Research Note

Inhibition of *Listeria monocytogenes* in Deli-Style Turkey Breast Formulated with Cultured Celery Powder and/or Cultured Sugar–Vinegar Blend during Storage at 4°C

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ABSTRACT

Fermentation-derived nitrite (NO₂) from vegetable sources is increasingly used as a “clean label” alternative to conventional NaNO₂. Previous results suggested that processed meats cured with NO₂ derived from a ‘natural’ source had lower antimicrobial activity than did meats produced with chemical NaNO₂; however, the differences were likely due to NO₂ concentration rather than source. The objective of this study was to compare the antilisterial properties of traditional and clean label alternative curing approaches when combined with antimicrobials in deli-style turkey. *Listeria monocytogenes* inhibition by NO₂ from synthetic and natural sources was validated in deli-style turkey (73 to 74% moisture, 1.8% salt, pH 6.4). Products were prepared with 0, 80, or 120 mg/kg NO₂ using purified NaNO₂ or cultured celery powder. Additional treatments were supplemented with 3.8% lactate-diacetate blend (LD) or 1% cultured sugar–vinegar blend (DF). Sliced cooked products were surface inoculated with *L. monocytogenes* at 3 log CFU/g, vacuum packaged, and stored at 4°C for 12 weeks. Results revealed an average 2.4-log increase in *L. monocytogenes* at 3 weeks in the control without antimicrobials, a 1.3-log increase at 4 weeks for both 80 mg/kg NO₂ treatments, and a 1.5-log increase at 6 weeks for the 120 mg/kg NO₂ treatments. No significant difference (P > 0.05) in growth inhibition was found between NO₂ sources when equivalent concentrations were added. In uncured turkey with 3.8% LD or 1% DF, growth was delayed until 6 weeks, whereas supplementation with LD or DF and 80 mg/kg NO₂ from either source delayed listerial growth through 12 weeks. This study confirmed that the concentration of NO₂, rather than the source, is a primary factor in enhancing the safety of ready-to-eat meats. Both conventional NO₂ treatments and a clean label solution consisting of a fermentation-derived antimicrobial combined with 80 mg/kg naturally derived NO₂ inhibited *L. monocytogenes* through 12 weeks of storage at 4°C.

Nitrite in meat products is well known as the agent responsible for the distinctive color and flavor of cured meats and has been used for effective control of the growth of pathogenic microorganisms such as *Listeria monocytogenes*, *Clostridium perfringens*, and *Clostridium botulinum* (15, 18, 19, 21, 27). However, many consumers believe that additive-free or natural versions of foods are more healthful, which has stimulated growth in the natural and organic foods sector (3, 17). In response to this market trend, the industry has focused efforts on production of ready-to-eat (RTE) meat and poultry products, which could be considered clean label and naturally cured but retain similar microbial safety characteristics as conventionally prepared products (16, 31).

As an alternative to conventional curing, which uses synthetically prepared sodium nitrite, manufacturers have incorporated a natural source of nitrate, typically celery juice, which is converted to nitrite by a nitrate-reducing starter culture (25). Sindelar et al. (25) found that residual nitrite concentrations in “natural” or alternative cured products were lower and more variable than those in conventional cured products. Manufacturers have recently begun substituting the more laborious in situ curing system with commercially available cultured celery juice or powder where the nitrate has been preconverted to nitrite by the supplier (21, 22). However, use of this system is limited by both cost and sensory considerations; therefore, concentrations of nitrite in these products are typically lower (e.g., 40 or 80 ppm of ingoing nitrite) than those found in conventional cured products (maximum of 156 ppm of ingoing nitrite in comminuted product).

Products cured with natural ingredients and lower concentrations of nitrite may be more susceptible to growth of pathogens unless adjunct antimicrobials are added (12, 20, 26). This decrease in antimicrobial activity is of particular concern when targeting the control of *L. monocytogenes* in refrigerated RTE meat and poultry products. The U.S. Department of Agriculture Food Safety Inspection Service (FSIS) maintains a zero tolerance policy...
for \textit{L. monocytogenes} but has established a risk-based sampling program based on inhibition of listerial growth (29). Formulating meat products to limit growth of \textit{L. monocytogenes} serves to reduce the risk to immunocompromised individuals, pregnant women, and the elderly and consequently qualifies for the FSIS Alternative 2 sampling program (5, 29).

