermometer (Omega Engineering Inc., Stamford, Conn.). Hot air is applied using three heating guns in parallel, blowing downward to deliver hot air to the beef trim surfaces.

Trim samples and inoculation procedures. To achieve uniformity, beef loins were cut into a uniform size (225 cm^2) and shape and used as trim samples. Loins were split through the middle to give one half with a predominantly fat cut surface and the other half with a predominantly lean cut surface. Trim was divided into two groups: beef trim lean (BTL) and beef trim fat (BTF). Trim pieces (BTL or BTF) were cut (15 by 15 by 2.5 cm), held at −20°C for up to 3 months, and thawed at 4°C before use.

A composite of three bovine fecal samples from different animals was used to ensure a consistent inoculation level. On each day of an experiment, feces were obtained from three cows fed a corn-silage ration containing no antibiotics. Fifty grams of each feces sample was mixed with 150 ml of sterile distilled water. The slurry was mixed for 2 min using a metal spatula; then it was passed through three layers of cotton cheesecloth (Kendall Co., Chicago, Ill.). The whole area of each BTL or BTF surface was inoculated with 8 ml of filtered bovine fecal suspension by spoon inoculation *(6, 13)*.

Testing of individual treatments: water, lactic acid, hot water, and hot air treatments. The following general procedure was used throughout all of the described experiments unless specifically noted. Different exposure treatments and times were accomplished by placing the inoculated meat samples on the conveyor belt and passing the entire tissue section under a single spray bar with four oscillating spray nozzles (situated in a perpendicular plane to the belt) for a specified number of passes. The chain table speed was set at 1 cm/s for all experiments. Each pass of the inoculated tissue under the spray bar resulted in each centimeter of length of the inoculated tissue being sprayed for approximately 1 s at the specified spray bar height, pressure, and spray composition.

Each of the six individual antimicrobial treatments was evaluated for its antimicrobial activities using BTL as the test material. For water washing, tap water (15 to 17°C, 65 psi) was sprayed on inoculated BTL at an oscillation rate of 60 strokes/min. Immediately after treatment, the BTL surface temperature was measured with an infrared, noncontact thermometer (Omega) held 3 cm from the BTL surface for 2 s. Five random locations were measured in a pattern covering most of the BTL surface area.

Lactic acid treatment for spray washing–inoculated BTL was applied as described above for one to five passes under the trim wash nozzle at 30 psi. Lactic acid (Sigma Chemical Co., St. Louis, Mo.) was mixed with tap water to 2% (vol/vol).

Hot water treatment of inoculated BTL was accomplished by placing the tissue on the conveyor belt and passing it one, two, or three times under a 30-psi spray of tap water set at 65, 71, 76, or 82°C. Combinations of temperatures and spray exposure times were separately tested to evaluate the microbial reductions.

Hot air treatment was applied using commercial hot air guns (Varytemp Heat Gun, Master Appliance Co., Racine, Wis.) preheated for 10 min before use. Inoculated BTL was treated at 371, 426, 454, 482, or 510°C for one to nine passes under spray. Maximum treatment time for each hot air temperature was the point in time at which visual evaluation of the BTL color indicated denaturation to brown cooked color. After treatments, the meat color was assessed as good or bad or acceptable or nonacceptable by five people. All experiments were replicated three times.

Application of combined antimicrobial treatments on inoculated BTL and BTF. Inoculation methods were evaluated before testing antimicrobial treatments. Eight milliliters of 1:2 diluted bovine feces was applied to BTL by spoon inoculation, and the BTL was incubated for 15 min at room temperature *(13, 14)* or overnight at 5°C. A comparison of a single water treatment of inoculated BTL incubated for each of these times was conducted. Based on this information, the method of inoculating trim sections the night before treatment and holding at 5°C was used for the rest of the experiments.

Each of six different multiple intervention treatments was evaluated. The six treatments and exposure times were as follows: control, water wash at 65 psi for five passes under the spray bar, water plus lactic acid (2% [vol/vol] lactic acid wash at 30 psi for three passes), combination 1 (comb 1; water plus hot water at 65°C at 30 psi for one-time passage plus hot air at 510°C for four passes plus lactic acid), combination 2 (comb 2; water plus hot water at 82°C for one pass plus hot air at 510°C for five passes plus lactic acid), and combination 3 (comb 3; water plus hot water at 82°C for three passes plus hot air at 510°C for six passes plus lactic acid). Within 1 h following treatment, a 25-cm^2 section of each treated sample was excised as described below. Each multiple intervention experiment was replicated four times.

