Journal of Food Protection, Vol. 69, No. 8, 2006, Pages 1808–1813 CORRECTED

Treatments Using Hot Water Instead of Lactic Acid Reduce Levels of Aerobic Bacteria and *Enterobacteriaceae* and Reduce the Prevalence of *Escherichia coli* O157:H7 on Preevisceration Beef Carcasses†

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MS 05-599: Received 30 November 2005/Accepted 28 March 2006/Corrected November 2006

ABSTRACT

Lactic acid has become the most commonly used organic acid for treatment of postevisceration beef carcasses. Many processors have also implemented 2% lactic acid washes on preevisceration carcasses. We previously demonstrated that hot water washing and steam vacuuming are effective carcass interventions. Because of the effectiveness of hot water, we compared its use with that of lactic acid as a preevisceration wash in a commercial setting. A commercial hot water carcass wash cabinet applying 74°C (165°F) water for 5.5 s reduced both aerobic plate counts and *Enterobacteriaceae* counts by 2.7 log CFU/100 cm^2 on preevisceration carcasses. A commercial lactic acid spray cabinet that applied 2% L-lactic acid at approximately 42°C (105 to 110°F) to preevisceration carcasses reduced aerobic plate counts by 1.6 log CFU/100 cm^2 and *Enterobacteriaceae* counts by 1.0 log CFU/100 cm^2. When the two cabinets were in use sequentially, i.e., hot water followed by lactic acid, aerobic plate counts were reduced by 2.2 log CFU/100 cm^2 and *Enterobacteriaceae* counts were reduced by 2.5 log CFU/100 cm^2. Hot water treatments reduced *Escherichia coli* O157:H7 prevalence by 81%, and lactic acid treatments reduced *E. coli* O157:H7 prevalence by 35%, but the two treatments in combination produced a 79% reduction in *E. coli* O157:H7, a result that was no better than that achieved with hot water alone. These results suggest that hot water would be more beneficial than lactic acid for decontamination of preevisceration beef carcasses.

Note: hot water pressure was previously reported as 700psi, but is now corrected and reads as ~20psi.

The pathogen *Escherichia coli* O157:H7 has been of concern to the meat processing industry for the last 20 years. In the early 1980s, cases of hemorrhagic colitis caused by *E. coli* O157:H7 were associated with consumption of undercooked ground beef *(25),* and a ground beef–related *E. coli* O157:H7 infection outbreak caused hundreds of illnesses and four deaths during 1992 and 1993 *(27).* In response to these events, the U.S. Department of Agriculture Food Safety and Inspection Service declared *E. coli* O157:H7 an adulterant in ground beef and required meat processors to establish hazard analysis critical control point (HACCP) plans *(21).* Since then, several interventions that focus on preventing carcass contamination and on decontaminating carcasses have been designed, tested, and put into use. These antimicrobial interventions, combined with strict hygiene practices, have significantly improved microbial quality of beef carcasses and reduced the incidence of *E. coli* O157:H7 in processing plants *(2, 3, 5, 20).*

The majority of *E. coli* O157:H7 organisms that contaminate beef carcasses during processing originate on the hides of cattle *(5, 8, 24).* Contamination is believed to occur during hide removal *(2, 5, 8, 24).* Processes that effectively clean the hides before removal have been effective interventions for lowering carcass microbial contamination, but they do not eliminate it completely *(8, 9, 24).* Therefore, the preevisceration carcass that has just had its hide removed also must be treated to reduce contaminating bacteria. In most commercial beef processing plants, the treatment used is an organic acid rinse. Numerous researchers have described the efficacy of a variety of organic acids for sanitizing whole carcass sides *(14, 16, 26),* and lactic acid has become the most commonly used organic acid in commercial practice. In modern beef processing plants, 2% lactic acid is applied to preevisceration carcasses via an online spray cabinet that warms the lactic acid to approximately 42°C (between 105 and 110°F).

