Validation of Food-Grade Salts of Organic Acids as Ingredients To Control *Listeria monocytogenes* on Pork Scrapple during Extended Refrigerated Storage\(^{\ddagger}\)

A. C. S. PORTO-FETT,\(^1\) S. G. CAMPA NO,\(^2\) J. E. CALL,\(^3\) B. A. SHOYER,\(^3\) L. YODER,\(^3\) K. GARTNER,\(^3\) L. TUFFT,\(^4\) A. OSER,\(^1\) J. LEE,\(^5\) AND J. B. LUCHANSKY\(^{3*}\)

\(^1\)Oser Technologies, Blacksville, West Virginia 26521; \(^2\)Hawkins Inc., Minneapolis, Minnesota 55413; \(^3\)U. S. Department of Agriculture, Agricultural Research Service, Wyndmoor, Pennsylvania 19038; \(^4\)Hatfield Quality Meats, Hatfield, Pennsylvania 19440, and \(^5\)Drexel University, Philadelphia, Pennsylvania 19104, USA

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**ABSTRACT**

This study was conducted to investigate control of *Listeria monocytogenes* on pork scrapple during storage at 4°C. In phase I, scrapple was formulated, with or without citrate-diacetate (0.64%), by a commercial processor to contain various solutions or blends of the following antimicrobials: (i) lactate-diacetate (3.0 or 4.0%), (ii) lactate-diacetate-propionate (2.0 or 2.5%), and (iii) levulinate (2.0 or 2.5%). Regardless of whether citrate-diacetate was included in the formulation, the subsequent addition of the targeted antimicrobials pathogen levels increased ca. 6.4 log CFU/g within the 50-day storage period. In the absence of citrate-diacetate but when the targeted antimicrobials were included in the formulation, pathogen numbers increased by ca. 1.3 to 5.2 log CFU/g, whereas when citrate-diacetate was included with these antimicrobials, pathogen numbers increased only by ca. 0.7 to 2.3 log CFU/g. In phase II, in the absence of citrate-diacetate, when the pH of the lactate-diacetate-propionate blend (2.5%) was adjusted to pH 5.0 or 5.5 pathogen numbers remained unchanged (\(\leq 0.5\) log CFU/g increase) over 50 days, whereas when citrate-diacetate was included with the lactate-diacetate-propionate blend adjusted to pH 5.0 or 5.5, pathogen numbers decreased by 0.3 to 0.8 log CFU/g. In phase III, when lower concentrations of the lactate-diacetate-propionate blend (1.5 or 1.94%) were adjusted to pH 5.5, pathogen numbers increased by ca. 6.0 and 4.7 log CFU/g, respectively, whereas when the mixture was adjusted to pH 5.0, pathogen numbers increased by \(\leq 0.62\) log CFU/g. Thus, scrapple formulated with lactate-diacetate-propionate (1.5 and 1.94% at pH 5.0) is an unfavorable environment for outgrowth of *L. monocytogenes*.

Scrap**p**le is a ready-to-eat (RTE) food containing meats and cereal products, sometimes referred to as a mush, that is quite popular in the northeastern United States, where it is especially enjoyed as a breakfast item. Scrapple typically comprises about 40% comminuted meat and meat by-products from swine but also could be made using bovine or poultry meats and/or by-products plus corn meal or flour derived from grains and/or soybeans (43). This mixture is then further seasoned, cooked, portioned into pans or loaves, chilled to 4°C, and then vacuum packaged for refrigerated retail display. Additional details on the origin and formulations of this tasty product, which according to some was derived from the “thriftiness and love of good eating” among early German settlers in Eastern Pennsylvan-ia, can be found elsewhere (17, 21). Although scrapple is quite popular among its many devotees, with over $15 million sold at retail in 2008 alone (13), relatively few data exist on the types, levels, and/or fate of either pathogenic or spoilage microbes associated with scrapple.

For the past 30 years, *Listeria monocytogenes* has been one of only a handful of foodborne pathogens responsible for numerous product recalls and appreciable numbers of human illnesses (32, 45). Recent figures from the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) established that the prevalence of this pathogen associated with RTE foods tested at random decreased from 4.61% in 1990 to 0.38% by 2009 (10). Although no reported illnesses have been associated with pork scrapple and only two relatively small recalls (ca. 350 lb [159 kg] [44] and <3,600 lb [1636 kg] [30]) due to contamination of scrapple with *L. monocytogenes* have been reported, we found that scrapple provides a very favorable environment for growth of this pathogen. Surface inoculation with even relatively few cells (ca. 2.0 log CFU/g) resulted in an increase in pathogen numbers to ca. 9.0 log CFU/g within 44 days of storage at 4°C (1). However, most respondents who participated in our informal survey...
reported (re)heating scrapple immediately before consuming it, and typical time-temperature conditions used by these consumers were more than adequate to kill relatively low numbers of *L. monocytogenes* that may be present (1).

