Microbiological Effects of Acid Decontamination of Beef Carcasses at Various Locations in Processing


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ABSTRACT

Hot (55°C), dilute (1% v/v) lactic acid was sprayed on beef carcass surfaces immediately after dehiding, after evisceration (immediately before chilling), or at both locations. Surface samples of carcasses were examined for total aerobic plate counts (APCs) and for the presence of Salmonella and Listeria. APCs of treated beef carcasses were lower (P<0.05) than those of control carcasses. APCs were determined both at slaughter day 0 (immediately after carcasses enter the chill room) and after 72 h postmortem. At day 0, reductions in log_{10} APC by more than 90% occurred when carcasses were treated with lactic acid after evisceration or both after dehiding and after evisceration. The effect of lactic acid decontamination was greatest on carcasses treated with lactic acid both after dehiding and after evisceration. No further reductions in APCs of carcasses were observed on samples taken 72 h postmortem. No difference in color between control and acid-treated carcasses was observed. All samples tested for the presence of Salmonella were negative. Listeria was detected in three samples from control carcasses only. Samples obtained from strip loins of acid-treated or control carcasses did not show any consistent pattern of differences in microbiological counts.

Additional data collected from carcasses sprayed with lactic acid in three different sized slaughter plants showed that irrespective of differences in size of slaughter facility, mean APCs of acid-treated carcasses were significantly (P<0.05) lower than those of control carcasses.

The shelf life of fresh meat is primarily determined by the number and types of microorganisms and the temperature of storage. The hide and gastrointestinal tract are major sources of microbial contamination on dressed carcasses. Proper slaughtering and dressing practices are critical factors in controlling the degree of initial contamination of fresh meat. In practice, however, even with the best possible slaughter and dressing practices, carcasses still contain microbial loads that will affect the shelf life of products produced from these carcasses. Several additional methods have been investigated to reduce initial surface bacterial contamination on carcasses (2,3,5,8,17,18,21). These include the use of cold or hot water washes or sprays (2,20,23), application of chlorinated water (2,11,14) and surface application of organic acids such as acetic, citric, lactic, propionic, ascorbic and formic (5,13,17,18,21,24-26). Among these, acetic and lactic acid have received increasing attention for decontamination purposes.

The bactericidal properties of lactic acid are well documented (7,17,24-26,28). According to Snijders et al. (26), lactic acid exerts both an immediate (bactericidal) and a delayed (bacteriostatic) effect that results in extended shelf life of meat. The immediate bactericidal effect of lactic acid decontamination on beef, veal and pork carcasses reduced the aerobic plate count (APC) by 1.5 log_{10} per cm². Spraying the surface of calf carcasses with 1.25% (v/v) lactic acid, Smulders and Woolthuis (25) reported the reduction in log_{10} APC (per cm²) by 0.8. Initial log_{10} APC (per cm²) of control carcasses were 3.0. This reduction in APC (log_{10} per cm²) increased to 1.3 at 14 d postmortem, indicating some delayed effects of lactic acid. In further studies these authors (25) investigated the microbiological condition of hot-boned cuts sprayed with 2% lactic acid followed by vacuum packaging. At 7 and 14 d postmortem, acid treatment plus vacuum packaging was significantly more effective at reducing bacterial counts than vacuum packaging alone.

Time and temperature of application also influences the efficacy of lactic acid. Spraying hot carcasses at 45 min postmortem (before chilling) with 1% (v/v) lactic acid was reported by Snijders et al. (26) to result in greater bacterial reduction than spraying chilled carcasses. Other authors (12,19) have reported that organic acids, such as acetic or sorbic acid, exert stronger microbicidal effects at 35°C than at temperatures of chilling and that acid treatment at elevated temperatures (53-55°C) is even more effective in reducing bacterial numbers (4,6). Acid decontamination of beef subprimals or top loin steaks was reported to have little or no effect on shelf life (1,9). These results and those of Hamby et al. (13), indicate that acid decontamination of fresh meat is probably more effective when employed as soon after slaughter as possible. Since some bacteria are present in a surface water-film on a hot carcass (in the early postmortem period) before becoming attached to the tissue or fat surface during chilling, early acid treatment may allow for easier removal (26).

