Inhibition of *Listeria monocytogenes* by Propionic Acid–Based Ingredients in Cured Deli-Style Turkey

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ABSTRACT

*Listeria monocytogenes* growth can be controlled on ready-to-eat meats through the incorporation of antimicrobial ingredients into the formulation or by postlethality kill steps. However, alternate approaches are needed to provide options that reduce sodium content but maintain protection against pathogen growth in meats after slicing. The objective of this study was to determine the inhibition of *L. monocytogenes* by propionic acid–based ingredients in high-moisture, cured turkey stored at 4 or 7°C. Six formulations of sliced, cured (120 ppm of NaNO₃) deli-style turkey were tested, including control without antimicrobials, 3.2% lactate-diacetate blend (LD), 0.4% of a liquid propionate-benzoate–containing ingredient, or 0.3, 0.4, and 0.5% of a liquid propionate–containing ingredient. Products were inoculated with 5 log CFU *L. monocytogenes* per 100-g package (3 log CFU/ml rinsate), vacuum-sealed, and stored at 4 or 7°C for up to 12 weeks; and populations were enumerated by plating on modified Oxford agar. As expected, the control without antimicrobials supported rapid growth, with >2 log average per ml rinsate increase within 4 weeks of storage at 4°C, whereas growth was observed at 6 weeks for the LD treatment. For both replicate trials, all treatments that contained liquid propionate or propionate-benzoate limited *L. monocytogenes* growth to an increase of <1 log through 9 weeks storage at 4°C. Sporadic growth (1-log increase) was observed in individual samples for all propionate-containing treatments at weeks 10, 11, and 12. As expected, *L. monocytogenes* grew more rapidly when products were stored at 7°C, but trends in relative inhibition were similar to those observed at 4°C. These results verify that propionate-based ingredients inhibit growth of *L. monocytogenes* on sliced, high-moisture, cured turkey and can be considered as an alternative to reduce sodium-based salts while maintaining food safety.

*Listeria monocytogenes* is estimated to cause approximately 1,600 listeriosis cases and 260 deaths in the United States each year, and total annual costs of foodborne listeriosis are approximately $1.1 billion (14, 20). In 2003, deli meats and frankfurters without growth inhibitors were identified among ready-to-eat foods as posing one of the greatest risks of illness or death per serving from *L. monocytogenes* since they are often consumed directly from the refrigerator without thorough reheating (25). Later risk assessments identified that use of growth inhibitors, location of slicing (at deli versus at manufacturing facility), and storage time were important factors affecting the relative risk of listeriosis-associated deaths (17, 21). Substantial progress has been made in reducing the incidence of *L. monocytogenes* in meat processing facilities; however, among food-pathogen pairs, the deli meats–*L. monocytogenes* pair ranks number three for cost of illness and loss of quality-adjusted life years and continues to be a major food safety concern (2).

The ability of *L. monocytogenes* to grow under refrigeration conditions further increases the risk of illness after consumption of contaminated food. Generally recognized as safe chemical antimicrobials such as lactates and diacetate are widely used to inhibit *L. monocytogenes* in processed meat formulations. Recently, antimycotic agents such as propionate and benzoate salts have been shown to exert significant antilisterial activity in both media and meat matrices, such as ready-to-eat uncured turkey and cured ham and bologna (8–13). Propionates were also found to be effective against gram-negative bacterial pathogens, such as *Escherichia coli* O157:H7, *Salmonella Typhimurium*, and *Campylobacter* spp., and against gram-positive pathogens, such as *Clostridium botulinum*, *Staphylococcus aureus*, and rope-forming *Bacillus subtilis* (4, 15, 16, 18, 19). In addition to the antimicrobial properties, these antimycotic agents contribute less sodium to the final food when compared to traditionally used sodium lactate because the addition levels are lower (7).

