Control of *Listeria monocytogenes* on Frankfurters with Antimicrobials in the Formulation and by Dipping in Organic Acid Solutions

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ABSTRACT

The antilisterial activity of sodium lactate (SL) and sodium diacetate (SD) was evaluated in a frankfurter formulation and in combination with a dipping treatment into solutions of lactic acid or acetic acid after processing and inoculation. Pork frankfurters were formulated with 1.8% SL or 0.25% SD or combinations of 1.8% SL with 0.25 or 0.125% SD. After processing, frankfurters were inoculated (2 to 3 log CFU/cm²) with a 10-strain composite of *Listeria monocytogenes* and left undipped or were dipped (2 min) in 2.5% solutions of lactic acid or acetic acid (23 ± 2°C) before vacuum packaging and storage at 10°C for 40 days. Total microbial populations and *L. monocytogenes*, lactic acid bacteria, and yeasts and molds were enumerated during storage. Sensory evaluations also were carried out on frankfurters treated and/or formulated with effective antimicrobials. The combination of 1.8% SL with 0.25% SD provided complete inhibition of *L. monocytogenes* growth throughout storage. Dipping in lactic acid or acetic acid reduced initial populations by 0.7 to 2.1 log CFU/cm², but during storage (12 to 20 days), populations on dipped samples without antimicrobials in the formulation reached 5.5 to 7.9 log CFU/cm². For samples containing single antimicrobials and dipped in lactic acid or acetic acid, *L. monocytogenes* growth was completely inhibited or reduced over 12 and 28 days, respectively, whereas final populations were lower (*P < 0.05*) than those in undipped samples of the same formulations. Bactericidal effects during storage (reductions of 0.6 to 1.0 log CFU/cm² over 28 to 40 days) were observed in frankfurters containing combinations of SL and SD that were dipped in organic acid solutions. Inclusion of antimicrobials in the formulation and/or dipping the product into organic acid solutions did not affect (*P > 0.05*) the flavor and overall acceptability of products compared with controls. The results of this study may be valuable to meat processors as they seek approaches for meeting new regulatory requirements in the United States.

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Ready-to-eat (RTE) meat or poultry products have been implicated as sources of *Listeria monocytogenes* infection in humans (6–8). Among 23 categories of RTE foods, deli meats and nonreheated frankfurters were identified as products of highest risk for listeriosis on a preserving basis (10). Because frankfurters may be consumed without cooking or reheating, there is a need for additional interventions to ensure the safety of the product until consumption. Given the widespread distribution of *L. monocytogenes* in nature (32), its ability to grow under unfavorable conditions (13), and the high (20%) fatality rate associated with listeriosis (18), regulatory agencies have established requirements for *L. monocytogenes* control in RTE meat and poultry products. Recently, the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture established an interim final rule for control of *L. monocytogenes* in RTE meat and poultry products (12). Federally inspected establishments that produce RTE meat or poultry products that support growth of this pathogen and are exposed to the environment after cooking are required to use one of three alternatives for *L. monocytogenes* control (12):

(i) Establishments are required to apply a postlethality treatment (may be an antimicrobial agent) to reduce or eliminate *L. monocytogenes* and an antimicrobial agent or process to limit or suppress growth of the pathogen. Establishments selecting this alternative are not required to perform testing on food contact surfaces.

(ii) Establishments are required to employ either a postlethality treatment or a growth inhibitor. Establishments choosing a postlethality treatment are not required to perform testing of food contact surfaces; however, if an establishment selects to employ an inhibitory agent or process then its sanitation program must include testing of food contact surfaces.