Sampling and bacterial enumeration. Samples were taken by excision of 5 by 5 by 0.5-cm-thick sections using an alcohol-flamed, 25 cm^2 template *(15)*. Excised samples were placed in stomacher bags (Spiral Biotech, Inc., Bethesda, Md.) with 50 ml of buffered peptone water (Difco Laboratories, Detroit, Mich.), which contained 0.1% Tween 20 (Sigma), then pummeled for 2 min using a model 400 stomacher (Tekmar, Inc., Cincinnati, Ohio). For all studies, appropriate sample dilutions were made in buffered peptone water, and the numbers of microorganisms were enumerated. Mesophilic aerobic bacteria (APC), psychrotrophic bacteria (PCT), presumptive lactic acid bacteria (PLAB), total fecal coliforms, and *Escherichia coli* biotype 1 were enumerated before treatment, after treatment, and after 1 and 7 days of storage at 4°C inside a large plastic container fitted with a snap-on cover, allowing for an airspace but not open to the direct refrigerator atmosphere. Both APC and PCT were enumerated using 3M Petrifilm aerobic count plates (3M, Inc., St. Paul, Minn.) incubated at 37°C for 48 h or at 15°C for 7 days, respectively. Coliforms and *E. coli* were enumerated using 3M Petrifilm *E. coli* count

TABLE 1. *The reduction of bovine fecal coliforms on BTL using each individual intervention with different exposure times*

Treatment times (passes under spray bar)	Log CFU/cm² reduction in coliform counts[a]								
		Hot air				Hot water[d]			
	Water[b]	454°C	482°C	510°C	Lactic acid[c]	65°C	71°C	76°C	82°C
1	1.1 ± 0.15	<0.1	<0.1	<0.1	0.73 ± 0.16	1.32 ± 0.11	1.53 ± 0.25	1.46 ± 0.21	1.42 ± 0.13
2	1.3 ± 0.14	<0.1	<0.1	<0.1	0.88 ± 0.16	1.48 ± 0.15	1.55 ± 0.14	1.86 ± 0.10	2.35 ± 0.34
3	1.6 ± 0.19	<0.1	0.25 ± 0.13	0.40 ± 0.15	1.00 ± 0.23	1.71 ± 0.25	1.72 ± 0.34	2.25 ± 0.28	2.78 ± 0.24
4	1.8 ± 0.21	<0.1	0.30 ± 0.16	0.60 ± 0.24	1.11 ± 0.18				
5	2.0 ± 0.14	0.10 ± 0.03	0.30 ± 0.21	0.88 ± 0.31					
6	2.0 ± 0.22	0.30 ± 0.04	0.40 ± 0.10	0.98 ± 0.08					
7	2.1 ± 0.13	0.40 ± 0.08	0.69 ± 0.04						
8	2.1 ± 0.09	0.63 ± 0.11							
9	2.1 ± 0.17	10							
10	2.0 ± 0.15								

[a] Initial coliform numbers are approximately 3.0 to 4.0 log CFU/cm².
[b] Water treatment, 65 psi at 15°C.
[c] 2% lactic acid spray wash treatment with 30 psi at 15°C.
[d] Hot water spray (65°, 71°, 76°, and 82°C) treatment at 30 psi.

plates. For the enumeration of PLAB, lactobacilli MRS agar (Difco) containing 0.02% sodium azide (Sigma) was used. MRS plates were incubated anaerobically in a Brewer Anaerobic Jar (BBL, Cockeysville, Md.) with anaerobic environment generation system (AnaeroGen, Oxoid, Hampshire, England) for 48 h at 30°C. The pH of untreated and treated trim was examined within 1 h of the experiment using a pH meter (Corning Scientific Products, Corning, NY). The pH values of three different spots were averaged and reported.

Statistical analysis. Bacterial counts were normalized per unit area, then $\log_{10}$ transformed and analyzed statistically by analysis of variance using the SAS General Linear Models procedure *(26)*. Means of four replicates were reported unless otherwise indicated. Differences among treatments were examined for level of significance by Duncan's multiple range test.

RESULTS

Optimizing individual antimicrobial treatments. Reductions of bovine fecal coliforms by water spray treatments (water and hot water) are reported in Table 1. Microbial reductions were similar with treatment times of 75 s or longer. Therefore, the optimum water treatment was chosen as 5 s at 65 psi. Hot water treatments were evaluated with water at temperatures of 65, 71, 76, or 82°C by the reduction in coliform populations. The initial coliform levels were approximately 3.0 to 4.0 log CFU/cm². The coliform reduction from 82°C treatments was greatest (Table 1); however, the trim surface color was changed to a cooked appearance as soon as the hot water contacted the trim surface. The maximum hot water treatment that resulted in visually acceptable meat color was found to be 65°C for one pass under the spray bar. After treatment with 82°C water for one, two, or three passes under the spray bar, the depths of cooked tissue were approximately 1, 2 to 3, or 3 to 4 mm, respectively, with concomitant reductions of 1.4, 2.4, and 2.8 log CFU/cm² after one, two, or three passes under spray bar treatments, respectively.

Minimum-exposure (65°C for one pass under spray bar), medium-exposure (82°C for one pass under spray bar), and maximum-exposure (82°C for three passes under spray bar) hot water treatments were chosen for further experiments. Coliform reduction for 65°C for one-time passage under spray bar water spray was less than the reduction obtained by washing with cold tap water.