The purpose of the present study was to evaluate the microbicidal effect of hot lactic acid sprayed on beef carcasses before chilling and to determine the most effective location for acid decontamination in slaughtering/dressing procedures. Microbiological condition and sensory characteristics of vacuum-packaged loins fabricated from acid-treated carcasses were also investigated.
Acid decontamination

Forty steers (approximately 450-kg each), were slaughtered two weeks apart in two replicate groups of 20 at a small packing plant in south Texas. The 20 steers in each group were assigned randomly to four treatment groups (5 steers per group) before slaughtering and then were treated as follows: 1% (v/v) lactic acid solution (55°C, pH 2.8) was sprayed (500 ml/35 s) in a fine mist from a distance of approximately 80 cm and at a pressure of 40 psi on carcasses (i) immediately after dehiding (before evisceration), (ii) after evisceration (immediately before chilling), or (iii) both after dehiding and after evisceration. A control group (iv) was not sprayed with acid. The acid solution was prepared from 88% L-lactic acid (Purac Inc., Arlington Heights, IL) mixed with sterile distilled water to 1% (v/v). The selection of acid concentration was based on the ranges of acid concentration frequently cited in the literature (18,24,28). For carcasses sprayed after dehiding (before evisceration), a total volume of 500 ml of lactic acid was sprayed over the entire surface of each carcass. After evisceration, each carcass was split lengthwise into two halves and showered with cold water to remove blood and bone dust. Carcass halves treated after evisceration were sprayed with a total of 500 ml of lactic acid for every carcass half (250 ml on each of the inside and outside surfaces of each carcass half) after the post-evisceration cold water shower.

Following treatment, all carcasses were moved immediately into the carcass chilling area (1 ± 0.5°C). Samples for aerobic plate count (APC), Salmonella and Listeria were obtained from both halves of the treated and untreated carcasses immediately upon entering the cooler. APCs also were determined after 72 h of cold storage. Samples were placed in sterile stomacher bags and transported to the Texas A&M University food microbiology laboratory under refrigeration (< 3 h).

A total of 40 strip loin short-cuts (institutional Meat Purchase Specifications #180) (16) was fabricated from both halves of the chilled treated and control carcasses in each replicate slaughter group. Each strip loin was placed in a separate bag (Cryovac® BH-620) with an oxygen transmission rate of 15-30 cc/m²/24 h at 22.8°C and a moisture vapor transmission rate of 6.27.75 g/m²/24 h at 37.8°C and 100% RH, and then vacuum packaged using a vacuum-packaging machine (Multivac AGH, 8941 Wolfertschwenden, Germany). All vacuum-packaged strip loins were delivered by refrigerated truck to the Rosenthal Meat Science and Technology Center at Texas A&M University where they were stored at 3 ± 1°C and examined at 3 and 14 d of storage after fabrication. Twenty vacuum-packaged strip loins (5 loins from each treatment) were selected randomly and sampled for APC (three 10-cm² x 2-mm combined samples from each strip loin) on each scheduled day of examination.

Microbiological analyses

Microbiological samples for aerobic plate counts (APCs) were obtained by excising 10-cm² surface areas (2 mm thick) with a sterile scalpel from five different locations (chuck, shank, rib, loin, and round) on each half of the treated and control carcasses immediately after entering the cooler (0 h) and at 72 h postmortem. The five 10-cm² surface areas obtained from each half of a carcass were combined for APC. For strip loins, three 10-cm² surface areas (2 mm thick) were obtained from each strip loin and combined.