We previously determined that hot water is a very effective carcass intervention *(17, 19)* and believe that it is the most effective intervention to reduce contamination of preevisceration carcasses. At our urging, some processors have adopted a hot water preevisceration carcass wash. However, not all processors are ready to commit to preevisceration hot water washes because of the lack of relevant published studies of its efficacy. Therefore, we compared hot water washes with lactic acid washes as preevisceration

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carcass treatments in a commercial beef processing plant. Because multiple hurdle interventions are expected to provide incremental increases in efficacy, we also evaluated the sequential application of hot water and then lactic acid.

MATERIALS AND METHODS

Experimental protocol. These experiments were carried out during 6 weeks in summer 2004. Four sample collection trips at 2-week intervals were made to a large midwestern beef processing plant. During each trip, 32 to 96 samples were collected to obtain a total of 256 samples for each of three treatments: hot water, lactic acid, and hot water plus lactic acid. The selected processing plant used both a preevisceration hot water wash cabinet (Chad Co., Olathe, Kans.) and a separate preevisceration lactic acid rinse cabinet (Chad Co.). The hot water wash cabinet was located before the lactic acid cabinet. The hot water wash cabinet was designed to deliver a 5.5-s wash at a processing speed of 380 head per h. The hot water wash was 74°C (165°F) and was applied with a nozzle pressure of ~20 lb/in^2 to preevisceration carcasses. The lactic acid cabinet sprayed 2% L-lactic acid at approximately 42°C (between 105 and 110°F) and at a pH of 2.4 ± 0.1 onto the surfaces of preevisceration carcasses.

Samples were collected directly from the processing line during normal production runs; therefore, all application parameters were fixed and could not be altered, with the exception of turning off either cabinet to determine the individual effects of lactic acid or hot water treatments. Before and during use, the concentration and pH of the lactic acid and the temperature and duration of the hot water treatment were verified. The verification of the hot water cabinet application parameters also included the measurement of carcass surface temperatures during treatment. A six-channel data logger (Datapaq, Wilmington, Mass.) with type-T thermocouples inserted 0.5 to 1 mm beneath the carcass fat surface was used to monitor the carcass surface temperature during hot water washing. Processing plant quality assurance personnel performed and provided results for all verification tests of lactic acid and hot water treatments and carcass surface temperatures.

Samples were collected from each subject carcass both before and after treatments. Samples to determine initial contamination levels were collected immediately after the hide was removed and before both the hot water and lactic acid treatments. A pretreatment sample was collected, and the carcass was tagged. A sample was then collected from the same carcass at a second point after the two treatments to measure their effects. The HACCP monitoring method proposed by Arthur et al. *(2)* was used to collect samples. Samples were collected from opposite sides of the same carcass pre- and posttreatment to prevent oversampling of the same areas and were collected alternately from leading and lagging sides of the carcass to avoid any bias in sample collection (e.g., carcass 1: leading side before treatment and lagging side after treatment; carcass 2: lagging side before treatment and leading side after treatment). Samples (8,000 cm^2) were collected from the midline brisket, foreshank, anus-hock, and top round surface areas of the carcass to avoid any disparity in the distribution of bacteria and pathogens on the carcass surface.

Sampling. All carcass samples were collected with sponges that were removed from Speci-Sponge Whirl-Pak bags (Nasco, Fort Atkinson, Wis.), wetted with buffered peptone water (Becton Dickinson, Sparks, Md.), and held separately from the Whirl-Pak bags, which were filled with 20 ml of a phosphate-buffered peptone solution (1% peptone containing 17 mM monobasic potassium phosphate and 72 mM dibasic potassium phosphate). This solution was used to neutralize any residual lactic acid absorbed during sampling of treated carcasses. Its use has been validated for recovering bacteria from samples with pH values as low as 2.0 while not interfering with cell counts or *E. coli* O157 recovery *(4)*. Each sample was collected using 10 bidirectional strokes of the sponge, and the sponge was turned over halfway through the process. After sample collection, each sponge was placed in a Whirl-Pak bag and massaged by hand to thoroughly mix the sample with the neutralizing buffered peptone water. All samples were collected and then placed on ice and transported to the laboratory to be processed.