Regardless of the apparently low risk of contamination, we thought it would be prudent to evaluate antilisterial ingredients for scrapple. Various food grade chemicals are antagonistic toward *L. monocytogenes* in foods when used as sprays and/or dip or bath applications and/or when added as an ingredient (6, 14, 24, 26, 28, 29, 37, 40). Perhaps the best example is addition of up to 4.8% sodium or potassium lactate (wt/wt), either alone or in combination with up to 0.25% sodium diacetate (wt/wt), to the formulation of red meat or poultry products (2). As an alternative to lactates, organic acids and their salts, such as levulinate and propionate, also have been evaluated as antilisterial agents in RTE meats (8, 36). Thus, the primary objective of this study was to evaluate the addition of food-grade antimicrobials as ingredients to control *L. monocytogenes* over the expected 50-day refrigerated shelf life of pork scrapple. The addition of antimicrobials also would lessen the likelihood that low numbers of the pathogen that may be present due to the rare event of postprocess contamination would amplify to levels that may cause illness, particularly for highly susceptible individuals.

**MATERIALS AND METHODS**

**Bacterial strains.** The five strains of *L. monocytogenes* (MFS2, MFS102, MFS104, MFS105, and MFS110) used as a cocktail to surface inoculate pork scrapple were confirmed, cultured, and maintained as described previously by Porto et al. (31).

**Manufacture of pork scrapple.** In each of two trials, freshly manufactured, commercially produced scrapple was formulated (pork stock, pork, pork liver, pork skin, yellow cornmeal, pork heart, whole wheat flour, pork tongue, salt, buckwheat, wheat flour, spices, dextrose, and flavoring) with or without sodium citrate–sodium diacetate (0.64%, wt/wt; Ional-Plus, WTI, Inc., Jefferson, GA) by a collaborating commercial processor.

In phase I, pork scrapple was formulated with and without citrate-diacetate and various solutions or blends of the following antimicrobials: (i) potassium lactate–sodium diacetate, 3.0 or 4.0% (vol/wt) of a 60% solution consisting of 56% potassium lactate and 4% sodium diacetate on a dry solids basis (wt/wt) (UltraLac KLS64, Hawkins, Inc., Minneapolis, MN); (ii) potassium lactate–potassium diacetate–potassium propionate, 2.0 or 2.5% (vol/wt) of a 60% solution consisting of 40% potassium lactate, 10% potassium diacetate, and 10% potassium propionate on a dry solids basis (wt/wt) (eLmSolute, Hawkins); and (iii) potassium levulinate, 2.0 or 2.5% (vol/wt) of a solution consisting of 50% potassium levulinate on a dry solids basis (wt/wt) (Hawkins). The pH of these antimicrobial solutions ranged from pH 6.0 to 7.5.

In phase II, pork scrapple was formulated with and without citrate-diacetate and the 2.5% (vol/wt) lactate-diacetate-propionate blend. For these experiments, the lactate-diacetate-propionate blend was prepared with and without a botanical-based flavor masking agent (≤1.0%, wt/wt; Hawkins). The pH of the lactate-diacetate-propionate blend, as measured in a dilute (3%, wt/wt) solution, was adjusted from pH 6.3 to pH 5.0 or pH 5.5 by the supplier. The botanical-based flavor masking agent was added to lessen the perceived metallic or bitter and vinegary aftertaste of the final product that some consumers associate with use of potassium salts and diacetate, respectively, when used as ingredients in some meat products.

In phase III, scrapple was formulated without citrate-diacetate but with the lactate-diacetate-propionate blend (1.5, 1.94, or 2.5%, vol/wt), with and without the botanical-based flavor masking agent, and adjusted to either pH 5.0 or pH 5.5.

The cooked and comminuted meats, stock, and dry ingredients were mixed and slowly brought up to and then subsequently tempered at ca. 82°C. Each resulting batch of scrapple (90 to 100 kg) was portioned into trays to form loaves (ca. 5 kg each; ca. 11 cm wide by ca. 6 cm high by ca. 64 cm long), to which the antimicrobial syrups were separately added. Each mixture was thoroughly mixed (ca. 5 min) by hand using a spatula, and the resultant product was chilled overnight to 4°C in a temperature-controlled room. The loaves of scrapple were subsequently transported on ice to the USDA Agricultural Research Service Eastern Regional Research Center (ARS, ERRC; Wyndmoor, PA), stored at 4°C for up to 2 h, sliced, and then inoculated.