(iii) Establishments will control the pathogen with sanitation measures only. Establishments that choose this option, however, are required to conduct testing of food contact surfaces to confirm the efficacy of sanitation procedures in the postlethality processing environment and to develop product-holding procedures when positive tests are obtained. Moreover, establishments that rely only on sanitation measures will be subject to the most frequent FSIS verification activity.
The use of appropriate concentrations and/or combinations of chemical compounds generally recognized as safe, such as various organic acids and their salts, may contribute to the safety of RTE meat products. The antilisterial activity of such compounds employed as dipping solutions (2, 13, 14, 19, 24, 26, 27) or as formulation ingredients (3–5, 9, 16, 17, 21–23, 26–31) has been investigated in various meat products. Results obtained from these studies may be valuable to the meat industry in its efforts to find effective methods for prevention or inhibition of *L. monocytogenes* growth in RTE products and meet the recent regulatory requirements (12). However, to our knowledge, only one study (23) has been published where inhibitory agents and a postlethality treatment were combined to control *L. monocytogenes* in an RTE meat product (alternative 1) (12). Results of this study (23) revealed that the combination of 1.8% sodium lactate (SL) with 0.25% sodium acetate, sodium diacetate (SD), or glucono-delta-lactone incorporated in the formulation inhibited *L. monocytogenes* growth in pork frankfurters for 120 days during storage at 4°C and that the antilisterial effect of the additives was enhanced when combined with postpackaging product heating. Additional studies are needed to examine whether and to what degree the antilisterial effectiveness of antimicrobials is enhanced by the application of postlethality treatments. Because irradiation is not permitted at this time for the treatment of RTE products, thermal pasteurization, high-pressure processing, and application (spraying or dipping) of organic acids are the most likely alternatives.

The aim of the present study was to investigate the antimicrobial effect of SL and SD added (singly or in combination) in the formulation of pork frankfurters (inhibitory treatment) in combination with immersion of the inoculated finished product into solutions of lactic or acetic acid (postlethality treatment). Product was stored at 10°C to simulate potential temperature abuse during transportation, retail sale, or consumer use. Sensory evaluations also were carried out to evaluate the influence of effective antilisterial treatments on the organoleptic properties of the product.

**MATERIALS AND METHODS**

**Preparation and treatment of pork frankfurters.** The basic frankfurter formulation (no antimicrobials included) consisted of fresh pork trimmings (82.2% wt/wt; approximately 30% fat), ice (10%), sodium chloride (2%), dextrose (2%), dry mustard (0.9%), corn syrup solids (2%), polyphosphate (0.4%; sodium tri-
FIGURE 2. Mean total microbial counts (TSAYE) on the surface of frankfurters with or without antimicrobials in the formulation that were inoculated with *Listeria monocytogenes* (2 to 3 log CFU/cm²) and then were left undipped (A) or were dipped into solutions of 2.5% lactic acid (B) or acetic acid (C), vacuum packaged, and stored at 10°C for 40 days. SL, sodium lactate; SD, sodium diacetate.

polyphosphate and sodium hexameta-phosphate, Heller, Inc., Bedford Park, Ill.), sodium nitrite (0.0156%), sodium erythorbate (0.05%), paprika (0.25%), onion powder (0.05%), garlic powder (0.05%), coriander (0.05%), and white pepper (0.05%) (3, 23). All spices and seasonings were purchased from AC Legg Co. (Birmingham, Ala.). Five formulation batches were prepared separately to contain (i) no antimicrobials (control); (ii) SL at 3% of a 60% (wt/wt) commercial product, equivalent to 1.8% pure SL (Purac, Inc., Lincolnshire, Ill.); (iii) 0.25% SD (Niacet, Niagara Falls, N.Y.); (iv) 1.8% SL combined with 0.25% SD; and (v) 1.8% SL combined with 0.125% SD. The meat and nonmeat ingredients of each batch were processed, extruded into 24-mm cellulose casings (Koch, Kansas City, Mo.), and cooked according to procedures described by Bedie et al. (3) and Samelis et al. (23). After cooking and smoking the frankfurters were showered with cool tap water for 5 min and stored overnight at 4°C. Frankfurters were then peeled manually and cut into pieces 10 cm in length (each having a surface area of 84.5 cm²). Frankfurters were weighed before and after cooking and cooling for determination of cooking yield.

**Product inoculation.** The 10-strain *L. monocytogenes* composite inoculum was prepared according to procedures described by Bedie et al. (3) and Samelis et al. (23, 24). The composite included Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b), and 103M (serotype 1a), all pork isolates; 558 (serotype 1/2, pork meat isolate); and PVM1, PVM2, PVM3, and PVM4 (pork variety meat isolates, serotype not known).

