Microbiological Quality of Beef Carcasses and Vacuum-Packaged Subprimals: Process Intervention during Slaughter and Fabrication

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ABSTRACT

Beef carcass sides (n = 9 per replicate) were sprayed with water (W), 200 ppm chlorine (C), or 3% (vol/vol) lactic acid (L) immediately after rail inspection and at the end of an 8-h spray-chill cycle, resulting in a total of nine different spray combinations. All treatment combinations involving chlorine and/or lactic acid reduced carcass contamination. The reductions in mean log10 CFU/cm2 for carcass aerobic plate count (APC) data ranged from 0.4 to 1.8. The treatment combination using lactic acid at both spray times (L+L) resulted in the greatest reduction. Additionally, treatment combinations involving lactic acid at either time and in combination with water or chlorine tended to reduce APCs more than those treatment combinations without acid. Browning of blood splashes was observed on carcasses sprayed with lactic acid and persisted until fabrication at 72 h postmortem. A companion study was designed, in conjunction with the carcass decontamination study, to evaluate effect of carcass treatment on the microbiological quality of subprimal subdivisions derived from treated carcasses. A facet of the subprimal study evaluated chlorine spray (200 ppm) and microwave radiation as approaches to improving subprimal shelf life and safety. Cuts taken from sprayed carcasses were vacuum packaged with or without intervention treatments, stored at 1 to 2°C and evaluated for both APC and pathogen populations at specified intervals of up to 120 days. These results demonstrated that neither carcass nor intervention treatment had any significant (P > 0.05), beneficial effect on the microbiological quality of subprimal cuts.

Key words: Acid, meat, beef, bacteria, safety, chlorine

Multiple opportunities exist for microbial contamination of fresh meat during slaughter and subsequent fabrication. Good sanitary practices during slaughter and fabrication have been effective in reducing bacterial contamination (1, 4, 5, 19). However, optimum reduction of bacterial contamination has not been achieved. Therefore, in addition to good sanitary practices, chlorine and more recently organic acids, lactic and acetic acid in particular, have been evaluated in an effort to improve the microbiological safety and shelf life of fresh meat. Organic acids are of particular interest because they are generally recognized as safe. The bactericidal properties of lactic acid are well documented (2, 12, 17, 18, 20, 21, 24). Woolthuis and Smulders (24) reported that a concentration of 1.25% lactic acid resulted in aerobic plate count (APC) reductions of 0.8 and 1.3 log10 CFU/cm2 on brisket and inside round regions, respectively. Smulders and Woolthuis (20) indicated that treatment of calf carcasses with 1.25% lactic acid reduced the APC by 0.8 log10 CFU/cm2 from initial counts of approximately 3.0 log10 CFU/cm2. This log10 reduction increased to 1.3 at 14 days postmortem, indicative of some delayed effects of lactic acid. Spray chlorination also has been evaluated as a method of controlling contamination in beef. Chlorinated water has been used successfully in the poultry industry for years (25). Emswiler et al. (6) reported that the reductions in log counts on beef forequarters washed with 100, 200, and 400 ppm chlorine solutions were 1.45, 1.64, and 1.84, respectively. However, chlorine normally is inactivated rather rapidly in an organic system such as meat.

To ensure that the U.S. beef industry will be competitive in the Asian beef export trade, it is necessary that the product have at least a 90-day shelf life so that purveyors and retailers have sufficient time to distribute and market the product. Because of this required shelf life, the potential utility of various antimicrobial agents at strategic points in animal processing and carcass fabrication warrants additional investigation. The objectives of this study were (i) to determine if chlorine, lactic acid, and water when used alone or in different combinations had any added advantages in reducing bacterial contamination of beef carcasses, (ii) to evaluate whether carcass treatments had residual effects on subprimals derived from treated carcasses when stored for up to 120 days at 4°C, and (iii) to determine if intervention treatment of subprimals with chlorine or microwave radiation had any additional decontamination effect. This study combines slaughter, fabrication, and vacuum storage conditions in a complete picture as opposed to evaluating each segment separately.
MATERIALS AND METHODS

