Antimicrobials in the Formulation To Control *Listeria monocytogenes* Postprocessing Contamination on Frankfurters Stored at 4°C in Vacuum Packages

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MS 01-128: Received 30 March 2001/Accepted 19 June 2001

ABSTRACT

Postprocessing contamination of cured meat products with *Listeria monocytogenes* during slicing and packaging is difficult to avoid, and thus, hurdles are needed to control growth of the pathogen during product storage. This study evaluated the influence of antimicrobials, included in frankfurter formulations, on *L. monocytogenes* populations during refrigerated (4°C) storage of product inoculated (10³ to 10⁴ CFU/cm²) after peeling of casings and before vacuum packaging. Frankfurters were prepared to contain (wt/wt) sodium lactate (3 or 6%, as pure substance of a liquid, 60% wt/wt, commercial product), sodium acetate (0.25 or 0.5%), or sodium diacetate (0.25 or 0.5%). *L. monocytogenes* populations (PALCAM agar and Trypticase soy agar plus 0.6% yeast extract [TSAYE]) exceeded 10⁶ CFU/cm² in inoculated controls at 20 days of storage. Sodium lactate at 6% and sodium diacetate at 0.5% were bacteriostatic, or even bactericidal, throughout storage (120 days). At 3%, sodium lactate prevented pathogen growth for at least 70 days, while, in decreasing order of effectiveness, sodium diacetate at 0.25% and sodium acetate at 0.5 and 0.25% inhibited growth for 20 to 50 days. Antimicrobials had no effect on product pH, except for sodium diacetate at 0.5%, which reduced the initial pH by approximately 0.4 U. These results indicate that concentrations of sodium acetate currently permitted by the U.S. Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) (0.25%) or higher (0.5%) may control growth of *L. monocytogenes* for approximately 30 days, while currently permitted levels of sodium lactate (3%) and sodium diacetate (0.25%) may be inhibitory for 70 and 35 to 50 days, respectively. Moreover, levels of sodium lactate (6%) or sodium diacetate (0.5%) higher than those presently permitted by the USDA-FSIS may provide complete control at 4°C of growth (120 days) of *L. monocytogenes* introduced on the surface of frankfurters during product packaging.

Sodium, potassium, or other salts of lactic, acetic, and other organic acids have demonstrated significant antimicrobial activity in broth or simulated foods (e.g., meat juices or slurries) under laboratory conditions (8, 13, 17, 27, 30, 31). Recent interest in use of lactates, mainly sodium lactate in its commercially available liquid (60% wt/wt) form, and sodium acetate as additives in processed meat formulations is associated with their potential to inhibit spoilage or pathogenic bacteria, especially *Listeria monocytogenes* (6, 16, 20, 24, 28, 29, 33, 35). Accordingly, sodium lactate has been incorporated as an additional hurdle parameter in mathematical models specifically designed to predict shelf life of cooked meat products (12), while sodium lactate or sodium acetate has also been incorporated as a hurdle against *L. monocytogenes* in mathematical models used to predict product safety (7, 18, 22).

Despite scientific evidence for the antilisterial activity of lactates (17, 29, 31), acetates (20, 28, 30), and other chemical compounds (2, 8, 13, 20), there is uncertainty regarding their effective concentrations in cured meat formulations. The antimicrobial effects of these additives, singly or in combination and in the presence of other antimicrobials, against *L. monocytogenes* depend on processing (pH, water activity, moisture, fat, nitrite, and salt content of the product) and storage (temperature and packaging atmosphere) conditions (8, 11, 18, 19, 22). Furthermore, the level of contamination of the meat product surface with *L. monocytogenes* is another important factor that may affect activity of these additives in challenge studies. For example, Wederquist et al. (35, 36) showed that, in decreasing order of effectiveness, 0.5% sodium acetate and 2% sodium lactate used alone extended the lag phase of artificially contaminated (2 to 3 log CFU/g) *L. monocytogenes* to approximately 35 days on vacuum-packaged turkey bologna when added singly in the formulation. Neither of those additives alone, however, inhibited growth after 70 to 90 days of storage at 4°C. Furthermore, combinations of sodium lactate (2%) and sodium acetate (0.5%) with potassium sorbate (0.26%) and sodium bicarbonate (1%) were less effective than when these chemicals were added singly, a finding attributed mainly to the increase of bologna pH to values >7.0 due to inclusion of sodium bicarbonate (35, 36). Qvist et al. (23) reported that 2% sodium lactate with 0.25% glucono-delta-lactone suppressed growth of *L. monocytogenes* on sliced, vacuum-packaged bologna at 5°C more than when either compound was used alone. Suppression of *L. monocytogenes* growth also occurred in vacuum-packaged bologna stored at 10°C when the product contained combinations of 2% sodium lactate with 0.25 or 0.5% glucono-