Bacterial counts. Each sponge sample bag was thoroughly massaged by hand three or more times, and then a 2.5-ml aliquot was removed for bacterial counts. Aerobic plate counts (APC) and *Enterobacteriaceae* counts (EBC) were determined with a Bactometer (bioMérieux, Hazelwood, Mo.) and Petrifilm (3M Microbiology, St. Paul, Minn.). The APC and EBC of all pretreatment samples were performed by impedance measurements of 1-ml samples in a Bactometer. Each Bactometer sample consisted of 0.1 ml of sample taken from the aliquot that was removed from the sponge bag and 0.9 ml of the appropriate Bactometer medium. The 10-fold dilutions for Bactometer APC were prepared with General Purpose Medium-Plus (bioMérieux) supplemented with 18 g/liter dextrose (for a final concentration of 2% dextrose), and the 10-fold dilutions for Bactometer EBC were prepared with Entero Medium (bioMérieux). The Bactometer incubated samples for 16 h at 37°C to determine the initial detection time (IDT) for each sample. IDTs were converted to log CFU per milliliter using standard curves derived for each test. The standard curves were obtained by performing quadratic regression analysis of IDTs and log CFU per milliliter, which had been determined previously with Petrifilm aerobic count plates for APC or Petrifilm *Enterobacteriaceae* count plates for EBC as the standards. The reliable lower limit of detection using the Bactometer is approximately 10 to 100 CFU/ml; therefore, all samples from treated carcasses also were directly plated to Petrifilm to determine APC and EBC that fell within this low range. One milliliter from each treated sample was directly plated from the aliquot removed from the sample bag to Petrifilm aerobic count plates or Petrifilm *Enterobacteriaceae* count plates. The plates were incubated for 16 h at 37°C, and colonies were counted manually.

***E. coli* O157 detection.** The procedure for detection of *E. coli* O157 followed the removal of the aliquot for APC and EBC determination. *E. coli* O157 detection consisted of enrichment of the remaining sample (sponge and liquid) in tryptic soy broth, immunomagnetic separation, and plating as described previously *(6, 7),* with minor modifications for plating as follows. Bacterial cells bound to the immunomagnetic separation beads were plated onto Difco sorbitol MacConkey agar (Becton Dickinson) plates supplemented with 0.05 mg/liter cefixime and 2.5 mg/liter potassium tellurite (Dynal, Lake Success, N.Y.) and onto *E. coli* O157 chromogenic agar plates. Chromogenic media were either CHROMagar O157 agar (CHROMagar, Paris, France) supplemented with 5 mg/liter novobiocin (Sigma, St. Louis, Mo.) and 1 mg/liter potassium tellurite or Rainbow Agar (Biolog, Hayward, Calif.) supplemented with 20 mg/liter novobiocin (Sigma) and 0.8 mg/liter of potassium tellurite (Sigma). The use of different chromogenic media was based solely on availability from the manufacturers. All plates were incubated at 37°C for 16 h, and suspect colonies (sorbitol negative on supplemented sorbitol MacConkey agar, characteristic magenta on supplemented CHROMagar, or characteristic blue on supplemented Rainbow agar) were confirmed to be *E. coli* O157 using DrySpot O157 latex agglutination tests (Oxoid, Ogdensburg, N.Y.).

TABLE 1. *Effects of lactic acid wash, hot water wash, and combined treatment on the aerobic plate counts (APC) for preevisceration carcasses*[a]

	Mean APC (log CFU/100 cm^2)		
	Lactic acid[b]	Hot water[c]	Both[d]
Before treatment[e]	6.1	6.2	6.4
After treatment[f]	4.5	3.5	4.2
Reduction[g]	1.6 C	2.7 A	2.2 B
P value[h]	0.0001	0.0001	0.0001

[a] Samples were taken from 8,000-cm^2 areas of preevisceration carcasses; $n = 253$ for each treatment. Standard error = 0.1.
[b] Two percent L-lactic acid was applied at ~42°C (between 105 and 110°F).
[c] Hot water (74°C [165°F]) was applied for 5.5 s.
[d] Hot water treatment was applied first, and then lactic acid treatment was applied.
[e] Before-treatment APC were determined using Bactometer (bioMérieux) detection times that were calibrated to Petrifilm for valid comparisons.
[f] After-treatment APC were determined using Petrifilm (3M Microbiology).
[g] Reductions followed by the same letter are not different ($P > 0.05$).
[h] *P* value for differences between before-treatment and after-treatment effects.