Carcass treatments

Fifteen steers (USDA Choice, Yield Grade 1 to 3) were slaughtered in three replicate groups of five steers each at the Kansas State University abattoir. For each replicate, five carcasses were split into right and left sides, producing ten sides. Nine of these sides were assigned randomly to three groups of three carcass sides each. Each group of three sides was manually sprayed with either deionized/distilled water (W), 200 ppm chlorine (C), or 3\% (vol/vol) lactic acid solution (L) immediately after rail inspection and immediately before entering the holding cooler (2°C). The spray was applied (ca. 1.3 liters per side) with a 7.5-liter, hand-held manual sprayer (Model 523 795, H. D. Hudson Mfg. Co.; Chicago, IL). The pressure was not measured; however, the volume of spray delivered was evaluated under different conditions of sprayer fill, strokes to pressurize the tank, and time to ensure that a standard volume of spray was delivered. While in the holding cooler, the nine sides were subjected to an 8-h, automated spray-chill protocol using potable water applied with a built-in system (Spray chiller, Model SN-11-03, Chester-Jensen Co., Cattaraugus, NY). Spray cycles consisted of a 30 s spray every 15 min. Immediately after the chill cycle, each carcass side was similarly sprayed again with either W, C, or L, resulting in a total of nine different spray combinations: W+W, W+C, W+L, C+W, C+C, C+L, L+W, L+C, and L+L. The first letter in each combination indicates the spray received after rail inspection, and the second letter indicates the spray received after the 8-h spray-chill cycle. Approximately 1.3 liters of spray solution (21°C) were sprayed per carcass side. The 3\% (vol/vol) lactic acid solution was prepared from an 85\% lactic acid solution (Fisher Scientific, St. Louis, MO) and mixed with deionized, distilled water for a final pH of 2.1. A 200 ppm chlorine solution was prepared by diluting chlorine water (Fisher Scientific) with deionized/distilled water for a final pH of 2.6. Neither the water nor the stock solutions were sterilized prior to making each solution.

To determine the effect of treatments on carcass microbial load, carcass surfaces were sampled for aerobic bacteriological populations just before the first set of spray treatments (following rail inspection) and after the second set of spray treatments that followed the 8-h spray chill cycle. The hindshank and the split surfaces of the sternum and neck were sampled on each of the nine sides. Each area was scored with a 3.81-cm-diameter core to a depth of ca. 2 mm and aseptically removed with a sterile scalpel. Two samples were obtained from each carcass location and combined, representing a total of 11.4 cm² sampled. Before sampling, any discoloration associated with the various treatments was noted visually by an experienced evaluator as a “brown” or “rust-colored” appearance.

Subprimal intervention treatments

All carcass sides immediately after microbiological sampling and following the second set of manual spray treatments were moved to a holding cooler (3°C) until fabrication. At approximately 72 h postmortem, each side from the carcass decontamination portion of these experiments was fabricated into six subprimals: clod, rib, striploin, top butt, inside round, and outside round. Separate cutting surfaces, clean knives, and clean gloves and aprons were used for each side. Because six subprimarial treatments were applied, it was necessary to use different subprimals from the same treated side. Although they were taken from different locations, the confounding of subprimal treatment with carcass location was alleviated by randomly assigning the subprimals to each of the six intervention treatments (Table 1). Each subprimal was then divided into eight subdivisions that were necessary to accommodate sampling at 4, 10, 15, 20, 30, 60, 90, and 120 days of vacuum storage. Each of the eight subprimal subdivisions received the treatment that had been assigned to that subprimal (Table 1). Subprimal cuts were randomly assigned to the following treatments: (1) vacuum packaged (VP); (2) manually sprayed with 200 ppm chlorine (21°C) (C) and then vacuum packaged (C+VP); (3) vacuum packaged and microwaved for 15 s per side of subprimal cut (VP+MW); (4) inoculated with 1 ml of approximately 10⁶ CFU/ml of an inoculum mixture containing four pathogens (P) and then vacuum packaged (P+VP); (5) (P+C+VP); and (6) (P+VP+MW). For treatment groups (2) and (5), each side of each subprimal cut was sprayed until the 200 ppm chlorine solution saturated the cut surface (ca. 5 s). To accomplish this, the subprimal was placed on a sterile rack supported by a plastic pan to collect residual spray solution. This residual solution was autoclaved and subsequently discarded. Chlorine spray was applied five minutes after inoculation with bacterial cultures for treatment (5).