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delta-lactone (23). In contrast, 2% sodium lactate used alone in a bologna formulation was ineffective against L. monocytogenes when storage was at 10°C and was effective for 28 days at 5°C (23). Moreover, these products were stored for up to 35 days (23), which is considerably less than the targeted commercial shelf life (i.e., 75 to 90 days) of refrigerated cooked meat products in the United States (35). In this study (35), the pathogen inoculation level was as low as 100 CFU/g (23), while Junger et al. (19) reported that 10 CFU/g of inoculated L. monocytogenes was inhibited by combinations of 2% sodium lactate with either 0.5% sodium acetate or 0.25% glucono-delta-lactone on sliced saveloy sausage stored under 80% N₂/20% CO₂ at 5 or 10°C for up to 28 days. Higher concentrations of antimicrobials may be needed in cured meat formulations when used singly or when the contamination levels are higher. For example, Weaver and Shelef (34) reported that 3%, but not 2%, sodium lactate prevented growth of inoculated L. monocytogenes in pork liver sausage at 5°C for 50 days. Blom et al. (6) showed that 2.5% sodium lactate combined with 0.25% sodium acetate were adequate to inhibit growth of L. monocytogenes introduced (3 log CFU/g) after heating on sliced, vacuum-packaged servelat sausage stored at 4 or 9°C, and cooked ham at 4°C, for 35 days. On ham stored at 9°C, this mixture extended the lag phase of the pathogen to 10 to 20 days but eventually did not inhibit growth (6). Thus, effective concentrations in combinations of lactates and acetates may become ineffective against L. monocytogenes in meat products with high moisture and low fat contents (5, 15, 25) following post-processing contamination with increased pathogen numbers and subsequent storage at elevated temperatures (6). Lactates, acetates, and other preservatives that are found to be effective in combination (6, 8, 19, 25) may be of reduced effectiveness when used as single antimicrobials in meat product formulations.

On this basis, more research is needed to examine inhibitory concentrations of sodium lactate and other additives as influenced by processing and storage conditions, when used as single or combined antimicrobial hurdles. Data from such research may be useful in efforts by the industry to control L. monocytogenes in meat products (4, 10, 32). In 2000, the U.S. Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) increased the permissible level of sodium lactate to 3% (i.e., 4.8% of a commercially available product) and approved the use of 0.25% sodium acetate and sodium diacetate as flavoring and antimicrobial agents in cured meat products (14). The USDA-FSIS also indicated (14) that concentrations of sodium acetate and sodium diacetate could be increased, while their approval for use as potassium salts could be considered after relevant scientific data become available. Therefore, the objective of this study was to evaluate currently permissible, and increased, levels of sodium lactate, sodium acetate, and sodium diacetate included in frankfurter formulations as antilisterial agents during refrigerated (4°C) storage of products inoculated after peeling of casings and before vacuum packaging.