In the United States, propionate and benzoate salts are approved for use in a variety of foods such as bread, baked goods, pizza crust, etc. (24). Until recently, both ingredients were excluded from use in meat and poultry products
without in-plant waivers approved by the U.S. Department of Agriculture (22), but these compounds have now been allowed for use in ready-to-eat meat and poultry products (23). Before these products enter the commerce stream, validation studies are required to confirm the antilisterial efficacy of the antimycotic agents. The objectives of the current study were to evaluate the inhibition of \textit{L. monocytogenes} and spoilage microflora on cured deli-style turkey manufactured with different proprietary liquid benzoate– and/or propionate-based ingredients and stored at 4 or 7°C for up to 12 weeks.

\section*{MATERIALS AND METHODS}

\textbf{Turkey product manufacture.} Six formulations of sliced, cured, deli-style turkey were tested, including a negative control without antimicrobials, 3.2\% lactate-diacetate blend (blend containing 56\% sodium lactate and 4\% sodium diacate; Purac, Lincolnshire, IL, or MAK Wood, Inc., Grafton, WI, for trials 1 and 2, respectively), and four additional test treatments with 0.3, 0.4, or 0.5\% proprietary ingredient PC (liquid sodium propionate described as propionic acid buffered with sodium hydroxide to a pH of 4.8 to 5.2; Kemin Industries, Des Moines, IA), or with 0.4\% proprietary ingredient PBC (liquid sodium propionate and sodium benzoate described as propionic acid and benzoic acid buffered with sodium hydroxide to a pH of 4.8 to 5.2; Kemin Industries). Meat treatments were produced and slicing was done in a pilot facility of a commercial manufacturer under good manufacturing practices by injecting whole muscle turkey breasts with brine solutions, which included water, modified food starch, carrageenan, sodium chloride, dextrose, sodium phosphate, turkey flavor, and sodium nitrite; antimicrobial solutions were added to the brine solution to achieve the final concentration as appropriate (Table 1). Target analytical specifications were 75 to 77\% moisture, 1.6 to 1.8\% NaCl, and pH 6.2 to 6.4 after cook, and 120 ppm of ingoing sodium nitrite (wt/wt total formulation). Turkey breasts were tumbled under vacuum at 4°C, cooked to 74°C (165°F) internal temperature, and chilled to 4°C. Chilled, sliced products were packaged and shipped to the Food Research Institute, University of Wisconsin–Madison under refrigeration. Products were stored at 4°C until inoculation and were used within 1 week after production. The study was replicated twice.

\textbf{Proximate and chemical analysis.} Triplicate uninoculated samples for each lot were assayed by the Food Research Institute for moisture (5 h, 100°C, vacuum oven method 950.46) (1), NaCl (measured as % \text{Cl}^-, \text{AgNO}_3 potentiometric titration, Mettler DL22 food and beverage analyzer, Columbus, OH), residual nitrite (colorimetric method 973.31) (1), and water activity (AquA Lab 4TE water activity meter, Decagon Devices, Pullman, WA). In addition, the pH (Accumet Basic pH meter and Orion 8104 combination electrode, Thermo Fisher Scientific, Waltham, MA) was measured on the slurry obtained by removing a representative of 10 g of the uninoculated samples and homogenizing it with 90 ml of deionized water using a lab blender (Stomacher 400, A.J. Steward, London, England).

\textbf{Microbial challenge testing: preparation of inocula.} \textit{L. monocytogenes} strains FSL-C1-109 (deli turkey isolate associated with illness, serotype 4b), LM 101 (hard salami isolate, serotype 4b), LM 108 (hard salami isolate, serotype 1/2a), LM 310 (goat’s milk cheese isolate associated with illness, serotype 4), and V7 (raw milk isolate, serotype 1) were grown individually in 10 ml of Trypticase soy broth (BBL, BD, Sparks, MD) at 37°C for 18 to 20 h. Cells were harvested by centrifugation (2,500 x g, 20 min) and suspended in 4.5 ml of 0.1% buffered peptone water (pH 7.2). Equivalent populations of each isolate were combined to provide a five-strain mixture of \textit{L. monocytogenes}. Populations of each strain and the mixture were verified by plating on trypticase soy agar and modified Oxford agar (\textit{Listeria} selective agar base, Difco, BD, Sparks, MD).

