

## Evaluation of Combination Treatment Processes for the Microbial Decontamination of Pork Trim<sup>†</sup>

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### ABSTRACT

Combination treatment processes for the microbial decontamination of pork trim were developed and evaluated. Lean pork trim tissue (LPT) and fat-covered pork trim tissue (FPT) inoculated with swine feces were treated with intervention processes as follows: (i) control (untreated), (ii) water (15°C, 120 s), (iii) water followed by lactic acid wash (15°C, 75 s), (iv) combination 1 (water plus hot water [65.5°C, 15 s] plus hot air [510°C, 60 s] plus lactic acid), (v) combination 2 (water plus hot water [82.2°C, 15 s] plus hot air [510°C, 75 s] plus lactic acid), and (vi) combination 3 (water plus hot water [82.2°C, 45 s] plus hot air [510°C, 90 s] plus lactic acid). Populations of aerobic bacteria, psychrotrophic bacteria, coliforms, *Escherichia coli*, and lactic acid bacteria were determined before and after treatment and at days 2 and 7 of 4°C storage. Regardless of the intervention treatment, lower microbial populations were observed on FPT than on LPT immediately after treatment and during the 7-day storage period. Both LPT and FPT treated with water plus lactic acid, combination 1, combination 2, and combination 3 had lower remaining populations of all microbial groups immediately after treatment than did water-treated samples. Populations of aerobic bacteria, coliforms, *E. coli*, and lactic acid bacteria on either LPT or FPT did not statistically increase during the 7-day storage period. On LPT, populations of psychrotrophic bacteria grew during 4°C storage but remained lower at day 7 on LPT treated by combinations 2 and 3 (2.29 and 1.89 log<sub>10</sub> CFU/cm<sup>2</sup>, respectively) than on LPT treated with water (4.07 log<sub>10</sub> CFU/cm<sup>2</sup>) or water plus lactic acid (3.52 log<sub>10</sub> CFU/cm<sup>2</sup>). Populations of psychrotrophic bacteria remained below detectable levels throughout the 7-day storage on FPT treated with water plus lactic acid or any of the three combination treatments. Treatment of pork trim with any of the combination treatments significantly ( $P < 0.05$ ) affected the color and emulsion stability of the ground pork. Water and water plus lactic acid were the most favorable treatments in reducing microbial populations on pork trim without affecting the quality attributes of the ground pork.

Consumer demand and awareness of microbial foodborne diseases have resulted in intensified efforts by the red meat industry and regulatory authorities to reduce the extent to which raw meat becomes contaminated with pathogenic microorganisms during the slaughtering process (12, 24, 28). Prevention of microbial contamination during the slaughtering and fabrication process is one of the most critical meat safety issues. Even when processed under ideal conditions, the production of wholesome meat products from normal, healthy animals presents many opportunities for contamination with a variety of bacteria, including low levels of some pathogens (18, 21, 28).

By means of current legislation established under the Pathogen Reduction/Hazard Analysis Critical Control Points (HACCP) systems final rule, the Food Safety and Inspection Service mandates that all meat and poultry products during the slaughtering or fabrication process be tested for generic *Escherichia coli* and *Salmonella* spp. (3). Al-

though not currently required by law, incorporation of pathogen reduction steps is strongly recommended to complement HACCP programs. A pathogen reduction step is a process that reduces or eliminates foodborne pathogens (3). These steps may use approved agents that have an antimicrobial effect on targeted pathogens. Many intervention processes to reduce or eliminate bacteria from the surface of carcasses have been studied. Some of these methods include live animal cleaning and washing, knife trimming, steam and hot water vacuuming, and spray washing with water at low or high pressures and temperatures or with chemical solutions (1, 8-10, 14, 16, 19, 23, 25, 26). Most of these intervention methods have been shown to be effective in reducing microbial contamination on carcasses. However, because of extensive handling during fabrication, recontamination of cuts and trim is unavoidable. The application of effective antimicrobial intervention strategies to cuts and trim may be useful to further improve microbial safety. Some studies have examined antimicrobial treatments of meat trim as a means to reduce bacterial pathogens and improve shelf life of the ground products (4, 13, 17, 22). Recently, the application of multihurdle processes for microbial decontamination of beef trim has been evaluated (13, 20, 22). The multihurdle process relies on the concept that if a single intervention treatment is effective in reduc-

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ing bacteria, then the combination of two or more intervention treatments may result in a synergistic or additive decontaminating effect (22). Thus, a multihurdle intervention process for decontamination of trim may provide a means of reducing microbial populations and providing ground meat products with increased shelf life and reduced potential for causing foodborne illness. Our objective was to develop combination intervention processes to reduce microbial contamination on pork trim without compromising the quality attributes, such as color and emulsion stability, of the resultant ground pork.

## MATERIALS AND METHODS

**Pork trim preparation.** Pork loins were obtained from the abattoir at the Roman L. Hruska U.S. Meat Animal Research Center (MARC), Clay Center, Neb. The pork trim samples were prepared by cutting pork loins in approximately 30-cm-long sections. Then, the pork loins were split in half along the length, resulting in half with a predominantly fat pork trim (FPT) cut surface and half with a predominantly lean pork trim (LPT) cut surface. Pork trim samples were stored at  $-20^{\circ}\text{C}$  until use. Before use, LPT and FPT samples were thawed overnight at  $4^{\circ}\text{C}$  and aseptically cut into 10 by 30-cm sections.