TABLE 2. *Effects of lactic acid wash, hot water wash, and combined treatment on the* Enterobacteriaceae *counts (EBC) for preevisceration carcasses*[a]

	Mean EBC (log CFU/100 cm^2)		
	Lactic acid[b]	Hot water[c]	Both[d]
Before treatment[e]	4.0	4.4	4.7
After treatment[f]	3.0	1.7	2.2
Reduction[g]	1.0 B	2.7 A	2.5 A
P value[h]	0.0001	0.0001	0.0001

[a] Samples were taken from 8,000-cm^2 areas of preevisceration carcasses; $n = 255$ for hot water treatment and $n = 256$ for lactic acid and combined (both) treatments. Standard error = 0.01.
[b] Two percent L-lactic acid was applied at ~42°C (between 105 and 110°F).
[c] Hot water (74°C [165°F]) was applied for 5.5 s.
[d] Hot water treatment was applied first, and then lactic acid treatment was applied.
[e] Before-treatment EBC were determined using Bactometer (bioMérieux) detection times that were calibrated to Petrifilm for valid comparisons.
[f] After-treatment EBC were determined using Petrifilm (3M Microbiology).
[g] Reductions followed by the same letter are not different ($P > 0.05$).
[h] *P* value for differences between before-treatment and after-treatment effects.

Statistical analyses. Carcass APC and EBC were log transformed and then analyzed for variance with the GLM procedures of SAS (SAS Institute, Inc., Cary, N.C.). The model included the main effect of treatment. For significant main effects ($P < 0.05$), least squares means separation was accomplished with the PDIFF option (a pairwise *t* test). Pairwise comparisons of frequencies of *E. coli* O157 detection were made with PROC FREQ and the Mantel-Haenszel chi-square analysis (SAS).

RESULTS AND DISCUSSION

In this study, the efficacies of hot water, lactic acid, and hot water followed by lactic acid for decontaminating preevisceration beef carcasses were compared in a commercial processing plant. The results are unique because to the best of our knowledge this is the first comparison of these treatments using production equipment under typical beef processing conditions. Direct comparison of the results with those of previous studies was difficult, but best efforts were made to compare our results with those from studies in which similar or comparable treatments were used with regard to lactic acid concentration, application temperatures, and experimental conditions such as use of inoculums and types of beef surfaces.

The efficacy of each treatment was determined by measuring general indicators of carcass cleanliness (APC and EBC) and the prevalence of *E. coli* O157:H7. A large variety of cattle from different production lots was represented in each treatment group by taking multiple sampling trips to collect the 256 samples for each treatment. The initial APC and EBC, therefore, differed ($P < 0.05$) between the groups, as did the prevalence of *E. coli* O157:H7, but because the same carcass was sampled before and after treatment, the effects of each treatment could be accurately monitored and the bacterial reductions resulting from each treatment could be compared. Immediately after hide removal, pretreatment mean APC ranged from 6.1 to 6.4 log CFU/100 cm^2 (Table 1) and mean EBCs ranged from 4.0 to 4.7 log CFU/100 cm^2 (Table 2). The pretreatment means for *E. coli* O157:H7 prevalence ranged from 19 to 31%. Thus, treatments were compared using log reductions from pretreatment values for APC and EBC and using percent reduction from pretreatment prevalences for *E. coli* O157:H7.

