Inhibition of *Listeria monocytogenes* Using Natural Antimicrobials in No-Nitrate-or-Nitrite-Added Ham

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**ABSTRACT**

Consumer demand for foods manufactured without the direct addition of chemical preservatives, such as sodium nitrite and organic acid salts, has resulted in a unique class of “naturally” cured meat products. Formulation with a natural nitrate source and nitrate-reducing bacteria results in naturally cured processed meats that possess traits similar to conventionally cured meats. However, previous research has shown that the naturally cured products are more susceptible to pathogen growth. This study evaluated *Listeria monocytogenes* growth on ham manufactured with natural curing methods and with commercially available clean-label antimicrobials (cultured sugar and vinegar blend; lemon, cherry, and vinegar powder blend) and assessed impacts on physicochemical characteristics of the product. Hams made with either of the antimicrobials supported *L. monocytogenes* growth similar to that in the traditionally cured control (*P* > 0.05). Hams made with prefermented celery juice powder had the lowest residual nitrite concentrations (*P* < 0.05), and when no antimicrobial was added, *L. monocytogenes* growth was similar to that of the uncured control (*P* > 0.05). Aside from residual nitrite and nitrate concentrations, few physicochemical differences were identified. These findings show that ham can be produced with natural curing methods and antimicrobials to provide similar *L. monocytogenes* inhibition and physicochemical traits as in traditionally cured ham.

Nitrate and nitrate have been used in meat processing for thousands of years and are responsible for the color, flavor and aroma, antioxidant, and antimicrobial properties associated with cured meats. Additionally, 4th century CE Chinese records describe potassium nitrate (saltpeter) placement under the tongue as a method to ease chest pain (7, 12), and early Chinese alchemists were investigating saltpeter since at least the 5th century CE (23). While benefits and uses of nitrite and nitrate have been identified throughout recorded history, fears began to develop about their healthfulness in the middle of the 20th century. In 1945, methemoglobinemia in infants (blue baby syndrome) was linked to high nitrate concentrations and bacterial contamination of well water (8); and, in 1964, carcinogenic n-nitrosamine compounds were isolated in herring meal used in herring meal produced with large amounts of sodium nitrite and were shown to have deleterious effects on animal health (9). These findings created a public concern about consuming cured meats that still persists, even though the National Academy of Science has clearly stated that nitrate is “neither carcinogenic nor mutagenic” and “evidence does not indicate that nitrite acts directly as a carcinogen” (13). More recently, researchers have also shown that nitrate, nitrite, and nitric oxide are important biological compounds that provide many healthful benefits (12).

These concerns, among others, have resulted in a growing segment of consumers who have begun seeking natural or organic foods. A recent survey by the Organic Trade Association (14) reported that 73% of U.S. households at least occasionally purchase organic foods. These respondents cited health benefits as the major reason for organic food purchases, and 47% indicated that they avoided artificial ingredients and preservatives in food to improve health. Even outside the natural or organic segments, processors are looking for clean-label alternative ingredients, those that have less “chemical sounding” names, to simplify the ingredient statements. Current U.S. Department of Agriculture (USDA) regulations for producing and labeling natural foods prohibit the use of artificial flavoring, color, chemical preservatives, or synthetic ingredients (25). Sodium nitrite and nitrate are among those ingredients not allowed in natural products. However, processed meats are currently manufactured without the addition of sodium nitrite but maintain characteristics similar to traditionally cured products by using natural nitrate or nitrite sources (18). Although similar product characteristics exist, USDA regulations require these products to be labeled as “uncured” and “no nitrate or nitrite added except those naturally occurring in... (added ingredients)” (27, 28). Ingredients high in nitrate, such as...
Compared to traditionally cured products, naturally cured meats may be more susceptible to pathogenic bacterial growth because of comparatively lower ingoing nitrite concentrations used. However, when compared with traditionally cured products, ingoing nitrite levels are typically lower in these naturally cured meats. Listeria monocytogenes is considered an adulterant in ready-to-eat processed meats, and many antimicrobials are not allowed in natural products and high mortality rates associated with listeriosis with this organism to grow during refrigerated storage. Because this ingredient qualifies as natural in the eyes of the USDA, all commercial ingredients were used at concentrations recommended by the supplier.