**Fecal inoculum preparation.** Inocula were prepared on each day of an experiment from fresh pork feces collected from three pigs. A three-animal composite fecal slurry was prepared by mixing 50 g of each fecal sample with 300 ml of sterile distilled water (1:2). The fecal slurry was mixed using a sterile tongue depressor for 2 min and filtered using a sterile filtered stomacher bag (Spiral Biotech, Bethesda, Md.). Ten milliliters of the filtered slurry was inoculated to the entire 10- by 30-cm surface of each LPT or FPT sample by spoon inoculation (11, 22) and incubated overnight at  $4^{\circ}\text{C}$  before experiments to approximate trim contamination incubation conditions that might occur in slaughter plants (22). This inoculum, applied to LPT or FPT, resulted in approximately 4 to 5 log CFU/cm<sup>2</sup> of total coliforms. The LPT or FPT was then subjected to the intervention strategies described below. Control samples received no treatments.

**Trim intervention chamber.** The trim intervention chamber used for this study was a custom-built chamber with adjustable speed, moving chain table, two adjustable spray units, and one hot air cabinet with three heat guns (Varitemp heat gun, Master Appliance Corp., Racine, Wis.); the trim intervention chamber is described in further detail by Kang et al. (22).

**Optimization of individual intervention treatments.** Inoculated LPT samples were treated with four individual intervention treatments, including water, lactic acid, hot water, and hot air at multiple exposure times and/or temperatures. Each intervention treatment was evaluated to determine the optimum parameters of exposure and temperature for maximum coliform reduction from the LPT.

Inoculated LPT was spray washed with water for 15 to 180 s (in 15-s increments) at 65 lb/in<sup>2</sup> and  $15^{\circ}\text{C}$ . Lactic acid (Sigma Chemical Co., St. Louis, Mo.) was applied by spraying the LPT surface with a 2% (vol/vol) lactic acid solution for 15 to 120 s (in 15-s increments) at 35 lb/in<sup>2</sup> and  $15^{\circ}\text{C}$ . Hot water (35 lb/in<sup>2</sup>) was sprayed at different temperatures ( $65.5^{\circ}\text{C}$  [ $150^{\circ}\text{F}$ ],  $71^{\circ}\text{C}$  [ $160^{\circ}\text{F}$ ],  $76.6^{\circ}\text{C}$  [ $170^{\circ}\text{F}$ ], and  $82.2^{\circ}\text{C}$  [ $180^{\circ}\text{F}$ ]) for 15 to 45 s (in 15-s increments). All spray washing treatments were done with an oscillation rate of 60 passes per min and a chain speed of 1 cm/s. Hot air was applied with the hot air guns preheated for 5

min before use. Inoculated LPT was treated with hot air at different temperatures ( $454.4^{\circ}\text{C}$  [ $850^{\circ}\text{F}$ ],  $482.2^{\circ}\text{C}$  [ $900^{\circ}\text{F}$ ], and  $510^{\circ}\text{C}$  [ $950^{\circ}\text{F}$ ]) for 15 to 90 s (in 15-s increments) with a chain speed of 1 cm/s. Each treatment was replicated three times.

**Evaluation of different combinations of treatments on LPT and FPT.** Based on results obtained in optimization experiments, treatments with minimum, medium, and maximum conditions of hot water and hot air to reduce coliform populations on pork trim were combined with maximum conditions of water and lactic acid to develop three combination treatments that combine water, followed by hot water, hot air, and lactic acid as the final spray washing treatment. Inoculated LPT and FPT were treated using a split-plot in time design with intervention treatments as the main plot and storage time as the split-plot in time factor. Six different intervention treatments were evaluated as follows: (i) control (untreated); (ii) water ( $15^{\circ}\text{C}$ , 65 lb/in<sup>2</sup>, 120 s); (iii) water followed by lactic acid wash ( $15^{\circ}\text{C}$ , 35 lb/in<sup>2</sup>, 75 s); (iv) combination 1 (water plus hot water [ $65.5^{\circ}\text{C}$ , 35 lb/in<sup>2</sup>, 15 s] plus hot air [ $510^{\circ}\text{C}$ , 60 s] plus lactic acid); (v) combination 2 (water plus hot water [ $82.2^{\circ}\text{C}$ , 35 lb/in<sup>2</sup>, 15 s] plus hot air [ $510^{\circ}\text{C}$ , 75 s] plus lactic acid); and (vi) combination 3 (water plus hot water [ $82.2^{\circ}\text{C}$ , 35 lb/in<sup>2</sup>, 45 s] plus hot air [ $510^{\circ}\text{C}$ , 90 s] plus lactic acid). Microbial populations were determined both before and after treatments and at days 2 and 7 of aerobic storage in plastic containers at  $4^{\circ}\text{C}$ . This experiment was replicated five times each for both LPT and FPT.

