Research Note

Inhibition of Listeria monocytogenes in Turkey and Pork-Beef Bologna by Combinations of Sorbate, Benzoate, and Propionate

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ABSTRACT

The control of Listeria monocytogenes was evaluated with ready-to-eat uncured turkey and cured pork-beef bologna with combinations of benzoate, propionate, and sorbate. Three treatments of each product type were formulated to include control with no antimycotic agents; a combination of 0.05% sodium benzoate and 0.05% sodium propionate; and a combination of 0.05% sodium benzoate and 0.05% potassium sorbate. Ingredients were mixed, stuffed into fibrous, moisture-impermeable casings, cooked to an internal temperature of 73.9°C, chilled, and sliced. The final product was surface inoculated with L. monocytogenes (4 log CFU per package), vacuum packaged, and stored at 4°C for 13 weeks. The antimycotic addition to the second and third uncured turkey treatments initially slowed the pathogen growth rate compared with the control, but populations of L. monocytogenes increased 5 log or more by 6 weeks. In contrast, the addition of antimycotic combinations in the cured bologna prevented growth of L. monocytogenes during the 13-week storage period at 4°C, compared with a more than 3.5-log increase in listerial populations in the control bologna, to which no antimicrobial agents had been added. These data suggest that low concentrations of antimycotic agents can prevent L. monocytogenes growth in certain ready-to-eat meats. Additional research is needed to define the levels needed to prevent growth of L. monocytogenes in high-moisture cured and uncured ready-to-eat meat and poultry and for gaining governmental approval for their use in such formulations.

The U.S. Department of Agriculture, Food Safety and Inspection Services (USDA-FSIS) 2003 Final Rule to “Control of Listeria monocytogenes in Ready-to-Eat Meat and Poultry Products” manages food safety risks by mandating that establishments incorporate strategies to control Listeria monocytogenes, such as postlethality treatments or use of growth inhibitors for Listeria on ready-to-eat (RTE) meat and poultry products (3). Products produced under alternative 1 and 2 of the Final Rule must be processed to eliminate L. monocytogenes and/or formulated to limit its growth such that there is no greater than a 1.0-log increase during product shelf life (18). Under these alternatives, approved food-grade antimicrobial agents can be used to prevent growth of L. monocytogenes in high-moisture, high-pH products and thus prevent additional outbreaks and recalls.

Currently, manufacturers incorporate lactate and diacetate into many formulations of RTE products (1, 2). However, although the addition of lactate and diacetate combinations inhibits growth of L. monocytogenes in cured (with sodium nitrite) meat and poultry products, these organic acid salts are less effective in uncured products (7, 11, 12, 16). Therefore, alternative food-grade antimicrobial sys-

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Although screening for antimicrobial activity in model meat systems such as slurries is rapid and efficient, the levels of fat, moisture, and nitrite may not be identical to those found in finished RTE meat and poultry products. Formulations identified as useful in screening studies may be more or less effective than in finished products. Therefore, efficacy of antimicrobial treatments must be validated in finished product.

The objective of the current study was to confirm whether 0.1% total antimycotic agents will inhibit growth of *L. monocytogenes* in meat systems manufactured under simulated commercial conditions. Concentrations of antimycotic agents for testing were chosen to be less than levels that have adverse affect on sensory profile, were shown to inhibit *L. monocytogenes* in model meat systems, and were within U.S. Food and Drug Administration regulatory limits (19). Test treatments included 0.1% total of benzoate-propionate or benzoate-sorbate in a low-fat, high-moisture, poultry product without nitrite (uncured turkey) and high-fat, intermediate-moisture, meat product with nitrite (cured pork-beef bologna). This study validates benzoate, propionate, and sorbate as anti-listerial ingredients and helps identify factors that affect activity in processed meat products to design future experiments.