**Inoculation of product.** The scrapple was placed onto sterile polystyrene foam packing trays (Koch Supplies, Kansas City, MO) and sliced (ca. 11 cm wide by ca. 6.0 cm high by 1.0 cm thick; ca. 80 ± 11 g per slice) with an alcohol-sterilized knife. Slices were placed onto packing trays and separately surface inoculated with 500 μl per face (1 ml total per slice) of the pathogen cocktail to an average target level of ca. 2.5 log CFU/g essentially as described previously (1). In each of the two trials and for each antimicrobial tested, triplicate scrapple samples were analyzed at each sampling interval.

**Microbiological analyses.** At each sampling interval during storage at 4°C for up to 50 days, *L. monocytogenes* was enumerated by transferring the inoculated scrapple slice to a sterile filter stomacher bag (type XX-C003, Microbiology International, Frederick, MD), adding 75 ml of sterile peptone water (0.1%; Difco, BD, Sparks, MD), macerating the slice by stomaching for 2 min (Stomacher 400, Seward, Cincinnati, OH), and plating the resulting slurry onto modified Oxford (Difco, BD) agar plates essentially as described (1). Total aerobic bacteria counts and total lactic acid bacteria (LAB) counts were determined on days 0 and 50 by spread plating 100 μl of the control slurry or dilutions thereof onto brain heart infusion (Difco, BD) and de Man Rogosa Sharpe (Difco, BD) agar plates, respectively. Typical colonies were counted, and bacterial numbers were expressed as log CFU per gram.

**Sensory evaluation.** A consumer acceptability test was performed by an untrained volunteer panel (n = 34). The test was conducted at the ERRC sensory laboratory on scrapple formulated (i) with citrate-diacetate, (ii) without citrate-diacetate, and (iii) without citrate-diacetate in combination with 2.5% lactate-diacetate-propionate (with and without the botanical-based flavor masking agent) adjusted to either pH 5.0 or pH 5.5. Samples were removed from the original package, sliced (ca. 11 cm wide by ca. 6.0 cm high by ca. 1.0 cm thick) with an alcohol-sterilized knife, and separately reheated without any added oil or butter on a nonstick electric skillet (model 169216, General Electric Housewares, Bentonville, AR) for 20 min (10 min each side) at the medium heat dial setting (ca. 300°F according to the manufacturer’s specifications). Each sample (½ slice) was served on a 6-in. (15.2-cm) Styrofoam plate that was labeled randomly with a three-digit number. Panelists were asked to rate each sample for overall
liking, liking of flavor, and liking of texture using a 9-point hedonic scale anchored from dislike extremely (1) to like extremely (9). Overall flavor intensity was measured using a 9-point intensity scale anchored from none (1) to extreme (9). Crackers without salt on the top and room temperature water were used as palate cleansers between tasting of the different samples. At the end of the evaluation, panelists also were asked to anonymously provide their demographic information (i.e., gender, age, race, and education) and answer a few scrapple-related questions (e.g., frequency of consumption).

**Proximate composition analyses.** Chemical analyses were performed on scrapple samples according to methods approved and described by the AOAC International (3) as conducted by a commercial testing laboratory. The analyses were performed on representative samples from each of two trials (200 g total) formulated (i) with citrate-diacetate, (ii) without citrate-diacetate, and (iii) without citrate-diacetate in combination with 1.5, 1.94, or 2.5% lactate-diacetate-propionate blend (formulated without the botanical-based flavor masking agent) adjusted to pH 5.0, pH 5.5, or pH 6.0. The analyses for potassium lactate, potassium diacetate, potassium propionate, and potassium levulinate were performed in a manner similar to the procedures outlined by Fernandez-Garcia and McGregor (11) and by Seman et al. (38) with the following modifications: (i) 50-g samples were extracted with 300 ml of distilled water, and (ii) filtrate was analyzed using a high-performance liquid chromatography apparatus (model 1090, series L, Hewlett Packard, Palo Alto, CA) equipped with a Rezex ROA–organic acid H+ (8%) column (300 by 7.8 mm; Phenomenex, Torrance, CA) with a run time of 40 min at 60°C.

**Statistical analyses.** Data were analyzed using version 9.1.3 of the SAS statistical package (SAS Institute Inc., Cary, NC). Means and standard deviations of pathogen numbers in pork scrapple were determined from three samples at each sampling interval for each of the two trials. Mean separations were performed using the Bonferroni least significant difference method to determine significant differences ($P \leq 0.05$) among means.

**RESULTS**

**Levels of indigenous flora.** The average initial levels of total aerobic bacteria and indigenous LAB (two trials; three replicates or slices per sampling interval per trial) on pork scrapple formulated with or without citrate-diacetate were 1.41 ± 0.34 and 1.82 ± 1.06 log CFU/g and 1.76 ± 0.33 and 1.19 ± 0.82 log CFU/g, respectively. After 50 days of storage at 4°C, the average levels of total aerobic bacteria and indigenous LAB for pork scrapple formulated with and without citrate-diacetate were 7.43 ± 1.80 and 7.23 ± 1.73 log CFU/g and 7.52 ± 0.91 and 6.85 ± 1.53 log CFU/g, respectively. The inclusion of 0.64% citrate-diacetate as an ingredient in scrapple did not result in any discernible differences in the levels of total aerobic bacteria or indigenous LAB compared with otherwise similar scrapple formulated without these antimicrobials.