When used at concentrations within regulatory limits, nitrite will slightly inhibit but not prevent growth of \textit{L. monocytogenes} unless used in combination with an adjunct antimicrobial such as lactate, diacetate, propionate, or benzoate (7–9, 15, 23, 24, 28, 30). However, to be consistent with clean label ingredient statements, an array of microbial fermentates, organic acid blends, and plant extracts are being investigated as antilisterial alternatives (16, 32). We hypothesized that when products are standardized for ingoing nitrite concentration, moisture, pH, and salt, the nitrite derived from a natural source will provide antilisterial activity equivalent to that of purified nitrite. Supplementation of alternatively cured (80 mg/kg ingoing nitrite) poultry formulations with a commercially available cultured sugar–vinegar blend should inhibit growth of \textit{L. monocytogenes} and qualify the product for Alternative 2 sampling program. The objectives of this study were (i) to determine whether the source of nitrite (purified versus natural) affects inhibition of \textit{L. monocytogenes} in RTE meat products standardized to the same ingoing level, (ii) to compare the antilisterial activity of a commercial cured sugar–vinegar blend in a RTE meat system with that of a conventional potassium lactate–sodium diacetate blend, and (iii) to determine whether nitrite derived from a natural source enhances the effect of adjunct clean label and conventional antimicrobials. Results from this study can be used to validate the use of similar turkey-based formulations as qualified for the Alternative 2 sampling program.

### MATERIALS AND METHODS

#### Ingredients

Boneless, skinless turkey breasts were received fresh from a local provisioner and held at 2°C. Cultured celery juice powder (CP; celery juice, lactic acid starter culture, maltodextrin, and corn starch; Accel 2000, Kerry Ingredients, Beloit, WI) contained sodium nitrite equivalent at 22,500 mg/kg. The clean label antimicrobial was a cultured sugar–vinegar blend (DF; vinegar, cultured dextrose, and dried corn syrup; Durafresh 2012, Kerry Ingredients), and the conventional antimicrobial utilized was a potassium lactate–sodium diacetate blend that contained an average of 56% potassium lactate and 4% sodium diacetate (KL-SD; OptiForm PD4, Purac America, Lenexa, KS).

#### Preparation of deli-style turkey breast

Nine test formulations (Table 1) of deli-style turkey breast were manufactured using good manufacturing practices in the Meat Science and Muscle Biology Laboratory (University of Wisconsin–Madison) essentially as previously described (16). An additional uncured control formulation containing no nitrite or antimicrobials (treatment 10) also was included. Treatments were prepared with 75% ground, boneless, skinless turkey breast and a 25% solution containing 2.0% modified food starch, 1.8% salt, 1.0% dextrose, 1.0% carrageenan, 0.4% sodium tripolyphosphate, and the appropriate concentration of antimicrobial (1.0% DF or 3.8% KL-SD blend, equivalent to 2.128% potassium lactate solids and 0.152% sodium diacetate), all added on a total formulation basis, and/or the appropriate concentration of nitrite (80 or 120 mg/kg nitrite, derived from either CP or purified sodium nitrite [conventionally cured; CC]) added on a meat block weight basis and the balance added as water. Concentrations chosen were based on typical industry usage levels. Formulations were adjusted to target 73% ± 1% moisture, 1.8% ± 0.2% salt, and pH 6.4 ± 0.1. Experiments were replicated twice on different days with each trial utilizing lots of products made from different sets of ingredients and inoculated with cultures of \textit{L. monocytogenes} grown on different dates.

Treatment chubs were then held overnight at 4°C (16 to 18 h) to allow nitrite-related curing reactions to occur because a cure accelerator was not used. The next day, treatment chubs were cooked to an internal temperature of 73.8°C, chilled, and sliced, and these prepared products were vacuum packaged and stored at 3 to 4°C for up to 3 days and then transferred to the Food Research Institute for inoculation and testing.