Cultures of four bacterial pathogens (Escherichia coli O157:H7, Listeria monocytogenes, Salmonella enteritidis, and Yersinia enterocolitica) were obtained from the Kansas State University food microbiology culture collection. A 16- to 18-h-broth culture of each pathogen was diluted in 0.1% peptone diluent (Difco Laboratories, Detroit, MI). Approximately equal populations (1 x 10⁷ CFU/ml) of each strain were used to prepare the four-pathogen inoculum. One milliliter of the inoculum was spread on each side of the subprimal cuts using a sterile bent glass rod. Approximately 5 min elapsed before subsequent treatment with either chlorine or microwave radiation. Both surfaces of the cut were treated similarly and for cuts requiring chlorine spray, the cuts were drained for 2 min before transfer to a Cryovac® B-620 bag (Cryovac Division, W.R. Grace Co., Duncan, SC) with sterile tongs. The bag specifications were an oxygen transmission rate of 4 cm²/100 in²/day at 22°C and 45% RH and a water-vapor transmission rate of 0.6 g/100 in²/day at 37.7°C and 90% RH. A Multivac Model A 300/16 vacuum packaging machine was used to package the individual cuts; it pulled an average vacuum of 599 ± 62 mm of Hg.

For microwave treatment, vacuum-packaged subprimal subdivisions were placed in a Kenmore microwave oven (Stock no. 88041) on the “high” setting (output wattage, 112.9 watts/h) for 15 s per side. The temperature was recorded on the edges and at the center for each cut upon removal from the oven using a surface temperature probe (Pocket-Probe Model MPSP6, Electronic Development Laboratories, Inc. Plainview, NY). Because sample geometry differed as a function of the subprimal source (Table 1) and because sample geometry affects heating rate in microwave ovens, temperature ranged from 5 to 80°C for sample edges and from 5 to 50°C for sample centers. Core samples for microbial analyses were always removed from the center of each subprimal subdivision to avoid the cooked edges in some subdivisions. Sample temperature was not allowed to equilibrate with room temperature before storage.

Following treatments, all vacuum-packaged, subprimal subdivisions were placed in a Kenmore microwave oven (Stock no. 88041) on the “high” setting (output wattage, 112.9 watts/h) for 15 s per side. The temperature was recorded on the edges and at the center for each cut upon removal from the oven using a surface temperature probe (Pocket-Probe Model MPSP6, Electronic Development Laboratories, Inc. Plainview, NY). Because sample geometry differed as a function of the subprimal source (Table 1) and because sample geometry affects heating rate in microwave ovens, temperature ranged from 5 to 80°C for sample edges and from 5 to 50°C for sample centers. Core samples for microbial analyses were always removed from the center of each subprimal subdivision to avoid the cooked edges in some subdivisions. Sample temperature was not allowed to equilibrate with room temperature before storage.

Following treatments, all vacuum-packaged, subprimal subdivisions were segregated by carcass treatment and stored in cardboard boxes at 1 to 2°C until evaluated for both APC and pathogen populations at 4, 10, 15, 20, 30, 60, 90, and 120 days. Before sampling, each subprimal subdivision was visually evaluated by trained personnel for any discoloration and the surface pH was measured with a pH5 pH meter (Beckman Instruments, Inc., Reston, VA) equipped with a flat-surface combination electrode (Fisher Scientific, St. Louis, MO). For microbial analyses, two 3.8-cm diameter cores were aseptically removed from each subprimal subdivision. These cores represented 45.6 cm² of area sampled since each core would contain two sampled areas of 11.4 cm².
TABLE I. Treatment assignment for subprimals obtained from each carcass treatment for each replicate.