**Efficacy of selected antimicrobials to control L. monocytogenes on pork scrapple.** In phase I, we evaluated different types and concentrations of antimicrobials to control L. monocytogenes on scrapple formulated with and without citrate-diacetate (Table 1). The antimicrobials evaluated were selected based on published reports and on our personal experience with their antilisterial activity in red meat and poultry products. In the absence of citrate-diacetate, inclusion of lactate-diacetate (4%) was significantly more effective ($P \leq 0.05$) for inhibiting outgrowth of L. monocytogenes than was lactate-diacetate-propionate (2 or 2.5%), levulinate (2.0 or 2.5%), or lactate-diacetate (3.0%). A significant synergistic effect ($P \leq 0.05$) was observed for citrate-diacetate in combination with the other food-grade chemicals compared with otherwise similar samples to which these food-grade chemicals were added separately as part of the formulation. The inclusion of citrate-diacetate in combination with 2.5% lactate-diacetate-propionate or 3.0 or 4.0% lactate-diacetate was significantly more effective ($P \leq 0.05$) for suppressing outgrowth of L. monocytogenes when compared with scrapple that was formulated with 2.0% lactate-diacetate-propionate or either concentration of levulinate. Without inclusion of lactate-diacetate-propionate, levulinate, or lactate-diacetate, levels of L. monocytogenes increased ca. 6.4 log CFU/g after 50 days of storage at 4°C, whereas when these same food-grade antimicrobials were included, pathogen numbers increased by ca. 1.3 to 5.2 log CFU/g (without citrate-diacetate) and by ca. 0.7 to 2.3 log CFU/g (with citrate-diacetate). These results established the antilisterial potential of blends of lactate-diacetate-propionate, levulinate, or lactate-diacetate as ingredients in scrapple. In the event of postprocess contamination, the inclusion of these chemical blends in the formulation would provide a less favorable environment for outgrowth of L. monocytogenes on scrapple.

**Viability of L. monocytogenes on pork scrapple formulated with and without citrate-diacetate in combination with the lactate-diacetate-propionate blend adjusted to different pH levels.** Because similar results were obtained for the lactate-diacetate and lactate-diacetate-propionate blends, in phase II we evaluated (based on cost, efficacy, and taste/texture considerations) lactate-diacetate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concn (% vol/wt)</th>
<th>Without citrate-diacetate</th>
<th>With citrate-diacetate (0.64%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.5</td>
<td>8.84 ± 0.01 A</td>
<td>8.90 ± 0.01 A</td>
</tr>
<tr>
<td>Lactate-diacetate-propionate</td>
<td>2.5</td>
<td>7.70 ± 0.08 AB</td>
<td>3.98 ± 0.75 DE</td>
</tr>
<tr>
<td>Levulinate</td>
<td>2.5</td>
<td>6.77 ± 0.22 BC</td>
<td>2.63 ± 0.23 BC</td>
</tr>
<tr>
<td>Lactate-diacetate</td>
<td>3.0</td>
<td>7.28 ± 0.12 BC</td>
<td>4.75 ± 0.37 D</td>
</tr>
<tr>
<td>Lactate-diacetate</td>
<td>4.0</td>
<td>6.09 ± 0.44 C</td>
<td>3.42 ± 0.31 DEF</td>
</tr>
<tr>
<td>Levulinate</td>
<td>4.0</td>
<td>7.04 ± 0.36 BC</td>
<td>2.58 ± 0.67 FG</td>
</tr>
<tr>
<td>Levulinate</td>
<td>4.0</td>
<td>3.79 ± 1.60 DEF</td>
<td>1.84 ± 0.13 G</td>
</tr>
</tbody>
</table>

*Initial L. monocytogenes level was ca. 2.5 log CFU/g. Values are the mean ± standard deviation of two trials (three replicates or slices per sampling interval per trial). Within rows and columns, means with the same letters are not significantly different ($P > 0.05$).*
to address the potential effect of pH on *L. monocytogenes* and the effect of a masking agent on possible taste concerns (Fig. 1). Levulinate was not selected for further analyses because it allowed a $\geq 1.0$-log increase in pathogen numbers (Table 1), whereas the other blends did not when evaluated in scrapple containing citrate-diacetate.

