Postprocess Control of *Listeria monocytogenes* on Commercial Frankfurters Formulated with and without Antimicrobials and Stored at 10°C

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**ABSTRACT**

The antilisterial effect of postprocess antimicrobial treatments on commercially manufactured frankfurters formulated with and without a 1.5% potassium lactate–0.05% sodium diacetate combination was evaluated. Frankfurters were inoculated (ca. 3 to 4 log CFU/cm²) with 10-strain composite *Listeria monocytogenes* cultures originating from different sources. The inocula evaluated were cells grown planktonically in tryptic soy broth plus 0.6% yeast extract (30°C, 24 h) or in a smoked sausage homogenate (15°C, 7 days) and cells that had been removed from stainless steel coupons immersed in an inoculated smoked sausage homogenate (15°C, 7 days). Inoculated frankfurters were dipped (2 min, 25 ± 2°C) in acetic acid (AA; 2.5%), lactic acid (LA; 2.5%), potassium benzoate (PB; 5%), or Nisaplin (commercial form of nisin; 0.5%, equivalent to 5,000 IU/ml of nisin) solutions, or in Nisaplin followed by AA, LA, or PB, and were subsequently vacuum packaged and stored for 48 days at 10°C. In addition to microbiological analyses, sensory evaluations were performed with uninoculated samples that had been treated with AA, LA, or PB for 2 min. Initial *L. monocytogenes* populations were reduced by 1.0 to 1.8 log CFU/cm² following treatment with AA, LA, or PB solutions, and treatments that included Nisaplin reduced initial levels by 2.4 to >3.8 log CFU/cm². All postprocessing treatments resulted in some inhibition of *L. monocytogenes* during the initial stages of storage of frankfurters that were not formulated with potassium lactate–sodium diacetate; however, in all cases, significant (*P < 0.05*) growth occurred by the end of storage. The dipping of products formulated with potassium lactate–sodium diacetate in AA or LA alone—or in Nisaplin followed by AA, LA, or PB—increased lag-phase durations and lowered the maximum specific growth rates of the pathogen. Moreover, depending on the origin of the inoculum, this dipping of products led to listericidal effects. In general, differences in growth kinetics were obtained for the three inocula that were used to contaminate the frankfurters. Possible reasons for these differences include the presence of stress-adapted subpopulations and the inhibition of the growth of the pathogen due to high levels of spoilage microflora. The dipping of frankfurters in AA, LA, or PB did not (*P > 0.05*) affect the sensory attributes of the product when compared to the control samples. The data generated in this study may be useful to U.S. ready-to-eat meat processors in their efforts to comply with regulatory requirements.

*L. monocytogenes* is a significant foodborne pathogen that causes listeriosis, a disease that can be life-threatening, with a fatality rate of 20% (23). Major listeriosis outbreaks have been linked to contaminated ready-to-eat (RTE) meat and poultry products, specifically frankfurters and poultry deli meats (6–9). In fact, non-reheated frankfurters and deli meats were categorized as high-risk products in a *L. monocytogenes* quantitative risk assessment of 23 RTE products (34). Because of the high fatality rate and the uncertainty of the infective dose of this pathogen, the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) has established a “zero-tolerance” policy for this pathogen on RTE products (26). In a further effort to control *L. monocytogenes* in RTE meat and poultry products, the USDA-FSIS established an interimfinal rule in 2003 (33). To comply with this interim final rule, establishments that produce RTE meat and poultry products that are exposed to the environment after thermal processing and that support the growth of *L. monocytogenes* are required to comply with one of three alternatives, based on the risk of the product, to prevent product adulteration with the pathogen. In brief, alternatives 1 and 2 require the use of a postlethality treatment (which may include antimicrobial agents) to reduce or eliminate *L. monocytogenes* and/or antimicrobial agents or processes to limit or suppress the growth of the pathogen throughout the shelf life of the product, whereas alternative 3 relies on sanitation measures and testing to control *L. monocytogenes* in the postlethality environment (33).

Although thermal processing has been estimated to reduce *L. monocytogenes* levels by 3 to 5 log cycles (21, 36), prevalence levels of 0.5 to 15.6% have been reported for various RTE products (16, 20, 35). Knowledge of the ability of *L. monocytogenes* to withstand adverse conditions, combined with reports that it is able to persist in food-processing environments for “months” and up to 10 years (10, 19, 32), indicates that meat products are most likely recontaminated when they are exposed to the manufactur-
ing environment during peeling, slicing, dicing, or packaging (32). An effective method for controlling L. monocytogenes in RTE meat and poultry products is the addition of antimicrobials to the formulation, especially when two or more compounds are used in combination (2–4, 15, 22, 24, 27, 30). Most RTE meat processors in the United States include sodium or potassium lactate, at levels of up to 2%, combined with 0.05 to 0.15% sodium diacetate in their formulations (32). Although postprocess antimicrobial dipping or spraying solutions are not currently applied in the industry, a number of studies have shown the potential effectiveness of such solutions in controlling L. monocytogenes during storage (2, 14, 15, 17, 18, 24, 25, 28). Very few (2, 24) of these studies, however, have evaluated control strategies based on antimicrobials being added to the formulation of the product and then treated by postprocess dipping or spraying. Such data could provide the industry with the option of using antimicrobials at lower concentrations and consequently minimize any negative effects to the sensory qualities of the products.