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- CT, carcass treatment; 1, water*water; 2, chlorine*water; 3, lactic acid*water; 4, water*chlorine; 5, chlorine*chlorine; 6, lactic acid*chlorine; 7, water*lactic acid; 8, chlorine*lactic acid; 9, lactic acid*lactic acid.
- C, clo; R, rib; S, strip; TB, top but; IR, inside round; OR, outside round.
- 1, vacuum packaged; 2, vacuum packaged after chlorine spray; 3, vacuum packaged and microwaved; 4, added pathogen, vacuum packaged; 5, added pathogen, chlorine spray, vacuum packaged; 6, added pathogen, vacuum packaged, and microwaved.

Microbiological analyses for carcass and subprimal intervention treatments

Core samples from carcass sides or vacuum-packaged subprimal cuts were placed in stomacher bags containing 25 ml of sterile Butterfield's phosphate buffer (pH 7.2) and massaged for 30 s using a Stomacher-400 (Tekmar Co., Cincinnati, OH). Appropriate tenfold dilutions were prepared in Butterfield's phosphate buffer and APCs were determined using pour or spiral plating (Spiral Plater Model DU-2, Spiral Systems, Inc., Cincinnati, OH) on plate-count agar (Difco). Plates were incubated at 37°C for 48 h before enumeration. After incubation of spiral plates, bacterial populations on PCA were enumerated using a Spiral Counter (Model 500A, Spiral Biotech, Bethesda, MD). Pour plates were enumerated with a Quebec Colony Counter (Model 3325, AO Scientific Instruments, Keene, NH). Bacterial populations were reported as log10 CFU/cm² for each sample.

After the homogenate was removed from each stomacher bag for APC analyses, 1 ml of the sample homogenate was serially diluted in Butterfield's phosphate buffer and pathogens were enumerated on selective media using a pour-plate method (23). Selective plating media used for the presumptive enumeration of \textit{E. coli} O157:H7, \textit{L. monocytogenes}, \textit{S. enteritidis}, and \textit{Y. enterocolitica} were McConkey sorbitol agar (MSA), modified oxford medium (MOX), xylose lysine deoxycholate (XLD) agar, and cefsulodin irgasan novobiocin (CNN), respectively. Plates were incubated at 37°C for 48 h before enumeration.

Statistical analyses

Data from enumeration of bacterial populations (log10 CFU/cm²) from intact carcasses were analyzed as a randomized complete-block design with carcass treatment as the whole-plot main effect and sampling location as the subplot main effect. To evaluate the residual effect of carcass treatment and the effect of subprimal treatment and length of vacuum storage, a randomized complete-block design was employed with carcass treatment as the whole-plot main effect and intervention treatment (subprimal cut treatment) as the subplot effect. Vacuum storage period as the sub-subplot effect. The latter design was adopted for analyses of APCs and pathogen data. The least-square procedures of SAS (14) were used to separate means in both analyses.

RESULTS AND DISCUSSION

Spray treatment effects on carcasses

A comparison of mean APCs (log10 CFU/cm²) for each carcass location before spray application showed that the hindshank had the highest (P < 0.05) and the neck had the lowest (P < 0.05) counts (Fig. 1). The higher counts for hindshanks were apparently due to greater fecal contamination from the hide at this location. Before treatment, the neck and sternum had 2.50 and 3.22 log10 CFU/cm², respectively, and these means were different (P < 0.05). Perhaps the sternum was contaminated when split during carcass evisceration. It is plausible that the saw would spread organisms from the carcass surface to the cut, split surface of the sternum. Following treatment, the neck and sternum had 2.07 and 2.25 log10 CFU/cm², respectively, and these means were not different (P > 0.05; Fig. 1). A greater reduction in APCs occurred for the sternum than for the neck, suggesting that the level of contamination prior to treatment influences the degree of decontamination following treatment when each sampling site is compared. This information emphasizes that strict sanitary practices should be exercised at all steps in the hide removal process.