Following hot air treatments (Table 1), the BTL colors started to change after eight passes under hot air guns with air at 454°C, seven passes with air at 482°C, and six passes with air at 510°C. Air at 510°C for six passes was the most effective hot air treatment for reducing bovine fecal coliforms on BTL (Table 1). Minimum, medium, and maximum treatments of 510°C for four passes, 510°C for five passes, and 510°C for six passes were selected for inclusion in multiple intervention regimens.

Coliform reduction by 2% (vol/vol) lactic acid treatment (applied at 30 psi, 12 to 15°C for three passes under spray bar) was 1.0 log CFU/cm² (Table 1). In the case of tap water treatment (12 to 15°C, 65 psi for three passes), the initial reduction was about 1.6 log CFU/ cm². The cooling effects of lactic acid to BTL heated by hot water were examined to find optimal treatment times, i.e., chain speed. The surfaces of meat trim were 65°C after 82°F hot water treatment for 1 s. Following lactic acid spray, the meat surface temperature was reduced to less than 20°C. Therefore, the chain speed of three passes was chosen as the operational lactic acid treatment.

For the combination treatments, the water and lactic acid treatments were the first and last interventions, respectively. Depending on the middle step (conditions of hot water and hot air), the three combinations were evaluated as comb 1 (minimum combination), comb 2 (medium combination), and comb 3 (maximum combination).

Evaluation of incubation times of fecal inocula. Following inoculation, different pretreatment holding times were evaluated to assess potential differences in the attach-

TABLE 2. *The differences of attachment and detachment abilities of coliforms on BTL between 15-min incubation at 25°C and overnight incubation at 5°C*

Treatments	Log CFU/cm^2 of coliform counts	
	15-min incubation at room temperature	Overnight incubation at 5°C
Before treatment	3.71 ± 0.06 A[a]	3.77 ± 0.05 A
After water washing treatment (65 psi at 15°C for five passes under the spray bar)	1.93 ± 0.24 C	2.40 ± 0.06 B

[a] Values in a column followed by different letters are statistically different ($P < 0.05$).

ment of fecal coliforms to BTL. Differences in resulting populations after wash were observed between the 15-min and the overnight 5°C incubation method ($P < 0.05$). Prewashing treatments were not significantly different ($P > 0.05$) between the two incubation methods (Table 2). We assumed that stomaching was forceful enough to detach microorganisms attached overnight at 5°C. However, significant differences ($P < 0.05$) between meat inoculated by the two methods (Table 2) were observed after treatment (water, 65 psi for five passes). Thus, for subsequent combination experiments, the method of allowing the inoculum to incubate overnight at 4°C was used for both BTL and BTF.

Combination antimicrobial treatments. After preliminary experiments, combined treatments (control, water, water plus lactic acid, comb 1, comb 2, and comb 3) were evaluated. Minimum, medium, and maximum conditions of hot water and hot air were combined for comb 1 (water for five passes under the spray bar plus hot water at 65°C for one-time passage plus hot air at 510°C for four passes under hot air gun plus 2% lactic acid for three passes), comb 2 (water for five passes plus hot water at 82°C for one-time passage plus hot air at 510°C for five passes plus 2% lactic acid for three passes), and comb 3 (water for five passes plus hot water at 82°C for three passes plus hot air at 510°C for six passes plus 2% lactic acid for two passes).

Based on preliminary experiments, the resulting microbial reduction from each treatment was evaluated, and the optimum conditions for color retention were tested. Differences between individual and combined treatments were observed. For example, after hot water treatment, the meat surface temperature was 65°C and remained at that temperature for some period, presumably still effecting a microbial reduction, before sampling. However, in the case of combined processes, after hot water and hot air treatment, the BTL was directly exposed to colder lactic acid solution (12 to 15°C), lowering the meat surface temperature and nullifying further microbial reduction attributable to heat. Therefore, in combined treatments, residual antimicrobial action of hot water or hot air treatments is nullified.

Surface pH values of the BTL and BTF both before treatment and for the untreated control are reported in Table 3. The pH of control BTL remained unchanged through the 7 days of storage at 5°C. In the case of treatment including lactic acid, the surface pH of BTL dropped to 3.70, 3.54, 3.55, and 3.47 from treatments of water plus lactic acid and of comb 1, 2, and 3, respectively, and was significantly lower than non–acid-treated BTL ($P < 0.05$). After 7 days of refrigerated storage, pH values increased to above 5.0.

In the case of BTF, the pHs decreased to 3.23, 3.14, 3.15, and 3.10 by treatments of water plus lactic acid and of comb 1, 2, and 3, respectively, and increased to 4.84 (water plus lactic acid), 4.67 (comb 1), 4.70 (comb 2), and 4.53 (comb 3) after 7 days of storage at 5°C. Rise in surface pH of BTF was slower than that of BTL surface pHs.