Samples were placed in sterile bags containing 100 ml of sterile 0.1% peptone diluent (Difco) and pummelled for 1 min in a Stomacher-400 (Tekmar® Company, Cincinnati, OH). APCs were determined by placing 1 ml (divided over four plates) of the sample homogenate and then 0.1 ml of the sample homogenate and appropriate tenfold dilutions of the same on preploured and dried tryptic soy agar (Difco, Detroit, MI) plates, followed by evenly spreading the samples on the surface of the plates with a sterile, bent, glass rod. Plates were incubated for 3 d at 25°C before counting and reporting the APC per cm² for each sample.

The presence of Salmonella was tested using the 3M® Report™ Salmonella Visual Immunoassay Test. Samples were obtained from each carcass half by excising a composite 25-cm² surface area with a sterile scalpel and forceps from three locations (chuck, loin, and round) on the surface of carcasses immediately after entering the cooler. Each 25-cm² sample was placed in a sterile stomacher bag containing 225 ml of lactose broth (J.T. Baker, Inc., Phillipsburg, NJ) and pummelled for 1 min in a Stomacher-400 (Tekmar® Company). This preenrichment mixture was transferred into a sterile screw cap container and incubated at room temperature for 1 h. The samples, with the cap loosened, were then incubated at 37°C for 18-22 h. After incubation, 1 ml of each sample was pipetted into a tube of sterile tetrationate broth (Difco) and into a tube of sterile selenite cystine broth (Difco) for selective enrichment. These tubes were incubated at 37°C for 16-20 h. After incubation, 1 ml each from the incubated tubes of selenite and tetrationate broth was transferred to two separate tubes containing 10 ml of sterile M-broth (Difco). The M-broth was incubated at 35°C for 18 h and then 0.5 ml from each of the two M-broth tubes was combined into a clean test tube which then was heated in boiling water for 15 min and cooled. Detection of Salmonella was accomplished according to the procedures in the 3M® Report™ kit. Appearance of a blue-green color in the well indicated the presence of Salmonella.

The presence of Listeria was tested by using the FSIS method for isolating Listeria monocytogenes from processed meat and poultry products (15). A surface area composite sample was obtained by excising a total of 25 cm² of the carcass surface from three locations (chuck, loin, and round) on each half of the treated
and control carcasses as described previously for *Salmonella* samples.

**Statistical analysis**

Differences in microbiological counts between treatments were compared by analysis of variance (22). When significant (P<0.05) differences were observed, mean separation was accomplished by the use of Duncan’s multiple range test (10).

**Commercial application of acid decontamination on beef carcasses**

In order to test the applicability and reproducibility of lactic acid decontamination under different slaughter conditions, beef carcasses were sprayed with hot (55°C), dilute (1% v/v) lactic acid in three different commercial plants in Texas: a large slaughter facility (1,250 head of fed beef per day), a small slaughter facility (125 head of fed beef per day), and a cow-slaughter facility (1,250 head of cows per day). The small slaughter facility was selected as the site for initial decontamination studies where the 40 steers were slaughtered in two groups of 20. In the other two plants, lactic acid decontamination was applied on 10 carcasses in each plant using two treatment groups (with and without acid treatment; the treated group was sprayed with lactic acid both after dehiding and after evisceration).

**RESULTS AND DISCUSSION**

Mean aerobic plate counts (APCs, log$_{10}$/cm$^2$) of control and acid-treated carcass surfaces (Table 1) were different (P<0.05) in every case 0 h after slaughter, and in all but one case after 72 h of storage. Mean log$_{10}$ APCs of lactic acid sprayed carcasses at day 0 (sampled immediately after entering the cooler) were lower usually by more than 1.0 (log$_{10}$ reduction ranged from 0.9 to 2.3) than those of control carcasses on which initial counts ranged from 2.6 to 3.9. Greatest reduction in log$_{10}$ APC occurred on carcasses treated with lactic acid after evisceration or both after dehiding and after evisceration. These treatments reduced initial bacterial numbers by more than 90%, thus confirming the role of lactic acid as an effective terminal decontaminant.

<table>
<thead>
<tr>
<th>Slaughter groups</th>
<th>Time of sampling (h post-slaughter)</th>
<th>Treatment $^b$</th>
<th>Order of means $^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>3.9$^d$</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>2.4</td>
<td>2.2</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>3.4</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>3.5</td>
<td>2.6</td>
</tr>
</tbody>
</table>

$^a$Each slaughter group consisted of 20 steers.