### MATERIALS AND METHODS

#### Bacterial strains.

Ten strains of L. monocytogenes, Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 103M (serotype 1a), 101M (serotype 4b), isolated from pork sausage, 558 (serotype 1/2, pork meat isolate), and PVM1, PVM2, PVM3, and PVM4 (pork variety meat isolates, serotype not known) (3), were used in this study. All strains were available as frozen (−30°C) cultures in Trypticase soy broth (BBL, Becton Dickinson Co., Cockeysville, Md.) with 0.6% yeast extract (TSBYE; Difco, Becton Dickinson Co., Sparks, Md.) plus 20% glycerol (Malhinkrodt Specialty Chemicals Co., Paris, Ky.) and were activated by transferring a loopful of stock culture in 10 ml of TSBYE at 30°C for 24 h. Working cultures were kept on Trypticase soy agar (BBL) plus 0.6% yeast extract (TSAYE) at 4°C and transferred monthly. Strains were subcultured twice in TSBYE (30°C, 24 h) before use in the experiments.

#### Preparation of pork frankfurters.

Fresh pork trimmings of approximately 30% fat were obtained from Swift Co., Greeley, Colo. The basil frankfurter mixture consisted of (% of total weight in the formulation): pork trimmings (82.2%), water as ice (10), dextrose (2), corn syrup solids (2), sodium chloride (2), dry mustard (0.9), polyphosphate (sodium tripolyphosphate and sodium hexametaphosphate. Heller, Inc., Bedford Park, Ill.) (0.4), 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). Spices and seasonings were purchased from AC Legg Co., Birmingham, Ala. The mixture was divided into batches of equal weight (9.1 kg), and the following compounds were added (wt/wt) to each batch as single antimicrobials in the formulation: sodium acetate (0.25 or 0.5%), Sigma Chemical Co., St. Louis, Mo.), sodium diacetate (0.25 or 0.5%), Nicelt, Niagara Falls, N.Y.), and sodium lactate (3 or 6%, 60 wt/wt, Purac Inc., Lincolnshire, Ill.). Another batch of the product mixture was processed without addition of antimicrobials to serve as the control.

The meat and other ingredients of each treatment batch were emulsified in a Meissner 35-liter bowl chopper (RMP, Kansas City, Mo.) at high speed for 3 to 5 min (3,000-rpm blade speed and 18-rpm bowl speed). The final temperature of the batter after mixing was 15.5°C. After mixing, the frankfurter batters were extruded (Zescomobile 80012, Eden Prairie, Minn.) into 24-mm-diameter, fibrous cellulose casings (Koch, Kansas City, Mo.) and weighed. Frankfurters were cooked in a smokehouse (Alkar, Lodi, Wis.) to a final internal temperature of 70°C. The cooking schedule was as follows. Initially, frankfurters were cooked in dry air for 30 min (smokehouse temperature, 80°C) followed by hot smoking (Zesti liquid smoke, Hickory Specialties Inc., Crossville, Tenn.) for 30 min. After smoking, frankfurters were cooked with steam for 30 min (smokehouse temperature, 60°C; relative humidity, 26%); then, the smokehouse temperature was increased to 80°C, and cooking was continued until the product reached its final internal temperature. After cooking, frankfurters were shored for 5 min with cool water and reweighed after overnight cooling at 4°C to determine the cooking yield. Then, the casings were removed manually, and frankfurters (11-cm length) were obtained, yielding an external surface of 83 cm². Peeled frankfurters were transferred to the microbiology laboratory for inoculation, vacuum packaging, storage, and testing (as described below) within 2 h.

#### Preparation of L. monocytogenes inoculum.

One-milliliter aliquots of TSBYE cultures (30°C for 24 h) of each L. monocytogenes strain were combined in a sterilized conical 15-ml tube (Nalgene, Nalge Co., Rochester, N.Y.), centrifuged (3,000 rpm for...
15 min), and washed with sterile phosphate-buffered saline. The phosphate-buffered saline was composed of 1.2 g of anhydrous Na$_2$HPO$_4$ (Sigma), 0.22 g of NaH$_2$PO$_4$·H$_2$O (Sigma), and 8.5 g of NaCl (Sigma) per liter, adjusted to pH 7.4 with 0.1 N HCl or NaOH and sterilized at 121°C for 15 min. The mixed culture was serially diluted with phosphate-buffered saline to a concentration capable of giving 10$^3$ to 10$^6$ CFU/cm$^2$ of frankfurters, when inoculated with 0.25 ml per frankfurter. To confirm the desired concentration of cells, the inoculum was serially diluted and plated on two microbiological media, TSAYE and PALCAM (Difco) agars, as will be described in following paragraphs.

