Microbiological and Sensory Tests of Beef Treated with Acetic and Formic Acids¹,²

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ABSTRACT

One-centimeter cubes of the semimembranous and adductor muscles of beef were inoculated with 5.2 × 10⁶ of Salmonella typhimurium, Shigella sonnei, Yersinia enterocolitica, Escherichia coli, Pseudomonas aeruginosa or Streptococcus faecalis. Exposure of the meat by dipping in 1.2% acetic acid for 10 s reduced average numbers recoverable of these bacteria by 65%. E. coli was the most resistant, losing 46% of its viable cells. One-half of the acetic acid was replaced with 0.046% formic acid without loss in effectiveness. The rate of increase of bacterial numbers on surfaces of animal carcasses includes some species as well as acidic solutions (2,3,6,11-13,15). An acid spray (2.0% acetic acid, 1.0% lactic acid, 0.25% citric acid and 0.1% ascorbic acid in water) tested on beef and sheep carcasses caused a slight darkening of the mutton and a mild acidic odor to both species for about 24 h (13).

The ideal method to decrease bacterial counts on meat would reduce bacterial numbers maximally, minimize discoloration of meat and cause no discernable off-flavor in cooked meat. The objective of this research was to determine which of four concentrations of acetic or combined acetic and formic acids best meet these requirements.

MATERIALS AND METHODS

Materials

Cultures acquired from our departmental collection were Salmonella typhimurium (ATCC 9148), Shigella sonnei, Yersinia enterocolitica (ATCC 23715), Escherichia coli, Pseudomonas aeruginosa, and Streptococcus faecalis.

Sanitizers were glacial acetic and formic acids of purified or certified grade (Fisher Scientific Co., St. Louis, MO). Acids were mixed with distilled water and used at 21 to 25°C. The plating medium for bacteria was Tryptic soy agar (Difco Laboratories, Detroit, MI).

Methods

Sensory tests of color of fresh meat. In the first test, 0.6, 1.2, 1.8 and 2.4% acetic acid solutions were used in four replications. Top round retail beef cuts, purchased on the day that solutions were applied, were selected for their bright redness and uniformity of color. Meat was cut into pieces (3 × 2 × 1 cm), dipped into a solution for 1 or 10 min and refrigerated at 5°C for 20 ± 2 h. Within each replication were identified and coded control samples of meat. Controls were dipped in distilled water for 1 or 10 min. All meat cubes were randomly placed within a set, except for the identified control sample. Each set contained five cubes that were displayed on Kraft paper under fluorescent lighting (two 48-in. Sylvania Cool White B4W lamps placed 2 m above samples). An experienced, lated meat. However, no acetate was used in the buffer, only phosphate and citrate. Although many methods may reduce bacterial counts, they could have a negative effect on color or flavor (8,11,13,15). Brownish discoloration developed on meat treated with formic acid at concentrations higher than 0.5% (15). An acid spray (2.0% acetic acid, 1.0% lactic acid, 0.25% citric acid and 0.1% ascorbic acid in water) tested on beef and sheep carcasses caused a slight darkening of the mutton and a mild acidic odor to both species for about 24 h (13).

Muscles of an animal are essentially sterile. Once the animal is slaughtered, bacterial contamination usually occurs and spoilage is expected. This is of major concern to the meat industry. Most industrial methods to reduce bacterial numbers on surfaces of animal carcasses include the use of sprays. Cold, hot (1-4,7,17,18) and chlorinated (2,3,6,11-13,15,16) waters and acidic solutions destroyed or removed bacteria of inocu-

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A 13-member sensory panel was instructed to give each piece of meat a number that best described its color. A scale from one to six was used: 1 = extremely bright red, 2 = moderately bright red, 3 = slightly bright red, 4 = slightly discolored, 5 = moderately discolored and 6 = extremely discolored. Panelists independently scored the samples on a score sheet, comparing the test samples with the control which they scored first.

In the second test, 0.6% acetic acid, 1.2% acetic acid, and 0.6% acetic acid combined with 0.046, 0.092, 0.184 and 0.23% formic acid solutions were used in six replications. Methods of application and sensory testing were as with the first sensory test. Color of each of seven pieces of meat was judged by each of 12 panelists.

Antimicrobial properties of sanitizing solutions. In the first study of effectiveness of sanitizers, meat inoculated with the six cultures was dipped in 1.2% acetic acid or in distilled water. Bacteria were grown on membrane filters (45-μm) that rested on pads (47-mm) saturated with 2 ml of double strength brain heart infusion broth. Filters, inoculated with a few drops of culture, were incubated at 32°C for 16 ± 2 h. Each filter was transferred to a widemouth bottle containing 100 ml of sterile 0.1% peptone broth. The bottle was shaken and the filter was removed with sterile forceps, then discarded. The suspension was adjusted by nephelometry to yield plate counts of 10^9 ml (0.5 absorbance at 520 nm; McFarland standard). This suspension was blended in a prechilled 1-L Waring blender jar at 17,000 rpm for 2 min then maintained at 5°C until needed.