TABLE 1. Ham formulations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ham (kg)</th>
<th>Water (kg)</th>
<th>Salt (kg)</th>
<th>Sugar (kg)</th>
<th>NaNO₂ (ppm)</th>
<th>Natural NO₂ (g)</th>
<th>Natural NO₃ (g)</th>
<th>Starter culture (g)</th>
<th>Lactate-diacetate (g)</th>
<th>Antimicrobial A (g)</th>
<th>Antimicrobial B (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>18.14</td>
<td>3.74</td>
<td>0.5</td>
<td>0.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>18.14</td>
<td>3.72</td>
<td>0.5</td>
<td>0.3</td>
<td>—</td>
<td>68.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>18.14</td>
<td>3.52</td>
<td>0.5</td>
<td>0.3</td>
<td>—</td>
<td>68.1</td>
<td>68.1</td>
<td>5.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>D</td>
<td>18.14</td>
<td>3.72</td>
<td>0.5</td>
<td>0.3</td>
<td>—</td>
<td>68.1</td>
<td>68.1</td>
<td>5.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>E</td>
<td>18.14</td>
<td>3.52</td>
<td>0.5</td>
<td>0.3</td>
<td>—</td>
<td>68.1</td>
<td>68.1</td>
<td>5.0</td>
<td>—</td>
<td>—</td>
<td>158.9</td>
</tr>
<tr>
<td>F</td>
<td>18.14</td>
<td>3.13</td>
<td>0.5</td>
<td>0.3</td>
<td>—</td>
<td>68.1</td>
<td>68.1</td>
<td>5.0</td>
<td>—</td>
<td>—</td>
<td>540.0</td>
</tr>
<tr>
<td>G</td>
<td>18.14</td>
<td>3.17</td>
<td>0.5</td>
<td>0.3</td>
<td>156</td>
<td>550</td>
<td>550</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>570.0</td>
</tr>
</tbody>
</table>

Notes:
- a Natural NO₂, Vegetable 504 (Florida Food Products, Inc.); natural NO₃, Vegetable 502 (Florida Food Products); starter culture, CS-Starter Culture299 Bactoferm (Staphylococcus carnosus, Chr. Hansen, Inc.); lactate-diacetate, Purasal Opti.Form PD.4 (Purac America); antimicrobial A, vinegar, lemon, and cherry powder blend (Vegetable 507); antimicrobial B, cultured sugar and vinegar blend (Verdad 55). —, ingredient not included in the treatment.
- b A, uncured control; B, natural nitrite, no antimicrobial; C, natural nitrite, antimicrobial A; D, natural nitrate, no antimicrobial; E, natural nitrate, antimicrobial A; F, natural nitrite, antimicrobial B; G, natural nitrate, antimicrobial B; H, traditionally cured control.
Treatments with natural nitrate and starter culture (treatments D, E, G) were placed in a single truck smokehouse (thermal processing unit, Maurer-Atmos, Reichenau, Germany, and Direct Digital Control System, Alkar-RapidPak, Lod, WI) for fermentation at 42°C for 2 h to allow for reduction of nitrate to nitrite. Conventionally cured control (H) and treatments with natural nitrite (treatments B, C, F) were placed in the smokehouse 90 min after beginning the fermentation to allow temperature to equilibrate prior to thermal processing. Treatment A (negative control) was processed in a separate smokehouse (food processing oven with direct digital control, Alkar-RapidPak) following the same thermal processing schedule, excluding fermentation. All products were heated to an internal temperature of 73.9°C. The hams were placed in a 0°C cooler overnight to stabilize. The next day, the hams were sliced (1.5 mm thick) using a fully automatic slicing machine (model A-500, Bizerba, Piscataway, NJ) and vacuum packaged (MINI Series, Ulma Packaging, Ball Ground, GA). Hams were then transferred to the Food Safety Research Laboratory or the analytical laboratory at Iowa State University to begin day 0 of the study. Three independent replications were produced.