Regardless of whether citrate-diacetate was included in the formulation, the lower the pH of the lactate-diacetate-propionate blend, the greater the inhibition and/or inactivation of *L. monocytogenes* (Fig. 1). In addition, for a given pH, inclusion of the botanical-based flavor masking agent in the blend did not affect the antilisterial activity ($P \leq 0.05$) of lactate-diacetate-propionate (data not shown). In the absence of citrate-diacetate, there were significant differences ($P \leq 0.05$) in pathogen viability when scrapple was formulated with the lactate-diacetate-propionate blend adjusted to pH 6.0 compared with formulations with the lactate-diacetate-propionate blend adjusted to pH 5.0 or pH 5.5. In contrast, when scrapple was formulated with citrate-diacetate, essentially no pH effect of the lactate-diacetate-propionate blend on *L. monocytogenes* was observed ($P \leq 0.05$). In the absence of citrate-diacetate, when scrapple was formulated with the lactate-diacetate-propionate blend adjusted to pH 5.5 or pH 6.0, pathogen numbers increased by 0.51 and 3.7 log CFU/g, respectively, after 50 days of storage at 4°C, whereas when the blend was adjusted to pH 5.0 pathogen numbers decreased by 0.44 log CFU/g. Likewise, when scrapple was formulated with citrate-diacetate in combination with the lactate-diacetate-propionate blend adjusted to pH 6.0, pathogen numbers remained relatively unchanged, whereas when the pH of the blend was adjusted to pH 5.0 or pH 5.5, pathogen numbers decreased by 0.82 and 0.29 log CFU/g, respectively, over the 50 days of refrigerated shelf life. These data established that in the absence of citrate-diacetate, the pH lowering effect of the lactate-diacetate-propionate blend had an appreciable effect on the viability of *L. monocytogenes* on scrapple.

Viability of *L. monocytogenes* on pork scrapple formulated without citrate-diacetate in combination with different concentrations of the lactate-diacetate-propionate blend adjusted to different pH levels. In phase III, we evaluated lower concentrations of the lactate-diacetate-propionate blend (1.5 and 1.94% in addition to 2.5%) adjusted to either pH 5.0 or pH 5.5 for antilisterial activity in scrapple (Fig. 2). In general, the lower the pH of the blend, the greater the inhibition and/or inactivation of the pathogen. As expected, inclusion of the masking agent in the blend did not have a significant effect ($P \leq 0.05$) on viability of *L. monocytogenes* on scrapple (data not shown). In general, the inclusion of 1.5, 1.94, or 2.5% blend adjusted to pH 5.0 was equally effective ($P \leq 0.05$) for suppressing the outgrowth of *L. monocytogenes* on scrapple. In contrast, when scrapple was formulated with lactate-diacetate-propionate at 1.5, 1.94, or 2.5% and adjusted to pH 5.5, pathogen numbers increased by 5.94, 4.69, and 0.56 log CFU/g, respectively. When scrapple was formulated with the lactate-diacetate-propionate blend (1.5, 1.94, or 2.5%) adjusted to pH 5.0, pathogen numbers increased by 0.56 log CFU/g over the 50 days of refrigerated shelf life. Thus, inclusion of lower concentrations of the lactate-diacetate-propionate blend, such as 1.5 and 1.94%, was possible without compromising product safety or shelf life when the blend was adjusted to pH 5.0.

Physical-chemical composition of selected pork scrapple formulations. Chemical analyses revealed only subtle differences in the levels of ash, carbohydrate, fat, moisture, protein, and salt content, acidity, or water activity
among scrapple formulated with and without 0.64% citrate-diacetate and with 1.5, 1.94, or 2.5% lactate-diacetate-propionate blend adjusted to pH 5.0 compared with that adjusted to pH 5.5. The higher the concentration and the lower the pH of the blend, the lower the pH of the scrapple. As expected, differences of ca. 0.8 to 1.4% in the potassium lactate concentration were observed between scrapple formulated with the lactate-diacetate-propionate blend adjusted to different pH levels compared with scrapple that was formulated without this food-grade chemical.

Sensory evaluation of scrapple. In general, panelists moderately liked all formulations of scrapple tested (average score of 7.5). About half of the panelists (17 individuals) consumed scrapple once a month or less, and 21% consumed it 2 to 4 times a week. No significant differences ($P \leq 0.05$) in overall liking, liking of flavor, liking of texture, and overall flavor intensity were observed between scrapple formulated with and without the lactate-diacetate-propionate blend. These results established that inclusion of the lactate-diacetate-propionate blend did not affect the acceptability of the product.