Most of the studies that target the control of L. monocytogenes on RTE meat products have used strains cultivated under optimal, or near-optimal, conditions for growth. Although the results obtained in these studies are of great value, such studies have not taken into consideration that the cells contaminating a product in the food-processing environment may have been exposed to environmental conditions that are often less than optimal. Studies (5, 11–13) have shown that lag-phase durations and growth rates of L. monocytogenes may be influenced by the preincubation conditions of the organism. Gay et al. (13) found that L. monocytogenes initially stored at 4°C for 4 weeks and then preincubated at 14°C (96 h) or 30°C (48 h) had lag-phase durations of <1 and 7.7 days, respectively, when subsequently subcultured and incubated at 14°C. However, investigations on the growth kinetics of L. monocytogenes cultured under different conditions before being used to inoculate RTE meat products are limited. In one study (12), the preincubation of L. monocytogenes strains at 4°C before the inoculation and storage (4°C) of bologna resulted in reduced lag phases when compared to strains preincubated at 20 and 37°C.

The objectives of this study were to compare the antilisterial activity, during storage at 10°C, of postprocess antimicrobial treatments applied as dipping solutions on commercially manufactured frankfurters formulated with and without potassium lactate–sodium diacetate combinations as ingredients and contaminated with inocula cultured to represent organisms of different ecological backgrounds. Furthermore, evaluations were carried out to determine the effect of selected postprocess antilisterial dipping treatments on the sensory properties of frankfurter products.

**MATERIALS AND METHODS**

**Bacterial strains and preparation of inocula.** A 10-strain composite of L. monocytogenes was used in this study and included Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b), and 103M (serotype 1a), all isolated from pork sausage; 558 (serotype 1/2, pork meat isolate); and PVM1, PVM2, PVM3, and PVM4 (pork variety meat isolates, serotypes not known). The strains were activated as described by Samelis et al. (27, 28); they were subsequently used to prepare three types of inocula, including cells grown planktonically in tryptic soy broth (Difco, Becton Dickinson, Sparks, Md.) supplemented with 0.6% yeast extract (Acumedia, Baltimore, Md.) (TSBYE) and in a smoked sausage homogenate (SSH), as well as detached cells from biofilms (BIO) formed on stainless steel coupons immersed in the same homogenate used for the SSH inoculum.

For preparation of the TSBYE inoculum, activated L. monocytogenes strains were subcultured (0.1 ml) in 10 ml of TSBYE and incubated at 30°C for 24 h. The strains were subsequently combined and centrifuged (Eppendorf, model 5810 R, Brinkmann Instruments, Inc., Westbury, N.Y.) at 4,629 × g for 15 min at 4°C. The harvested cells were washed with 10 ml of phosphate-buffered saline (PBS, pH 7.4; 0.2 g KH₂PO₄, 1.5 g of Na₂HPO₄·7H₂O, 8.0 g of NaCl, and 0.2 g of KCl in 1 liter of distilled water); centrifuged, as previously described; and resuspended in 100 ml of fresh PBS. The washed culture was then serially diluted in PBS to obtain a target inoculum level of 3 to 4 log CFU/cm² when 0.25 ml of inoculum was applied over the entire surface of each frankfurter sample.

The SSH and BIO inocula were cultured in a homogenate prepared from a commercially manufactured, fully cooked smoked sausage (formulated without potassium lactate and sodium diacetate as ingredients). The ingredients of the sausage included pork, salt, corn syrup, dextrose, flavoring, sodium erythorbate, sodium nitrite, and water. The product was received within 2 days of its production and stored at −30°C for <90 days. To prepare the homogenate, sausage was thawed overnight at 4°C, cut into 7-cm lengths, placed in WhirlPak bags (Nasco, Modesto, Calif.) with 40 ml of sterile distilled water, and homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min. The resultant slurry was passed through cheesecloth to separate the solid and liquid phases of the homogenate and then further diluted 1:3 (vol/vol) with sterile distilled water. Portions (40 ml) of the homogenate were placed in sterile centrifuge tubes (Oak Ridge, Nalge Nunc International, Rochester, N.Y.), each containing a stainless steel coupon (2 by 5 cm, type 304, no. 2b finish, 0.08 mm thick) that had been previously cleaned as described by Stopforth et al. (31). The homogenate was then inoculated with 1 ml of a 10-fold dilution of the 10-strain composite of L. monocytogenes and incubated at 15°C for 7 days. Cells growing planktonically in the homogenate (SSH inoculum) were appropriately diluted in PBS and used for the inoculation (0.25 ml) of frankfurters to obtain a target inoculum level of 3 to 4 log CFU/cm². Biofilm cells (BIO inoculum) on the stainless steel coupons were harvested (31), and 1.2 ml of the undiluted detached cell mixture (concentration of approximately 6 log CFU/ml) was used for product inoculation.