**Product inoculation and sampling.** Peeled frankfurters were placed under a biosafety cabinet and surface inoculated with the *L. monocytogenes* composite as follows. For each sample, two frankfurters were aseptically transferred into vacuum bags (20 by 25 cm, 3-mil std barrier, nylon/PE vacuum pouch, Koch), and 0.25 ml of composite inoculum was deposited along each frankfurter inside the bag. The inoculum was spread over the surface of frankfurters by swirling the sample by hand from the outside of the bag. Treatments with uninoculated or inoculated frankfurters, without antimicrobials, were also prepared and served as controls. Frankfurters were vacuum packaged (Multivac, Germany) at 80 mm Hg and stored at 4°C for up to 120 days. Samples were analyzed microbiologically, and pH determinations were obtained immediately after inoculation (day 0) and at days 10, 20, 35, 50, 70, 90, and 120. Also, frankfurter samples were analyzed, as described below, for water activity, moisture, and fat content.

**Microbiological analyses.** On each sampling day, three samples (of two frankfurters/bag) from each treatment were transferred into individual sterile stomacher bags (Whirl-Pak, Nasco), mixed with 100 ml of 0.1% buffered peptone water (Difco), and pumped in a stomacher (Masticator IUL Instruments, Barcelona, Spain) for 30 s. For each sample, serial decimal dilutions were prepared and then plated by spreading 0.1 ml in duplicate on TSAYE and PALCAM agar plates. Colonies on plates were counted after incubation at 30°C for 48 h. PALCAM was used to selectively enumerate *L. monocytogenes*, while TSAYE was used as a nonselective medium to evaluate potential differences in recovery of the inoculated pathogen on TSAYE, compared to PALCAM, and to give an overall picture of contaminants able to form colonies at 30°C, calculated on the basis of the frankfurter surface (i.e., 166 cm$^2$; two frankfurters/bag) by 83 cm$^2$ of frankfurters, when inoculated with 0.25 ml per frankfurter. To confirm the desired concentration of cells, the inoculum was serially diluted and plated on two microbiological media, TSAYE and PALCAM (Difco) agars, as will be described in following paragraphs.

**Chemical analyses.** The pH of each sample was determined by immersing a pH electrode (Denver Instruments, Arvada, Colo.) in the stomacher bag after samples were plated. An Accumet 50 digital pH meter (Fisher Scientific, Houston, Tex.) was used for the measurement. Moisture and fat contents were determined according to the AOAC International (1) official methods 950.46 and 960.39, respectively, for meat and meat products. The water activity ($a_w$) was determined with a Rotronic $a_w$ meter (Quick Instrument Corp., Huntington, N.Y.), according to the manufacturer’s instructions, and AOAC International (1) official method 978.18 for the preparation of the standard $a_w$ curve. Also, the cooking yield (%) of each treatment was determined based on the weights of the product before and after cooking.

**Data analysis.** All experiments were replicated three different times and, for each replicate, three individual samples were analyzed on each sampling day. The data were converted to log CFU/cm$^2$ and analyzed using the General Linear Model procedure of SAS (26). Independent variables included treatment and time as well as treatment-time interactions. Means and standard deviations were calculated, and, when F-values were significant at the $P \leq 0.05$ level, mean differences were separated by the Least Significant Difference procedure (26).

**RESULTS AND DISCUSSION**

In frankfurters without antimicrobials in the formulation, inoculated (3.2 to 3.4 log CFU/cm$^2$) *L. monocytogenes* exceeded ($P < 0.05$) 6 log CFU/cm$^2$ at 20 days and eventually increased above 8 logs with prolonged storage at 4°C (Table 1). This result was consistent with findings of previous studies describing the ability of the pathogen to proliferate (>5 log CFU/g or cm$^2$) in frankfurters and other cured meat products prepared without antimicrobials, when introduced on their surface after heating (5, 9, 15, 35, 36).