#### Proximate analysis

TriPLICATE uninoculated samples for each treatment were analyzed for moisture (5 h, 100°C, vacuum oven...
Ten grams of each uninoculated sample was removed before rinsing in buffer for pH testing as described above.

**Statistical analysis.** Two trials were conducted to compare the antilisterial effect of alternative curing using CP versus CC using purified sodium nitrite and a natural fermentate compared with a potassium lactate–sodium diacetate blend. Data are expressed as mean ± standard deviation of the two replications. A general linear model procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC) was used to evaluate differences (P < 0.05) in the physicochemical properties of meat samples. Means were separated using the REGWQ grouping method. To assess the effect of treatments on inhibition of *L. monocytogenes*, a repeated measure design was used. An analysis of variance for repeated measures was performed using the MIXED procedure of SAS. The effect of replication was blocked to reduce the contribution of replications to the overall variability. Compound symmetry was used as the covariance structure. The differences between means were tested by Tukey’s method at a 5% level of significance.

**RESULTS AND DISCUSSION**

**Proximate analysis.** Values for moisture, pH, and salt for all treatments were similar to those expected in commercial production and were within acceptable limits of target ranges (Table 2). The pH was 6.39 to 6.49 (6.43 ± 0.03) across all treatments. The mean moisture for the three KL-SD treatments (71.90 ± 0.21%) was lower than that of the remaining treatments (74.17 ± 0.23%) probably because of the removal of water from the brines to compensate for the 3.8% KL-SD addition on a whole formulation basis. Similarly, the mean a_w for KL-SD treatments (0.963 ± 0.001) was approximately 0.010 units lower than that for other treatments (0.974 ± 0.002). Salt concentrations also were variable, with the CC formulations containing slightly higher salt (2.06% ± 0.13% NaCl) than the CP treatments (1.85% ± 0.06%) or uncured treatments (1.83% ± 0.08%), regardless of KL-SD addition. The increased salt concentrations in the CC samples resulted in higher overall water-phase salt values (2.73 ± 0.09) compared with all other formulations (2.45 ± 0.09). Mean residual nitrite values after cooking for formulations with 80 mg/kg were 52.6 ± 5.9, and recovered values for formulations with 120 mg/kg were 68.3 ± 5.5, which is consistent with conversion of nitrite to nitric oxide in product without a cure accelerator. The 50 to 70% recovery rate immediately after formulation is consistent with the recovery efficiency previously described (2). No significant differences in residual nitrite concentrations were found between the CC and CP treatments at either concentration of added nitrite.

**Control of indigenous spoilage microorganisms.** Populations of lactic acid bacteria were typically below the level of detection (<1 log CFU/ml of rinse) throughout the study regardless of treatment or replicate. Infrequent packages (19 of 294 uninoculated sample packages tested) had lactic acid bacteria at >2.0 log CFU/ml of rinse (data not shown), and this contamination was not correlated with any specific formulation. For those treatment groups for which sporadic samples with high counts of spoilage
bacteria, the pH, odor, and appearance remained unchanged throughout the sampling period, and changes in populations of background microflora did not appear to have any effect on growth or inhibition of *L. monocytogenes*.

**Control of *L. monocytogenes***. Results from this study indicate that the concentration of nitrite, rather than the source, is the primary factor in enhancing the safety of RTE meat products (Table 3). The addition of 80 or 120 mg/kg ingoing nitrite derived from either CP or CC significantly inhibited growth of *L. monocytogenes* (*P* < 0.05) compared with the uncured (0 mg/kg nitrite) control without antimicrobials at 3 through 8 weeks of storage at 4°C. No significant difference in growth of *L. monocytogenes* was found on turkey breast formulated with either CP or purified nitrite (CC) when both were standardized to equivalent ingoing nitrite levels of 80 or 120 mg/kg (*P > 0.05*). At 3 weeks, *L. monocytogenes* had not grown (<0.5-log increase) on any of the four nitrite-only treatments compared with a 2.39 ± 0.63-log increase in the no-nitrite, no-antimicrobial control. At 4 weeks, populations of *L. monocytogenes* increased 0.97 ± 0.60 and 1.32 ± 1.24 CFU/ml for the 80 mg/kg nitrite CP and CC treatments, respectively, a mean ≤0.6-log increase was observed for the 120 mg/kg nitrite treatments, and a 4.39 ± 0.31-log increase was observed for the no-nitrite control. At 6 weeks, populations of *L. monocytogenes* increased 2.28 ± 0.75 and 2.34 ± 0.53 log CFU/ml for the 80 mg/kg nitrite CP and CC treatments, respectively, and 1.24 ± 0.46 and 2.20 ± 0.71 log CFU/ml for the 120 mg/kg nitrite CP and CC treatments, respectively. All samples of the four nitrite treatments supported a >2-log increase at 8 weeks of storage at 4°C with no significant difference (*P > 0.05*) in the levels of *L. monocytogenes* for the 80 mg/kg CP versus CC treatments or for the 120 mg/kg CP versus CC treatments.