\textbf{Inoculation and testing.} Slices were surface inoculated with \textit{L. monocytogenes} to provide approximately 5 log CFU/100-g package (equivalent to 3 log CFU/ml of rinsate when using 100 ml of rinsate for testing). For each package, a total 0.5 ml of liquid inoculum was distributed over the top surface of the slices, and slices were stacked such that the inoculum was between the slices (typically 10 slices per package, range 97 to 105 g per package). Inoculated products were vacuum packaged (Multivac AGW, Sepp Haggenmuller KG, Wolfertschwenden, Germany) in gas-impermeable pouches (3 mil high barrier EVOH pouches, Deli 1 material, gas-impermeable pouches (3 mil high barrier EVOH pouches, Deli 1 material, oxygen transmission 2.3 cm\(^2\)/cm\(^2\)/24 h at 24°C; water transmission 7.8 g/cm\(^2\)/24 h at 37.8°C and 90\% relative humidity; WinPak, Winnipeg, Manitoba, Canada), and samples for each treatment were divided to store at either 4 or 7°C. Uninoculated samples without additional moisture were similarly packaged and stored at both temperatures. Triplicate uninoculated samples for each treatment were assayed for changes in \textit{L. monocytogenes} populations, and duplicate uninoculated samples were assayed for changes in lactic acid bacteria and pH at zero time, and at 4, 6, 8, 9, 10, 11, and 12 weeks storage at 4°C, and at 2, 4, 5, 6, and 7 weeks at 7°C. Bacterial populations were determined in rinsate obtained after adding 100 ml of sterile Butterfield phosphate buffer to each package and massaging the contents externally by hand for about 3 min. \textit{L. monocytogenes} was enumerated by surface plating serial (1:10) dilutions of rinsate on duplicate plates of modified Oxford agar. Sampling was discontinued early for a formulation if \textit{L. monocytogenes} growth occurred (e.g., >2-log increase). In order to determine the effect of the experimental treatments on the

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\textbf{Formulation} & \textbf{\% moisture} & \textbf{\% NaCl} & \textbf{pH} & \textbf{a\(_{w}\)} & \textbf{Residual nitrite (ppm)} \\
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Control & 76.62 ± 0.56 & 1.71 ± 0.04 & 6.44 ± 0.10 & 0.978 ± 0.004 & 37.9 ± 8.6 \\
0.3\% PC & 77.02 ± 0.68 & 1.74 ± 0.06 & 6.30 ± 0.12 & 0.977 ± 0.003 & 28.1 ± 9.8 \\
0.4\% PC & 76.54 ± 0.49 & 1.69 ± 0.03 & 6.20 ± 0.12 & 0.977 ± 0.003 & 27.9 ± 11.6 \\
0.5\% PC & 76.18 ± 0.09 & 1.66 ± 0.02 & 6.13 ± 0.10 & 0.977 ± 0.002 & 43.8 ± 6.5 \\
0.4\% PBC & 76.44 ± 0.58 & 1.79 ± 0.00 & 6.38 ± 0.09 & 0.973 ± 0.006 & 42.0 ± 8.1 \\
3.2\% LD & 75.57 ± 0.20 & 1.78 ± 0.05 & 6.40 ± 0.14 & 0.970 ± 0.001 & 40.1 ± 18.1 \\
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\caption{\textit{Analyzed values of moisture, salt (NaCl), pH, water activity, and residual nitrite in sliced turkey formulations\(^a\)}}
\end{table}

\(^a\) Values are expressed as average ± standard deviation for two replicate trials (\(n = 6\)). a\(_{w}\), water activity; PC, liquid sodium propionate; PBC, liquid sodium propionate and sodium benzoate; LD, lactate-diacetate blend.
growth of spoilage microorganisms that may ultimately affect the growth of *L. monocytogenes*, changes in pH and populations of competitive microflora were evaluated on unincubated samples. The pH was measured on a 10-g subsample as described above. Populations of lactic acid–producing bacteria were assayed for the remaining portion of the unincubated samples by plating rinsate on all purpose Tween (APT) agar (Difco, BD) with 0.002% bromoresol purple (25°C, 48 to 72 h). Counts were not completed on APT agar for inoculated samples because the agar is not selective and does not differentiate between populations of *L. monocytogenes* versus lactic acid–producing and other spoilage bacteria.