**DISCUSSION**

In a recent study, it was estimated that ethnic foods account for $1 of every $7 spent on groceries; sales of ethnic food in the United States exceeded $2.2 billion in 2009 and is likely to increase by 20% by 2014 (35). Scrapple is a regionally popular, RTE ethnic mushlike product that accounted for at least $15 million in retail sales in 2008 (13). Although the vast majority of scrapple is sold vacuum packaged and refrigerated, some is sold frozen and some is sold canned. Regardless of how scrapple is sold, subsequent contamination at retail and/or by consumers can occur. Because scrapple has a relatively high water activity (0.970 to 0.987), a relatively high moisture content (63 to 70%), a relatively neutral pH (5.4 to 6.4), and a low salt concentration (ca. 1.0 to 2.0%), as reported both previously (1) and here, it was not surprising that pork scrapple supported rapid and appreciable growth of L. monocytogenes in our inoculated-package challenge study. Although concerted efforts by food safety professionals in industry, academia, and government have significantly lowered the prevalence of L. monocytogenes in certain foods and accordingly have reduced the occurrence of listeriosis (10, 25), it remains possible to recover L. monocytogenes from a variety of RTE foods, including scrapple, albeit at relatively low frequencies and levels (22, 23, 30, 33, 44, 46). Although the pathogen is eliminated during cooking and processing, if present L. monocytogenes can be reintroduced onto the surface of RTE meats such as scrapple when the finished product is exposed to the food processing environment before packaging and/or is mishandled or left uncovered after opening at retail or within a consumer’s refrigerator (7, 9, 43). Thus, because scrapple has an anticipated shelf life of about 50 days and can support amplification of L. monocytogenes even when the product is properly stored and/or refrigerated, further research was warranted to investigate scrapple formulations that would
We evaluated different types and concentrations of several antimicrobials for antilisterial activity in pork scrapple. Although for the brand of scrapple evaluated herein both sodium citrate and sodium diacetate were listed as ingredients on the product label (presumably added because of their purported antilisterial properties), the results of this study and our previous study (1) revealed that at the concentrations used, these food-grade chemicals were not antagonistic toward *L. monocytogenes*. In retrospect, it may have been erroneous to assume that these compounds would exhibit antilisterial properties, as tacitly implied by the supplier. In another study, buffered sodium citrate (ca. 1.0%) added to cooked ham allowed for growth of *L. monocytogenes* (41). Likewise, Kouassi and Shelif (22) evaluated the effect of organic acid salts on intracellular metabolism of *L. monocytogenes* and reported that sodium citrate was not as effective as the sodium salts of propionate, acetate, or lactate for suppressing growth for 24 h at 35 °C in a minimal culture medium supplemented with yeast extract.

In agreement with results reported by other investigators (4, 5, 27, 38, 39, 48), we also reported that lactates prevented outgrowth of *L. monocytogenes* on meat products, such as frankfurters, during refrigerated storage (31, 33). However, the concentrations of lactate and diacetate commonly used by the food industry as antilisterial ingredients in RTE meat and poultry products may be cost prohibitive and/or may have undesirable effects on the taste of the finished product, as experienced by some consumers. Thus, in recent years several articles have been published in which the authors evaluated the salts of other organic acids for antilisterial activity in RTE meats. For example, Thompson et al. (42) reported that inclusion of 1 and 2% levulinate in bologna and turkey breast roll, respectively, suppressed outgrowth of *L. monocytogenes* for up to 90 days at 4 °C without adversely affecting the sensory quality. Similarly, Vasavada et al. (47) reported that inclusion of 1.4% sodium levulinate as an ingredient in fresh pork and turkey sausage was more effective than 1.4% sodium lactate for suppressing growth of the indigenous flora after 15 days of storage at 2 °C. Likewise, Islam et al. (19, 20) found that when turkey frankfurters or slices of chicken luncheon meat were surface treated separately with the sodium salts of benzoate, propionate, sorbate, or diacetate, numbers of *L. monocytogenes* were initially reduced by ca. 1.0 to 2.0 and 0.1 to 1.3 log CFU/g, respectively, with an additional reduction in pathogen numbers by ca. 0.3 to 0.6 and 0.2 to 1.0 log CFU/g, respectively, after 14 days of storage at 4 °C. As another example, Glass et al. (16) evaluated the effectiveness of up to 0.3% potassium sorbate, sodium benzoate, and sodium propionate, or combinations thereof, as ingredients in sliced uncured turkey breast and hams and reported that these antimicrobials suppressed outgrowth of *L. monocytogenes* for up to 84 days at 4 °C with negligible effects on sensory attributes. In another study, Glass et al. (15) found that inclusion of 0.05% sodium benzoate and 0.05% sodium propionate as ingredients in uncured turkey

