Combined Effects of Lactic Acid and Nisin Solution in Reducing Levels of Microbiological Contamination in Red Meat Carcasses

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ABSTRACT

Changes in bacterial counts on beef carcasses at specific points during slaughter and fabrication were determined, and the effectiveness of nisin, lactic acid, and a combination of the lactic acid and nisin in reducing levels of microbiological contamination was assessed. Swab samples were obtained from the surfaces of randomly selected beef carcasses. Carcasses were swabbed from the neck, brisket, and renal site after skinning, splitting, and washing. Treatments involving lactic acid (1.5%), nisin (500 IU/ml), or a mixture of nisin and lactic acid were applied after the neck area was washed. A control group was not sprayed. Results indicated that the highest prevalence of aerobic plate counts (APCs), total coliforms, and *Escherichia coli* was found in the neck site after splitting, and the lowest level of microbial contamination was found after skinning. Washing with water did not significantly reduce the bacterial load. The largest reduction in APCs, total coliforms, and *E. coli* occurred on carcasses treated with a mixture of nisin and lactic acid. A mixture of nisin and lactic acid can be applied to beef carcasses through spray washing and can reduce bacterial populations by 2 log units.

Red meat processors are actively looking for reasonable interventions that minimize the risk of introducing undesirable microorganisms and bacterial pathogens from contaminated raw carcasses into processed meats. Sanitary manufacturing practices during slaughter and fabrication have been effective in reducing bacterial contamination; however, optimum reduction has yet to be achieved.

Organic acids and bacteriocins are of particular interest as decontaminants. The bactericidal properties of lactic acid have been well documented. Smulders and Woolthuis reported that when calf carcasses were treated with 1.25% lactic acid, a reduction in the aerobic plate count (APC) was observed. The effectiveness of an organic acid in reducing populations of meatborne pathogens varies with the concentration of acid used, the temperature of the acid and the carcass, the contact time, the spray application pressure, the point at which the sanitizer is used in the slaughtering-skinning-evisceration process, the tissue type, and the sensitivity of the target organism to the specific acid. The antibacterial effects of lactic acid and acid mixtures (acetic acid with lactic or propionic acid) against gram-negative organisms are generally more extensive than their effects against gram-positive organisms.

Numerous reports have examined the use of nisin to inhibit spoilage and pathogenic bacteria in foods and beverages. Nisin is a small antimicrobial polypeptide produced by some strains of *Lactococcus lactis*. It inhibits the growth of a broad spectrum of gram-positive microorganisms, including the pathogens *Clostridium botulinum*, *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*. *L. monocytogenes* is widely distributed in nature, and the association of this pathogen with meat and the abattoir environment is well established. Incidence rates of *L. monocytogenes* in raw meat vary considerably, and rates as high as 92% have been reported. Researchers have found that under certain conditions, when used with chelating agents, nisin inhibits *Salmonella* species and *Escherichia coli* O157:H7 and other gram-negative microorganisms. Ariyapitipun et al. reported that vacuum-packaged fresh beef treated with 2% polylactic acid, 2% lactic acid, and a combination of each acid with nisin significantly lowered the population of psychrotrophic aerobic bacteria and mesophilic *Enterobacteriaceae* compared with that observed for no treatment or treatment with water or nisin alone. The objectives of this study were (i) to determine the bacterial loads in the necks, chests, and renal sites of beef carcasses after skinning, splitting, and washing; (ii) to measure the effectiveness of conventional washing in reducing contamination; and (iii) to evaluate the effectiveness of a combined treatment of nisin and lactic acid in the reduction of bacterial counts in carcasses.

MATERIALS AND METHODS

Sampling of carcasses. Two bovine slaughterhouses were chosen for the collection of samples. Both plants were visited...
weekly, on Tuesday and Wednesday, for six months. The average ambient temperature at the plants was 30°C.

**Determination of the slaughter area and the carcass site with the most extensive contamination.** This study was conducted in two phases. During the first phase, 67 bovine carcasses were selected at random. Samples were taken as the carcasses passed by on line, so as to avoid the disruption of normal production as much as possible. Samples were collected at three points in the slaughter operation (after skining, after splitting, and after washing) and from three carcass sites (the neck, the brisket, and the renal [flank] site).