Two frankfurters from each treatment were transferred into a vacuum bag (15 by 20 cm, 3 mil standard barrier; nylon/PE vacuum pouch, Koch) and inoculated (0.25 ml from the appropriate dilution applied on each frankfurter) in a biological safety cabinet. The frankfurters were then massaged to spread the inoculum uniformly over the product surface. The inoculated frankfurters were allowed to stand for 15 to 30 min at 5°C to encourage bacterial attachment before they were treated with organic acid solutions and/or vacuum packaged (two frankfurters per bag).

**Immersion in organic acid solutions.** Frankfurters from each treatment were removed from the bags in which they were inoculated and dipped into 2.5% solutions of organic acids (at 23 ± 2°C) for 2 min or vacuum packaged in new vacuum bags (two frankfurters per bag; Hollymatic Corp., Countryside, Ill.) without treatment. The organic acids used were lactic acid (LA; Sigma Chemical Co., St. Louis, Mo.) and acetic acid (AA; Mallinckrodt and Baker, Inc., Phillipsburg, N.J.). Ten frankfurters per treatment were dipped into 1 liter of the acid solution and stirred occasionally for 2 min. The pH of the 2.5% solutions of LA and AA were 2.00 ± 0.02 and 2.50 ± 0.02, respectively. The pH of the acid solutions was monitored after dipping, and the solutions were changed when a >0.5 increase in pH was observed. After dipping, the frankfurters were drained, placed into a new vacuum bag (Koch), vacuum packaged (Hollymatic Corp.), and stored at 10°C.
FIGURE 3. Mean populations of lactic acid bacteria (MRS agar) on the surface of frankfurters with or without antimicrobials in the formulation that were inoculated with *Listeria monocytogenes* (2 to 3 log CFU/cm²) and then were left undipped (A) or were dipped into solutions of 2.5% lactic acid (B) or acetic acid (C), vacuum packaged, and stored at 10°C for 40 days. SL, sodium lactate; SD, sodium diacetate. Lactic acid bacteria counts were variable; counts obtained on day 20 for control samples were below the detectable limit of the analysis (~0.2 log CFU/cm²) in both replicates of the experiment.

**Microbiological analyses.** Samples were tested on days 0, 4, 8, 12, 20, 28, and 40 for total microbial populations on tryptic soy agar (TSA; Difco, Becton Dickinson, Sparks, Md.) supplemented with 0.6% yeast extract (YE; Acumedia, Baltimore, Md.); for *L. monocytogenes* on PALCAM agar (Difco, Becton Dickinson); for lactic acid bacteria (LAB) on deMan Rogosa Sharpe (MRS) agar, pH 5.5 (International BioProducts, Bothell, Wash.); and for yeasts and molds on rose bengal chloramphenicol (RBC) agar (Difco, Becton Dickinson). The pH of MRS agar was adjusted to 5.5 with 5 N hydrochloric acid (Mallinckrodt and Baker) to make the medium more selective for the enumeration of LAB. Each sample (two frankfurters) was placed in a sterile bag (Whirl-Pak, Nasco, Modesto, Calif.) with 50 ml of 0.1% buffered peptone water (Difco, Becton Dickinson) and shaken 30 times as described in the U.S. Meat and Poultry Inspection Regulation (11). Decimal dilutions were made with 9 ml of 0.1% buffered peptone water, and 0.1 ml of each sample was surface plated (PALCAM, TSAYE, RBC). For LAB, 1 ml of the dilution was added to 10 ml of molten (45°C) MRS agar. After setting, a 10-ml overlay of the molten agar was added to each plate. Plates were incubated for 72 h at 25°C (TSAYE, MRS, RBC) or for 48 h at 30°C (PALCAM).