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Ash (g/100 g)</th>
<th>Carbohydrates (g/100 g)</th>
<th>Fat (g/100 g)</th>
<th>Moisture (g/100 g)</th>
<th>Protein (g/100 g)</th>
<th>Salt (g/100 g)</th>
<th>Water activity</th>
<th>pH</th>
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<tr>
<td>Citrate-diacetate</td>
<td>1.74 ± 0.04</td>
<td>9.56 ± 0.28</td>
<td>6.98 ± 0.17</td>
<td>69.45 ± 0.08</td>
<td>1.49 ± 0.15</td>
<td>0.37 ± 0.02</td>
<td>0.977 ± 0.01</td>
<td>5.38 ± 0.02</td>
</tr>
<tr>
<td>Lactate-diacetate</td>
<td>2.20 ± 0.04</td>
<td>9.72 ± 0.26</td>
<td>7.02 ± 0.17</td>
<td>70.45 ± 0.08</td>
<td>1.49 ± 0.15</td>
<td>0.37 ± 0.02</td>
<td>0.977 ± 0.01</td>
<td>5.38 ± 0.02</td>
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<tr>
<td>Propionate-diacetate</td>
<td>2.50 ± 0.04</td>
<td>10.62 ± 0.21</td>
<td>9.25 ± 0.28</td>
<td>68.65 ± 0.23</td>
<td>1.49 ± 0.15</td>
<td>0.37 ± 0.02</td>
<td>0.977 ± 0.01</td>
<td>5.38 ± 0.02</td>
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<tr>
<td>No antimicrobial</td>
<td>1.41 ± 0.04</td>
<td>9.56 ± 0.28</td>
<td>6.98 ± 0.17</td>
<td>69.45 ± 0.08</td>
<td>1.49 ± 0.15</td>
<td>0.37 ± 0.02</td>
<td>0.977 ± 0.01</td>
<td>5.38 ± 0.02</td>
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</tbody>
</table>

**TABLE 2.** Physical-chemical analyses of pork scrapple formulated with and without citrate-diacetate-propionate blend adjusted to different pH levels.
and pork-beef bologna suppressed the growth of *L. monocytogenes* during storage for 91 days at 4°C.

In prefatory studies, we evaluated several antimicrobials for their effect on cells of *L. monocytogenes* that were surface inoculated onto slices of pork scrapple. As expected, pathogen numbers increased by at least 6.0 log CFU/g over 50 days at 4°C on scrapple formulated with or without citrate-diacetate. Some antimicrobials, such as cranberry powder (Mak AMC, Mak Wood Inc., Grafton, WI) had appreciable antilisterial activity at 1.7% (pH 5.1) but not at 0.85% (pH 5.6) ≤0.2 log CFU/g increase over 50 days at 4°C; data not shown). Other antimicrobials, such as cranberry seed extract (Mak Disperse, Mak Wood) or dried citrus peel pulp (pH 7.0; CitrusSmart, Alorama Corporation, La Belle, FL), allowed for appreciable growth similar to that of control treatments (data not shown). Unfortunately, cranberry powder was not very soluble for inclusion in scrapple, and it caused some reddish purple coloration of the product and/or compromised its texture. For these reasons, cranberry powder, cranberry seed extract, and dried citrus peel pulp were excluded from further evaluation. In contrast, when used alone, citrate-diacetate was generally ineffective, but when used in combination with lactate-diacetate (3 or 4%), lactate-diacetate-propionate (2.5%), or levulinate (2.5%) citrate-diacetate acted synergistically, at least in part, because of a decrease in the pH of scrapple (from ca. pH 6.4 to ≤6.0) that was directly attributable to the pH-altering effect of the antimicrobial blends. Addition of 0.85% cranberry powder also contributed to a decrease in the pH of scrapple (to 5.6), but unlike the salts of organic acids, an antimicrobial effect was not observed. Thus, lowering pH alone may not always be sufficient to inhibit or eliminate *L. monocytogenes*. Greater antilisterial activity may be achieved by adjusting the pH of solutions containing certain salts of organic acids used as ingredients to lower the pH of scrapple, which in turn generates more of the undissociated forms of these respective organic acids in the formulation. Because of cost considerations, it may only be feasible to include a single antimicrobial as an ingredient for scrapples. Therefore, because citrate-diacetate used alone was ineffective, because the lactate-diacetate-propionate blend was as effective as the lactate-diacetate blend but at a lower concentration, and because levulinate even when used in combination with citrate-diacetate allowed for a >1.0-log CFU/g increase in pathogen levels, the lactate-diacetate-propionate blend was selected for further evaluation at lower concentrations and lower pH levels. The expected result was that outgrowth of *L. monocytogenes* would be suppressed when the pH of the lactate-diacetate-propionate blend was adjusted to pH 5.0 or pH 5.5 because this low-pH ingredient in turn lowered the pH of the scrapple to ≤6.0.