$^b$C= no treatment (Control); D= 1% (55°C) lactic acid sprayed after dehiding; E= lactic acid sprayed after evisceration; F= lactic acid sprayed after dehiding and after evisceration.

$^c$Mean values in the same row and underscored by a common line do not differ significantly (P>0.05).

$^d$Individual means in each treatment are based on 10 surface samples; each surface sample represents a composite sample of 50 cm$^2$ from five different locations on each side of every carcass.

After 72 h of cold storage (Table 1), log$_{10}$ APC per cm$^2$ of treated carcasses were lower (range 0.6 to 2.1) than those of comparable control carcasses. This reduction was slightly less than that of samples taken immediately after the carcasses entered the cooler. It has been reported (24) that the inhibitory action of lactic acid, which is believed to be primarily caused by the decrease of pH, decreases after an extended storage in the chill room. In a study on microbial decontamination of calf carcasses by 1.25 % (v/v) lactic acid solution, Woolthuis and Smulders (28) reported a pH drop of more than three units 1.5 h after treatment. After overnight storage, they reported that the pH had increased by approximately two units but still remained significantly (P<0.001) below that of the control; however, at 72 h postmortem, the surface pH of acid-treated carcasses had increased to a value similar (P>0.05) to that observed initially. The pH drop attained and the period during which it is maintained depend on the amount of acid and the buffering capacity of food (24). Although in the present investigation the mean APCs of samples from acid-treated carcasses were still significantly lower (P<0.05) than those of the control samples, additional decreases were not observed. No delayed action of lactic acid resulting in further reduction in APC of the carcasses was observed after 72 h of cold storage.

The greatest reduction of bacterial numbers was observed on carcasses treated with lactic acid both after dehiding and after evisceration (Treatment F in Table 1); however, this reduction was in most cases not significantly (P>0.05) greater than that achieved by a single spray immediately after evisceration (Treatment E in Table 1). Lactic acid treatment immediately after dehiding (before evisceration), Treatment D in Table 1, reduced the APC the least of all treatments. Therefore, a single lactic acid spray after evisceration (before chilling) seems appropriate and more practical from an economic standpoint. Similar results were reported by earlier workers (27) on lactic acid decontamination of broiler carcasses treated (immersed) with as many as three acid treatments in succession, namely, after defeathering, after evisceration, and after air chilling. The greatest effect of lactic acid decontamination on bacterial colonization was found when carcasses had been treated just before air chilling. All carcass surface samples tested for the presence of *Salmonella* were negative. Presence of *Listeria* was detected in three samples, all of which were from control carcasses. Further studies would be necessary to confirm the effect of lactic acid on *Listeria* spp. present on carcass surfaces.

Mean APCs (log$_{10}$ per cm$^2$) of boneless strip loins from acid-treated and control carcasses, sampled after 3 and 14 d of vacuum-packaged storage, did not differ (P>0.05) (Table 2). Also, mean scores of sensory attributes, such as lean color, odor and overall acceptability of strip loins from control and treated carcasses, did not differ (P>0.05) (Table 3). However, mean scores of sensory attributes of loins from treated carcasses were often (in 6 of 9 instances) judged more acceptable than those of comparable controls. One possible explanation for the lack of significant differences in APC and/or sensory attributes between loins from acid-treated and control carcasses in the present study could...
TABLE 2. Mean aerobic plate counts (log_{10}/cm²) of beef loins at 3 and 14 d of vacuum-packaged storage after fabrication.