**Product inoculation.** Frankfurters were supplied by a commercial manufacturer and were inoculated within 5 days of production. The formulation of the 97% fat-free frankfurters consisted of pork, water, modified food starch, hydrolyzed soy and potato protein, corn syrup, salt, dextrose, flavorings, potassium chloride, autolyzed yeast, sodium phosphates, smoke flavoring, paprika, sodium erythorbate, and sodium nitrite. The manufacturer formulated one set of frankfurters to contain no antimicrobials as ingredients; the other set was formulated with 1.5% potassium lactate and 0.05% sodium diacetate. Frankfurters (2.5 cm in diameter) were cut in half (ca. 6 cm in length; 57 cm²) and were subsequently inoculated as described by Bedie et al. (4).
Postprocess antimicrobial treatments. Following inoculation, frankfurters were immersed in antimicrobial solutions for 2 min. The treatments included (i) no dipping (control); (ii) 2.5% acetic acid (AA; Mallinckrodt, Paris, Ky.); (iii) 2.5% lactic acid (LA; Sigma, St. Louis, Mo.); (iv) 5% potassium benzoate (PB; Sigma-Aldrich, Milwaukee, Wis.); (v) 0.5% Nisaplin (a commercial form of nisin; Aplin & Barret, Dorset, UK), equivalent to 5,000 IU/ml of nisin; (vi) Nisaplin followed by AA; (vii) Nisaplin followed by LA; and (viii) Nisaplin followed by PB. All treatments were applied to frankfurters inoculated with L. monocyto- genes cells of TSBYE origin, whereas the treatments applied to products inoculated with the cells of SSH and BIO origin included (i) no treatment (control); (ii) LA; (iii) Nisaplin; and (iv) Nisaplin followed by LA.

The solutions were made up in sterile distilled water, except for Nisaplin (which was prepared in 0.02 N HCl), and were applied at room temperature (25 ± 2°C). The pH values of the solutions were 2.59 ± 0.01 (AA), 2.08 ± 0.00 (LA), 7.47 ± 0.22 (PB), and 1.70 ± 0.02 (Nisaplin). All treatments were applied by immersing approximately 20 frankfurters in 1.5 litters of solution for 2 min and then draining for approximately 1 min. For sequential treatments, frankfurters were immersed in the first solution and then drained for 1 min before being immersed in the second solution. After draining, three frankfurters from each treatment were placed in a vacuum bag (15 by 22 cm, 3 mil std barrier, nylon/polyethylene vacuum pouch, Koch, Kansas City, Mo.), vacuum packaged (Hollymatic Corp., Countryside, Ill.), and stored at 10°C for 48 days.

Microbiological analyses. Triplicate samples were microbiologically analyzed on days 0, 4, 8, 12, 20, 28, 36, and 48 of storage. One frankfurter from each vacuum bag was aseptically transferred to a sterile WhirlPak bag, to which 30 ml of 0.1% buffered peptone water (Difco, Becton Dickinson) was added, and vertically shaken 30 times (2, 28). After serially diluting each sample in 0.1% buffered peptone water, 0.1 ml was surface plated onto tryptic soy agar (Difco, Becton Dickinson) supplemented with 0.6% yeast extract (TSAYE) and PALCAM agar (Difco, Becton Dickinson) for the enumeration of total microbial and L. monocyto- genes populations, respectively. Colonies were counted after incubation at 25°C for 72 h (TSAYE) and at 30°C for 48 h (PALCAM agar).

pH and aw measurements. For pH measurements, a second frankfurter from the same sample was diluted 10-fold (wt/wt) with distilled water and homogenized (Masticator, IUL Instruments) for 2 min. Measurements of the slurry were taken with a Denver Instruments (Arvada, Colo.) pH meter and glass electrode. Water activity (aw) values (AquaLab model series 3, Decagon Devices Inc., Pullman, Wash.) were determined for day 0 samples only. Performance verification standards composed of saturated salt solutions (Decagon) were used to check the calibration of the machine before use. Samples were cut into pieces <0.5 cm and placed evenly in an AquaLab sample cup, which was then placed inside the sample drawer of the instrument. All aw measurements were taken at room temperature (25 ± 2°C).