Temperature changes and color evaluation of BTL and BTF treated by six different antimicrobial regimens are presented in Table 4. The meat color of BTL was much more sensitive to the effects of multiple and single interventions than that of BTF. The color of BTL treated by comb 1, 2, and 3 deteriorated rapidly and was unacceptable. However, the meat color of comb 1 reverted to an acceptable red color after 1 day of incubation at 4°C. Temperatures of heat-treated meat increased to 18 to 20°C following treatments then decreased.

Microbial reduction following antimicrobial treatments. The initial APC level of approximately 5.1 log CFU/cm^2 was reduced ($P < 0.05$) by 1.0 to 1.5 log CFU/cm^2 by treatments compared with control (Fig. 1a). The aerobic bacteria population on beef surfaces treated with water only began to increase after 1 day of cold storage and by 7 days had attained a population of approximately 5.51 log CFU/cm^2 (Fig. 1a). By 7 days aerobic bacteria on BTL treated with water plus lactic acid and comb 1, 2, and 3 had achieved populations of 4.95, 4.87, 4.78, and 4.70 log CFU/cm^2, respectively.

The numbers of PCT were strongly reduced by initial washing treatments with water, water plus lactic acid, and comb 1, 2, and 3 treatments. Significantly higher numbers of PCT were reduced by water plus lactic acid and by comb 1, 2, and 3 compared with water ($P < 0.05$). However, the numbers of PCT were also starting to increase after 7 days of storage at 5°C (Fig. 1b).

After 7 days of storage, the numbers of coliforms treated by water plus lactic acid and by comb 1, 2, and 3, were significantly less than control and water treatment (Fig. 1c). The numbers of biotype 1 *E. coli* present were also initially reduced by 1.5 to 2.0 log CFU/cm^2 by all treatments except the control. After 7 days of storage at 4°C, the numbers of presumptive *E. coli* on BTL treated by water plus lactic acid and comb 1, 2, and 3 were also significantly less than control and water treatments ($P < 0.05$) (Fig. 1d).

TABLE 3. *pH changes of BTL and BTF tissues through 7 days of incubation at 5°C*

Treatments	pH changes of BTL[a]				pH changes of BTF[b]			
	Before	After treatment	After 1-day storage	After 7-day storage	Before	After treatment	After 1-day storage	After 7-day storage
Control	5.90 ± 0.01 A[f]	5.92 ± 0.02 A	5.87 ± 0.04 A	6.14 ± 0.24 A	6.01 ± 0.14 A	6.03 ± 0.22 A	6.01 ± 0.24 A	6.71 ± 0.60 A
Water[d]	5.91 ± 0.02 A	5.97 ± 0.02 A	5.84 ± 0.04 A	6.01 ± 0.17 A	5.92 ± 0.07 A	6.34 ± 0.25 A	6.18 ± 0.33 A	6.73 ± 0.74 A
Water plus lactic acid[b]	5.91 ± 0.02 A	3.70 ± 0.01 B	4.88 ± 0.08 B	5.28 ± 0.07 B	6.01 ± 0.24 A	3.23 ± 0.15 B	4.09 ± 0.26 B	4.84 ± 0.31 B
Comb 1[c]	5.90 ± 0.03 A	3.54 ± 0.06 C	4.92 ± 0.03 B	5.19 ± 0.07 B	5.96 ± 0.06 A	3.14 ± 0.18 C	4.00 ± 0.21 B	4.67 ± 0.27 B
Comb 2[d]	5.93 ± 0.03 A	3.55 ± 0.12 C	4.83 ± 0.16 B	5.10 ± 0.07 B	5.94 ± 0.06 A	3.15 ± 0.20 C	3.97 ± 0.03 B	4.70 ± 0.18 B
Comb 3[e]	5.92 ± 0.01 A	3.47 ± 0.08 C	4.77 ± 0.14 B	5.13 ± 0.06 B	5.99 ± 0.18 A	3.10 ± 0.17 C	3.94 ± 0.13 B	4.53 ± 0.20 B

[a] Water washing at 65 psi for five passes.
[b] Water wash plus 2% (vol/vol) lactic acid wash at 30 psi for three passes.
[c] Water wash plus 65°C hot water at 30 psi for one-time passage plus hot air at 510°C for four passes under hot air gun plus lactic acid.
[d] Water wash plus 82°C hot water for one-time passage plus hot air at 510°C for five passes plus lactic acid.
[e] Water wash plus 82°C hot water for three passes plus hot air at 510°C for six passes plus lactic acid.
[f] Values in a column followed by different letters are statistically different ($P < 0.05$).

The numbers of PLAB were monitored through all treatments. The numbers of PLAB were significantly reduced by all treatments compared with control values ($P < 0.05$). Initially, the reductions by water plus lactic acid and comb 1, 2, and 3 were significantly higher than control and water treatments ($P < 0.05$). No significant differences between water plus lactic acid and comb 1, 2, and 3 treatments occurred to initially control PLAB in BTL ($P > 0.05$). After 7 days of storage at 5°C, the numbers of PLAB started to increase regardless of any treatments (Fig. 1e).