**Physical and chemical properties.** The determination of cooking yields (%) of frankfurters formulated with or without antimicrobials was based on product weight before and after cooking and chilling (3). The pH of samples before storage was measured by placing approximately 40 g of product in a sterile bag (Whirl-Pak) and homogenizing for 120 s at 8.0 strokes per s with distilled water (1:10) (Masticator, IUL Instruments, Barcelona, Spain). The pH was then determined (Accumet 50, Fisher Scientific, Pittsburgh, Pa.) by immersing a pH electrode (Denver Instruments, Arvada, Colo.) in the bag containing the homogenate. Fat and moisture contents of samples for all treatment groups were determined according to the AOAC International official methods (950.46.B and 960.39, respectively) (1). The water activity of frankfurters was determined using an Aw Quick (Rotronic Instrument Corp., Huntington, N.Y.) water activity meter. Frankfurters of each treatment were portioned into small pieces, and approximately 5 g of each sample was placed in a plastic sample cup that was then placed in the vapor chamber. The water activity reading was obtained approximately 5 min later.

**Sensory evaluation.** Sensory analysis was performed to evaluate the effect of treatments on the sensory properties of frankfurters formulated with or without antimicrobials and/or treated with organic acid solutions. After cooking and cooling, frankfurters were peeled manually, cut into 3- to 6-cm pieces, either left undipped or immersed into 2.5% solutions of LA or AA for 2 min, and vacuum packaged. Samples were coded with
FIGURE 4. Mean populations of yeasts and molds (RBC agar) on the surface of frankfurters with or without antimicrobials in the formulation that were inoculated with *Listeria monocytogenes* (2 to 3 log CFU/cm²) and then were left undipped (A) or were dipped into solutions of 2.5% lactic acid (B) or acetic acid (C), vacuum packaged, and stored at 10°C for 40 days. SL, sodium lactate; SD, sodium diacetate.

An untrained panel of 25 consumers from the Department of Animal Sciences at Colorado State University was used to evaluate frankfurters for appearance, odor, flavor, and overall acceptability on a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely). The color (1 = extremely pale; 9 = extremely dark) and texture (1 = extremely soft; 9 = extremely firm) of the frankfurters also were evaluated. For purposes of this study, frankfurters were not heated before they were tasted, but panelists were given the option of not eating the unheated samples. All of the panelists opted to taste the frankfurters.

**Statistical analysis.** The experiment was conducted twice, and for each replicate three individual samples (two frankfurters each) were analyzed on each sampling day and for each treatment.

**TABLE 1. Sensory evaluation scores from an untrained panel of tasters for pork frankfurters with or without antimicrobials in the formulation**

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Control</th>
<th>1.8% SL + 0.25% SD</th>
<th>1.8% SL + 0.125% SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.8 ± 1.4 A</td>
<td>5.3 ± 1.4 A</td>
<td>5.7 ± 1.2 A</td>
</tr>
<tr>
<td>Color&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.2 ± 0.9 A</td>
<td>5.5 ± 1.2 B</td>
<td>5.4 ± 1.0 B</td>
</tr>
<tr>
<td>Odor&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.5 ± 1.2 A</td>
<td>5.3 ± 1.2 A</td>
<td>5.5 ± 1.5 A</td>
</tr>
<tr>
<td>Flavor acceptability&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.5 ± 1.6 A</td>
<td>5.8 ± 1.4 A</td>
<td>6.0 ± 1.7 A</td>
</tr>
<tr>
<td>Texture&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.0 ± 1.9 A</td>
<td>5.6 ± 1.5 A</td>
<td>6.0 ± 1.5 A</td>
</tr>
<tr>
<td>Overall acceptability&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.6 ± 1.4 A</td>
<td>5.6 ± 1.3 A</td>
<td>6.0 ± 1.1 A</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are means ± standard deviations; n = 25. SL, sodium lactate; SD, sodium diacetate. Means with different letters within a row are significantly different (*P* < 0.05).

<sup>b</sup> 1, dislike extremely; 9, like extremely.

<sup>c</sup> 1, extremely pale; 9, extremely dark.