**Sampling and microbial analysis.** At each sampling time, a 5 by 5 by 0.5-cm sample from LPT or FPT was taken by excision using an alcohol-flamed 25 cm<sup>2</sup> template. The sample was placed into a sterile stomacher bag (Spiral Biotech, Inc.) containing 25 ml of sterile buffered peptone water (Difco, Becton Dickinson Microbiology Systems, Sparks, Md.) with 0.1% Tween 20 (Sigma) and pummeled in a Model 400 stomacher (Tekmar, Inc., Cincinnati, Ohio) for 2 min. Appropriate sample dilutions were made in buffered peptone water. In the optimization experiments, total coliform populations on LPT treated with water, lactic acid, hot water, and hot air were enumerated both before and immediately after treatment. For the evaluation of the combination treatments, microbial populations of mesophilic aerobic bacteria, psychrotrophic bacteria (PCT), total coliforms, *E. coli*, and presumptive lactic acid bacteria (PLAB) were enumerated both before and after treatment and at days 2 and 7 of storage at  $4^{\circ}\text{C}$ . Petrifilm aerobic count plates (3M Microbiology Products, St. Paul, Minn.), incubated at  $37^{\circ}\text{C}$  for 48 h and at  $9^{\circ}\text{C}$  for 7 days, were used to enumerate aerobic bacteria and PCT, respectively. Coliforms and *E. coli* were enumerated using Petrifilm *E. coli*/coliform count plates (3M) incubated at  $35^{\circ}\text{C}$  for 24 h. Counts for PLAB were determined by spread plating on pre-poured Lactobacilli MRS agar plates (Difco Laboratories, Detroit, Mich.) containing 0.02% sodium azide. The MRS plates were anaerobically incubated in a BBL GasPak jar (Becton Dickinson) with AnaeroGen (Oxoid Ltd., Basingstoke, Hampshire, England) for 48 h at  $30^{\circ}\text{C}$ . Diluted as described, the lowest level of detection for bacterial populations plated on Petrifilm was 1 CFU/cm<sup>2</sup>, and the lowest level of detection for PLAB on MRS plates was 10 CFU/cm<sup>2</sup>.

**pH determination.** The surface pH of LPT and FPT was determined using a flat surface combination probe (Corning model 440, Corning Inc., Corning, N.Y.) before and after treatment and at days 2 and 7.

**Color evaluation.** Fresh, uninoculated, commercial pork trim pieces of irregular shape and size (no larger than approximately 10 by 20 by 2.5 cm) and of 80% lean and 20% fat content were

obtained from the MARC abattoir. The pork trim was subjected to the combination treatments as described above, stored at 4°C for 24 h, and then ground with a commercial grinder (Davpol Enterprises Inc., New York, N.Y.). The trim samples were ground first through a 1-cm grinder plate, and then half of the ground pork (approximately 500 g) of each untreated and treated sample was sampled and subjected to the emulsion stability test as described below. The other half of the ground pork was ground again through a 0.5-cm grinder plate and used to make patties (150 g) for color measurement. A Hunter Miniscan XE Plus Model no. 45/O-L (Hunter Associates Laboratory, Inc., Reston, Va.) was used to evaluate L\* (lightness), a\* (redness), and b\* (yellowness) values. Three readings were taken randomly from each of three patties made from each untreated and treated ground pork sample and then averaged. This study was conducted with two replicates; each replicate was done on separate days, resulting in at least six readings.

**Emulsion stability test.** Emulsions were prepared by mixing ground pork with 2% sodium chloride (Sigma), 0.4% phosphate blend (Vitaphos, First Spice Mixing Company, Inc., San Francisco, Calif.), and 20% flaked ice in a food processor (Quick N' Easy FP1200, Black & Decker, Towson, Mass.). Ground pork, sodium chloride, phosphate blend, and half of the flaked ice were chopped for 30 s, and then the remainder of the ice was added and chopped for another 30 s. The emulsion stability test of ground pork emulsions prepared from untreated and treated samples was done in triplicate, according to the method of Townsend et al. (30).

**Statistical analysis.** Bacterial counts for each treatment were converted to log<sub>10</sub> CFU/cm<sup>2</sup> values and analyzed statistically for analysis of variance (ANOVA) using the General Linear Model (GLM) procedure for repeated measurements (27). Means were separated using the least significant difference test (PROC MIXED) at 0.05 probability level. Data from the Hunter color values and emulsion stability test were analyzed by ANOVA using the GLM procedure. Differences among treatments were examined for level of significance (*P* < 0.05) by Tukey-Kramer Multiple Comparisons Test.

**RESULTS**

**Optimization of intervention treatments.** The microbiological effects of various intervention treatments were evaluated to develop three combination treatments that combined optimum exposure times of water and lactic acid treatments with minimum, medium, and maximum conditions of temperature and exposure time of hot water and hot air treatments to reduce coliform populations on pork trim. Reductions in total coliforms on inoculated LPT attributable to each intervention treatment at different exposure times and/or temperatures are shown in Table 1. Log reductions were calculated as the difference between populations of total coliforms (log<sub>10</sub> CFU/cm<sup>2</sup>) before treatment and remaining populations (log<sub>10</sub> CFU/cm<sup>2</sup>) immediately after treatment. Initial coliform populations on fecal inoculated LPT before treatment averaged 4.51 ± 0.48 log<sub>10</sub> CFU/cm<sup>2</sup>. Spray washing inoculated LPT with water for 15 to 180 s was effective in reducing total coliforms. Reduction of coliforms ranged from 1.46 to 2.52 log cycles immediately after spray treatments with water, with higher reductions at longer exposure times. No significant (*P* > 0.05) differences were found after spray washing LPT with