In the case of BTF, the microbial changes due to interventions showed different patterns compared with BTL. Greater reductions occurred for all microorganisms tested (Fig. 2a). Initially, the numbers of APC were reduced by 2.8 and 2.9 log units treated by comb 2 and comb 3 on BTF, respectively, with significant differences compared with other treatments ($P < 0.05$). In the case of water-treated BTF, the APC counts were increased from 4.9 to 6.3 log CFU/cm^2 after 7 days of incubation at 5°C. The trend of PCT populations of water-treated BTF was similar to the APC pattern (Fig. 2b). However, through incubation at 5°C, the numbers of PST treated by water plus lactic acid and by comb 1, 2, and 3 decreased even further ($P < 0.05$) during 7 days.

The coliform population patterns differed between BTL and BTF (Fig. 2c). Treatment comb 1, 2, and 3 on BTF significantly reduced the numbers of coliforms by an approximately 4.0-log reduction ($P < 0.05$), with significant differences compared with control, water, and water plus lactic acid treatments. Water and water plus lactic acid treatments were not significantly different ($P > 0.05$) in reducing the coliform population on BTF (1.6- and 1.7-log reductions, respectively). However, after 7 days of 4°C incubation, coliform populations of water plus lactic acid–treated BTF decreased by 4.0 log CFU/cm^2, respectively. With the exception of water treatment, all other treatments reduced coliform levels on BTF. The patterns of *E. coli* population reductions reflected coliform reductions (Fig. 2d). Treatment with comb 1, 2, and 3 initially reduced the numbers of *E. coli* by approximately 3.5 log CFU/cm^2 compared with the treatment with water and water plus lactic acid, which initially reduced the numbers of *E. coli* to 1.7 and 2.5 log CFU/cm^2, respectively. After 7 days of incubation, the numbers of *E. coli* were further reduced to 4.0 log units by water plus lactic acid treatment. *E. coli* reductions from water plus lactic acid and comb 1, 2, and 3 were not statistically different ($P > 0.05$) after 7 days of 4°C storage. In the case of PLAB populations on BTF, the reduction was not as great as the coliform or *E. coli* (Fig. 2d) reduction. However, the numbers of PLAB treated by lactic acid also continued to decrease through the 7 days at 4°C incubation period, whereas the populations of PLAB on water and control BTF continuously increased ($P < 0.05$) (Fig. 2e).

DISCUSSION

To date, antimicrobial intervention processes have focused on either the carcass stage of production (organic acid sprays, hot water, steam) or ground beef (irradiation).

TABLE 4. *Color evaluation and mean of temperatures of BTL and BTF tissues through 7 days of incubations at 5°C*[a]

	BTL								BTF							
	Before treatment		After treatment		1-Day storage		7-Day storage		Before treatment		After treatment		1-Day storage		7-Day storage	
Treatments	Color	°C	Color	°C	Color	°C	Color	°C	Color	°C	Color	°C	Color	°C	Color	°C
Control	Acc.	5.0	Acc.	17.5 A[g]	Acc.	5.0	Acc.	5.0	Acc.	5.0	Acc.	14.7 A	Acc.	5.0	Acc.	5.0
Water[b]	Acc.	5.0	Acc.	17.8 A	Acc.	5.0	Acc.	5.0	Acc.	5.0	Acc.	15.0 A	Acc.	5.0	Acc.	5.0
Water plus lactic acid[c]	Acc.	5.0	Acc.	18.3 AB	Acc.	5.0	Acc.	5.0	Acc.	5.0	Acc.	15.3 B	Acc.	5.0	Acc.	5.0
Comb 1[d]	Acc.	5.0	N-A.	18.8 AB	Acc.	5.0	Acc.	5.0	Acc.	5.0	Acc.	15.8 B	Acc.	5.0	Acc.	5.0
Comb 2[e]	Acc.	5.0	N-A.	18.8 AB	N-A.	5.0	Acc.	5.0	Acc.	5.0	Acc.	17.8 B	Acc.	5.0	Acc.	5.0
Comb 3[f]	Acc.	5.0	N-A.	20.5 B	N-A.	5.0	N-A.	5.0	Acc.	5.0	N-A.	19.0 B	Acc.	5.0	Acc.	5.0

[a] Acc., acceptable color as determined by visual evaluation; N-A., not acceptable color as determined by visual evaluation.
[b] Water washing at 65 psi for five passes.
[c] Water wash plus 2% (vol/vol) lactic acid wash at 30 psi for three passes.
[d] Water wash plus 65°C hot water at 30 psi for one-time passage plus hot air at 510°C for four passes under hot air gun plus lactic acid.
[e] Water wash plus 82°C for hot water one-time passage plus hot air at 510°C for five passes plus lactic acid.
[f] Water wash plus 82°C hot water for three passes plus hot air at 510°C for six passes plus lactic acid.
[g] Values in a column followed by different letters are statistically different ($P < 0.05$).