**Statistical analysis.** Populations of *L. monocytogenes* (log CFU per milliliter of rinsate) reported are average values and standard deviations for triplicate samples and two separate trials for each test formulation (*n* = 6). Growth of microbes is reported as log increase over the average zero time populations. Differences between the experimental treatments and the control without antimicrobials were analyzed by one-way and two-way analysis of variance (ANOVA) at each sampling interval (Minitab 14.1, State College, PA). All statistically significant differences reported were at the *P* < 0.05 level.

**RESULTS AND DISCUSSION**

Average values of each formulation for moisture, pH, NaCl, water activity (aw), and nitrites are reported in Table 1. Across all treatments, the average analytical value for moisture was 76.47% ± 0.61%; NaCl, 1.73% ± 0.08%; pH, 6.30 ± 0.15; aw, 0.976 ± 0.004; and residual nitrite, 37.1 ± 11.7 ppm. Because all treatments had similar proximate analysis, differences in growth are attributed to antimicrobial inclusion.

Populations of indigenous spoilage microflora were different between the two trials, and presence was sporadic among samples. However, in neither trial did competitive microflora correlate with pathogen growth or inhibition (data not shown). In both trials, the populations of microorganisms isolated on APT agar at zero time were less than levels detectable by direct plating (<1 log CFU/ml rinsate). In trial 1, in packages in which indigenous microflora were present, they typically grew to high populations (>4 log CFU/ml rinsate) within the first 2 to 4 weeks of 4 and 7°C incubation, suggesting that the antimicrobials did not inhibit spoilage. In trial 2 no significant growth of spoilage microbes was observed for any of the treatments. Most trial 2 samples contained populations at a less than detectable level (<1 log CFU/ml rinsate) throughout the 12-week study, suggesting that either little recontamination occurred during the slicing of the test material or the type of microbes present did not grow under the conditions of this study. For both trials, the pH values typically remained stable for all treatments throughout the test period. Therefore, inhibition of *L. monocytogenes* observed for PC, PBC, and LD treatments of this study was likely due to the antimicrobial treatment itself, rather than to interference from competitive microflora.

The average *L. monocytogenes* inoculum for all samples across both trials was 3.11 ± 0.59 log CFU/ml rinsate. The inhibition of *L. monocytogenes* in the various treatments is reported in Figure 1A and 1B for products stored at 4 and 7°C, respectively. As observed in other studies, *L. monocytogenes* grew rapidly on the treatments with negative control without antimicrobials. When stored at 7°C, the negative control supported an average 4.02 ± 1.32 log increase at 2 weeks, whereas populations of the pathogen increased an average 2.93 ± 0.91 and 4.80 ± 0.57 log at 4 and 6 weeks, respectively, when stored at 4°C. These results are similar to increases predicted by the OptiForm 2007 Model (Purac America, Lincolnshire, IL) for a similar product that was formulated with 76.6% moisture, 1.71% NaCl, and pH 6.4 and that contained nitrite; the model predicts a 4-log increase in approximately 2 to 3 weeks (17 to 20 days) when stored at 7°C and a 3-log increase in approximately 4 to 5 weeks (29 to 36 days) when stored at 4°C.

Large variation in microbial inhibition was observed in the high-moisture, cured turkey supplemented with 3.2% LD and stored at 4°C. In trial 1, the LD treatment (75.5% moisture, 1.82% NaCl, and pH 6.5) supported a 0.74 ± 0.40- and 4.08 ± 1.15-log increase after 4 and 6 weeks of storage at 4°C, respectively; whereas, in trial 2 (75.8% moisture, 1.7% NaCl, and pH 6.3), a 0.41 ± 0.34-log increase was observed at 6 weeks, but >2.5-log increase was noted at 8 weeks at 4°C. The OptiForm 2007 model predicted a 1-log increase for a product with similar analytical values in approximately 8 to 11 weeks (55 to 76 days) of storage at 4°C. Additional study may be required to determine the effect of meat species, different strains of *L. monocytogenes*, and high moisture values on limits of the model or if undetected fluctuations in storage temperatures could have contributed to the enhanced growth rate.