Figure 1. Influence of carcass location on mean APC (log10 CFU/cm²) before and after spray treatments with water, chlorine, and lactic acid, either alone or in combination (*P < 0.05; LS mean standard error, SE, before any treatment, 0.10; and LS mean SE after decontaminant spray following tail inspection and spray chilling, 0.16). Means within before- and after-treatment groups with the same letter are not different.
The mean APCs of carcasses after spray treatment showed that all treatment combinations involving chlorine and/or lactic acid reduced carcass contamination. The reduction in mean log_{10} CFU/cm^2 ranged from 0.4 to 1.8 (Fig. 2). The L+L treatment combination resulted in a greater reduction (P < 0.05) as compared to W+W and C+C. Additionally, treatment combinations that included lactic acid as a component tended to have greater decontaminating effects than those without acid, suggesting that lactic acid was the agent with the greater propensity for reducing contamination of fresh meat in this study. The bacteriocidal effect of lactic acid has been attributed to a direct inhibitory effect of H+ ions (18) and an attempt by the organism to maintain homeostasis through maintenance of normal pH, thereby depleting energy necessary for cell proliferation (8).

The bactericidal effects of chlorine and organic acids have been studied and are well documented in a review by Dickson and Anderson (4). The results from our experiment indicated that a synergistic effect between chlorine and lactic acid did not exist. Application of lactic acid solution alone seemed more bactericidal than its combination with chlorine or water. Perhaps the rapid inactivation of chlorine in an organic system such as meat might have affected the efficacy of the chlorine wash (6). Before any treatment, carcasses assigned to the W+W treatment had lower initial counts (Fig. 2). The increase in APCs for these carcasses after treatment indicates that water had no bacteriostatic effect, allowing the growth of indigenous organisms. Relative to color changes, the primary discoloration was associated with L+L as browning of blood splashes on carcass surfaces that persisted until fabrication. No objectionable color was observed for water and chlorine treatments.

Effects of carcass and subprimal intervention treatments on subprimal cuts

An additional facet of this study evaluated the effectiveness of carcass and subsequent subprimal intervention treatments on the microbiological quality of subprimal cuts. Because there was no carcass treatment by subprimal treatment (P > 0.05) interaction, the results of these main effects are presented separately in Fig. 3 and 4, respectively. The effect of carcass treatments on the total bacterial populations (Fig. 2) did not persist to the subprimal level when subprimal cuts from treated carcasses were sampled at specific intervals for up to 120 days of vacuum-packaged storage (P > 0.05), demonstrating that carcass sprays had no residual effect at the subprimal level. Similar results were reported by Prasai et al. (13) on the effect of lactic acid decontamination of beef carcasses on the microbiological characteristics of strip loins. In this study, by 60 days, all subprimal cuts had counts that ranged from 6.5 to 7.0 log_{10} CFU/cm^2 (Fig. 3). This observation indicated that although the importance of presenting a "clean" carcass for fabrication should not be underestimated, multiple opportunities that exist during fabrication and handling for recontamination determine the microbiological quality of meat during extended storage.

The APC data for subprimal cut intervention showed no treatment effects (P > 0.05) on microbial reduction during extended storage (Fig. 4). We had hypothesized, when initiating this study, that an intervention treatment of subprimal cuts with either chlorine or microwave radiation would improve their shelf life and limit pathogen growth.