In fabricating operations, trim undergoes considerable handling by several personnel, thereby making contamination transfer likely. Therefore, applications of a final antimicrobial intervention, before grinding, should effect a reduction of background contamination, potentially distributed to a greater proportion of the trim and eventually to the ground beef. Trimming is the last stage of processing before grinding, so it is also the last practical site for antimicrobial interventions before grinding. The contaminated surface meat of trim is further "diluted" by the overwhelming amount of sterile meat from the interior of the carcass. The opposite is also true; i.e., contaminated surface tissue becomes intermixed with previously sterile tissue. Therefore, applying antimicrobial processes to trim offers a means to reduce the final source of surface contamination before grinding.

The processes presented in this article were developed using pieces of trim that were uniform in size, approximate lean or fat surface composition, and inoculum distribution. For such processes to find application in an actual trim production facility, engineering research must develop the means to deliver the antimicrobial process to both the top and undersides of trim that is highly irregular in size, shape, and composition.

A process designed to achieve reductions in enteric and coliform bacterial counts offers a means of reducing or inhibiting enteric pathogens, such as enterohemorrhagic *E. coli,* included in the contaminating population. Using a process that can achieve a predetermined reduction and inhibition of bacterial numbers, processors are assured that as long as process parameters are monitored, maintained, and controlled, consistent microbial decontamination is achievable. The main process steps comprising this multihurdle approach, chain speed and acid concentration, can be readily monitored in real time.

The most obvious difference between applying decontaminating interventions to carcasses versus trim is that carcasses are still covered with an intact fascia tissue that is somewhat protective to the underlying muscle, whereas trim surfaces are cut-exposed muscle tissues that are highly sensitive to heat and other denaturants. Recognizing the limits of the multihurdle process is critical in achieving a final ground product that is still acceptable to consumers yet has undergone the desired microbial reduction. Data presented in this article clearly demonstrate that there are process parameters or boundaries that can be crossed, beyond which the color quality of the resulting ground beef rapidly deteriorates. However, within boundaries (comb 1) greater than 1 $\log_{10}$ CFU/cm^2, coliform reduction is achievable with minimal loss of color quality.

The multihurdle approach to inhibiting microbial contaminants is applied routinely in other foods *(18, 21).* Research has also shown that sublethally injured bacteria are more susceptible to antimicrobial food processes and microbial inhibitors *(25)* than are those unstressed. Therefore, we have attempted to take advantage of a series of sublethal stresses, which together effect an overall greater microbial reduction than when applied alone and impart only minimal color quality losses.

Previous workers *(6, 14, 27)* have studied the effects of interventions applied to carcass tissues and the subsequent changes in microbial populations of the ground beef made from the treated carcass tissues. In brief, no major changes in the microbial progression of the resulting ground beef were observed, and there was no unchecked growth of inoculated pathogens, including *E. coli* O157 and salmonellae, under the controlled conditions of those studies. The current experimental data follow a similar trend in that the APC, PLAB, PCT, and coliform counts followed a growth progression similar to the control treatment group, albeit starting from lower initial posttreatment populations.

Our data indicate that on the inoculated adipose trim surfaces a clear reduction and inhibition of all tested bacterial groups after 7 days of storage from the combined