**MATERIALS AND METHODS**

**Preparation of inocula.** *Listeria monocytogenes* strains Scott A (clinical isolate, serotype 4b), LM 101 (hard salami isolate, 4b), LM 108 (hard salami isolate, 1/2a), LM 310 (goat milk cheese isolate, 4), and V7 (raw milk isolate, 4) were grown individually in 10 ml of Trypticase soy broth (BBL, Becton Dickinson, Sparks, Md.) at 37°C for 16 to 18 h. Cells were harvested by centrifugation (2500 × 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 *L. monocytogenes* to yield a target level of 4 log CFU/50-g package. Populations of each strain and the mixture were verified by plating on Trypticase soy agar and modified Oxford agar (Listeria Selective Agar base, Difco, Becton Dickinson, Sparks, Md.).

**Production of RTE meat and poultry products.** Two antimicrobial combination systems were evaluated by using uncured turkey and cured pork-beef bologna formulations. Treatments included control with no antimycotic agents added; 0.05% sodium benzoate with 0.05% sodium propionate; and 0.05% sodium benzoate with 0.05% potassium sorbate.

Products were manufactured in a USDA-inspected processing laboratory at the Meat Science and Muscle Biology Lab, University of Wisconsin–Madison, and were formulated to simulate commercial product by using the Least Cost Formulation Program (Least Cost Formulation, Ltd., Virginia Beach, Va.). Batter (4.54-kg batch) for control turkey product was prepared to include ground turkey breast (3.25 kg), water (1.13 kg), salt (77.2 g), sodium tripolyphosphate (13 g; 0.04% on meat-block basis), and commercial spice mix (88.3 g). Control bologna batter included ground lean pork (2.08 kg), pork fat (1.13 kg), beef fat (0.56 kg), water (0.58 kg), salt (68.2 g), commercial spice mixture (104 g), sodium tripolyphosphate (15.3 g), commercial cure mixture (9.57 g to yield 0.0156% sodium nitrite), and sodium erythorbate (1.7 g). For each treatment, food-grade sodium benzoate (Ashland Chemical Co., Columbus, Ohio), sodium propionate (Spectrum Chemical Mfg. Corp., Gardena, Calif.) or potassium sorbate (JLM Chemicals, Inc., Blue Island, Ill.) (0.05% wt/wt) was added when applicable.

Ground turkey or meat and all of the nonmeat ingredients were processed for 3 min (temperature maintained at less than 12.8°C) in a cutter/mixer (model VCM 12, Stephan Machinery Corp., Columbus, Ohio) under a full vacuum. Batter was stuffed into fibrous, moisture-impermeable casings (65 mm) and cooked to an internal temperature of 73.9°C in a water bath. Products were chilled with cold water for 10 min before being placed in a walk-in cooler (4°C) for overnight storage. The casings were manually removed, and the product was then sliced with a sanitized Globe Gravity Feed Deli Slicer (Globe Slicing Machine Co., Inc., Stamford, Conn.) to deliver approximately 25 to 30 g per slice.

**Inoculation and microbial testing.** Products were transferred to the Food Research Institute for surface inoculation with *L. monocytogenes*. For each sampling unit, two slices were inoculated by spreading 0.2 ml of the *L. monocytogenes* mixture over the surfaces with a pipette. Final concentration of *L. monocytogenes* was targeted to yield approximately 4 log CFU per package (two slices per package; total approximately 50 to 60 g). Slices were vacuum packaged (Multivac AGW, Sepp Hagemüller KG, Wolfertschwenden, Germany) in gas-impermeable pouches (3-mil high-barrier EVOH pouches, Deli 1 material, oxygen transmission 2.3 cm³/100 in²/24 h at 24°C; water transmission 7.8 g/cm², 24 h at 37.8°C and 90% relative humidity; WinPak, Winnipeg, Manitoba, Canada), and stored at 4°C for up to 13 weeks. Triplicate inoculated samples for each treatment were assayed at time 0, and after 1, 2, 3, 4, 6, 9, and 13 weeks of storage at 4°C for changes in *L. monocytogenes* populations. In addition, duplicate uninoculated samples were assayed at each sampling interval for changes in pH and lactic acid bacteria populations.