For each replication, a top round beef roast, comprised of mainly the semimembranosus and adductor muscles, was purchased and stored frozen (-20°C) for not longer than 48 h. The meat was thawed slightly (2 h at 25°C), cut into 1-cm cubes using sterile equipment, placed in a sterile beaker, and stored at 5°C not longer than 6 h.

Sterile paperclips (plated steel wire), bent into an S-shape, were inserted individually into the meat pieces. Cubes of meat were hung inside a laminar flow hood to dry slightly to facilitate the adsorption of the bacterial suspension.

Each of the six sides of the meat cube was inoculated with 10 μl of bacterial suspension; five cubes per culture. Two uninoculated cubes were used as controls; one for sterility of meat and the other for sterility of sanitizing solution. All cubes were hung at 23°C for 15 min to allow bacteria to attach. Three inoculated cubes were dipped individually for 10 s in separate sterile 1.2% acetic acid solutions. A fourth cube was dipped for 10 s in sterile distilled water. A fifth cube was not dipped. All cubes were then hung in a humid environment for 20 ± 2 h at 5°C. Each cube of meat was placed individually into a chilled, sterile Waring blender jar, and 100 ml of 0.1% peptone broth was added. This mixture was blended at 17,000 rpm for 60 s and plated. Plates were incubated at 32°C and counted at 48 h. There were four replications per microorganism.

In the second study, antimicrobial efficacies were determined for 1.2% acetic acid and 0.6% acetic acid plus 0.046% formic acid. Time of exposure was 1, 10 or 100 s. E. coli suspension was prepared as in the first study. Each of four replications involved 14 cubes rather than 7. Ten cubes were inoculated onto all six sides with 10 μl of suspension. Four uninoculated cubes were used as controls: one each for sterility of meat, distilled water, 1.2% acetic acid, and 0.6% acetic acid plus 0.046% formic acid. All cubes were hung at 23°C for 15 min in a humid environment after inoculation of the 10. Of the 10 inoculated cubes, three each were dipped into 1.2% acetic acid, 0.6% acetic acid plus 0.046% formic acid, and distilled water. One, undipped, was used as the unsanitized control. One each of set of three cubes treated with the above solutions, was exposed for 1, 10 and 100 s. As in the previous study, all cubes of meat were hung in a humid environment for 20 ± 2 h at 5°C before being plated. Counts were determined as before.

Sensory panel on the flavor of baked ground beef. A sensory panel on flavor of baked ground beef was employed after application of 1.2% acetic acid or 0.6% acetic acid plus 0.046% formic acid solutions.

A 7-kg top round boneless roast was used for each of five replications. The meat was stored frozen (-20°C) until needed. Each roast was thawed at 5°C for 16 h and refrigerated at 1°C for not longer than 32 h. The roast was sliced into 1-cm thick steaks. Bacon hooks were inserted into each steak. Steaks were dipped for 60 s as follows: ten in distilled water, five in 1.2% acetic acid and five in 0.6% acetic acid plus 0.046% formic acid. The steaks were hung inside plastic bags and refrigerated at 1°C for 20 ± 1 h. Perimeters of dipped steaks were trimmed, discarding scraps, so area of exposure to sanitizer was always 2 sq cm/cm² of volume. Treated, trimmed meat was ground (3-mm aperture diam). Grinder parts were washed between each treatment. Ground meat (200 g) from each treatment was packed into 400-ml beakers and covered with foil. Six, three and three beakers, respectively, contained meat treated (before grinding) with distilled water, 1.2% acetic acid, and 0.6% acetic acid plus 0.046% formic acid.

For each of five replications, combinations of three beakers each were placed randomly into four different conventional electric ovens 5 min apart. Twenty-five min at 149°C were required for the internal temperature of the meat to reach 71°C. Baked meat was mixed thoroughly and spooned (about 14 g) into randomly numbered blue glass serving cups (75 ml).

A triangle test was employed in comparing flavors of meat. A triangle consisted of three samples in serving cups seated in a pan (19 × 9 × 5.5 cm) containing sand to a depth of about 3 cm. The cups and pans with sand had been preheated (60°C). Permutations of sample order were randomly assigned to judges. Each triangle included a control dipped in distilled water, either as a single or duplicate sample. Samples treated with either 1.2% acetic acid or 0.6% acetic acid plus 0.046% formic acid completed the triangle. An experienced 8-member panel was instructed to taste the three samples, rinsing the mouth with odor-free water before each sample, and to circle the two randomly coded numbers corresponding to samples that were identical in flavor. Panelists were served four triangles daily with a 5-min break after the second triangle. A red light was used in the panel room to obscure slight differences in color which may have existed among samples.