Sensory evaluation. Sensory evaluations were performed on frankfurters that were formulated without potassium lactate–sodium diacetate and that (i) received no dipping treatment (control) or (ii) were dipped for 2 min (25 ± 2°C) in 2.5% AA, 2.5% LA, or 5% PB. After application of the chemical treatments, frankfurters were drained (1 min), placed in vacuum bags (oxygen transmission rate of 3 to 6 cm3/m2/24 h/atm [4.4°C, 0% relative humidity], Cryovac North America, Duncan, S.C.), vacuum pack-

aged, and then stored at 5°C until the next day, when they were evaluated. To identify treatment groups, samples were coded with random three-digit numbers. An untrained panel of 25 consumers was recruited from the Department of Animal Sciences at Colorado State University (Fort Collins). A nine-point hedonic scale, where 1 = dislike extremely and 9 = like extremely, was used to evaluate the appearance, odor, flavor, and overall acceptability of the products. The color (1 = extremely pale; 9 = extremely dark) and texture (1 = extremely soft; 9 = extremely firm) of the products were also evaluated. For purposes of this study, the frankfurters were not heated before being evaluated. Even though the panelists were given the option of not eating the unheated frankfurters, they all chose to continue with the evaluation.

Statistical analysis. Microbiological data (n = 3) were converted to log CFU per square centimeter before preliminary statistical analysis of fixed effects by the mixed model procedure of SAS (29), which indicated that counts were dependent on the type of culture medium (TSAYE and PALCAM agar). Therefore, the data were reanalyzed using an 8 (treatments) × 8 (sampling times) factorial design. All statistical analyses were conducted with the SAS procedure for fixed effects and all interactions between fixed effects. The sensory evaluation data (n = 25) were also analyzed by the mixed model procedure of SAS. Least-squares means in bacterial populations between treatments and sampling times, along with sensory attribute scores, were separated by the analysis of variance mixed model procedure of SAS. All differences were reported at a significance level of α = 0.05.

In addition, the logarithm of the L. monocytogenes counts was modeled as a function of time using the model by Baranyi et al. (1). For curve fitting, the in-house program DMFit (Institute of Food Research, Reading, UK), which was kindly provided by Dr. J. Baranyi, was used. The Baranyi model is based on four parameters: (i) a parameter expressing the lag phase; (ii) a parameter that expresses the maximum specific growth rate per day (μmax); (iii) a parameter that represents the lower asymptote (γ0), which corresponds to the initial bacterial counts (log CFU per square centimeter); and (iv) a parameter that represents the upper asymptote (γυm), which corresponds to the maximum bacterial counts (log CFU per square centimeter) when the growth curve forms an upper plateau of the curve at the stationary phase of growth.

RESULTS AND DISCUSSION

Effect of postprocess antimicrobial dipping treatments on initial L. monocytogenes populations. Regardless of the presence or absence of potassium lactate–sodium diacetate in the formulation of frankfurters or the origin of the inoculum, similar trends in the reductions of initial L. monocytogenes populations were obtained for the different postprocess antimicrobial dipping treatments (Figs. 1 and 2). The AA, LA, or PB treatments, applied on their own, led to initial reductions of 1.0 to 1.8 log CFU/cm2. For treatments that included Nisaplin, higher initial reductions of the pathogen were obtained. More specifically, Nisaplin applied alone reduced initial L. monocytogenes populations by 2.4 to >3.8 log CFU/cm2, and almost identical reductions were obtained for treatments of Nisaplin followed by AA, LA, or PB (reductions of 2.4 to >3.6 log CFU/cm2), which indicates that the antimicrobial effects obtained were mainly due to the Nisaplin treatment. In a previous study (14) in which the same postprocess antimicrobial treatments
were applied to bologna and ham slices, similar reductions of initial \( L. \) \textit{monocytogenes} populations were reported.

**Effect of antimicrobials in ingredient formulation and postprocess antimicrobial treatments on survival and growth of microbial populations during storage.** Because only frankfurters inoculated with the TSBYE inoculum were subjected to all the different postprocess dipping treatments, only the results for this inoculum are discussed in this section. \( L. \) \textit{monocytogenes} populations on frankfurters that did not contain potassium lactate–sodium diacetate in the ingredient formulation and that were not subjected to any postprocess antimicrobial treatments (control) increased \((P < 0.05)\) from 3.2 log CFU/cm\(^2\) (day 0) to 7.1 log CFU/cm\(^2\) in 8 days at 10°C (Fig. 1). Similar findings...
were reported by Barmpalia et al. (2) for pork frankfurters stored at 10°C. In this study, all postprocess treatments applied to the product without potassium lactate–sodium diacetate additives resulted in the inhibition of the pathogen during the initial part of the storage period, compared to the control (undipped), with lag-phase durations ranging from 2.2 days (Nisaplin applied alone) to 16.1 days (Nisaplin followed by AA) (Table 1). Although the pathogen eventually grew ($P < 0.05$) in all cases, the most effective treatments were AA applied alone and Nisaplin followed by AA or PB, with levels reaching 4.6, 3.2, and 4.7 log CFU/cm², respectively, by the end of storage (day 48), compared to the other treatments, in which pathogen levels were $>7.0$ log CFU/cm² by day 36 (Fig. 1). In a previous study (14), when the same postprocess antimicrobial treatments were applied to bologna and ham slices, which did not contain antimicrobials in their ingredient formulations, listericial effects were obtained for all the treatments, except for Nisaplin applied on its own, during a 48-day storage period at 10°C. The difference in the results obtained in these two studies is most likely due to the different nature of the products. Specifically, in the case of frankfurters, coagulated protein forming the surface of frankfurter “skin” may have restricted the uptake of the antimicrobial solutions, whereas in bologna and ham, the cut surfaces of the slices probably allowed a better absorption of the antimicrobial solutions into the product, leading to longer-lasting antimicrobial effects. Alternatively, or in addition, differences in the shape of the products (frankfurter links versus bologna and ham slices) may have resulted in different residual oxygen levels in vacuum bags that, in turn, may have affected the growth ability of the pathogen during storage. Product $a_w$ values did not appear to be responsible for the differences observed, because these values were similar for bologna and frankfurters formulated without potassium lactate–sodium diacetate and were higher for ham (data not shown).