Data from this study also revealed that supplementation of uncured deli-style turkey breast with 1% DF provided the same level of inhibition of *L. monocytogenes* growth as did addition of 3.8% KL-SD (*P > 0.05*) (Table 4). No growth of *L. monocytogenes* was observed in either the uncured DF or KL-SD treatments at 4 weeks storage at 4°C, whereas the uncured control without antimicrobials supported a 4.4-log increase during the same time interval (Tables 3 and 4). Populations of *L. monocytogenes* increased by means of 1.4 and 1.6 log CFU/ml in the uncured 3.8% KL-SD and 1% DF treatments, respectively, by 6 weeks and by means of 1.8 and 2.1 log CFU/ml, respectively, by 8 weeks.

As expected, nitrite derived from both CP and CC sources significantly enhanced (*P < 0.05*) the antimicrobial activity of lactate-diacetate in RTE meat products when added at 80 mg/kg on a meat block basis (Table 4). After 12 weeks of storage, populations of *L. monocytogenes* increased by only 0.24 ± 0.29 and 0.74 ± 0.22 log CFU/ml in the 3.8% KL-SD+CP and 3.8% KL-SD+CC treatments, respectively. No significant difference (*P > 0.05*) was noted at any point between these two treatments throughout the 12-week sampling interval, and no significant difference (*P > 0.05*) was noted between the 1%
TABLE 3. Populations of L. monocytogenes in deli-style turkey breast formulated with nitrite derived from two sources and stored at 4°C for up to 8 weeks

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Wk 0</th>
<th>Wk 1</th>
<th>Wk 2</th>
<th>Wk 3</th>
<th>Wk 4</th>
<th>Wk 6</th>
<th>Wk 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 mg/kg NO₂, CP</td>
<td>3.10 ± 0.16 A ab</td>
<td>2.88 ± 0.24 A a</td>
<td>2.85 ± 0.41 A a</td>
<td>3.59 ± 0.77 B bc</td>
<td>4.06 ± 0.60 A bc</td>
<td>5.37 ± 0.75 B e</td>
<td>6.48 ± 0.50 B f</td>
</tr>
<tr>
<td>80 mg/kg NO₂, CC</td>
<td>3.07 ± 0.08 A a</td>
<td>2.97 ± 0.28 A a</td>
<td>2.89 ± 0.30 A a</td>
<td>3.03 ± 0.19 A a</td>
<td>4.41 ± 1.24 B b</td>
<td>5.42 ± 0.53 B c</td>
<td>6.32 ± 0.34 B d</td>
</tr>
<tr>
<td>120 mg/kg NO₂, CP</td>
<td>3.11 ± 0.15 A ab</td>
<td>2.95 ± 0.16 A a</td>
<td>2.90 ± 0.32 A a</td>
<td>3.21 ± 0.19 A bc</td>
<td>3.71 ± 0.55 A b</td>
<td>4.46 ± 0.46 A c</td>
<td>6.16 ± 0.37 A d</td>
</tr>
<tr>
<td>120 mg/kg NO₂, CC</td>
<td>3.08 ± 0.21 A ab</td>
<td>2.71 ± 0.17 A a</td>
<td>2.89 ± 0.23 A a</td>
<td>2.87 ± 0.17 A a</td>
<td>3.58 ± 0.73 A b</td>
<td>5.29 ± 0.71 B c</td>
<td>5.62 ± 0.49 A c</td>
</tr>
<tr>
<td>Uncured control, no antimicrobialsb</td>
<td>2.79 ± 0.09 A a</td>
<td>2.71 ± 0.11 A a</td>
<td>3.44 ± 0.13 A a</td>
<td>5.19 ± 0.63 C b</td>
<td>7.19 ± 0.31 C c</td>
<td>9.08 ± 0.07 C d</td>
<td>8.90 ± 0.08 C d</td>
</tr>
</tbody>
</table>