<sup>d</sup> 1, extremely soft; 9, extremely firm.
TABLE 2. Sensory evaluation scores from an untrained panel of tasters for pork frankfurters with or without antimicrobials in the formulation and dipped or not dipped in a 2.5% solution of LA

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Undipped control</th>
<th>Control</th>
<th>1.8% SL</th>
<th>0.25% SD</th>
<th>1.8% SL + 0.25% SD</th>
<th>1.8% SL + 0.125% SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>6.1 ± 1.2 A</td>
<td>5.6 ± 1.1 AB</td>
<td>5.6 ± 1.5 AB</td>
<td>5.3 ± 1.3 B</td>
<td>5.0 ± 1.5 B</td>
<td>5.6 ± 1.3 AB</td>
</tr>
<tr>
<td>Colorb</td>
<td>5.2 ± 1.6 A</td>
<td>5.0 ± 1.5 A</td>
<td>4.6 ± 1.3 A</td>
<td>4.5 ± 1.5 A</td>
<td>5.4 ± 1.7 A</td>
<td>5.2 ± 1.5 A</td>
</tr>
<tr>
<td>Odorb</td>
<td>6.1 ± 1.3 A</td>
<td>5.6 ± 1.0 AB</td>
<td>5.4 ± 1.0 BC</td>
<td>5.4 ± 1.3 BC</td>
<td>4.8 ± 1.4 C</td>
<td>5.5 ± 1.2 AB</td>
</tr>
<tr>
<td>Flavor acceptabilityb</td>
<td>5.8 ± 1.3 A</td>
<td>6.0 ± 1.5 A</td>
<td>6.0 ± 1.1 A</td>
<td>5.8 ± 1.7 A</td>
<td>6.2 ± 1.5 A</td>
<td>6.2 ± 1.4 A</td>
</tr>
<tr>
<td>Textured</td>
<td>6.3 ± 1.7 A</td>
<td>6.3 ± 1.5 A</td>
<td>5.6 ± 1.8 AB</td>
<td>5.6 ± 1.6 AB</td>
<td>5.2 ± 1.5 B</td>
<td>5.8 ± 1.5 AB</td>
</tr>
<tr>
<td>Overall acceptabilityb</td>
<td>5.9 ± 1.2 A</td>
<td>6.0 ± 1.0 A</td>
<td>6.0 ± 1.1 A</td>
<td>5.9 ± 1.4 A</td>
<td>5.9 ± 1.5 A</td>
<td>6.0 ± 1.5 A</td>
</tr>
</tbody>
</table>

a Values are means ± standard deviations; n = 25. SL, sodium lactate; SD, sodium diacetate; LA, lactic acid. Means with different letters within a row are significantly different (P < 0.05).
b 1, dislike extremely; 9, like extremely.
c 1, extremely pale; 9, extremely dark.
d 1, extremely soft; 9, extremely firm.
(n = 6). The microbiological data were converted to log CFU/cm² based on the sample surface (169 cm²; two frankfurters per bag) and the volume of buffer peptone water (50 ml) added to each sample. Data were analyzed using the mixed procedure of SAS (25). Independent variables included treatment, time, and dipping and the interactions of treatment × time, dipping × time, treatment × dipping, and treatment × time × dipping. The sensory evaluation data (n = 25) were analyzed using the mixed procedure of SAS. Means and standard deviations for microbiological and sensory evaluation data were calculated, and the mean differences were separated with the least significant difference procedure at the significance level of 95%.

RESULTS AND DISCUSSION

Physical and chemical properties. Cooking yields ranged from 88.0 ± 1.2% (1.8% SL combined with 0.125% SD) to 94.6 ± 0.5% (1.8% SL) (data not shown). The cooking yield of control samples was 92.9 ± 1.2%. Frankfurters that contained SL combined with 0.125% or 0.25% SD had lower cooking yields (P < 0.05) compared with controls and other formulation treatments. The average moisture and fat contents were not different (P > 0.05) among treatments (data not shown). Moisture contents ranged from 51.9 ± 1.6% (control) to 53.1 ± 1.5% (1.8% SL combined with 0.125% SD). Control samples (no antimicrobials in the formulation) had the lowest moisture content, which resulted in a higher fat content (25.2%), compared with treated frankfurters, whereas the lowest fat content (23.1 ± 1.3%) was observed in samples that contained 0.25% SD.