As expected (2, 4, 27), potassium lactate–sodium diacetate in frankfurter ingredient formulations inhibited the growth of L. monocytogenes during storage (Fig. 2 and Table 2). For control (undipped) frankfurters, a lag-phase duration of 10.1 days and a maximum specific growth rate ($\mu_{\text{max}}$) of 0.154 day$^{-1}$ were obtained for the pathogen on products with antimicrobials as ingredients, compared to no lag phase and a $\mu_{\text{max}}$ value of 0.485 day$^{-1}$ on undipped products without antimicrobials as ingredients (Tables 1 and 2). Also, $Y_{\text{end}}$ values for L. monocytogenes on undipped products containing potassium lactate–sodium diacetate were lower than on products formulated without the antimicrobials ($Y_{\text{end}} = 6.6$ and 8.1 log CFU/cm², respectively) (Tables 1 and 2).

The majority of dipping treatments (AA or LA alone, or in sequence with Nisaplin, as well as Nisaplin followed by PB) applied to frankfurters formulated with potassium lactate–sodium diacetate caused death of the pathogen during storage (Fig. 2 and Table 2). L. monocytogenes levels on samples treated with AA, LA, or Nisaplin followed by AA were reduced ($P > 0.05$) by 0.3 to 0.5 log CFU/cm² by day 48, and populations on frankfurters treated with Nisaplin followed by LA or PB were reduced by $>0.5$ log.

### Table 1: Growth kinetics of L. monocytogenes in commercial frankfurters

<table>
<thead>
<tr>
<th>Inoculum origin</th>
<th>Postprocess antimicrobial treatment</th>
<th>Lag-phase duration (days ± SE)</th>
<th>Maximum specific rate ($\mu_{\text{max}}$, days$^{-1}$ ± SE)</th>
<th>$Y_0$ (log CFU/cm²)$^a$</th>
<th>$Y_{\text{end}}$ (log CFU/cm²)$^b$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSBYE$^c$</td>
<td>Control</td>
<td>---$^d$</td>
<td>0.485 ± 0.017</td>
<td>3.2</td>
<td>8.1</td>
<td>0.992</td>
</tr>
<tr>
<td></td>
<td>Acetic acid (2.5%)</td>
<td>15.6 ± 1.9</td>
<td>0.108 ± 0.015</td>
<td>2.2</td>
<td>4.6</td>
<td>0.946</td>
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<td>Lactic acid (2.5%)</td>
<td>9.9 ± 0.8</td>
<td>0.373 ± 0.027</td>
<td>1.5</td>
<td>8.1</td>
<td>0.989</td>
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<td>Potassium benzoate (5%)</td>
<td>2.4 ± 1.1</td>
<td>0.182 ± 0.008</td>
<td>1.8</td>
<td>7.5</td>
<td>0.985</td>
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<td>Nisaplin (0.5%)</td>
<td>2.2 ± 0.9</td>
<td>0.366 ± 0.023</td>
<td>-0.3</td>
<td>8.1</td>
<td>0.982</td>
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<tr>
<td></td>
<td>Nisaplin (0.5%) + acetic acid (2.5%)</td>
<td>16.1 ± 3.1</td>
<td>0.487 ± 0.378</td>
<td>-0.1</td>
<td>2.8</td>
<td>0.963</td>
</tr>
<tr>
<td></td>
<td>Nisaplin (0.5%) + lactic acid (2.5%)</td>
<td>7.3 ± 0.7</td>
<td>0.496 ± 0.034</td>
<td>0.0</td>
<td>7.9</td>
<td>0.988</td>
</tr>
<tr>
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<td>Nisaplin (0.5%) + potassium benzoate (5%)</td>
<td>12.6 ± 1.3</td>
<td>0.569 ± 0.184</td>
<td>-0.3</td>
<td>4.3</td>
<td>0.963</td>
</tr>
<tr>
<td>SSH$^e$</td>
<td>Control</td>
<td>---</td>
<td>0.395 ± 0.027</td>
<td>4.0</td>
<td>7.8</td>
<td>0.971</td>
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<tr>
<td></td>
<td>Lactic acid (2.5%)</td>
<td>6.7 ± 0.7</td>
<td>0.553 ± 0.081</td>
<td>2.4</td>
<td>7.6</td>
<td>0.981</td>
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<td>Nisaplin (0.5%)</td>
<td>---</td>
<td>0.401 ± 0.019</td>
<td>0.1</td>
<td>7.7</td>
<td>0.987</td>
</tr>
<tr>
<td></td>
<td>Nisaplin (0.5%) + lactic acid (2.5%)</td>
<td>6.5 ± 0.6</td>
<td>0.402 ± 0.024</td>
<td>0.6</td>
<td>7.6</td>
<td>0.990</td>
</tr>
<tr>
<td>BIO$^f$</td>
<td>Control</td>
<td>---</td>
<td>0.409 ± 0.018</td>
<td>2.4</td>
<td>7.9</td>
<td>0.986</td>
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<td>Lactic acid (2.5%)</td>
<td>7.0 ± 0.5</td>
<td>0.388 ± 0.017</td>
<td>0.9</td>
<td>7.6</td>
<td>0.995</td>
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<td>Nisaplin (0.5%)</td>
<td>5.0 ± 0.9</td>
<td>0.464 ± 0.040</td>
<td>0.0</td>
<td>7.7</td>
<td>0.979</td>
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<td>Nisaplin (0.5%) + lactic acid (2.5%)</td>
<td>11.1 ± 1.0</td>
<td>0.602 ± 0.069</td>
<td>-0.2</td>
<td>7.3</td>
<td>0.986</td>
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</tbody>
</table>

