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Use of Calcium Alginate to Immobilize Antimicrobial Agents on Beef Tissue

Gregory R. Siragusa and James S. Dickson1,2

Introduction

Even under the best of slaughtering and processing conditions, beef carcasses will become naturally contaminated with some bacteria from the animal’s hide, hair, hooves, and the abattoir environment. This contamination is mostly composed of bacteria which are harmless, but which can ultimately cause spoilage of the beef. The shellfife of raw beef is largely determined by the numbers and types of these bacteria. Since some bacterial contamination will always be present on beef, it is desirable to reduce these numbers to decrease the rate of spoilage, increase refrigerated shellfife, and further ensure the microbiological safety of raw beef before consumption.

Any methods to reduce this bacterial contamination would greatly benefit the beef processing industry. Methods are currently used in the red meat industry to decontaminate the carcass. These include spraying of water or antimicrobial agents on the carcasses. Sprays include the use of dilute chlorine in the spray chill water or the application of dilute foodgrade acid sprays on the carcass before chilling. These acids are usually either acetic acid or lactic acid, both of which are commonly consumed food ingredients.

Any methods which would enhance the antimicrobial effect of the acid or antimicrobial agent would be a significant improvement to beef production. We have developed the idea of applying food grade acids (acetic and lactic acids) into an edible gel coating which could be sprayed onto the carcass surface before entering the chilling chamber. Gel coatings have been shown to decrease the amount of moisture loss of the carcass. Incorporating the antimicrobial agent in an edible gel on the carcass would possibly help reduce moisture loss and simultaneously reduce the amount of spoilage bacteria. Any methods which decrease the amount of spoilage bacteria will also reduce the numbers of any pathogenic bacteria which may be present such as Listeria, Salmonella, and pathogenic Escherichia coli. The purpose of this research was to test the use of alginate edible gels to coat a layer of antimicrobial agent on the carcass surface to reduce the numbers of bacteria.

Procedure

Materials and Methods. Sterilized lean and fat beef tissue sections (3.6 in² total surface area) were inoculated with the foodborne pathogen Listeria monocytogenes (Lm). Tissue samples were dipped in a solution of 1% sodium alginate. Food grade acids (2% acetic and 1.7% lactic acids) were prepared in solutions of calcium chloride. Calcium chloride causes a gel to form when applied to the carcass surface before entering the chilling chamber. Gel coatings have been shown to decrease the amount of moisture loss of the carcass. Incorporating the antimicrobial agent in an edible gel on the carcass would possibly help reduce moisture loss and simultaneously reduce the amount of spoilage bacteria. Any methods which decrease the amount of spoilage bacteria will also reduce the numbers of any pathogenic bacteria which may be present such as Listeria, Salmonella, and pathogenic Escherichia coli. The purpose of this research was to test the use of alginate edible gels to coat a layer of antimicrobial agent on the carcass surface to reduce the numbers of bacteria.

Microbiological and Data Analysis. Samples were taken at days 0, 1, 3, and 7. Listeria was enumerated by blending the sample in acid neutralizing solution, diluting this sample, and plating on two different bacteriological growth media to enumerate the bacteria. Bacterial counts were converted to log10 per total piece of tissue. The amount of reduction in the bacterial population was calculated as the difference in log10 bacteria per tissue section from day 0 to the sampling date.

Results

Table 1 shows the log reductions in bacterial counts from day 0 to day 7 at 40°F on lean beef tissue. Organic acids immobilized or incorporated into a gel decreased the numbers of Listeria monocytogenes attached to the beef tissue more than the acids applied without alginate. This effect was not observed in the first three days of storage (see Figure 1). Listeria monocytogenes grew on the tissue that was untreated, treated with calcium chloride, or treated with alginate and no acid. The largest reduction in numbers was caused by the application of acetic acid in a calcium alginate gel. Lactic acid at a lower concentration than acetic (1.7% vs 2.0%) caused a comparable but lower reduction than did acetic (Table 1). Counts determined by using Tryptic Soy agar represent the total of bacteria which are metabolically healthy and possibly injured by the effect of the acids. Counts determined on Oxford Listeria agar represent the portion of the bacterial population which is only metabolically healthy (Table 1). This difference is important to the food microbiologist when testing food because the use of only a selective agar (e.g., Oxford Listeria agar) to count a specific species of bacteria can result in underestimating the actual number. Calcium chloride or alginate by itself did not significantly affect the population of the test bacteria.