TABLE 1. Reductions in total coliforms on inoculated LPT immediately after treatment with water, lactic acid, hot air, and hot water<sup>a</sup>

Time (s)	Log <sub>10</sub> CFU/cm <sup>2</sup> reduction <sup>b</sup>									
	Water at 15°C		Lactic acid (2%) at 15°C <sup>c</sup>		Hot air		Hot water			
	15°C	15°C <sup>c</sup>	454.4°C	482.2°C	510°C	65.5°C	71°C	76.6°C	82.2°C	
15	1.46 (0.21) A <sup>d</sup>	0.97 (0.19) A	0.13 (0.10) A	0.17 (0.03) A	0.23 (0.16) A	1.28 (0.32) A	1.41 (0.01) A	1.56 (0.08) A	1.68 (0.31) A	
30	1.62 (0.30) A	1.06 (0.26) A	0.24 (0.11) A	0.16 (0.03) A	0.18 (0.09) A	1.45 (0.06) A	1.75 (0.04) B	1.88 (0.02) B	1.88 (0.06) AB	
45	1.79 (0.19) AB	1.31 (0.08) AB	0.21 (0.18) A	0.22 (0.08) AB	0.22 (0.12) A	1.63 (0.30) A	1.81 (0.18) B	2.04 (0.21) B	2.29 (0.35) B	
60	1.90 (0.07) AB	1.60 (0.15) BC	0.15 (0.10) A	0.20 (0.14) AB	0.26 (0.20) AB					
75	1.97 (0.02) AB	1.97 (0.35) C	0.21 (0.15) A	0.33 (0.06) AB	0.41 (0.05) AB					
90	2.14 (0.18) AB	1.80 (0.12) BC	0.23 (0.16) A	0.54 (0.27) B	0.66 (0.24) B					
105	2.37 (0.33) B	1.92 (0.19) C								
120	2.50 (0.40) B	1.92 (0.16) C								
135	2.41 (0.32) B									
150	2.51 (0.28) B									
165	2.52 (0.20) B									
180	2.52 (0.20) B									

<sup>a</sup> Average initial total coliform counts were 4.51 ± 0.48 log<sub>10</sub> CFU/cm<sup>2</sup>.

<sup>b</sup> Mean taken from three replicates; values in parentheses are standard deviations.

<sup>c</sup> Water, lactic acid, and hot water were applied at 65, 35, and 35 lb/in<sup>2</sup>, respectively.

<sup>d</sup> Means with the same letter within a column are not significantly different (*P* < 0.05).

water for more than 120 s. Spray treatments with 2% lactic acid (15°C, 35 lb/in<sup>2</sup>) for 15 to 120 s resulted in population reductions of coliforms ranging from 0.97 to 1.97 log cycles. Maximum reduction of total coliforms with lactic acid was observed up to 1.97 log cycles at 75 s. Therefore, the optimum exposure times for water and lactic acid treatments chosen for the combination treatments were 120 and 75 s, respectively. Greater reductions of total coliforms were observed when inoculated LPT was sprayed with hot water at higher temperatures and longer exposure times and ranged from 1.28 to 2.29 log cycles. Based on these results, hot water sprays of 65.5°C for 15 s, 82.2°C for 15 s, and 82.2°C for 45 s were chosen as the minimum, medium, and maximum exposure times and temperatures, respectively, for use in the combination processes. Inoculated LPT treated with hot air at 454.4°C, 482.2°C, and 510°C for 15 to 90 s (in 15-s increments) resulted in reduction of coliforms ranging from 0.13 to 0.66 log cycles. Although reductions of coliforms on some hot air-treated samples differed significantly ( $P < 0.05$ ) but not practically, hot air treatments of 510°C for 60, 75, and 90 s were chosen as minimum, medium, and maximum exposure times for use in the combination processes.

**Evaluation of the combination treatments.** The effects that the different intervention treatments had on the LPT and FPT surface pH during the 7-day period of this study are shown in Table 2. Surface pH on LPT and FPT after treatment with water plus lactic acid, combination 1, combination 2, and combination 3 resulted in significantly lower pH ( $P < 0.05$ ) than control and water. Although pH on FPT treated with water plus lactic acid, combination 1, combination 2, and combination 3 increased slightly during the 7-day study period, it remained significantly ( $P < 0.05$ ) lower than control and water-treated samples. This may be due to the low buffering capacity of the fat tissue.

The effects of the intervention treatments on microbial populations of LPT are shown in Figure 1A through 1E. All intervention treatments were significantly ( $P < 0.05$ ) effective in reducing microbial populations on LPT compared with control (untreated samples). The mean aerobic bacteria population on all pork trim samples before treatment was  $4.82 \pm 0.32 \log_{10}$  CFU/cm<sup>2</sup>. Treatment of LPT with intervention treatments, including water plus lactic acid (2.39  $\log_{10}$  CFU/cm<sup>2</sup>), combination 1 (2.31  $\log_{10}$  CFU/cm<sup>2</sup>), combination 2 (2.18  $\log_{10}$  CFU/cm<sup>2</sup>), and combination 3 (2.23  $\log_{10}$  CFU/cm<sup>2</sup>) resulted in significantly ( $P < 0.05$ ) lower aerobic bacteria populations immediately after treatment than with water wash alone (3.05  $\log_{10}$  CFU/cm<sup>2</sup>). Regardless of treatment, populations of aerobic bacteria did not significantly ( $P > 0.05$ ) increase throughout the 7-day study period. Similarly, water alone resulted in remaining PCT populations of 2.68  $\log_{10}$  CFU/cm<sup>2</sup> immediately after treatment, whereas PCT populations remaining on LPT samples treated with water plus lactic acid, combination 1, combination 2, and combination 3 were 1.67, 1.37, 1.11, and 1.05  $\log_{10}$  CFU/cm<sup>2</sup>, respectively. However, only in samples treated with combination 2 and combination 3 of the intervention treatments studied did populations