$^a$ Lower asymptote estimated by the Baranyi et al. model (1).
$^b$ Upper asymptote estimated by the Baranyi et al. model (1).
$^c$ Planktonically grown in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE; 30°C, 24 h).
$^d$ No lag phase observed.
$^e$ Planktonically grown in a smoked sausage homogenate (SSH; 15°C, 7 days).
$^f$ Biofilm cells removed from stainless steel coupons immersed in an inoculated smoked sausage homogenate (BIO; 15°C, 7 days).
TABLE 2. Survival and growth kinetics of *Listeria monocytogenes* (*n* = 3) cultured under various conditions before inoculation on the surface of commercially manufactured frankfurters formulated with 1.5% potassium lactate and 0.05% sodium diacetate and subsequently left untreated or immersed for 2 min in antimicrobial solutions, alone or in sequence, then vacuum packaged and stored at 10°C for 48 days

<table>
<thead>
<tr>
<th>Inoculum origin</th>
<th>Postprocess antimicrobial treatment</th>
<th>Lag-phase duration (days ± SE)</th>
<th>Maximum specific rate (µmₚₙₓ, day⁻¹ ± SE)</th>
<th>Y₀ (log CFU/cm²)¹</th>
<th>Y_end (log CFU/cm²)²</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSBYE⁴⁻ Control</td>
<td>10.1 ± 2.4</td>
<td>0.154 ± 0.027</td>
<td>3.2</td>
<td>6.6</td>
<td>0.916</td>
<td></td>
</tr>
<tr>
<td>Acetic acid (2.5%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Lactic acid (2.5%)</td>
<td>—</td>
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<td></td>
</tr>
<tr>
<td>Potassium benzoate (5%)</td>
<td>23.0 ± 2.7</td>
<td>0.092 ± 0.013</td>
<td>1.9</td>
<td>1.9</td>
<td>0.881</td>
<td></td>
</tr>
<tr>
<td>Nisaplin (0.5%)</td>
<td>21.2 ± 1.7</td>
<td>0.198 ± 0.017</td>
<td>0.8</td>
<td>0.4</td>
<td>0.572/</td>
<td></td>
</tr>
<tr>
<td>Nisaplin (0.5%) + acetic acid (2.5%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.8</td>
<td>0.572/</td>
<td></td>
</tr>
<tr>
<td>Nisaplin (0.5%) + lactic acid (2.5%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.2</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Nisaplin (0.5%) + potassium benzoate (5%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.2</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>SSH⁵⁻ Control</td>
<td>4.3 ± 1.7</td>
<td>0.149 ± 0.020</td>
<td>3.2</td>
<td>6.2</td>
<td>0.950</td>
<td></td>
</tr>
<tr>
<td>Lactic acid (2.5%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.7</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Nisaplin (0.5%)</td>
<td>7.9 ± 2.6</td>
<td>0.131 ± 0.016</td>
<td>—0.1</td>
<td>3.9</td>
<td>0.936</td>
<td></td>
</tr>
<tr>
<td>Nisaplin (0.5%) + lactic acid (2.5%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>BIO⁶⁻ Control</td>
<td>14.0 ± 3.3</td>
<td>0.149 ± 0.020</td>
<td>0.1</td>
<td>—</td>
<td>0.867</td>
<td></td>
</tr>
<tr>
<td>Lactic acid (2.5%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.7</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Nisaplin (0.5%)</td>
<td>36.5 ± 2.4</td>
<td>0.360 ± 0.071</td>
<td>—0.2</td>
<td>—</td>
<td>0.959</td>
<td></td>
</tr>
<tr>
<td>Nisaplin (0.5%) + lactic acid (2.5%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

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¹ Positive maximum specific rate value indicates growth of *L. monocytogenes*; negative value indicates inhibition or listericidal effects.