Statistical analysis. Analysis was performed using Statistical Analysis System (SAS) software. Analysis of variance, employing a randomized complete block design, was used on the data compiled from the sensory tests on color and bacterial studies. Mean differences were ascertained using Fisher’s least significant difference (P ≤ .05) (19). Percentage reductions in bacteria counts were analyzed in the linear statistical model that contained the effect of replication, treatment, bacteria and the interaction of treatment × bacteria. The same statistical design was used in the second bacterial study and with all color tests. However, the linear statistical model contained the effect of replication, treatment, time and the interaction of treatment × time.
TREATING BEEF WITH ACETIC AND FORMIC ACIDS

RESULTS AND DISCUSSION

Sensory tests of the color of fresh meat

After exposure to sanitizers for 1 min, only meat treated with 0.6% acetic acid did not differ significantly (P > 0.05) in color from the control (Table 1). Meat treated with 1.2% acetic acid did not differ in color from meat treated with 0.6% acetic acid. However, 1.8 and 2.4% acetic acid caused significantly more discoloration than 0.6% acetic acid. After dipping for 10 min, all samples differed in color from the control. Samples exposed to 0.6% or 1.2% acetic acid were not significantly different in color, yet their degree of discoloration was significantly less than that of meat exposed to either 1.8 or 2.4% acetic acid.

Quartey-Papafio et al. (15) used formic, acetic and propionic acids as sanitizers for beef, and screened them for antimicrobial efficacy and effect on meat color. Solutions of 2% formic acid or 1% formic plus 1% acetic acids were most effective, destroying 84% and 73%, respectively, of the test cultures. However, meats turned brown within a few seconds after spraying with formic acid in concentrations of 0.5% and greater.

In our second sensory test of color of fresh meat, formic acid in low concentrations (up to 0.23%) was combined with 0.6% acetic acid. Mean scores for color of meat dipped for 1 min in water or 0.6% acetic acid did not differ significantly (Table 2). All other treatments caused significant color changes compared with the control. Yet there was no significant difference in color scores between samples dipped in 0.6% acetic acid or in 0.6% acetic acid plus 0.046% formic acid (P > 0.05).

After dipping for 10 min, the control was significantly more red than any of the treated samples. Meat treated with 0.6 or 1.2% acetic acid, or with 0.6% acetic acid plus 0.046% formic acid had a mean score of 3.5 (between “slightly bright red” and “slightly discolored”). Formic acid, when used in concentrations greater than 0.046% in combination with 0.6% acetic acid, averaged a score of 4.1 (greater than “slightly discolored”).

Antimicrobial properties of sanitizing solutions

Numbers of bacteria on untreated control samples averaged $5.2 \times 10^6$, much higher than numbers of these bacteria expected on fresh meat. Dipping in distilled water decreased numbers an average of 41.5%, and dipping in 1.2% acetic acid decreased numbers from 45.8% with E. coli to 77.2% for P. aeruginosa (Table 3). Bacteria which were not recovered, after application of a sanitizer, were either removed, destroyed, or injured. Cause of reduction in numbers was not determined. Percentages of S. thphimurium, S. sonnei, Y. enterocolitica, P. aeruginosa or S. faecalis recovered from meat dipped in 1.2% acetic acid did not differ significantly (P > 0.05). However, E. coli was significantly more resistant than the other bacteria.

In the second study, there was no significant difference between 1.2% acetic acid solution and 0.6% acetic acid plus 0.046% formic acid solution in their ability to reduce the number of E. coli from the surface of meat at 1, 10 and 100 s of exposure to the sanitizers (Table 4). However, these two sanitizers reduced the bacterial

TABLE 1. Mean sensory scores for color of fresh meat treated with acetic acid.

<table>
<thead>
<tr>
<th>Dipping solution</th>
<th>Time in dipping solution</th>
<th>1 min</th>
<th>10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (H2O)</td>
<td></td>
<td>1.6b</td>
<td>1.9a</td>
</tr>
<tr>
<td>0.6% A</td>
<td></td>
<td>2.1b</td>
<td>2.9b</td>
</tr>
<tr>
<td>1.2% A</td>
<td></td>
<td>2.4b</td>
<td>3.2b</td>
</tr>
<tr>
<td>1.8% A</td>
<td></td>
<td>2.8c</td>
<td>3.9c</td>
</tr>
<tr>
<td>2.4% A</td>
<td></td>
<td>3.6d</td>
<td>5.2d</td>
</tr>
</tbody>
</table>

1Total mean scores were based on a six point scale (1 = extremely bright red, 6 = extremely discolored).
2Four replications.
3A = acetic acid.
4Values with a common superscript within a row are not significantly different (P > 0.05).