In contrast, supplementing cured turkey with 0.3, 0.4, and 0.5% PC or 0.4% PBC consistently limited growth of *L. monocytogenes* to <1-log increase for all samples tested through 9 weeks storage at 4°C for both replicate trials (Fig. 1A). Populations of *L. monocytogenes* stored at 4°C for 10 weeks and beyond demonstrated variability between samples within replicate trials, supporting ≥1-log increase in at least one sample each during the remainder of the trial. The sporadic increases in *L. monocytogenes* may be attributed to various factors, including, but not limited to, sample-to-sample variation in distribution of moisture, salt, or antimicrobial within the matrix, and robustness of the various *L. monocytogenes* strains used in this study.

Because of the variation of populations, one-way ANOVA of population data for the 0.3, 0.4, and 0.5% PC and 0.4% PBC treatments revealed no significant differences (*P* > 0.05) among the four treatments through 12 weeks of storage at 4°C. Data suggest that there is a weak correlation between the concentration of PC (0.3, 0.4, and 0.5%) versus time for inhibition in high-moisture turkey products. Regardless, each of the four propionate-liquid treatments significantly delayed growth of *L. monocytogenes* compared to the control without antimicrobials throughout the study and controlled growth to <1 log for 9 weeks of storage at 4°C.

As expected, *L. monocytogenes* grew faster on cured turkey stored at slight abuse temperature (7°C), but trends in relative inhibition were similar to those observed at 4°C.
No growth of the pathogen was detected at 2 weeks for any of the propionate-containing treatments, whereas an average 4.02 ± 1.32 and 2.38 ± 0.25 log increase was observed for the no antimicrobial and LD controls, respectively. Growth (defined as >1-log average increase) was delayed until weeks 4, 5, and 7 for the 0.3, 0.4, and 0.5% PC treatments and week 4 for the product supplemented with 0.4% PBC.

Previous studies conducted in our laboratory revealed that 0.2% sodium propionate solids (0.152% propionic acid equivalent) inhibited growth of *L. monocytogenes* in cured ham (average 73.7% moisture, 2.59% NaCl, pH 6.39, 35 ppm of residual NO₂, 156 ppm of added sodium nitrite) stored at 4 °C for 12 weeks, whereas 0.1% sodium benzoate solids (0.084% benzoic acid equivalent) provided comparable protection (11). Greater concentrations of antimicrobials were required to inhibit listerial growth in uncured, deli-style turkey. The more consistent inhibition by sodium propionate treatment in the Glass et al. (12) publication compared with the 0.3 or 0.4% PC treatments in this study may be attributed to the lower moisture, higher salt, and higher added sodium nitrite in the cured ham for the 2007 study. Insufficient data is available to assess the effect of meat type (pork or ham versus turkey).

Although propionate and benzoate are typically added to foods to inhibit molds, growing evidence demonstrates their efficacy in inhibiting bacterial growth. Benzoate has been reported to have a number of detrimental effects on microbes, including alteration of the permeability of microbial cell membranes, reduction of glucose consumption and growth in yeast, inhibition of amino acid uptake in molds, and inhibition of some microbial enzyme systems, including the citric acid cycle and oxidative phosphorylation (5). Propionate has been reported to decrease RNA, DNA, protein, lipid, and cell wall synthesis in bacteria (6). *Aspergillus* takes up propionate and forms propionyl coenzyme A, which inhibits glucose metabolism, thereby depressing growth (3).
Comparison of results from this study with previous studies and predictive models suggests that moisture, pH, NaCl, added nitrite, storage temperature, and, perhaps, meat type are significant factors in determining the efficacy of various antimicrobials. Although this study demonstrates that liquid propionate–based ingredients inhibit growth of L. monocytogenes in high-moisture, cured, ready-to-eat meat products for 9 weeks at 4°C, it is incumbent upon the manufacturers to validate the efficacy of the antimicrobials in their specific product prior to use. As suggested by the growth in the LD control in this study, high moisture values (>73%) and storage temperatures (>4°C) may accelerate listerial growth under otherwise inhibitory conditions.

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