The processing plant that participated in this study used a 2% lactic acid spray as a preevisceration carcass intervention. This lactic acid spray reduced ($P < 0.05$) carcass APC by 1.6 log CFU/100 cm^2 (Table 1) and EBC by 1.0 log CFU/100 cm^2 (Table 2). The prevalence of *E. coli* O157:H7 was reduced by 35% ($P = 0.01$) after lactic acid treatment (Table 3). Before treatment, 79 of 256 carcasses harbored *E. coli* O157:H7, and after treatment 50 of 256 still harbored *E. coli* O157:H7. Samples from all treated carcasses were collected near the exit of the lactic acid cabinet using a neutralization buffer; therefore, the effect of lactic acid may have been attenuated by the sampling methodology. To determine whether attenuation was a possibility, a second set of samples for APC was collected just before the next intervention, which in this case was steam vacuuming approximately 2.5 min further down the production line. The increased time of exposure to lactic acid up to this point had no effect on APC; APC between this point and the site of sampling at the exit of the lactic acid cabinet were not different ($P > 0.05$, data not shown). Therefore, we concluded that the lactic acid treatment

TABLE 3. *Effects of lactic acid wash, hot water wash, or combined treatment on the prevalence of* E. coli *O157:H7 on preevisceration carcasses*[a]

	% (no.) of carcasses positive for *E. coli*		
	Lactic acid[b]	Hot water[c]	Both[d]
Before treatment	31 (79)	27 (69)	19 (48)
After treatment	20 (50)	5 (14)	4 (9)
Reduction (%)[e]	35 B	81 A	79 A
P value[f]	0.01	0.001	0.001

[a] Carcass samples ($n = 256$ per treatment) were tested for *E. coli* O157:H7 by culture isolation.
[b] Two percent L-lactic acid was applied at ~42°C (between 105 and 110°F).
[c] Hot water (74°C [165°F]) was applied for 5.5 s.
[d] Hot water treatment was applied first, and then lactic acid treatment was applied.
[e] Reductions followed by the same letter are not different ($P > 0.05$).
[h] *P* value for differences between before-treatment and after-treatment effects.

would not have had a greater effect if given additional time on the surface of the carcass.

Lactic acid has been described as an effective intervention on cold or chilled beef carcasses *(13, 18, 22)* and on hot beef carcasses *(10, 12, 15),* even though these surfaces can be very different. In those studies, the concentration of lactic acid was either 2 or 4% and the application temperatures were approximately 32°C (90°F) or 55°C (131°F). The reductions in reported bacterial counts varied greatly depending on the inoculum used. For valid comparisons to be made, two different sets of data based on the inoculum used must be distinguished. In one set of studies, large reductions of greater than 4 log CFU (APC, *Salmonella* Typhimurium, *E. coli* O157:H7, and coliforms) were found when laboratory strains were used to inoculate beef surfaces *(10, 12, 13).* In the other set of studies, smaller reductions of 0.8 to 1.2 log CFU were found when natural contamination was evaluated *(15, 22).* The studies in the second set are more similar to our study because the inoculated surfaces received 6 to 7 log CFU/cm^2 (8 to 9 log CFU/100 cm^2), whereas naturally occurring contamination levels, as measured by APC and mesophilic bacteria counts, were reported to be 3.8 and 3.2 log CFU/cm^2, respectively (5.8 and 5.2 log CFU/100 cm^2). The reduction we observed following treatment with 2% lactic acid was similar to the previously reported reductions of 1.6 log CFU for naturally contaminated surfaces *(15, 22).*

The hot water wash used alone was superior to the lactic acid wash for reducing preevisceration carcass contamination. Water at 74°C applied for 5.5 s reduced both APC and EBC by 2.7 log CFU/100 cm^2. Compared with the lactic acid wash, the hot water wash reduced APC by an additional 1.1 log CFU and EBC by an additional 1.7 log CFU. In previous studies of hot water washes of postevisceration carcasses and chilled beef, similar reductions have been obtained: 2.1 to 2.9 log CFU for APC *(11, 17)* and 2.7 to 3.3 log CFU for coliforms *(10, 11),* a group that represents the majority of *Enterobacteriaceae* on carcass surfaces *(9).*