Preparation of inocula. L. monocytogenes strains H7969, H7764, H7769, H7762, and Scott A were obtained from the Food Safety Research Laboratory at Iowa State University. These strains were each isolated from previous foodborne illness outbreaks involving this organism. Each L. monocytogenes strain was cultured separately in Trypticase soy broth supplemented with 0.6% yeast extract (TSBYE, Difco, BD, Sparks, MD) for 24 h at 35°C. A minimum of two consecutive 24-h transfers of each strain to fresh TSBYE were performed prior to each experiment. A 250-ml bottle of TSBYE was inoculated with 1 ml from each of the five L. monocytogenes strains and was incubated at 35°C for 24 h to reach the stationary phase. The total concentration of the five-strain mixture of L. monocytogenes was approximately 10^3 cells per ml. A 10-ml aliquot was removed from the inoculated broth and dispensed into bottles with 90 ml of 0.1% peptone to achieve a 1:10 dilution. This diluted culture (five-strain mixture) was used to inoculate samples of ham.

Sample inoculation. While in the Food Safety Research Laboratory, 25-g samples of ham were placed in vacuum bags (12.70 by 40.64 cm; Cryovac Packaging, Duncan, SC). A 0.1-ml aliquot of the diluted (10^-1) culture was then aseptically transferred onto the ham in each bag for the various treatments. The cell concentration at inoculation was approximately 10^6 cells per gram. The bags were then vacuum sealed, massaged gently for 1 min to disperse the inoculum evenly over the surface, and stored at 4°C throughout the duration of the 35-day study.

L. monocytogenes analysis. L. monocytogenes sampling was conducted on days 0, 7, 14, 21, 28, and 35. On the appropriate day, one package for each treatment was opened aseptically. Sampling was achieved by performing an initial 1:5 dilution using a diluter (ASAP diluter, Spiral Systems Inc., Cincinnati, OH). Each sample was homogenized in a sterile Whirl-Pak stomacher bag (Nasco, Ft. Atkinson, WI) for 1 min in the laboratory blender (Stomacher 400, Seward Medical, London, UK). The product was further serially diluted, according to the sample date. An aliquot of 0.1 ml of the appropriate dilution was surface plated on modified Oxford medium base supplemented with modified Oxford antimicrobial supplement (Difco, BD). All inoculated agar plates were incubated at 35°C. After 24 to 48 h, the plates were removed and colonies typical of L. monocytogenes were enumerated on duplicate plates. Numbers of bacterial colonies were converted to log CFU per gram.

Analytical analysis. Packaged samples were held in dark storage at 4°C in a walk-in cooler. Samples were analyzed for residual nitrite, pH, CIE L*, a*, and b* on days 0, 8, 14, 21, 28, and 35. Samples from days 0, 8, 21, and 35 were frozen (−30°C) for up to 70 days before being analyzed for residual nitrite. Water activity and proximate composition (fat, moisture, protein, and ash) were also analyzed on day 0. Samples were also collected after mixing of the meat batches and after fermentation; they were also evaluated for residual nitrite, nitrate, and pH. Color was measured as described below, and then samples were homogenized using a food processor (model KFP715, KitchenAid, St. Joseph, MI) to prepare for remaining analyses. Residual nitrite determination was conducted using the AOAC official method 973.31 (4). Residual nitrate was measured using high-performance liquid chromatography (HPLC) (29, 30) as described and modified by Ahn and Maurer (1). The pH of ham samples was determined in a 9:1 water:sample slurry (Inlab Solids Pro probe; MultiSeven pH meter, Metler Toledo Inc., Columbus, OH). Product color was measured at four random locations using CIE L*, a*, and b*, Illuminante A, 10° standard observer, and a 1.27-cm port (LabScan XE, HunterLab, Reston, VA). Water activity was determined using an Aqualab Series 3 water activity meter (Decagon, Pullman, WA). Moisture (3), fat (2), and protein (5) were determined by AOAC official methods 950.46, 960.39, and 992.15, respectively. Ash was calculated by difference. When not indicated, all analyses were conducted in duplicate.

Statistical analysis. The general linear models procedure of Statistical Analysis System (version 9.2, SAS Institute Inc., Cary, NC) was used for statistical analysis. L. monocytogenes growth was analyzed for treatment effects with day. Analytical data was analyzed for treatment; and, where applicable, day and treatment × day interactions were also analyzed. Where significant effects (P < 0.05) were found, means separation was conducted using LSMEANS function of SAS and Fisher’s least significant difference adjustment for pathogen growth and Tukey’s honestly significant difference adjustment for physicochemical traits.