² Lower asymptote estimated by the Baranyi et al. model (1).

³ Upper asymptote estimated by the Baranyi et al. model (1); no value could be estimated when the upper part of the growth curve ceased without forming an upper asymptote that corresponded to the stationary phase.

⁴ Planktonically grown in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE; 30°C, 24 h).

⁵ No lag phase or shoulder period (for treatments that inhibited or reduced *L. monocytogenes* populations) observed.

⁶ No curve fitting because no growth observed, or slight decreases in *L. monocytogenes* populations.

⁷ Planktonically grown in a smoked sausage homogenate (SSH; 15°C, 7 days).

⁸ Biofilm cells removed from stainless steel coupons immersed in an inoculated smoked sausage homogenate (BIO; 15°C, 7 days).

CFU/cm² and reached undetectable (less than −0.2 log CFU/cm²) levels. These findings are important, especially to the RTE meat industry, because they indicate that the application of postprocess antimicrobial treatments to frankfurters formulated with potassium lactate–sodium diacetate brings about both reductions in the initial *L. monocytogenes* levels and bacteriostatic effects during storage, even at an abusive temperature of 10°C. The results of our study, which are in agreement with previous findings (2, 24), demonstrate that *L. monocytogenes* on frankfurters can be effectively controlled by the use of strategies that combine both antimicrobial formulation ingredients and postprocess treatments. Barmpalia et al. (2) reported listericidal effects on stored (10°C), pilot-plant–made frankfurters containing sodium lactate (1.8%) and sodium diacetate (0.125 or 0.25%) that had undergone postprocess dipping treatments (2 min) in 2.5% AA or LA. In this study, although the single treatments of PB or Nisaplin permitted the growth (*P < 0.05*) of the pathogen on frankfurters containing antimicrobial ingredients, longer lag-phase durations, smaller *µ*mₚₙₓ values, and lower final (day 48) levels of *L. monocytogenes* were obtained than on corresponding products formulated without antimicrobial additives (Figs. 1 and 2 and Tables 1 and 2). More specifically, following lag-phase durations of 23.0 and 21.2 days for samples dipped in PB or Nisaplin, respectively, the *L. monocytogenes* levels reached by day 48 were 4.2 and 6.0 log CFU/cm², respectively (Fig. 2).

The growth of total microbial populations, as detected on TSYE (data not shown), showed patterns similar to the growth of populations on PALCAM agar, which indicates that the predominant population on these samples was *L. monocytogenes*. Treatments that effectively controlled *L. monocytogenes* (cells of TSBYE origin) during storage also inhibited the growth of the natural flora on those products. At the end of storage (data not shown), total microbial populations of 0.5 to 2.3 log CFU/cm² were obtained on frankfurters containing potassium lactate–sodium diacetate and treated postprocess with AA or LA applied on their own or with the three sequential treatments.

Effect of inoculum origin on the survival and growth of *L. monocytogenes* during storage. In general, the data indicated that there were differences in the growth kinetics of the three inocula (TSBYE, SSH, and BIO) used in this study (Tables 1 and 2). Notable differences in the survival and growth of the three inocula were obtained for frankfurters formulated with potassium lactate–sodium diacetate and dipped in Nisaplin followed by LA (Table 2 and Fig. 2). In this particular case, *L. monocytogenes* cells of BIO origin grew (*P < 0.05*) to a level of 4.0 log CFU/cm² following a lag phase of 36.5 days, whereas cells of
TSBYE and SSH origin did not grow on the product at all. A possible explanation for this could be that the cells of BIO origin contained a stress-adapted subpopulation, which, when exposed to the stressful environment of frankfurters containing antimicrobial additives and treated with Nisaplin followed by LA, permitted their proliferation, albeit after a long lag phase. This hypothesis, however, should be further investigated. Notable differences between the different inocula were also obtained for undipped (control) frankfurters formulated with antimicrobial additives where similar $\mu_{\text{max}}$ values were obtained (0.123 to 0.154 day$^{-1}$); however, L. monocytogenes cells of TSBYE and SSH origin exhibited lag-phase durations of 10.1 and 4.3 days, respectively, whereas no lag phase was obtained for the cells of BIO origin (Table 2 and Fig. 2). Furthermore, cells of SSH and BIO origin, which were cultivated at a lower temperature (15°C, 7 days) before inoculation onto products, generally had shorter lag-phase durations than cells of TSBYE origin, which were incubated at a higher temperature (30°C, 24 h) before contamination of the product. These findings are in agreement with a previous study (12) that reported shorter lag-phase durations for L. monocytogenes preincubated at 4°C before inoculation onto bologna than for cells preincubated at 20 and 37°C. These results are of interest because they indicate that the history or ecological background of the cells contaminating a product affects the growth kinetics of the organism during storage.