The pH of control samples (day 0) was 6.07 ± 0.04 (data not shown). Treatments that caused pH reductions on day 0 were 0.25% SD (5.54 ± 0.05) and 1.8% SL combined with 0.25% SD (5.58 ± 0.01). The pH values for samples that contained 1.8% SL and 1.8% SL combined with 0.125% SD were 6.05 ± 0.07 and 6.00 ± 0.00, respectively. Dipping in LA reduced the pH by 0.3 to 0.4 pH units, and dipping in AA caused a reduction of 0.2 to 0.4 units. The samples that contained 0.25% SD and were dipped in AA or LA had the lowest initial pH values of 5.47 ± 0.06 and 5.69 ± 0.08, respectively.

The water activity of samples ranged from 0.951 ± 0.001 (samples formulated with 1.8% SL) to 0.972 ± 0.015 (samples formulated with 0.25% SD and dipped in AA) (data not shown). The latter samples, and the undipped samples that contained 0.25% SD and the control samples

TABLE 3. Sensory evaluation scores from an untrained panel of tasters for pork frankfurters with or without antimicrobials in the formulation and dipped or not dipped in a 2.5% solution of AA

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Undipped control</th>
<th>Control</th>
<th>1.8% SL</th>
<th>0.25% SD</th>
<th>1.8% SL + 0.25% SD</th>
<th>1.8% SL + 0.125% SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>5.5 ± 1.5 A</td>
<td>5.0 ± 1.5 A</td>
<td>5.2 ± 1.0 A</td>
<td>5.2 ± 1.2 A</td>
<td>5.1 ± 1.3 A</td>
<td>5.4 ± 1.3 A</td>
</tr>
<tr>
<td>Colorb</td>
<td>4.7 ± 1.6 AB</td>
<td>4.2 ± 1.5 A</td>
<td>4.7 ± 1.1 AB</td>
<td>5.2 ± 1.6 B</td>
<td>4.9 ± 1.2 B</td>
<td>5.0 ± 1.2 B</td>
</tr>
<tr>
<td>Odorb</td>
<td>5.3 ± 1.2 A</td>
<td>5.3 ± 1.2 A</td>
<td>5.2 ± 1.1 A</td>
<td>5.2 ± 0.9 A</td>
<td>4.9 ± 1.7 A</td>
<td>5.4 ± 1.0 A</td>
</tr>
<tr>
<td>Flavor acceptabilityb</td>
<td>5.5 ± 1.6 A</td>
<td>5.8 ± 1.4 AB</td>
<td>5.3 ± 1.6 A</td>
<td>5.8 ± 1.6 AB</td>
<td>5.9 ± 1.8 B</td>
<td>6.0 ± 1.4 B</td>
</tr>
<tr>
<td>Textured</td>
<td>6.4 ± 1.6 A</td>
<td>6.3 ± 1.4 A</td>
<td>6.0 ± 1.7 A</td>
<td>5.3 ± 1.4 A</td>
<td>5.9 ± 1.6 A</td>
<td>6.2 ± 1.6 A</td>
</tr>
<tr>
<td>Overall acceptabilityb</td>
<td>5.6 ± 1.3 A</td>
<td>5.5 ± 1.7 A</td>
<td>5.5 ± 1.4 A</td>
<td>5.7 ± 1.5 A</td>
<td>5.5 ± 1.6 A</td>
<td>6.0 ± 1.5 A</td>
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b 1, dislike extremely; 9, like extremely.
c 1, extremely pale; 9, extremely dark.
d 1, extremely soft; 9, extremely firm.
dipped in LA (water activities of 0.967 ± 0.011 and 0.967 ± 0.009, respectively), had significantly higher water activity values (P < 0.05) than did undipped control samples (0.956 ± 0.003). The results of this study are in agreement with previous findings by Samelis et al. (23) in indicating that although combinations of antimicrobials did not decrease and sometimes even increased the water activity of the product, they provided effective inhibition of *L. monocytogenes*.