<table>
<thead>
<tr>
<th>Slaughter groups</th>
<th>Time of sampling (days post-fabrication)</th>
<th>Treatment</th>
<th>Order of means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C  D  E  F</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>5.7  5.2  5.0  4.9</td>
<td>C  D  E  F</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>7.2  7.4  7.3  7.0</td>
<td>D  E  C  F</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>4.7  4.6  4.7  4.8</td>
<td>F  E  C  D</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>6.7  6.5  6.6  6.5</td>
<td>C  E  F  D</td>
</tr>
</tbody>
</table>

aEach slaughter group consisted of 20 steers.
bC=no treatment (Control); D=l% (55°C) lactic acid sprayed after dehiding; E=lactic acid sprayed after evisceration; F=lactic acid sprayed after dehiding and after evisceration.
cMean values in the same row and underscored by a common line do not differ significantly (P>0.05).
dIndividual means in each treatment are based on five surface samples; each surface sample represents a composite sample of 30 cm² from 3 different locations on each of the five beef loins.

TABLE 3. Mean scores of sensory attributes of beef loin sections at 14 d of vacuum-packaged storage (3 ± 1°C) after fabrication.

<table>
<thead>
<tr>
<th>Slaughter</th>
<th>Days of storage</th>
<th>Sensory attributes</th>
<th>Treatment</th>
<th>Order of means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lean color</td>
<td>Acceptability</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C  D  E  F</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>14</td>
<td>5.4</td>
<td>5.6  5.8  5.8</td>
<td>E  F  D  C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Odor</td>
<td>3.4  2.4  2.1  2.8</td>
<td>C  F  D  E</td>
</tr>
<tr>
<td>II</td>
<td>14</td>
<td>4.8</td>
<td>4.4  5.6  4.8</td>
<td>E  C  F  D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Odor</td>
<td>4.6  5.2  6.0  4.6</td>
<td>E  D  F  C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.4  2.2  3.6  2.2</td>
<td>E  D  F  C</td>
</tr>
</tbody>
</table>

Individual means in each treatment are based on 5 scores obtained from five replicate beef loin sections. Means for lean color and acceptability scores are based on 8-point scale (8=extremely desirable/acceptable, 1=extremely undesirable), and on 5-point scale for odor scores (5=no off-odor, 1=unacceptable off-odor).

be the degree and nature of recontamination during handling and fabrication which may have masked any significant continuing effect of lactic acid decontamination. In each case the mean APC increased considerably after loin fabrication as shown by the APC for 72-h carcass samples and day 3 strip loin samples.

Irrespective of differences in size of slaughter plants, mean APCs for samples from carcasses sprayed with lactic acid were significantly lower (P<0.05) than those of control carcasses (Table 4). In each plant, mean log_{10} reductions in APC were greater than 90% with log_{10} reductions ranging from 1.3 to 1.9 per cm². In summary, lactic acid decontamination of beef carcasses after dehiding and again before chilling significantly reduced the microbial counts. The microbiological quality of subprimals depends most likely to a large extent on the degree of secondary contamination. Therefore, along with lactic acid decontamination, it is essential that good manufacturing practices are applied during fabrication to extend the shelf life of the product and improve microbiological safety.

TABLE 4. Mean aerobic plate counts (log_{10}/cm²) of control and acid-treated beef carcass surfaces immediately after entering the cooler (0 h).

<table>
<thead>
<tr>
<th>Slaughter plants</th>
<th>Slaughter groups</th>
<th>Time of sampling (h post-slaughter)</th>
<th>Treatment</th>
<th>Order of means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow-slaughter facility</td>
<td>I</td>
<td>0</td>
<td>2.6  1.3  1.3</td>
<td>C  F</td>
</tr>
<tr>
<td>Large slaughter facility</td>
<td>I</td>
<td>0</td>
<td>3.4  1.9  1.9</td>
<td>C  F</td>
</tr>
<tr>
<td>Small slaughter facility</td>
<td>I&amp;II</td>
<td>0</td>
<td>3.7  1.8  1.8</td>
<td>C  F</td>
</tr>
</tbody>
</table>

Individual means in each treatment are based on 10 (20 in case of small slaughter facility) surface samples; each sample represents a composite sample of 50 cm² from five different locations on each side of every carcass.

ACKNOWLEDGMENTS

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REFERENCES