At 6%, sodium lactate was bacteriostatic, or even bactericidal ($P < 0.05$), throughout storage (120 days), whereas frankfurters prepared with 3% sodium lactate (i.e., the currently permitted level by the USDA-FSIS) were stored for 90 days at 4°C before a significant ($P < 0.05$) increase in pathogen populations occurred, while counts never reached 8 log CFU/cm$^2$ (Table 1). In contrast, 0.25% (i.e., the currently permitted level by the USDA-FSIS) and 0.5% sodium acetate permitted significant ($P < 0.05$) growth of *L. monocytogenes* at 35 and 50 days of storage, respectively. Compared to the inoculated control samples, however, 0.5% and, to a lesser extent, 0.25% sodium acetate slowed the growth of *L. monocytogenes* and depressed ($P < 0.05$) its population density during storage of frankfurters (Table 1). Sodium diacetate was more effective than sodium acetate against *L. monocytogenes*, because at equal concentrations of 0.25% (i.e., the currently permitted level by the USDA-FSIS), the former antimicrobial extended the lag phase by approximately 15 days compared to the latter. Practically, though, at 0.25%, sodium diacetate permitted growth ($P < 0.05$) of *L. monocytogenes* at 50 days of storage at 4°C. At 0.5%, however, sodium diacetate had a strong bacteriostatic, or even bactericidal ($P < 0.05$), effect from days 0 to 120, which was comparable to that of 6% sodium lactate (Table 1).

Plating on either PALCAM (Table 1) or TSAYE agar (Table 2) resulted in comparable results leading to similar conclusions. It was observed that the concentrations of antimicrobials that inhibited or slowed growth of *L. monocytogenes* on PALCAM (Table 1) also did so on TSAYE (Table 2), a medium that appeared to enhance recovery of the pathogen in the effective treatments, as it lacked the selective antimicrobial agents of PALCAM. Indeed, it was observed that TSAYE permitted growth of higher ($P < 0.05$) bacterial populations than PALCAM at 70 to 120
days at 4°C in treatments with sodium lactate (3 or 6%) and sodium diacetate (0.5%) following a period of 10 to 50 days of storage, during which both media detected similar counts (Tables 1 and 2). Although a major part of the 70- to 120-day populations of the above treatments on TSAYE (Table 2) were natural flora (e.g., mainly gram-positive, catalase-negative colonies tentatively characterized as lactic acid bacteria), it could be observed that the numbers of L. monocytogenes colonies among the total TSAYE counts were higher than those on PALCAM (Table 1). In other words, PALCAM appeared to overestimate the bactericidal effects of 6% sodium lactate and 0.5% sodium diacetate compared to TSAYE, indicating that part of the pathogen cells were not killed but were injured by the antimicrobials and, thus, could recover only on the nonselective medium. This observation is important, because the effectiveness of antimicrobial agents or other food preservation methods is often determined by plating on selective media; thus, injured cells may be overlooked or underestimated, despite their potential to repair damages, and proliferate in foods to potentially become a risk. Further studies with antibiotic-resistant inocula or with meat products having their surfaces sterilized by various techniques (e.g., UV light, steam, etc.) postprocessing could be useful to accurately determine the injury effect of lactates and acetates added to the formulation on L. monocytogenes.

Consistent with the above findings, the inoculated controls and the treatments of low antilisterial effectiveness developed comparably high numbers of colonies on TSAYE (Table 2) and PALCAM (Table 1), which were