a CP, added nitrate derived from cultured celery powder; CC, conventionally cured with purified nitrite.
b Values are mean ± standard deviation for two replicates (n = 6 except as noted). Within a column, means followed by the same uppercase letter are not significantly different (P ≥ 0.05). Within a row, means followed by the same lowercase letter are not significantly different (P ≥ 0.05).
c Results for the uncured control without antimicrobials are based on only one replication (n = 3).

TABLE 4. Populations of L. monocytogenes in deli-style turkey breast formulated with adjunct antimicrobials alone or in combination with nitrite and stored at 4°C for up to 12 weeks

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Wk 0</th>
<th>Wk 2</th>
<th>Wk 4</th>
<th>Wk 6</th>
<th>Wk 8</th>
<th>Wk 10</th>
<th>Wk 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cured control, 3.8% KL-SD, CC</td>
<td>3.41 ± 0.13 A a</td>
<td>3.29 ± 0.07 A a</td>
<td>3.19 ± 0.12 A a</td>
<td>3.66 ± 0.45 A ab</td>
<td>3.77 ± 0.49 A bc</td>
<td>4.15 ± 0.71 B b</td>
<td>4.25 ± 0.22 B b</td>
</tr>
<tr>
<td>3.8% KL-SD, no cure</td>
<td>3.42 ± 0.10 A a</td>
<td>3.34 ± 0.18 A a</td>
<td>3.45 ± 0.29 A a</td>
<td>4.79 ± 0.56 A b</td>
<td>5.24 ± 0.33 A b</td>
<td>6.20 ± 0.65 A c</td>
<td>7.89 ± 0.02 A d</td>
</tr>
<tr>
<td>1.0% DF, no cure</td>
<td>3.16 ± 0.35 A a</td>
<td>2.95 ± 0.56 A a</td>
<td>3.46 ± 0.30 A a</td>
<td>4.76 ± 0.25 A b</td>
<td>5.29 ± 0.16 A bc</td>
<td>5.94 ± 0.37 A c</td>
<td>7.69 ± 0.62 A d</td>
</tr>
<tr>
<td>1.0% DF, CP</td>
<td>3.43 ± 0.08 A a</td>
<td>3.33 ± 0.08 A a</td>
<td>3.14 ± 0.19 A a</td>
<td>3.75 ± 0.62 A bc</td>
<td>3.66 ± 0.36 A b</td>
<td>4.45 ± 0.83 B c</td>
<td>4.19 ± 0.27 B bc</td>
</tr>
<tr>
<td>3.8% KL-SD, CP</td>
<td>3.47 ± 0.04 A a</td>
<td>3.40 ± 0.22 A a</td>
<td>3.17 ± 0.13 A a</td>
<td>3.19 ± 0.17 A a</td>
<td>3.42 ± 0.19 A a</td>
<td>3.71 ± 0.34 A a</td>
<td>3.73 ± 0.29 A a</td>
</tr>
</tbody>
</table>

a Nitrite (80 mg/kg, meat block basis) was used as a curing agent in some treatments. CP, added nitrite derived from cultured celery powder; CC, product conventionally cured with purified nitrite.
b Values are mean ± standard deviation for two replicates (n = 6). Within a column, means followed by the same uppercase letter are not significantly different (P ≥ 0.05). Within a row, means followed by the same lowercase letter are not significantly different (P ≥ 0.05).
DF + CP treatment and the two cured KL-SD treatments, even though the cured KL-SD treatments had slightly higher salt-in-moisture than did the cured DF treatment.