Bacterial populations were determined in rinse material obtained after adding 100 ml of sterile Butterfield phosphate buffer to each package and massaging the contents externally by hand for about 3 min (6, 7). *L. monocytogenes* was enumerated for the triplicate inoculated samples by surface plating serial dilutions of rinse material on modified Oxford agar. Select colonies were confirmed as *L. monocytogenes* by Gram stain, tumbling motility, CAMP test, hemolysis on Trypticase soy agar with sheep blood, and biochemical analysis by MICRO-ID Listeria (Remel, Lenexa, Kans.). Changes in populations of lactic acid bacteria were assayed with uninoculated samples by plating rinse material on plate count agar with brom cresol purple (25°C, 48 to 72 h). Colony counts were converted to log CFU per milliliter rinse for data analysis.

**Proximate analysis.** Triplicate samples for each treatment were analyzed within 24 h after production for moisture (5 h, 100°C, vacuum oven method 950.46) (4), pH (1:10 dilution for 10 g homogenized portion, Accumet Basic pH meter and Orion 8104 combination electrode), NaCl (measured at % Cl⁻, AgNO₃ potentiometric titration, Brinkman Metrohm auto titrator, Herisau, Switzerland), and nitrite (Colorimetric Method 973.31) (4). Fat and protein levels were not analyzed for this study, but formulation was adjusted to target approximately 4 and 30% fat and 18 and 12% protein for turkey and pork-beef bologna, respectively.

**Statistical analysis.** Data for changes in populations of *L. monocytogenes* were analyzed by two-way analysis of variance to determine significant differences (*P < 0.05*) at each weekly sampling interval between meat-cure types (uncured turkey and cured pork-beef bologna) and among antimicrobial treatments by Minitab version 14.2 statistical software (Minitab Inc., State College, Pa.). One-way analysis of variance was used to analyze popula-
TABLE 1. Chemical analysis of uncured turkey and cured pork-beef bologna before inoculation with L. monocytogenes (measured within 24 h after cooking and chilling; average of three samples for each treatment)

<table>
<thead>
<tr>
<th>Meat type</th>
<th>Treatment</th>
<th>Moisture (%)</th>
<th>Salt (%)</th>
<th>Water activity</th>
<th>pH</th>
<th>Residual NaNO2 (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey</td>
<td>Control</td>
<td>76.61</td>
<td>2.00</td>
<td>0.963</td>
<td>6.19</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Benzoate-sorbate</td>
<td>75.53</td>
<td>2.07</td>
<td>0.961</td>
<td>6.23</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Benzoate-propionate</td>
<td>75.27</td>
<td>2.00</td>
<td>0.962</td>
<td>6.13</td>
<td>NA</td>
</tr>
<tr>
<td>Pork-beef</td>
<td>Control</td>
<td>57.44</td>
<td>2.13</td>
<td>0.956</td>
<td>6.13</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>Benzoate-sorbate</td>
<td>56.23</td>
<td>2.16</td>
<td>0.956</td>
<td>6.14</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>Benzoate-propionate</td>
<td>56.91</td>
<td>2.14</td>
<td>0.956</td>
<td>6.07</td>
<td>10.3</td>
</tr>
</tbody>
</table>

* NA, none added.

Populations of L. monocytogenes at each weekly sampling interval to compare the effect of antimycotic treatments with the control formulations of each meat-cure type.

RESULTS AND DISCUSSION

Proximate analyses for finished product (summarized in Table 1) suggest that the addition of low levels of sorbate, benzoate, or propionate has no obvious effect on basic product makeup compared with the control product; however, yield was not measured in this study. In addition to the expected differences of nitrite, the uncured turkey and the cured pork-beef bologna varied in proximate analysis for moisture, pH, salt, and water activity. Average pH for turkey treatments were 0.04 to 0.09 pH units higher than comparable pork-beef bologna treatments. Average moisture values for turkey were higher than for the pork-beef bologna, ranging approximately 75.3 to 76.6% treatments and 56.2 to 57.4%, respectively. Salt levels typically were 0.1% lower and water activity levels were 0.05 units higher in the turkey products compared with the pork-beef bologna treatments.