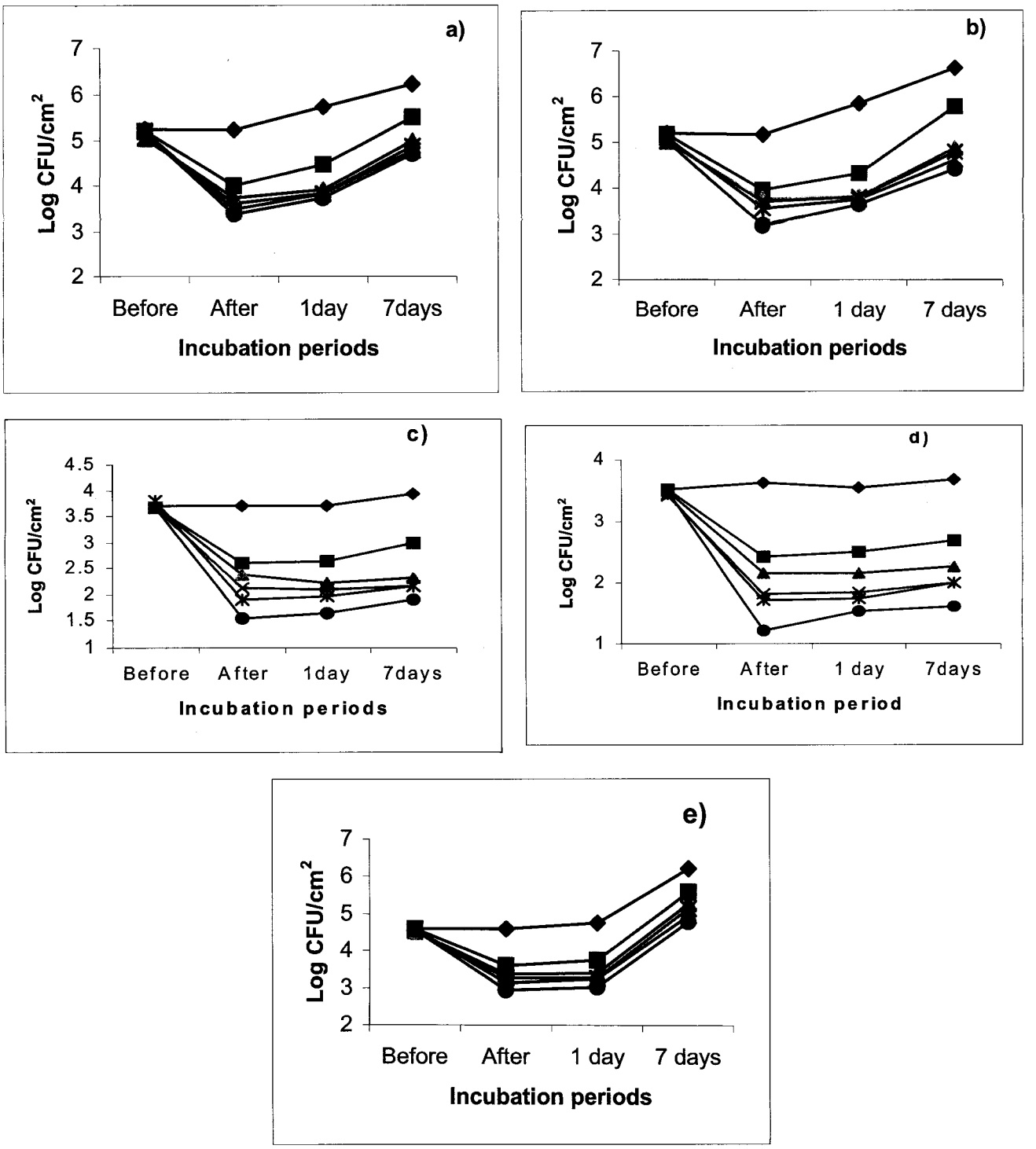

FIGURE 1. *Effects of antimicrobial processes and water wash interventions on inoculated BTL on the initial microbial population (before) and the population immediately after treatment (after) and subsequent outgrowth of APC (a), PCT (b), coliforms (c), generic* E. coli *(d), and PLAB (e) populations during storage at 5°C aerobically for 7 days.* ♦, *control;* ■, *water spray;* ▲, *water and lactic acid spray;* ***X***, comb 1; *, comb 2; ●, comb 3.

antimicrobial processes (comb 1, comb 2, and comb 3) occurred. In the case of BTL tissues, the coliform and *E. coli* biotype 1 populations dropped initially, then remained static throughout the 7-day storage period. The remaining test groups (APC, PCT, and PLAB) approached control levels after 7 days of refrigerated incubation, indicating that these samples were approaching a normal microbial progression for aerobically stored refrigerated beef *(13)*.

As reported previously *(6, 12)*, microbial reductions were greater from adipose tissues than from lean tissues. This phenomenon has been reported for both carcass tissues with intact surface fascia and currently for lean and adipose