Also of interest was that, at the end of storage, the SSH cell levels on frankfurters formulated with potassium lactate–sodium diacetate and dipped in Nisaplin (on its own) were 3.9 log CFU/cm$^2$, whereas the levels of cells of TSBYE and BIO origin on corresponding products were 6.0 and 5.5 log CFU/cm$^2$, respectively (Fig. 2). All samples inoculated with L. monocytogenes cells of SSH origin had high levels of natural flora (5.8 to 6.6 log CFU/cm$^2$ on day 48), even for postprocess antimicrobial treatments where no growth of the pathogen was obtained (data not shown). The source of the natural flora was most likely in the preparation of this particular inoculum, and their presence most likely suppressed the growth of the pathogen on the SSH-inoculated samples, specifically those dipped in Nisaplin. This finding indicates that the presence of natural microflora may affect the ability of L. monocytogenes to grow to high levels.

Chemical and physical analyses. On day 0, similar pH values (6.23 ± 0.04 and 6.26 ± 0.03) were obtained for untreated frankfurters formulated with and without potassium lactate–sodium diacetate, respectively (data not shown in tabular form). Expectedly, dipping of frankfurters in the organic acid treatments, alone or following treatment with Nisaplin, led to reductions in the pH of the product. Specifically, regardless of the product formulation and origin of the inoculum, pH reductions of 0.29 to 0.85 were obtained for samples dipped in AA or LA alone, and pH reductions of 0.43 to 0.85 were obtained for samples treated with sequential treatments. In a similar study (14), the same postprocess treatments resulted in pH reductions of 1.31 to 1.41 for bologna samples and 1.75 to 2.12 for ham samples. Again, these differences are most likely due to the nature of the products (sausage-type products versus thinly sliced deli meats), with the deli meats absorbing more of the treatment solutions. In this study, the pH values on day 0 for the frankfurters dipped in PB remained unchanged, whereas treatments of Nisaplin or Nisaplin followed by PB led to reductions of 0.04 to 0.17 pH units. At the end of storage, reductions in product pH values, compared to day 0 samples, of up to 1.65 pH units were observed for samples with microbial levels of approximately 8 log CFU/cm$^2$.

On day 0, the $a_w$ values of control (undipped) frankfurters formulated with and without potassium lactate–sodium diacetate were 0.948 ± 0.001 and 0.963 ± 0.004, respectively (data not shown in tabular form). Following the application of the postprocess antimicrobial dipping treatments, $a_w$ values were 0.954 to 0.956 (for products formulated with potassium lactate–sodium diacetate) and 0.964 to 0.970 (for products formulated without potassium lactate–sodium diacetate).

Sensory analysis. Sensory evaluations were performed for antimicrobial dipping treatments that had completely inhibited the growth of L. monocytogenes during storage. These treatments included AA or LA alone or in sequence with Nisaplin and Nisaplin followed by PB. However, it was decided not to evaluate the sensory effects of sequential treatments, because AA, LA, and PB were applied after Nisaplin; consequently, the sensory qualities of the products would be mainly affected by the final treatments.

The panelists who tasted the unheated frankfurters were primarily college students (80%) aged 21 to 34 years (83.3%), 60% of whom were men. Most of the panelists (73.9%) indicated that they liked to eat frankfurters and that they cooked (80%) the product before consuming it. Furthermore, it was established that 40% of them ate frankfurters one to three times per month and that 48% ate the product one to six times per year.

Under the conditions of this study, dipping of frankfurters in AA, LA, or PB did not ($P > 0.05$) affect any of the sensory attributes tested for, compared to the undipped control (Table 3). Because an untrained panel was used to perform the sensory analysis, the standard deviations were expectedly high (1.0 to 2.1). For each of the sensory attributes evaluated, frankfurters dipped in PB received the lowest mean hedonic scores compared to the undipped control samples (0.2 to 0.9 lower scores). Similar results were reported by Barmpalia et al. (2), who dipped pork frankfurters in 2.5% AA or LA for 2 min and found similar or higher ($P > 0.05$) sensory scores compared to undipped frankfurters. In another study (14), bologna and ham dipped in AA, LA, or PB (2 min) received lower sensory scores than undipped controls, especially for ham dipped in PB. Furthermore, it was reported that products treated with AA resulted in the lowest scores for odor, flavor, and overall acceptability compared to the control samples (14).

In summary, our results indicate that dipping frankfurters in postprocess antimicrobial treatments that include Nisaplin results in 2.4 to >3.8 log CFU/cm$^2$ reductions in
initial _L. monocytogenes_ populations. Furthermore, by applying postprocess antimicrobial dipping treatments to frankfurters formulated with potassium lactate–sodium diacetate, inhibitory and listericidal effects were obtained during storage at 10°C. This approach may be refined and validated for commercial application.

**ACKNOWLEDGMENTS**

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**REFERENCES**