In the case of pure fat tissue, whether or not the acid was applied in an alginate gel made no difference on the reduction of Listeria monocytogenes counts based on Tryptic Soy agar. In the case of selective counts determined by Oxford Listeria agar, there was some increased reduction due to the alginate application method, however, this was not a statistically significant difference. Overall, the reduction in bacterial counts on fat tissue started at day 0 and continued through day 7 (Figure 2).

Discussion

Immobilizing organic acids in alginate gels and applying to inoculated beef tissue enhanced the antimicrobial effect of the acids on lean beef tissue. Overall, the bactericidal effect of acid sanitizers immobilized in alginate gels was much more pronounced on lean beef tissue. Applying organic acids in alginate to pure fat tissue did not enhance the antimicrobial action of the acids. This is a problem which might be overcome by using either different antimicrobial agents, such as other food grade organic acids, mixtures of these acids or higher concentrations of these agents. In addition, using other gelling agents may offer advantages. However, in the case of Listeria, the organism did readily multiply on fat tissue.

The exact mechanism by which alginate gel application of acids enhanced the killing effect of the acids is not known. It is hypothesized that the gel offers a means of maintaining the acid in a moist environment, which is necessary for the inhibitory action of the acid.

1Siragusa is a microbiologist, Meats Research Unit, MARC, and Dickson is a research food technologist, Meats Research Unit, MARC.
2The full report of this work was published in J. Food Sci. 57:293-296, 1992.
Using alginate/acid coatings might offer an alternative to spray chilling. The carcass would be encapsulated in a highly moist gel coating, and heat would still be conducted off of the carcass in the carcass chilling chamber. Also, moisture loss would be reduced since the gel retains water. The use of alginate gels as coatings to reduce moisture loss in red meat carcasses has already been demonstrated. This method might potentially be used to increase the shelf life of sub-primal beef cuts or used in other segments of the beef processing. Additional research is needed to determine the effectiveness of other antimicrobial compounds when immobilized in alginate or other gelling agents applied to beef carcasses. Research is underway to test the process on carcasses.

Table 1—Log reduction in viable counts of L. monocytogenes on lean beef tissue after 7 days at 5°C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TSA YE Agar</th>
<th>Oxford Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alginatethe</td>
<td>Alginatethe</td>
</tr>
<tr>
<td>Aceticb</td>
<td>1.5</td>
<td>1.56</td>
</tr>
<tr>
<td>Lacticb</td>
<td>1.26</td>
<td>2.08</td>
</tr>
<tr>
<td>Alginatethe controlc</td>
<td>0.01</td>
<td>-0.23</td>
</tr>
<tr>
<td>Control tissuef</td>
<td>-0.61</td>
<td>-1.16</td>
</tr>
</tbody>
</table>

a Difference in counts within each treatment between day 0 and day 7.

b See Materials and Methods section for explanation of treatments.

c Alginatethe control, Alginatethe no acid applied in alginatethe dip. Alginatethe control, no Alginatethe = CaCl2 dip only, no acid or Alginatethe applied.

d Control tissue = inoculated, untreated lean tissue.

Figure 1—Reduction and growth of Listeria monocytogenes on lean beef tissue held at 40°F and treated with acetic acid applied by immobilizing in a calcium alginate gel. Bacterial counts were determined using Tryptic Soy agar medium.

Figure 2—Reduction of Listeria monocytogenes on beef fat held at 40°F treated with acetic acid applied by immobilizing in a calcium alginate gel. Bacterial counts were determined using Tryptic Soy agar medium.