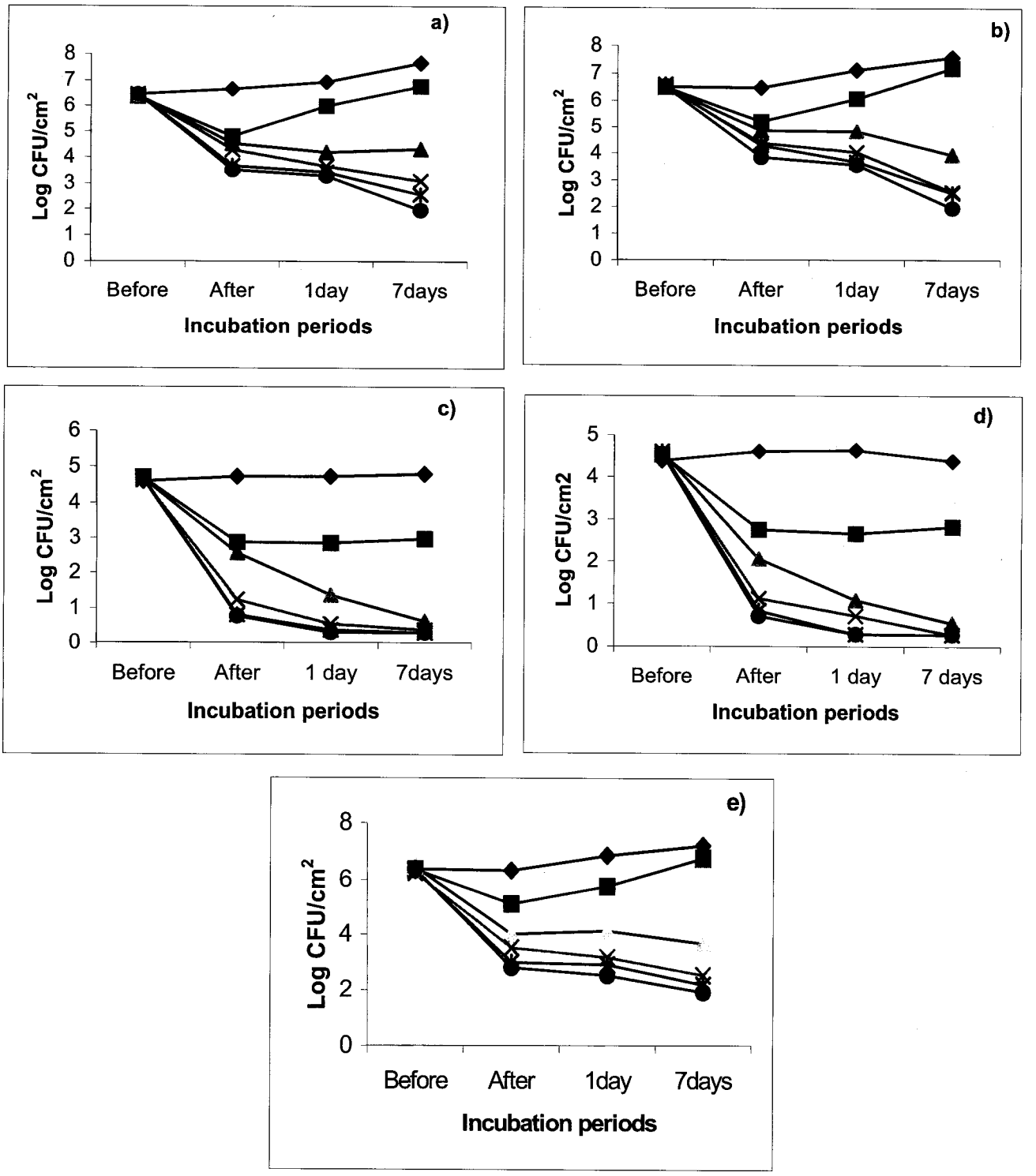

FIGURE 2. *Effects of antimicrobial processes and water wash interventions on inoculated BTF on the initial population (before) and the population immediately after treatment (after) and subsequent outgrowth of APC (a), PCT (b), coliforms (c), generic* E. coli *(d), and PLAB (e) populations during storage at 5°C aerobically for 7 days.* ♦, *control;* ■, *water spray;* ▲, *water and lactic acid spray;* X, comb 1; *, comb 2; ●, comb 3.

trim without an intact covering (i.e., cut surfaces). Interestingly, less microbial attachment to adipose tissues relative to lean-covered tissues has been reported *(6, 12, 27)*. Concurrently, adipose tissues remain poised at a lower pH following organic acid application than do lean-covered tissues *(6)*. Therefore, whether this phenomenon is a function of greater physical removal of microorganisms from adipose-covered tissue, greater in situ inactivation, less initial attachment, or a combination of all three is yet to be specifically answered. However, the current data seem to indicate it is possibly a function of the combination of conditions (surface chemistry and pH) at the tissue surface. Currently, no data exist to address the hypothesis that association of microbial cells with fluidized lipid (from warm-

ing the lipid content of the meat surface) influences attachment or entrapment. It has been reported that the surface topography *(28)* and the presence of a microfilm of water *(30)* exert distinct non–species-specific or bacterial phenotype–specific effects on microbial adherence to muscle foods.

Finishing the multihurdle intervention process with a lactic acid application not only reduces the trim surface temperature following the hot water spray but also provides an effective residual antimicrobial barrier. This residual antimicrobial effect has previously been demonstrated in beef tissues following application at the carcass stage of production *(6, 14)*. Because this is the final point of antimicrobial application and no further washing of the meat trim will occur, it is an ideal site for applying other food antimicrobials. Including antimicrobial interventions that effect minimal quality loss but exert an additive microbial reduction offers a framework strategy to develop other trim pathogen reduction processes. For instance, altering the concentration of lactic acid might confer a greater residual inhibitory effect than was observed in this set of experiments. Other interventions for trim have included shortwave UV irradiation *(29, 31)*.

Collectively, our data indicate that, at the trim stage, a multihurdle antimicrobial process can reduce inoculated levels of coliform bacteria on both lean and adipose cut surface trim, offering an immediate reduction with a residual inhibitory effect up to at least 7 days of 4°C refrigeration after processing. The currently reported process retains favorable color quality and does not greatly alter the normal microbial progression of the trim. Although the development of a multihurdle antimicrobial process would require further process engineering and development, this research provides the conceptual starting point and experimental design within which to develop such antimicrobial regimens for meat trim stock.

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