TABLE 2. Mean sensory scores for color of fresh meat treated with acetic and/or formic acid.

<table>
<thead>
<tr>
<th>Dipping solution</th>
<th>Time in dipping solution</th>
<th>1 min</th>
<th>10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (H2O)</td>
<td></td>
<td>1.6b</td>
<td>1.9a</td>
</tr>
<tr>
<td>0.6% A</td>
<td></td>
<td>2.2b</td>
<td>3.3b</td>
</tr>
<tr>
<td>1.2% A</td>
<td></td>
<td>2.9b</td>
<td>3.6b</td>
</tr>
<tr>
<td>0.6% A + 0.046% F</td>
<td></td>
<td>2.7b</td>
<td>3.6b</td>
</tr>
<tr>
<td>0.6% A + 0.092% F</td>
<td></td>
<td>3.1c</td>
<td>4.0c</td>
</tr>
<tr>
<td>0.6% A + 0.184% F</td>
<td></td>
<td>3.1c</td>
<td>4.0d</td>
</tr>
<tr>
<td>0.6% A + 0.230% F</td>
<td></td>
<td>3.4c</td>
<td>4.5d</td>
</tr>
</tbody>
</table>

1Total mean scores were based on a six point scale (1 = extremely bright red, 6 = extremely discolored).
2Six replications.
3A = acetic acid, F = formic acid.
4Values with a common superscript within a row are not significantly different (P > 0.05).

TABLE 3. Percent reduction in counts of six cultures inoculated onto cubes and dipped 10 s in 1.2% acetic acid or distilled water.

<table>
<thead>
<tr>
<th>Culture</th>
<th>1.2% Acetic Acid</th>
<th>Distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella typhimurium</td>
<td>73.3b,x</td>
<td>42.1b,x</td>
</tr>
<tr>
<td>Shigella sonnei</td>
<td>62.2b,x</td>
<td>33.3b,x</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>69.7b,x</td>
<td>43.1b,x</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>45.8b,x</td>
<td>40.7b,x</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>77.2b,x</td>
<td>43.6b,x</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>62.1b,x</td>
<td>44.9b,x</td>
</tr>
</tbody>
</table>

1Four replications.
2Values with a common superscript within a row are not significantly different (P > 0.05).
3Values with a common superscript within a column are not significantly different (P > 0.05).
TABLE 4. Percentage reductions in numbers of Escherichia coli recovered from cubes of meat dipped in sanitizer or water for 1, 10 or 100 s.

<table>
<thead>
<tr>
<th>Time exposed to sanitizer (seconds)</th>
<th>Percent reduction in bacterial number1</th>
<th>0.6% Acetic Acid</th>
<th>0.046% Formic acid</th>
<th>Distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>14\textsuperscript{a,x}</td>
<td>22\textsuperscript{x}</td>
<td>-4\textsuperscript{b,x}</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>53\textsuperscript{a,y}</td>
<td>51\textsuperscript{a,y}</td>
<td>33\textsuperscript{b,y}</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>77\textsuperscript{a,z}</td>
<td>74\textsuperscript{a,z}</td>
<td>45\textsuperscript{b,y}</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Four replications.
\textsuperscript{a,b}Values with a common superscript within a row are not significantly different (P>.05).
\textsuperscript{x,y}Values with a common superscript within a column are not significantly different (P>.05).

counts significantly (P<.05) more than did distilled water. The two sanitizers reduced bacterial counts of inoculated meat more in the first 10 s than in the last 90 s.

Sensory panel on the flavor of baked ground beef

The sensory panel found no significant difference in flavor between meat dipped in 0.6% acetic acid plus 0.046% formic acid before grinding and baking and meat dipped in distilled water before grinding and baking. However, the panel differentiated between meat dipped in 1.2% acetic acid, ground and baked and meat dipped in distilled water, ground and baked.

CONCLUSION

It was concluded from these experiments, that meat treated with a solution of 0.6% acetic acid plus 0.046% formic acid did not differ significantly in color or in degree of reduced E. coli counts from meat treated with a solution of 1.2% acetic acid. When the latter solution was applied to beef, the resultant flavor was differentiated from the flavor of the beef control, whereas the flavor of beef dipped in the former solution was not. Hence, of the sanitizers tested, 0.6% acetic acid plus 0.046% formic acid was superior.

ACKNOWLEDGMENT

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REFERENCES