**Microbiological data.** Under the conditions of this study, populations of *L. monocytogenes* in untreated frankfurters (inoculated controls, no antimicrobials in the formulation and no dipping) exceeded 7.0 log CFU/cm² after 12 days (Fig. 1). The addition of 1.8% SL or 0.25% SD in the formulation resulted in slower *L. monocytogenes* growth (P < 0.05) compared with that observed in untreated (no antimicrobials in the formulation and no dipping) samples. At a concentration of 0.25%, SD appeared more effective than 1.8% SL because the SD treatment resulted in a more extended lag phase and in slower growth (P < 0.05) compared with the SL treatment. These results are in agreement with those of Mbandi and Shelef (16), who noted the superior antilisterial activity of SD (0.1 or 0.2%) compared with that of SL (1.8 or 2.5%). Combinations of antimicrobials in the formulation caused significantly greater inhibition of growth (P < 0.05) than that observed when antimicrobials used singly. In samples that contained 1.8% SL and 0.25% SD, growth was completely prevented throughout storage (Fig. 1), whereas 1.8% SL and 0.125% SD resulted in complete inhibition of *L. monocytogenes* growth during the first 8 days of storage; subsequent growth was significantly reduced (P < 0.05) compared with that observed in samples containing single antimicrobials. Dipping of frankfurters in solutions of LA or AA caused reductions (P < 0.05) of 0.7 to 2.1 and 0.7 to 1.7 log CFU/cm², respectively, in the initial *L. monocytogenes* counts. However, *L. monocytogenes* counts in control samples (no antimicrobials in the formulation) that were dipped in LA or AA reached approximately 8 log CFU/cm² in 28 to 40 days, suggesting that although immersion in LA or AA may reduce initial populations and then cause inhibition during storage, eventually counts may reach high levels. In contrast, in samples that were formulated with antimicrobials, *L. monocytogenes* counts in the dipped products were lower (P < 0.05) than those in undipped samples of corresponding formulations throughout storage. Dipping of samples formulated with either combination of SL and SD into LA or AA resulted in listericidal effects. These findings are of importance because they indicate that inclusion of antimicrobials in the formulation combined with dipping of the finished products into antimicrobial solutions resulted in reduction of initial populations and inhibitory or even bactericidal effects during product storage.

Trends in microbial growth on TSAYE (Fig. 2) were similar to those observed on PALCAM for all treatments, especially during the first days of storage, suggesting that the majority of colonies found on TSAYE were *L. monocytogenes*. The higher counts obtained on TSAYE, mainly during the last days of storage, may have reflected growth of spoilage organisms. Total microbial populations (TSAYE) in uninoculated and inoculated controls (non-dipped) reached 6.1 and 8.3 log CFU/cm², respectively, in 28 to 40 days (Fig. 2).

LAB were the predominant natural flora found in the product (Fig. 3). The acidified MRS agar used in this study is generally selective for *Lactobacillus* spp., which are the LAB expected to predominate in meat products. Changes in LAB populations during storage were variable, suggesting that LAB counts were influenced by factors such as levels of initial contamination, cross-contamination during product handling, and the effectiveness of the treatments. LAB proliferated (P < 0.05) in untreated samples (inoculated controls) and in samples containing 0.25% SD, where populations reached 6.8 and 6.7 log CFU/cm², respectively, in 28 to 40 days. In uninoculated control samples, final LAB populations on day 40 reached 4 log CFU/cm², whereas total microbial counts in the same samples on the same day reached 6.1 CFU/cm² (Fig. 2). In samples dipped in LA, all formulation treatments seemed to allow LAB growth compared with the controls (dipped in LA) (Fig. 3); however, it cannot be concluded that growth of *L. monocytogenes* in control samples (dipped in LA) inhibited growth of LAB because the opposite effect (effective antilisterial treatments also inhibited LAB more effectively compared with controls) was observed in samples dipped in AA (Fig. 3).