**Description and application of treatments.** During the second phase, 192 carcasses were selected at random. On the basis of the results of the first phase, treatments were applied after the neck area was washed with cold water. Carcasses were treated with (i) lactic acid (1.5% [vol/vol], pH 2.5) prepared from an 85% DL-lactic acid stock solution (J. T. Baker Chemical Co, Phillipsburg, N.J.), (ii) nisin (500 IU/ml; Nisaplin, Aplin and Barlett Ltd., Beaminstorh, UK), and (iii) a mixture of nisin (500 IU/ml) and lactic acid (1.5%, vol/vol). A control group of carcasses was not sprayed. The acid concentration used in this study was based on those cited in the literature (26–28), and the nisin concentration used was based on preliminary testing (data not shown).

Eight carcasses per day were assigned randomly to the four treatment groups (two carcasses per treatment). Treatments were applied with a 1-liter handheld manual sprayer in a fine mist from a distance of approximately 50 cm. The temperature of the solution was 25°C. The pressure was not measured; however, the volume of spray was evaluated to ensure that a standard volume of spray was delivered. The neck area (inside and outside) was sprayed with a total of 50 ml of the appropriate treatment solution. Following treatment, all carcasses were immediately moved into the carcass chilling area (1 to 2°C) for 24 h.

**Samples taken after skinning**

**Samples taken after splitting**

**Samples taken after washing**

<table>
<thead>
<tr>
<th></th>
<th>Neck</th>
<th>Brisket</th>
<th>Renal site</th>
<th>Neck</th>
<th>Brisket</th>
<th>Renal site</th>
<th>Neck</th>
<th>Brisket</th>
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<tbody>
<tr>
<td>APC</td>
<td>1.7 A</td>
<td>1.8 A</td>
<td>1.9 A</td>
<td>4.1 C</td>
<td>3.4 B</td>
<td>3.5 B</td>
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<td>3.4 B</td>
<td>3.4 B</td>
</tr>
<tr>
<td>CT</td>
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<td>0.9 A</td>
<td>0.9 A</td>
<td>2.8 C</td>
<td>2.1 B</td>
<td>2.3 c</td>
<td>3.0 c</td>
<td>2.9 c</td>
<td>2.4 C</td>
</tr>
<tr>
<td>EC</td>
<td>0.4 A</td>
<td>0.3 A</td>
<td>0.5 A</td>
<td>1.7 B</td>
<td>1.3 B</td>
<td>1.4 B</td>
<td>1.4 B</td>
<td>1.2 B</td>
<td>1.4 B</td>
</tr>
</tbody>
</table>

*Values shown are log CFU/cm². Means with different letters in the same row are significantly different (*P* < 0.05; least significant difference test).

**RESULTS AND DISCUSSION**

**Bacterial loads in the necks, briskets, and renal sites of carcasses after skinning, splitting, and washing.** Because microorganisms are not distributed evenly over the carcass, the choice of sampling sites was an important aspect of the surface microbial counts. Table 1 shows differences between counts for different carcass sites and reveals significant differences (*P* < 0.05) between bacterial counts at the three sampling points. The highest prevalence of APCs, total coliforms, and *Escherichia coli* was found after the carcasses were split. The highest aerobic bacterial count was found for the neck area after splitting (4.1 log CFU/cm²). The reduction in the mean APC, total coliforms, and *Escherichia coli* after washing was not statistically significant.

These results indicate that each process is positively associated with bacterial counts. The lowest levels of microbiological contamination were found after carcasses were skinned. The splitting process further increased levels of contamination and remained a significant factor in high levels of contamination on carcasses at the end because splitting is carried out after skinning and evisceration. In the beef slaughter and dressing process, the holding pen,
TABLE 2. Mean aerobic plate counts (APCs), total coliforms (CT), and E. coli (EC) for control, lactic acid–, nisin-, and mixture-treated carcasses (n = 48)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Lactic acid</th>
<th>Nisin</th>
<th>Mixture</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>APC</td>
<td>3.3 A</td>
<td>4.2 B</td>
<td>3.6 A</td>
<td>3.1 C</td>
</tr>
<tr>
<td>CT</td>
<td>3.2 A</td>
<td>4.1 B</td>
<td>3.2 A</td>
<td>1.4 B</td>
</tr>
<tr>
<td>EC</td>
<td>1.1 A</td>
<td>1.3 A</td>
<td>1.1 A</td>
<td>0.5 B</td>
</tr>
</tbody>
</table>