TABLE 2. Surface pH of treated LPT and FPT stored aerobically for 7 days at 4°C

Treatment	Surface pH values <sup>a</sup>					
	Before treatment		After treatment <sup>b</sup>		Day 7	
	LPT	FPT	LPT	FPT	LPT	FPT
Control	5.79 (0.09) A <sup>c</sup>	6.69 (0.22) A	5.83 (0.09) A	6.72 (0.21) A	5.86 (0.08) A	6.32 (0.14) A
Water	5.77 (0.13) A	6.69 (0.20) A	5.96 (0.18) A	7.23 (0.07) B	5.73 (0.08) A	6.50 (0.17) A
Water plus lactic acid <sup>d</sup>	5.77 (0.20) A	6.71 (0.21) A	3.47 (0.09) B	2.55 (0.17) C	5.28 (0.05) B	4.48 (0.14) B
Combination 1 <sup>e</sup>	5.85 (0.10) A	6.73 (0.13) A	3.28 (0.09) C	2.45 (0.08) C	5.10 (0.21) BC	4.44 (0.07) B
Combination 2 <sup>f</sup>	5.81 (0.09) A	6.74 (0.12) A	3.20 (0.04) C	2.47 (0.13) C	5.14 (0.16) BC	4.39 (0.16) B
Combination 3 <sup>g</sup>	5.81 (0.08) A	6.70 (0.21) A	3.25 (0.15) C	2.46 (0.14) C	5.08 (0.19) C	4.42 (0.20) B

<sup>a</sup> Values in parentheses are standard deviations.

<sup>b</sup> Surface pH was taken immediately after treatment.

<sup>c</sup> Means with the same letter in a column are not significantly different ( $P < 0.05$ ).

<sup>d</sup> Water wash (15°C, 65 lb/in<sup>2</sup>, 120 s) followed by 2% lactic acid wash (15°C, 35 lb/in<sup>2</sup>, 75 s).

<sup>e</sup> Water plus hot water wash (35 lb/in<sup>2</sup>, 65.5°C, 15 s) plus hot air (510°C, 60 s) plus lactic acid.

<sup>f</sup> Water plus hot water wash (35 lb/in<sup>2</sup>, 82.2°C, 15 s) plus hot air (510°C, 75 s) plus lactic acid.

<sup>g</sup> Water plus hot water wash (35 lb/in<sup>2</sup>, 82.2°C, 45 s) plus hot air (510°C, 90 s) plus lactic acid.

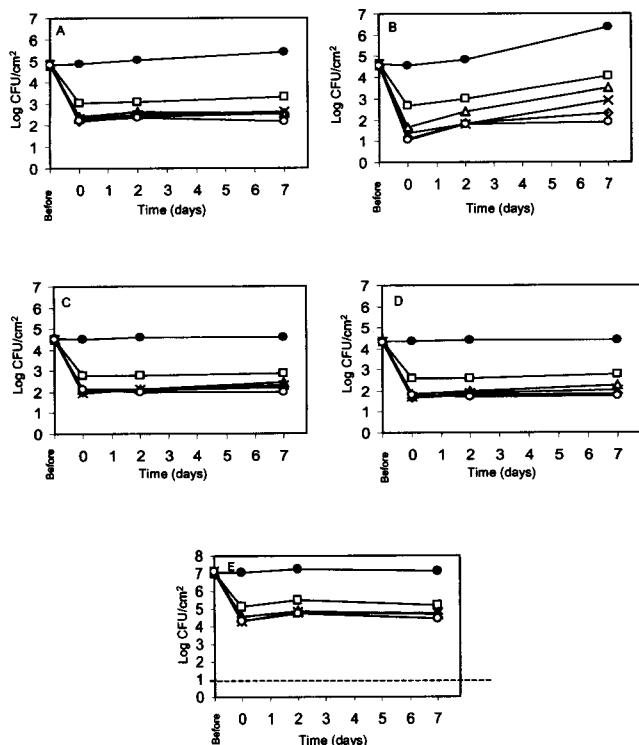


FIGURE 1. Effects of the combination processes on microbial populations on inoculated LPT immediately after treatment (day 0) and on days 2 and 7 of storage at 4°C. (A) Mesophilic aerobic bacteria, (B) psychrotrophic bacteria, (C) total coliforms, (D) *E. coli*, and (E) PLAB. ●, control; □, water spray; △, water followed by lactic acid spray; X, combination 1 (water plus hot water [65.5°C, 35 lb/in<sup>2</sup>, 15 s] plus hot air [510°C, 60 s] plus lactic acid); ◇, combination 2 (water plus hot water [82.2°C, 35 lb/in<sup>2</sup>, 15 s] plus hot air [510°C, 75 s] plus lactic acid); ○, combination 3 (water plus hot water [82.2°C, 35 lb/in<sup>2</sup>, 45 s] plus hot air [510°C, 90 s] plus lactic acid). The horizontal dotted line at 1.00 log<sub>10</sub> CFU/cm<sup>2</sup> in Figure 1E denotes the minimum detection level.