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>35</th>
<th>50</th>
<th>70</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>No antimicrobial</td>
<td>3.2 Va,b</td>
<td>4.8 Vc</td>
<td>6.2 Vb</td>
<td>7.8 Va</td>
<td>8.2 Va</td>
<td>8.3 Va</td>
<td>8.2 Va</td>
<td>8.2 Va</td>
</tr>
<tr>
<td>Sodium acetate (0.25%)</td>
<td>3.3 Vd</td>
<td>3.0 Wd</td>
<td>3.8 Wd</td>
<td>5.2 Wc</td>
<td>6.6 Wb</td>
<td>7.1 Wab</td>
<td>7.6 Wab</td>
<td>7.9 Va</td>
</tr>
<tr>
<td>Sodium acetate (0.50%)</td>
<td>3.3 Vd</td>
<td>2.6 Wdc</td>
<td>3.2 Wde</td>
<td>4.2 Wxcd</td>
<td>4.9 Xb</td>
<td>5.8 Xab</td>
<td>5.8 Xab</td>
<td>6.2 Wa</td>
</tr>
<tr>
<td>Sodium diacetate (0.25%)</td>
<td>3.2 Vd</td>
<td>2.5 Wde</td>
<td>3.3 Wcde</td>
<td>3.7 Xcd</td>
<td>4.4 Xbc</td>
<td>5.2 Xab</td>
<td>5.6 Xab</td>
<td>5.5 Wa</td>
</tr>
<tr>
<td>Sodium diacetate (0.50%)</td>
<td>3.4 Va</td>
<td>2.4 Wab</td>
<td>2.7 Wab</td>
<td>2.6 Xab</td>
<td>2.8 Yab</td>
<td>1.8 Zb</td>
<td>1.7 Zb</td>
<td>1.6 Zb</td>
</tr>
<tr>
<td>Sodium lactate (3%)</td>
<td>3.2 Vb</td>
<td>2.4 Wd</td>
<td>2.7 Wd</td>
<td>2.9 Xcd</td>
<td>3.5 Ybcd</td>
<td>3.9 Yabc</td>
<td>4.5 Ya</td>
<td>4.1 Xab</td>
</tr>
<tr>
<td>Sodium lactate (6%)</td>
<td>3.2 Va</td>
<td>2.4 Wab</td>
<td>2.7 Wab</td>
<td>2.7 Xab</td>
<td>2.6 Yab</td>
<td>2.4 Zab</td>
<td>2.0 Zb</td>
<td>1.8 Zb</td>
</tr>
</tbody>
</table>

\( a\) ABCDE: means within a row lacking a common letter are significantly different (\(P < 0.05\)).
\( b\) VWXYZ: means within a column lacking a common letter are significantly different (\(P < 0.05\)).
shown to be *L. monocytogenes* based on tests described previously. Accordingly, the uninoculated controls with no additives developed significantly (*P* < 0.05) lower bacterial populations on TSAYE (e.g., average counts of 4.5 to 5.3 log CFU/cm² at 90 to 120 days) during storage at 4°C (data not shown) compared to inoculated controls (Table 2). Several previous studies (12, 17, 24, 28, 29) have indicated that sodium lactate, sodium diacetate, and sodium acetate may also decrease the growth rate or select specific species of acetate-resistant, homofermentative spoilage bacteria in cured meat products to delay visible spoilage defects under commercial conditions. Additional studies with commercially prepared, naturally contaminated meat products are needed to evaluate the antimicrobial concentrations effective against *L. monocytogenes* in this study, for their effects against various groups of spoilage microorganisms (e.g., lactic acid bacteria, psychrophilts, anaerobes, etc.) and on product shelf life.

The above results indicate that, in decreasing order of effectiveness, the currently permissible levels (14) for sodium lactate (3%, corresponding to 4.8% of the 60% commercial product), sodium diacetate (0.25%), and sodium acetate (0.25%) appeared to be sufficient to control growth of *L. monocytogenes* introduced on the surfaces of meat products after heating and before vacuum packaging for at least 70, 35, and 20 days of storage at 4°C, respectively. Specifically for sodium lactate (3%), the results of this study are consistent with an average growth of 0.88 log CFU/g only reported in inoculated (4 to 5 log CFU/g) with *L. monocytogenes* liver sausage containing 3% sodium lactate at 50 days of storage at 5°C (34). By doubling the concentration of sodium lactate from 3 to 6% or that of sodium diacetate from 0.25 to 0.5%, the effect of these compounds was shifted to listericidal (*P* < 0.05) in frankfurters stored at 4°C for more than 70 to 90 days (Table 1). Previous studies have also indicated listericidal effects of 0.5% sodium diacetate in turkey slurries (27), while 4% sodium lactate has demonstrated a pronounced inhibition of *L. monocytogenes* in fresh meats treated with antimicrobials (21, 31).