The results of this study confirm that the use of a curing agent, whether from a natural source or synthetically derived by a purification process, is an important factor for enhancing the safety of deli-style turkey and that the concentration of nitrite, rather than the source, is the driving factor behind delaying the growth of *L. monocytogenes*. These results are similar to those of a study in which the antimicrobial effect of celery juice concentrate was investigated in ham (11). When the investigators in that study adjusted for pH differences, the antilisterial activity of nitrite derived from cultured celery was equivalent to that of synthetic nitrite. However, unlike the results from that study, in our experiments higher pH values were not detected as a result of the addition of CP. This discrepancy might be due to the lower physical amount of CP needed to achieve target concentrations because the CP used in our study had a higher nitrite concentration (22,500 mg/kg) than did the celery juice concentrate used in the previous study (10,000 to 15,000 mg/kg), which in turn resulted in a reduced effect on pH. The pH differences between the two studies also may be due to the higher initial pH associated with the fresh turkey breast versus fresh pork; in the former case, the addition of the CP likely had less of an impact on the meat system pH because of the smaller pH difference between the raw materials and the ingredient. Although the pH of the uncured 1% DF (6.39 ± 0.04) samples was significantly lower than that of the uncured control treatment (6.49 ± 0.00), the pH values among the other treatments were not significantly different. Hence, pH was not considered a confounding factor for determining the effect of nitrite in our study.

Sodium nitrite extends the lag phase of *L. monocytogenes* in RTE meats in a concentration-dependent manner (4, 6), and the antilisterial effect is potentiated when nitrite is used in combination with lactate and diacetate (7, 23). Subsequent studies have confirmed that nitrite also enhances the antilisterial effect of other conventional antimicrobials, such as potassium sorbate, sodium propionate, and sodium benzoate, particularly when used in RTE meat products with lower moisture or pH values (8, 10, 16, 24). In this study, populations of *L. monocytogenes* increased by >1 log CFU/ml on uncured turkey supplemented with 3.8% KL-SD alone and stored 6 weeks at 4°C, but a <1-log mean increase was observed in turkey supplemented with 3.8% KL-SD and 80 ppm of nitrite and stored at the same temperature for 12 weeks.

Clean label antimicrobials include vinegar (acetic acid) or fermentation by-products that contain organic acid blends, i.e., predominantly lactic acid but also potentially acetic or propionic acids, or other antimicrobial compounds depending on the starter culture, substrate, and culture conditions (13). These compounds are “natural” versions of sodium or potassium lactate, sodium diacetate, and sodium propionate, which are commonly used in RTE meat and poultry products produced in the United States and inhibit *L. monocytogenes* when used in combination with nitrite, moisture, pH, salt, and temperature controls (7, 8, 14, 16, 23, 26). Although product data sheets frequently specify that cultured sugars contain organic acids, peptides, and other naturally derived fermentation metabolites, the types and concentrations of each usually are not reported. Therefore, commercial preparations may contain different ratios of lactate, propionate, acetate, or bacteriocins, which will result in different levels of antimicrobial activity. Using a first-generation cultured sugar–vinegar blend (CSV), Sullivan et al. (26) reported a <1-log increase of *L. monocytogenes* on alternative-cured ham supplemented with 2.8% CSV and stored at 4°C for 28 days, whereas our laboratory found similar growth rates in alternative-cured ham supplemented with 3.0% CSV through 4 weeks but a 2-log increase by 6 weeks (16). In our study, the 1% concentration of the newer generation CSV (DF) inhibited *L. monocytogenes* growth for 10 to 12 weeks in an alternative-cured (80 ppm of nitrite) turkey product with moisture and pH values similar to those of the ham.

In this study, no significant difference (*P < 0.05*) was found between purified nitrite and vegetable-based natural sources of nitrite as an antimicrobial for *L. monocytogenes* when test products were standardized for nitrite, moisture, pH, and salt. Nitrite used in combination with either a commercial clean label antimicrobial (DF) or a conventional antimicrobial blend (KL-SD) added at effective levels further delayed the growth of *L. monocytogenes*. As with other formulation-safe foods, microbial inhibition in processed meat products depends on product moisture, pH, salt, *a*_w_, nutrient availability, type and concentration of antimicrobial ingredients, and interaction of the antimicrobial agents with the food components. Therefore, the manufacturer must confirm the efficacy of these additives in each particular application.

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