of PCT remain statistically the same throughout the 7-day storage period. In a pattern similar to that observed for aerobic bacteria, populations of coliforms, *E. coli*, and PLAB were more significantly ( $P < 0.05$ ) reduced by water plus lactic acid, combination 1, combination 2, and combination 3 treatments than by water alone.

Microbial populations on FPT were significantly ( $P < 0.05$ ) reduced by all treatments (Fig. 2A through 2E). Greater reductions in microbial populations were observed on FPT-treated samples than on LPT-treated samples. Any of the combination treatments (water plus lactic acid, combination 1, combination 2, or combination 3) reduced populations of aerobic bacteria, PCT, coliform, *E. coli*, and PLAB on FPT better than water alone. Aerobic bacteria populations on fecally inoculated FPT before treatment averaged  $4.74 \pm 0.12$  log<sub>10</sub> CFU/cm<sup>2</sup>. Immediately following water wash, water plus lactic acid, combination 1, combination 2, and combination 3, remaining aerobic bacteria populations were 2.52, 1.18, 0.84, 0.93, and 0.13 log<sub>10</sub> CFU/cm<sup>2</sup>, respectively. Of the various combination treatments examined in this study, combination 3 reduced aer-

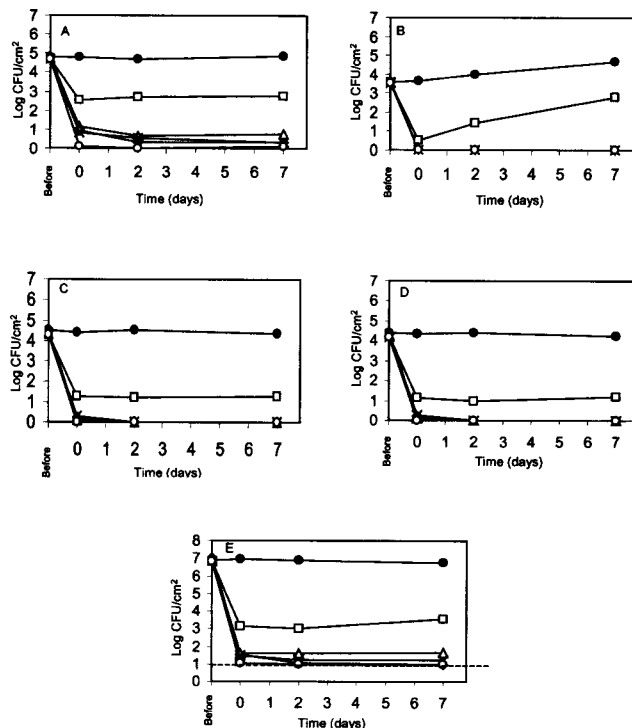


FIGURE 2. Effects of the combination processes on microbial populations on inoculated FPT immediately after treatment (day 0) and on days 2 and 7 of storage at 4°C. (A) Mesophilic aerobic bacteria, (B) psychrotrophic bacteria, (C) total coliforms, (D) *E. coli*, and (E) PLAB. ●, control; □, water spray; △, water followed by lactic acid spray; X, combination 1 (water plus hot water [65.5°C, 35 lb/in<sup>2</sup>, 15 s] plus hot air [510°C, 60 s] plus lactic acid); ◇, combination 2 (water plus hot water [82.2°C, 35 lb/in<sup>2</sup>, 15 s] plus hot air [510°C, 75 s] plus lactic acid); ○, combination 3 (water plus hot water [82.2°C, 35 lb/in<sup>2</sup>, 45 s] plus hot air [510°C, 90 s] plus lactic acid). The horizontal dotted line at 1.00 log<sub>10</sub> CFU/cm<sup>2</sup> in Figure 1E denotes the minimum detection level.

obic bacteria to the greatest extent and only 0.11 log<sub>10</sub> CFU/cm<sup>2</sup> remained after the 7-day storage period. PCT populations were reduced to below detectable levels (<1 CFU/cm<sup>2</sup>) after treatment with water plus lactic acid, combination 1, combination 2, and combination 3 and remained below detectable levels throughout the 7-day storage period, whereas the numbers of PCT significantly ( $P < 0.05$ ) increased on water-treated FPT (from 0.5 to 2.76 log<sub>10</sub> CFU/cm<sup>2</sup>). Initial levels of coliforms and *E. coli* on FPT before treatment were  $4.37 \pm 0.18$  and  $4.26 \pm 0.19$  log<sub>10</sub> CFU/cm<sup>2</sup>, respectively. Both coliforms and *E. coli* were significantly ( $P < 0.05$ ) reduced to very low levels following treatment with water plus lactic acid (0.13 log<sub>10</sub> CFU/cm<sup>2</sup> coliforms, 0.11 log<sub>10</sub> CFU/cm<sup>2</sup> *E. coli*), combination 1 (0.26 log<sub>10</sub> CFU/cm<sup>2</sup> coliforms, 0.26 log<sub>10</sub> CFU/cm<sup>2</sup> *E. coli*), and combination 2 (0.24 log<sub>10</sub> CFU/cm<sup>2</sup> coliforms, 0.21 log<sub>10</sub> CFU/cm<sup>2</sup> *E. coli*). At day 7, both coliforms and *E. coli* were below detectable levels in all the intervention treatments except water-treated samples (1.30 log<sub>10</sub> CFU/cm<sup>2</sup> coliforms, 1.19 log<sub>10</sub> CFU/cm<sup>2</sup> *E. coli*).