Among the organic acid salts tested as single antimicrobials in the formulation, sodium diacetate (0.25% and 0.5%) was the only one that significantly (*P* < 0.05) reduced the pH of frankfurters at the beginning of storage (day 0) (Table 3). Other workers have also observed a comparable reduction in meat pH when 0.3 to 0.5% sodium diacetate was added to ground beef or beef slurry (30) or to turkey slurries (27). Notably, the above studies indicated that the increased antilisterial activity in meat slurries with diacetate was due to the compound itself, to its synergistic effects with other antimicrobials, or both and not just to *P* < 0.05 reductions in product pH after storage occurred in inoculated controls, followed by samples with 0.25% sodium acetate, reflecting the prolific growth (>7.0 logs) and acidifying activity of the pathogen in those samples. In contrast, the pH of all other treatments was not reduced (Table 3), reflecting the suppression of *L. monocytogenes* growth to levels <7.0 log CFU/cm² throughout (120 days) storage.

As expected, antimicrobials in the formulation did not significantly (*P* > 0.05) affect the cooking yield, moisture content, or fat content of frankfurters, which ranged on average from 89.2 to 92.7%, from 51.4 to 54.5%, and from 23.0 to 25.6%, respectively, across treatments (Table 4). Sodium lactate and, to a much lesser extent, sodium diacetate significantly (*P* < 0.05) affected the water activity (aw) of the product after cooking (Table 4). It seems that the reductions in aw caused by sodium diacetate correlate with the significant (*P* < 0.05) reductions in product pH after

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### Table 3. Mean (n = 9; SD) pH values of frankfurters prepared with antimicrobials in the formulation, inoculated with *Listeria monocytogenes*, vacuum packaged, and stored at 4°C

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage at 4°C (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>No antimicrobial</td>
<td>6.33 YA,a,b</td>
</tr>
<tr>
<td>Sodium acetate (0.25%)</td>
<td>6.35 YAB</td>
</tr>
<tr>
<td>Sodium acetate (0.50%)</td>
<td>6.36 YABC</td>
</tr>
<tr>
<td>Sodium diacetate (0.25%)</td>
<td>6.03 ZBCD</td>
</tr>
<tr>
<td>Sodium diacetate (0.50%)</td>
<td>5.87 ZBC</td>
</tr>
<tr>
<td>Sodium lactate (3%)</td>
<td>6.23 YB</td>
</tr>
<tr>
<td>Sodium lactate (6%)</td>
<td>6.31 YABC</td>
</tr>
</tbody>
</table>

*a*bCDE: means within a row lacking a common letter are significantly different (*P* < 0.05).

*bCDE: means within a column lacking a common letter are significantly different (*P* < 0.05).
TABLE 4. Means (n = 6; SD) for processing and chemical characteristics of frankfurters prepared with antimicrobials in the formulation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>% cooking yield</th>
<th>% moisture</th>
<th>% fat</th>
<th>Water activity ($a_w$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No antimicrobial</td>
<td>92.25 w</td>
<td>52.59 w</td>
<td>24.96 w</td>
<td>0.972 w</td>
</tr>
<tr>
<td>Sodium acetate (0.25%)</td>
<td>91.55 w</td>
<td>51.54 w</td>
<td>23.49 w</td>
<td>0.964 wx</td>
</tr>
<tr>
<td>Sodium acetate (0.50%)</td>
<td>89.89 w</td>
<td>52.74 w</td>
<td>24.97 w</td>
<td>0.969 wx</td>
</tr>
<tr>
<td>Sodium diacetate (0.25%)</td>
<td>92.67 w</td>
<td>54.45 w</td>
<td>23.42 w</td>
<td>0.963 x</td>
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<tr>
<td>Sodium diacetate (0.50%)</td>
<td>89.16 w</td>
<td>52.70 w</td>
<td>25.63 w</td>
<td>0.962 x</td>
</tr>
<tr>
<td>Sodium lactate (3%)</td>
<td>90.83 w</td>
<td>53.06 w</td>
<td>22.97 w</td>
<td>0.946 y</td>
</tr>
<tr>
<td>Sodium lactate (6%)</td>
<td>90.55 w</td>
<td>51.36 w</td>
<td>23.38 w</td>
<td>0.933 z</td>
</tr>
</tbody>
</table>

a wxyz: means within a column lacking a common letter are significantly different (P < 0.05).