Our findings are in general agreement with those of others who have reported that a decrease in the pH of the food or growth medium greatly enhanced the effectiveness of an antimicrobial. Ryser and Marth (36) reported that 0.3% propionate, with and without acidifying agents, was more effective against *L. monocytogenes* in cold-pack cheese when the pH of the cheese (ca. pH 5.2) was adjusted to ca. pH 5.0. Similar results were reported by El-Shenawy and Marth (8), who found that addition of up to 0.3% sodium propionate in tryptose broth adjusted to pH 5.0, but not pH 5.6, was effective for inhibiting the growth of *L. monocytogenes* at 4°C. Weak organic acids such as lactic, acetic, and propionic acids can be either charged or uncharged, depending on the protonation state of their acidic group, which in turn is determined by the pKa (the pH at which 50% of the total acid is undissociated) of the acidic group and the pH of the environment surrounding it. Although the antimicrobial mode of action of weak acids is not fully understood, the undissociated (uncharged) form of certain weak acids is lipid permeable and can diffuse into the cytoplasm of the bacterial cell, resulting in detrimental osmotic effects, including disruption of membrane function and metabolic processes within the cytoplasm (18).

Decreasing the pH of scrapple effectively increases the amount of uncharged acid in the environment and enhances the diffusion of the protonated acid into the bacterial cytoplasm, with resulting deleterious effects on *L. monocytogenes*. Thus, our results established that in addition to preventing outgrowth of the pathogen during storage, lowering the pH of the blend allowed for use of lower concentrations of the attendant antimicrobial without compromising the safety or quality of the product and without adding cost, presumably by liberating more undissociated acid to inhibit *L. monocytogenes*.

Chemical analyses revealed that the pork scrapple evaluated herein is similar in composition to the scrapples evaluated in our previously published study (1), particularly with respect to ash, moisture, protein, acidity, water activity, and pH (data not shown). Inclusion of different combinations and types/levels of antimicrobials as ingredients to control *L. monocytogenes* did not result in appreciable differences in the overall proximate composition of scrapple prepared with the original recipe that contained citrate-diacetate. Thus, panelists were not expected to discern significant differences (P ≥ 0.05) in overall acceptance, appearance, texture, or flavor intensity among experimental treatments in comparison to the original recipe. Because we anticipated that panelists might find some of the new formulations objectionable, notably those containing potassium (i.e., metallic or bitter) or diacете (i.e., vinegar), an otherwise similar set of products was formulated to include a botanical-based flavor masking agent in the lactate-diacetate-propionate blend. Again, panelists were not able to discern significant differences (P ≥ 0.05) in taste based on whether a masking agent was used in the formulation. These data confirm that decisions to modify the formulation of pork scrapple could be based on the antilisterial efficacy of the antimicrobial ingredients without concern for compromising the taste or texture of the product.

This study is the first and only study in which food-grade chemicals used to control outgrowth of *L. monocytogenes* on scrapple have been comparatively validated. It is also the only study of its kind to report a scientifically controlled taste test of scrapple. As expected from our prior work (1), scrapple formulated with or without citrate-diacetate supported growth of the pathogen during extended refrigerated storage. Some of the antimicrobials tested in
prefatory studies were excluded from further evaluation because of problems and concerns associated with solubility, product texture or sensory attributes, and/or cost or availability. Nonetheless, our data supported the hypothesis that inclusion of a lactate-diacetate-propionate blend as an ingredient for scrapple provides a less favorable environment for outgrowth of *L. monocytogenes* over the expected 50-day shelf life. In fact, inclusion of the lactate-diacetate-propionate blend (1.94% adjusted to pH 5.0) extended shelf life to 75 days (data not shown) without affecting appearance and presumably without negatively impacting the taste or texture of the product. Although salts of organic acids are quite effective for suppressing outgrowth of *L. monocytogenes* during an extended refrigerated shelf life, they are not typically effective for initial pathogen lethality. Therefore, future studies may be directed toward developing and validating interventions that used either alone or in combination will deliver both initial pathogen lethality and subsequent inhibition during the shelf life.

Apart from being tasty, nutritious, and well liked by consumers, scrapple may serve as a model meat or food product for evaluating antilisterial agents because (i) scrapple is made in relatively small batches and thus lends itself to custom formulations, and (ii) *L. monocytogenes* also grows quite well on the product, and thus efficacy of test interventions can be readily manifested and easily measured. Based on the results presented here, a petition has been submitted to the USDA-FSIS requesting approval for the use of potassium propionate in combination with potassium lactate and potassium diacetate as an antimicrobial ingredient in certain RTE meat products. Although not currently approved by the FSIS, potassium propionate is included in Table 3 of the Food and Agriculture Organization of the United Nations—World Health Organization Codex General Standard for Food Additives (12) and is approved for use as a preservative in processed comminuted meat, poultry, and game meats. Studies are ongoing to refine the composition, concentration, and/or pH of the lactate-diacetate-propionate blend for applications in other foods to lower the chances of someone acquiring foodborne listeriosis. The results of the present study indicate that, compared with the original recipe, inclusion of the lactate-diacetate-propionate blend (1.5 or 1.94% adjusted to pH 5.0) as an ingredient in pork scrapple can suppress pathogen outgrowth and appreciably extend shelf life without detrimental effects on quality.

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