In a pattern similar to that of aerobic bacteria populations, remaining populations of PLAB in FPT samples

TABLE 3. Effects of the antimicrobial intervention treatments on the color and emulsion stability of ground pork trim

Treatment	Hunter color values			Emulsion stability <sup>a</sup>		
	L*	a*	b*	Fat	Liquid	Total <sup>b</sup>
Control	59.72 A <sup>c</sup>	14.49 A	12.75 A	0.55 A	4.38 A	4.93 A
Water	61.72 A	13.85 AB	15.98 B	0.47 A	4.35 A	4.82 A
Water plus lactic acid <sup>d</sup>	56.09 B	12.59 BC	15.23 B	0.79 AB	5.88 A	6.67 A
Combination 1 <sup>e</sup>	59.98 A	12.48 C	16.27 B	1.23 BC	10.82 B	12.05 B
Combination 2 <sup>f</sup>	61.32 A	11.70 C	16.12 B	1.56 C	19.08 C	20.64 C
Combination 3 <sup>g</sup>	60.50 A	12.73 BC	16.17 B	1.65 C	23.67 D	25.32 D

<sup>a</sup> Volume of emulsion components released (ml per 100 g of emulsion).

<sup>b</sup> Total volume of fat and liquid released.

<sup>c</sup> Means with the same letter within a column are not significantly different ( $P < 0.05$ ).

<sup>d</sup> Water wash (15°C, 65 lb/in<sup>2</sup>, 120 s) followed by 2% lactic acid wash (15°C, 35 lb/in<sup>2</sup>, 75 s).

<sup>e</sup> Water plus hot water wash (35 lb/in<sup>2</sup>, 65.5°C, 15 s) plus hot air (510°C, 60 s) plus lactic acid.

<sup>f</sup> Water plus hot water wash (35 lb/in<sup>2</sup>, 82.2°C, 15 s) plus hot air (510°C, 75 s) plus lactic acid.

<sup>g</sup> Water plus hot water wash (35 lb/in<sup>2</sup>, 82.2°C, 45 s) plus hot air (510°C, 90 s) plus lactic acid.

treated with water plus lactic acid (1.60 log<sub>10</sub> CFU/cm<sup>2</sup>), combination 1 (1.48 log<sub>10</sub> CFU/cm<sup>2</sup>), and combination 2 (1.55 log<sub>10</sub> CFU/cm<sup>2</sup>) immediately after treatment were significantly ( $P < 0.05$ ) lower than in water-treated samples (3.16 log<sub>10</sub> CFU/cm<sup>2</sup>). Of the intervention treatments studied, only samples treated with combination 2 and combination 3 had nondetectable levels (<10 CFU/cm<sup>2</sup>) of PLAB after the 7-day storage period.

Mean instrumental color values as affected by the different intervention treatments are shown in Table 3. After treatment, ground commercial pork trim treated with combinations 1, 2, and 3 did not affect L\* values but decreased a\* and increased b\* values compared with the control sample ( $P > 0.05$ ). The samples treated with water plus lactic acid had lower L\* values compared with control, water, and combinations 1, 2, and 3 but similar a\* and b\* values compared with combinations 1, 2, and 3. Treatment of pork trim with water did not affect L\* and a\* values but resulted in higher b\* values compared with the control. Visual observation of the ground pork patties showed negligible effects on the color and appearance in water-treated samples compared with the control. Pork trim treated with water plus lactic acid resulted in a darker red color with a mushy appearance. Small, brown, cooked, open spots were observed in ground pork patties treated by any of the combination treatments and were likely due to the use of heat (hot water and hot air) in these treatments.

No significant differences ( $P > 0.05$ ) were found on pork emulsion stabilities among the samples treated with control, water, and water plus lactic acid (Table 3). All combination treatments (1, 2, and 3) significantly ( $P < 0.05$ ) increased the liquid and fat released from these emulsions compared with the control, water, and water plus lactic acid treatments.

The commercial pork trim samples were weighed both before treatment and immediately after treatment to determine the fluid gained by the pork trim as a result of the spray washing treatments. An increase in weight of 2.2, 3.7, 4.7, 4.5, and 3.3% was found in commercial pork trim treated with water, water plus lactic acid, combination 1, combination 2, and combination 3, respectively.

## DISCUSSION

The application of intervention strategies has been extensively studied for reducing bacterial populations on carcasses. Most of these intervention methods have been shown to improve the microbiological safety and quality of carcasses. Since recontamination of meat during carcass breaking and further processing is inevitable, the added safety margins from whole carcass decontamination are decreased in the fabricated trim product. Therefore, as reasoned previously, it is perhaps of great importance to apply antimicrobial interventions to pork trim before grinding. Data on antimicrobial interventions for trim are limited (4, 13, 17, 22).