formulation (i.e., the functionality of the frankfurter batter to bind the water added was reduced upon the lowering of pH closer to the isoelectric point of meat proteins) (Table 3). In contrast, at 3 and 6%, sodium lactate reduced $a_w$ to 0.94 and 0.93, respectively (Table 4), while the pH of those frankfurters was not reduced (P > 0.05) compared to the control treatment. These results confirm that lactates, in addition to the specific activity of the lactate anion, may act as humectants to bind water, thus decreasing the $a_w$ of the emulsified meat and thereby inhibiting or retarding microbial growth in cooked meat products, without affecting the product pH (11, 17, 29, 31). Specifically, Chen and Shelef (11) showed that 3% lactate with 2% sodium chloride in a meat model system with 55% moisture inhibited *L. monocytogenes*, while 4% sodium lactate singly suppressed growth at >55% and inhibited growth in samples with 25 to 55% moisture ($a_w$, 0.964). Most likely, the intrinsic conditions in frankfurters with 3 or 6% sodium lactate, also containing 2% salt, were comparable to those of the model system, which confirms reduced or no growth of the pathogen (Table 1).

In summary, when 3 to 4 log CFU/cm² of *L. monocytogenes* was introduced on the surface of peeled frankfurters stored at 4°C in vacuum packages, permissible levels of additives for processed meats tested provided inhibition of the pathogen ranging widely from 20 to 70 days. Sodium lactate at 3% and sodium acetate at 0.25% were the most and least effective additives, respectively, while sodium diacetate at 0.25% provided intermediate antilisterial activity. However, by doubling the concentrations of sodium lactate and sodium diacetate from 3 and 0.25% to 6 and 0.5%, respectively, growth of *L. monocytogenes* was completely inhibited for more than 90 days. These findings support the possibility of the USDA-FSIS (14) increasing maximum levels allowed to prevent growth of *L. monocytogenes*, especially those of acetate salts. Such increases, however, should only be decided following scientific evaluation of potential sensory and other defects. In this study, 6% sodium lactate resulted in a product with increased elasticity, while 0.5% sodium diacetate was associated with a more vinegar-like smell and easier breakage of frankfurters compared to the respective treatments with the permissible levels of 3 and 0.25%. Although these effects were not obvious, consumer panel testing and technological product evaluation are required prior to requesting further increases in the permissible levels of sodium lactate or diacetate. Moreover, based on previous studies (6, 19, 23) and recent data from our laboratory (personal communication), further increases in permissible levels may not be needed if sodium lactate is used in combination with sodium acetate, sodium diacetate, or glucono-delta-lactone in cured meat formulations. Importantly, the inhibition observed in this study (Tables 1 and 2) may be lower in other types of meat products or at storage temperatures higher than 4°C. Thus, rather than using higher levels of single antimicrobials, it may be advisable to use combinations of lower levels following evaluation in meat products of interest. Additional studies are needed to evaluate the antilisterial activity of different combinations of additives in different products and at abusive temperatures (i.e., >4°C). These studies should be extended to determine the effects of additives on other groups and types of microorganisms and on product shelf life. In conclusion, the potential exists for meat researchers and the USDA-FSIS to help meat processors develop formulations with greater antimicrobial activity, provided that any adverse effects on product quality are recognized and corrected.

**ACKNOWLEDGMENTS**

Funding for this study was provided by the National Pork Producers Council, by the USDA-CSREES, and by the Colorado Agricultural Experiment Station. John Samelis was a recipient of a NATO postdoctoral research grant from the Hellenic Ministry of National Economy, Athens, Greece.
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