Populations of lactic acid bacteria were typically less than detectable levels (<1 log CFU/ml rinse) throughout the testing interval, regardless of the treatment or product type, with infrequent packages having 2.5 log CFU/ml rinse (data not shown). Samples with high counts of spoilage bacteria did not correlate with acid production and pH reduction.

Data revealed that low levels (0.1% total) of benzoate-propionate or benzoate-sorbate will inhibit growth of L. monocytogenes in certain RTE meat products, with significantly greater delay (P < 0.05) observed in intermediate-moisture, cured bologna than in high-moisture, uncured turkey throughout the 13-week sampling interval (Fig. 1). L. monocytogenes did not grow on cured pork-beef bologna supplemented with either antimycotic combination and stored at 4°C for 13 weeks, compared with a 3.5-log CFU per package pathogen increase in the control pork-beef bologna during the same testing interval. Both test treatments were significantly more effective in preventing growth of L. monocytogenes than the control bologna without antimycotic agents throughout the storage period (P < 0.05) and conformed to the USDA-FSIS compliance guidelines for alternative 2, which specifies that the formulation will support no more than a 1-log increase of L. monocytogenes during the product shelf life (18).

Adding 0.1% combined antimycotic agents inhibited, but did not prevent, L. monocytogenes growth on uncured turkey compared with the control formulation. Populations of L. monocytogenes increased 4 log CFU per package in the turkey control at 2 weeks’ storage compared with 2.1- and 1.3-log CFU per package increase in listerial populations at 2 weeks’ storage for the benzoate-sorbate and benzoate-propionate treatments, respectively. The benzoate-propionate treatment provided the greatest protection, resulting in statistically significantly lower populations (P < 0.05) than the control turkey through the 6-week sampling interval, whereas the benzoate-sorbate treatment had significantly lower populations only through 4 weeks. Populations of L. monocytogenes increased from approximately 3.7 log CFU per package to more than 8 log CFU per package for all turkey treatments during 6 weeks of storage at 4°C. Although inhibition was statistically significant (P < 0.05), these treatments were not sufficient to be considered antimicrobial under alternative 2 (18).

This study confirmed our previous research and other published reports that sorbate, benzoate, and propionate can delay or prevent growth of L. monocytogenes in processed meats compared with similar formulations without antimycotic agents (8–10, 15). As has been found for other antimicrobial systems such as lactate-diacetate combinations, greater inhibition was observed with cured meat products having intermediate moisture than with high-moist-

FIGURE 1. Growth of L. monocytogenes on sliced turkey (without nitrite) and pork-beef bologna (with 156 ppm sodium nitrite) manufactured with combinations of antimycotic agents, inoculated with 4 log CFU L. monocytogenes per package and stored at 4°C for 13 weeks. Standard deviation is designated by error bars.

Turkey: control (●); 0.05% benzoate with 0.05% sorbate (■); 0.05% benzoate with 0.05% propionate (▲). Bologna: control (○); 0.05% benzoate with 0.05% sorbate (□); 0.05% benzoate with 0.05% propionate (△).
ture uncured products (7, 16). Consequently, moisture and nitrite should be considered when developing safety systems for RTE meats and poultry with low levels of antimicrobial agents. On the basis of data from the previous studies in meat slurries, increased concentrations of antmycotic agents may be required to prevent pathogen growth in uncured products (8).

Combinations of low levels of antmycotic agents have an advantage over use of higher concentrations of individual agents in that they are more likely to be below the threshold for adverse flavor changes, but will still inhibit pathogen growth. Additional research is being completed in our laboratory to identify the minimum antimicrobial levels required to prevent \textit{L. monocytogenes} growth in high-moisture cured ham and uncured turkey products. Although sorbate, benzoate, and propionate are not currently approved for use in processed meat formulations, confirming their efficacy and safety in a variety of products should be useful in a successful petition for regulatory approval as antilisterial agents in RTE meats. Data from such studies will provide manufacturers with additional formulation options to inhibit growth of \textit{L. monocytogenes} during extended shelf life.

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REFERENCES