After the hot water wash, the prevalence of *E. coli* O157:H7 on preevisceration carcasses was reduced by 81%. Before treatment, 69 carcasses were positive for *E. coli* O157:H7, and after treatment only 14 remained positive. It would be interesting to have quantified the *E. coli* O157:H7 that remained on the carcasses after treatment; however, feasible methods for enumeration were not available at the time of this study. Water at 74°C previously reduced *E. coli* O157:H7 by 2.6 log CFU *(17),* and water at a higher temperature, 95°C (203°F), reduced *E. coli* O157:H7 by 3.7 log CFU *(12).*

During hot water treatment, the most important factor related to reducing the bacteria on the carcass is the temperature achieved at the carcass surface rather than the temperature of the water used. Various efficacies of hot water washes have been reported, and the differences can be attributed to the carcass surface temperature attained. For instance, in two similar studies of 95°C water washes, carcass surface temperatures of 70°C (158°F) and 82°C (180°F) were attained. The surface temperature of 70°C resulted in a 2.0-log reduction in APC and a 2.7-log reduction of coliforms *(19).* The surface temperature of 82°C resulted in a 4- to 4.9-log reduction of bacteria *(11).* The hot water wash cabinet used for preevisceration carcasses in our experiments applied 74°C water for 5.5 s. This treatment raised the carcass surface temperature to 70°C for 3.5 s and reduced both APC and EBC by 2.7 log CFU/100 cm^2. Previous reports and our data demonstrate that hot water is a very effective intervention for reducing surface contamination on preevisceration beef carcasses.

Multiple hurdle interventions have been described to provide incremental increases in efficacy *(2, 3).* Therefore the combination of a hot water wash followed by a lactic acid rinse was expected to have this effect. However, the application of both interventions was no more effective than application of hot water alone for reducing EBC and *E. coli* O157:H7 prevalence ($P > 0.05$). The application of both interventions was slightly less effective than application of hot water alone for reducing APC ($P < 0.05$). The combined treatments reduced APC 2.2 log CFU, whereas hot water alone reduced APC an additional 0.5 log CFU. This unexpected effect on APC likely occurred by chance; it was not observed for EBC or *E. coli* O157:H7 results. The lack of greater reductions in bacteria for the combined lactic acid plus hot water treatment compared with treatment with hot water alone may have been due to the cooling effect of the lactic acid. The lactic acid sprayed on the carcasses after the hot water wash was significantly cooler than the carcass surface and thus may have hastened the cooling of the carcass surface, thereby reducing the effect of hot water by an amount equivalent to that provided by the lactic acid. In previous studies of organic acids, factors such as the temperature of the acid solution had a profound effect on the magnitude of the observed bacterial reductions *(1, 23).* The temperature of the lactic acid spray in this experiment was approximately 42°C. The surface temperatures of the carcasses it was applied to were either ap-

proximately 30°C (86°F) when no hot water treatment was used or approximately 63°C (145°F) when hot water treatment was used. These temperature variables also may have contributed to the unexpected results of the combined treatments.

Most of the previous studies of hot water and lactic acid treatments of beef were performed in a laboratory setting or under laboratory conditions using model carcass washers or simulated sprays. The effects of hot water and/or lactic acid were usually determined for artificial inoculums of feces and pathogens placed on the carcass surface or carcass surface tissues. Our studies were performed in the production environment with current commercial equipment, and we measured the effectiveness of hot water and lactic acid for reducing carcass contamination that occurs during normal processing. One earlier study was performed in a processing plant environment, but only 30 samples were evaluated for each treatment *(15).* We sampled 768 carcasses at the processing plant, 256 for each treatment, so statistical evaluations could be made. Preevisceration carcass contamination was reduced to a greater extent ($P < 0.05$) by the use of a hot water wash than by the use of a lactic acid spray and was no better than the use of the two treatments sequentially. It was not determined whether the greater efficacy of hot water compared with lactic acid carried over to the final carcass in the sales cooler, but it would be logical to assume that the greater efficacy of hot water early in processing would be beneficial to the microbiological quality of the final product.

ACKNOWLEDGMENTS

We are grateful to the participating processing plant and its personnel for cooperation in these experiments. We thank Bruce Jasch, Kim Kucera, Ann Goding, and Gregory Smith for their technical support and Carol Grummert for her secretarial skills.

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