Colonies observed on RBC agar were exclusively yeasts. In general, no major growth of yeasts was observed throughout storage in untreated or treated samples (Fig. 4). The highest final counts (P < 0.05), approximately 2 log CFU/cm², were obtained from all nondipped samples that contained SD, either alone or in combination with SL, and from samples that contained 0.125% SD combined with SL and that were dipped in LA.

The results of this study are in agreement with those of other studies performed in our laboratory (3, 23) regarding the effectiveness of antimicrobials incorporated in the formulation of the product. However, in this study we also evaluated the effect of antimicrobials added to the formulation in combination with antimicrobials applied as dipping solutions after processing.

Results indicated that combinations of two antimicrobials in the formulation provided greater inhibition of *L. monocytogenes* growth compared with antimicrobials used individually, whereas when two antimicrobials in the formulation were combined with postprocessing dipping in organic acid solutions listericidal effects were observed. Thus, the antilisterial effect of combination treatments used in the formulation of the product appeared to increase when the product was dipped into organic acid solutions.

**Sensory analysis.** Panelists were primarily college students (77.8%), and high percentages were males (62.6%) and persons between the ages of 21 and 35 (76.8%). About 10.1% reported that they eat frankfurters once a week or more often, 42.4% eat them one to three times a month, 44.5% eat them one to six times a year, and 3% reported
that they never eat frankfurters. The majority of the panelists (81.9%) reported that they cook frankfurters before eating them.

Under the conditions of this study, addition of antimicrobials in the formulation of frankfurters (Table 1) did not affect the appearance, odor, flavor, texture, and overall acceptability of the samples ($P > 0.05$) compared with the controls. Samples containing 1.8% SL and 0.125% SD received the highest scores for flavor (6.0) and overall acceptability (6.0). Dipping in LA (Table 2) did not affect the flavor and the overall acceptability of products with or without antimicrobials in the formulation ($P > 0.05$). The lowest scores for flavor (5.8) were obtained for undipped control samples and samples containing 0.25% SD. Samples dipped in AA (Table 3) had similar or even higher scores for flavor and overall acceptability compared with those received by undipped control samples ($P > 0.05$). The highest scores for flavor were given to samples containing 1.8% SL and 0.125% SD, followed by samples with 1.8% SL and 0.25% SD (6.0 and 5.9, respectively).

The results of this study indicated that dipping into LA or AA did not cause negative effects on the flavor acceptability of frankfurters, and products with combination treatments received the highest scores for flavor compared with controls and samples that contained single antimicrobials. Dipping into AA did not have adverse effects on odor. However, dipping into LA seemed to have a negative effect on odor, especially for samples that contained 1.8% SL and 0.25% SD. Because the antilisterial effects of either 1.8% SL plus 0.25% SD or 1.8% SL plus 0.125% SD followed by dipping into organic acid solutions were similar (as indicated above), there may be no need for using the increased amount (0.25%) of SD in the formulation of the product. Combinations of antimicrobials used at reduced levels appeared to have fewer negative effects on the sensory properties of the products than did single antimicrobials used at increased concentrations.

Papadopoulos et al. (20) reported that although 3% and 4% SL contained in cooked beef rounds resulted in higher cooking yields and improved color, these additives resulted in mild throat irritations and sodium aftertastes for panelists. In another study, Blom et al. (4) found that the combination of 2.5% SL and 0.25% sodium acetate included in the formulation of servelat sausage and cooked ham resulted in a sensory-acceptable product. However, although panelists rated treated and untreated ham equally, they seemed to prefer servelat sausage without antimicrobials in the formulation, suggesting that acceptability is product dependent. Thus, it is not clear whether or how treatments used in the present study may affect the sensory characteristics of other meat products.

The results indicated that under the conditions of this study, treatments that combined incorporation of SL and SD (singly or in combination) in the formulation of the product and dipping in LA or AA solutions provided effective control of L. monocytogenes in frankfurters stored at 10°C. The results of this study may be useful to the RTE meat product industry in its efforts to meet the recent regulatory requirements (12) for control of L. monocytogenes. However, levels and conditions of use of the antimicrobials tested in this study must be refined and validated for use in specific product types and formulations.

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REFERENCES