In the initial optimization experiment, it was observed that greater reductions of total coliforms were found in water-treated samples than lactic acid-treated samples at the same exposure time. This may be due to the higher spray pressure applied in water-treated (65 lb/in<sup>2</sup>) samples than in lactic acid-treated (35 lb/in<sup>2</sup>) samples. In previous studies, several parameters have been shown to influence the decontaminating efficacy of intervention methods on meat (5, 29). These include water temperature, spray pressure, tissue type, chemicals used, time of exposure, and method of application (immersion, rinsing, or spray washing). Although hot water was more effective in reducing coliform populations than water, it also resulted in a cooked color on the surface of the LPT. Hot air was less effective for reducing coliforms from LPT than water, lactic acid, and hot water; however, the use of hot air in the multihurdle process not only may result in another inactivation step for bacteria, but also may serve to remove the excess fluid from the surface of meat. Since no individual intervention treatment is 100% effective, an approach involving a combination of methods has been recommended (6, 10, 20, 22, 29). It has been shown that a combination of intervention strategies may result in additional reduction and may create more hurdles to microbial growth, therefore providing a wider margin of safety. Castillo et al. (2) found that a water wash followed by a hot water wash was more effective in

removing *Salmonella* Typhimurium and *E. coli* O157:H7 from various carcass surface regions than a water wash alone. In another study, beef trim treated with hot water plus lactic acid resulted in significant reductions in *E. coli* O157:H7, *Salmonella* Typhimurium, and aerobic bacteria with log reductions of 1.1, 1.8, and 1.5, respectively (13). They also observed that the quality characteristics in the final ground meat product, such as color and odor, were not affected by the treatment. In another study, Kang et al. (22) found that a combination of treatments (water wash followed by hot water, then hot air, and a final wash with a 2% lactic acid) was more effective than individual treatments in reducing microbial populations on fecally inoculated beef trim immediately following treatments and during a 7-day storage period.

In this study, each individual intervention treatment was evaluated and then combined into three different combination treatments to achieve a greater microbial reduction than when any individual treatment was applied alone. As expected, regardless of any treatment, microbial reductions were greater on FPT than LPT. These results are in agreement with previous studies that have shown that bacteria are reduced to a greater extent from adipose tissue than from lean tissue (8, 9, 21). Although similar microbial populations for samples treated with water plus lactic acid, combination 1, combination 2, and combination 3 during the 7-day storage period were expected, the low microbial populations for water-treated samples were not. This may be due to the drying effect observed on the surface of the pork trim during the 7-day storage period at aerobic conditions. Freezing, thawing, and storage of the pork trim under aerobic conditions caused a drying effect on the surface of the pork trim. Thus, the lack of moisture on the surface of the pork trim may explain why microbial populations were the same on water-treated samples during the 7-day storage period. Lactic acid applied in the combination treatments has been shown to afford some residual antimicrobial activity on meat surface tissue over time in other studies (11, 28). However, water treatment should not afford any residual antimicrobial activity. Many studies have reported the antibacterial efficacy of dilute solutions of organic acids (10–12, 28). Cutter and Rivera-Betancourt (7) found that spray washing beef tissue with an organic acid solution (2% acetic acid or 2% lactic acid) resulted in greater reductions of *Salmonella* Typhimurium DT104, *E. coli* O157:H7, *E. coli* O111:H8, and *E. coli* O26:H11 than spray washing with water or hot water. No significant differences were found in bacteria populations after treatment and long-term vacuum packaged storage at 4°C for 35 days. In another study, Dorsa et al. (11) reported that an acetic acid or lactic acid wash was effective in reducing aerobic bacteria, *E. coli* O157:H7, *Listeria innocua*, *Clostridium sporogenes*, pseudomonads, and lactic acid bacteria in beef carcass tissue and inhibited their growth for the 21-day storage period of the study.

In summary, the antimicrobial combination intervention processes were effective in reducing microbial populations on pork trim. However, this study showed that although using a hot water wash in any of the combination

treatments was effective in reducing microbial contamination, it also negatively affected the color and emulsion stability of the ground pork, which are important quality attributes in products prepared with ground meat. Emulsion stability, a measure of how well the salt-soluble proteins can maintain the meat emulsion of dispersed fat particles in water during a heat process, is an important functional property of the ground meat used to make processed meats and sausages such as frankfurters or bologna (15). Decreases in emulsion stability result in increased loss of fat and water from the product during cooking, resulting in lower product yield and unacceptable texture and appearance. In addition, treating pork trim with any of the intervention treatments resulted in increased weight of the trim due to fluid retention. Therefore, using a hot air treatment as the final step in the combination treatments may provide a means to remove the excess fluid gained by the pork trim as a result of the spray wash treatments. More studies are needed to determine if the use of hot air as the only heat source in any of the combination processes affects the color and emulsion stability of the ground pork. Future experiments will examine additional combination intervention processes on commercial pork trim that do not incorporate hot water washes, and the effects on bacterial populations and quality attributes will be determined. In addition, the use of hot air treatment as the final step in combination intervention processes will be examined.

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