*Values shown are log CFU/cm². Means with different letters in the same row are significantly different (P < 0.05; least significant difference test). Day 1, initial count before spraying; day 2, final count after 24 h.*

The carcass skinning area, and the evisceration area have been identified as probable introduction points for major contamination (2). Evisceration can be carried out with minimal contamination of the carcass provided the intestinal tract is not ruptured or punctured. Special care must be taken during evisceration to not disrupt the gastrointestinal tract and contaminate the carcass. On the other hand, it is generally recognized that workers and their implements are important vehicles through which contamination is transferred from the hide to the carcass (25, 27). This finding demonstrates the need to determine the quantitative contribution of different processing practices to levels of microbiological contamination, as well as the quantitative contribution of any proposed changes. Nortjie and Naude (23) and Roberts (25) reported considerable variability in the levels of microbiological contamination at different red meat carcass sites.

The neck was a critical site because of the high prevalence of bacteria found there after the final wash in the slaughter process, and, since carcasses are kept in a vertical position, the contaminated material drains toward this site. This result agreed with that of Jericho et al. (19), who suggested that because of carcass geometry, counts are significantly affected at the neck by a general flow of wash water down the carcass. A survey of slaughter operations in Norway showed that the brisket, forerib, flank loin, and round sites of beef carcasses consistently had higher total bacterial counts than other carcass sites sampled (20). Ingram and Roberts (18, 25) noted that several sites on a carcass should be sampled to determine the true nature of bacterial contamination.

Effect of conventional washing. Bacterial counts before and after carcass washing (Table 1) bring the efficacy of the washing process into serious doubt. At best, the washing of carcasses with cold potable water brings about a redistribution of microbial contamination over the carcass surface. Redistribution of bacterial contamination on carcasses rather than removal of bacterial contamination from carcasses by cold water washes has previously been alluded to (10, 12).

Effect of treatments on bacterial counts, total coliforms, and E. coli. Treatment with lactic acid reduced APCs, coliform counts, and E. coli counts (P < 0.05). The reduction in these count with nisin treatment was not significant. The largest reduction in APCs, total coliforms, and E. coli occurred on carcasses treated with a mixture of nisin and lactic acid (Table 2). This treatment reduced initial bacterial numbers by almost 2 log units. E. coli was reduced to below the minimum detection level (0.2 log/cm²) by a mixture of nisin and lactic acid. For control carcasses, the mean APC values increased after 24 h.

The results of this study indicate that treatment of carcass surfaces with a mixture of nisin and lactic acid was more effective in the decontamination of beef carcass surfaces than was treatment with lactic acid or nisin alone. Our results indicate that lactic acid exerts a bactericidal effect on meat, and with nisin this effect was enhanced. Treatment with nisin alone was the least effective decontamination treatment evaluated; it is not surprising that nisin alone was not effective in slowing growth, because the majority of the microorganisms in question were gram negative. The cell membrane is the site of action for many small bacteriocins of lactic acid bacteria (9, 13, 16).

An acidic condition of the solution in which nisin is used may enhance the antimicrobial effect. This study shows that nisin with lactic acid may be useful in controlling microbial contamination. This treatment reduces the APCs on meat surfaces by almost 2 log CFU/cm² in 24 h, and it reduces total coliform counts by >2 log CFU/cm².

Treatments with lactic acid and with a mixture of nisin and lactic acid significantly reduced levels of aerobic bacteria. The results of our experiment seem to indicate that an additive or synergistic effect exists for lactic acid and nisin. The application of the mixture of lactic acid and nisin was more bactericidal than any single antimicrobial treatment alone. Therefore, the treatment of bovine carcasses with this mixture can considerably minimize the contamination of meat with microorganisms.

To date, there have been no reports that address the application of a mixture of nisin and lactic acid to red meat carcasses by spray washing. In this study, we demonstrated that a mixture of nisin and lactic acid can be applied to red meats through spray washing and that bacterial populations can be reduced by 2 log units. In conclusion, the sanitization of beef carcasses with nisin and lactic acid may be a useful method to inhibit spoilage bacteria and thus extend the shelf life of red meat.

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REFERENCES