![Figure 2. Effect of water (W), chlorine (C), and lactic acid (L), either alone or in combination, on mean APC (log_{10} CFU/cm^2) from carcasses (P < 0.05; LS mean standard error, SE, before any treatment. 0.19 and LS mean SE after decontaminant spray following rail inspection and spray chilling, 0.26). Means within before and after treatment groups with the same letter are not different.](http://meridian.allenpress.com/jfp/article-pdf/58/6/633/1665628/0362-028x-58_6_633.pdf)

![Figure 3. Aerobic plate counts of subprimal cuts as affected by length of storage and carcass treatment (W, water; C, chlorine; and L, lactic acid). All treatment comparisons were not different (P > 0.05).](http://meridian.allenpress.com/jfp/article-pdf/58/6/633/1665628/0362-028x-58_6_633.pdf)
inoculation, microwave-treated cuts sampled at the initial stages of storage tended to have numerically higher counts than nonmicrowaved samples (Fig. 4). Microwave treatment of inoculated cuts elevated the temperature of these cuts and perhaps this temperature rise in combination with the insulating properties of the cardbox during storage minimized the inhibitory effect of refrigeration temperatures. By day 60, however, the range was 6.5 to 7.1 log$_{10}$ CFU/cm$^2$, which is a narrow range, considering the total microbial load at day 60. Although the initial contamination level is important, the number and type of bacteria that can flourish under vacuum packaging may be more important in determining the final bacterial population associated with prolonged storage. Additionally, the rapid inactivation of chlorine in an organic system (6) and the extensive variation of temperature observed during microwaving may explain the ineffectiveness of treatments applied in this study.

In addition to controlling growth of spoilage organisms, controlling pathogen growth is an important facet of this study because of human health implications. In this study, S. enteritidis did not grow during vacuum storage. Listeria (L. monocytogenes) counts of 0.5 log at day 4 (Fig. 5) declined initially to 0.3 log CFU/cm$^2$ by day 15 followed by slow growth. In most instances, Listeria counts in samples treated with microwaves (P+VP+MW) were numerically higher throughout the storage period compared to other treatments (i.e., P+VP or P+C+VP). L. monocytogenes counts in microwaved treated samples (Fig. 5) ranged from 0.3 log$_{10}$ CFU/cm$^2$ at day 15 of vacuum storage to 0.9 log$_{10}$ CFU/cm$^2$ by day 60, and between days 30 and 90, the counts were different (P < 0.05) from P+VP or P+C+VP treatments. L. monocytogenes growth in P+VP and P+C+VP was slow and were not different (P > 0.05) between these treatments. In all cases, L. monocytogenes counts declined from day 60 and reached 0.1 to 0.4 log$_{10}$/cm$^2$ by day 120. This decline in L. monocytogenes may be associated with the growth of lactic acid bacteria and accumulation of their metabolites.

Even if lactic acid bacteria were not enumerated in this study, it has been well documented that lactic acid bacteria are the predominant microflora in vacuum-packaged beef (15, 16, 22). Lactic acid bacteria are also reported to produce a diverse group of bacteriocins that are responsible for antagonism of other gram-positive bacteria (10). Several strains of lactic acid bacteria are reported to be antagonistic to L. monocytogenes (9, 11).

Figure 5. Listeria counts of subprimal cuts as affected by length of storage and subprimal treatment (VP, vacuum packaged; C, 200 ppm chlorine spray; MW, microwaved; and P, pathogen added).

Although treatment of carcasses with lactic acid was effective in reducing bacterial contamination on carcasses, neither the carcass treatments nor the intervention treatments had any significant (P > 0.05) effect on the microbiological quality of subprimal cuts. Even though we used separate cutting surfaces, washed knives, and changed gloves and aprons between individual sides, fabrication procedures still appear to be critical factors in microbial contamination and may mask any significant continuing effect of carcass decontamination. In addition, the initial load of microorganisms may determine the shelf life and safety of refrigerated, vacuum-packaged, fresh meat. In
addition to intensive sanitary fabrication procedures, a
strict storage environment, especially storage temperature,
might be critical to minimize microbial contamination/
growth in fresh meat.

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