RESULTS AND DISCUSSION

L. monocytogenes growth. Treatments with clean-label antimicrobials (C, E, F, and G) and the natural nitrate and starter culture treatment (D) had L. monocytogenes growth similar (P > 0.05) to the traditionally cured control (H) over 35 days of storage at 4°C (Fig. 1). Treatments A (uncured control) and B (natural nitrite, no antimicrobial) showed similar growth throughout and were significantly greater (P < 0.05) than all treatments except C on day 28 and C and D on day 35. Although still statistically similar to the traditional cured product, treatments C (natural nitrite, antimicrobial A) and D (natural nitrate, no antimicrobial) showed a trend for greater growth on day 35 (P = 0.0563 and 0.0826, respectively). All other treatments (E, F, G) had growth similar to the traditionally cured control (H). Schrader (16) found that these antimicrobials reduced L. monocytogenes growth on naturally cured frankfurters, with antimicrobial B exhibiting greater inhibitory effects and major differences in growth that were observed by day 28 (of 120 days total) of storage at 4°C. However, in comparing the same natural curing agents and antimicrobials in similar treatment combinations, Jackson and others (11) found that the natural curing method used to produce naturally cured ham and frankfurters had a greater influence than did
antimicrobials on inhibiting Clostridium perfringens. These differences in pathogen inhibition between the organisms are likely explained by differences in nitrite tolerance (24). The antimicrobials used in this study contain natural compounds similar to those commonly used in processed meats. Vinegar, cultured sugar, and lemon are natural alternatives to organic acid salts often used in the industry, and they have well-documented antimicrobial properties (22). Cherry powder contains high levels of ascorbic acid (21), which functions as a cure accelerator by reducing nitrite to nitric oxide and increasing the rate and extent of the curing reaction.

Physicochemical traits. Physicochemical traits of the ham can be found in Table 2. Ingoing nitrite concentration was one of the greatest differences among treatments. Preliminary research determined that about 50 ppm of ingoing nitrite can be achieved with the prefermented celery juice powder (0.4% addition). The natural nitrate and starter culture treatments (D, E, G) had initial nitrate concentrations of 94.7 to 99.0 ppm; following bacterial reduction, 14.3 to 16.5 ppm of nitrate remained. This nitrate reduction is equivalent to ingoing sodium nitrite concentrations of 64.5 to 74.0 ppm. However, this likely underestimates actual ingoing nitrite; nitrate reformation occurs during meat curing (10), which could then be re-reduced to nitrite.

Lower residual nitrate concentrations in treatment D suggest that both antimicrobials may have slowed the bacterial reduction of nitrate in treatments E and G, although to a much lesser extent than found by Schrader in emulsified sausages (16). The decreased concentration of ingoing nitrite could explain the reduced L. monocytogenes inhibition in the preconverted celery juice treatment (B) relative to the treatment with celery juice and starter culture samples (D) when no antimicrobial was added. This is supported by Xi et al. (31), who found that greater ingoing nitrite resulted in decreased L. monocytogenes growth. Qvist and Bernbom (15) have shown that greater ingoing nitrite concentrations may also increase the effectiveness of organic acids used for Listeria control, although no differences were observed in our study.

No significant (P > 0.05) treatment × day interactions were found for any physicochemical traits measured. Residual nitrite concentrations were the highest in natural nitrate treatments (D, E, G), followed by the traditionally cured control (H). Prefermented celery juice treatments (B, C, F) had the lowest residual nitrite. Terns et al. (21) and Xi et al. (31) both reported that the addition of cherry powder reduced residual nitrite, and our results also show a reduction in residual nitrite in treatments containing cherry powder. As expected, residual nitrite declined over time (data not shown); as long as residual nitrite is not depleted to
TABLE 2. Effect of curing treatment and antimicrobial on means of physicochemical properties of ham products during production and storage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Residual nitrite (ppm)</th>
<th>pH</th>
<th>Residual nitrate (ppm)</th>
<th>pH</th>
<th>Residual protein</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.8 ± 0.3</td>
<td>6.09 ± 0.16</td>
<td>1.61 ± 0.12</td>
<td>6.21 ± 0.12</td>
<td>6.61 ± 0.12</td>
<td>6.11 ± 0.12</td>
</tr>
<tr>
<td>B</td>
<td>2.48 ± 0.24</td>
<td>6.12 ± 0.16</td>
<td>1.37 ± 0.12</td>
<td>6.21 ± 0.12</td>
<td>6.61 ± 0.12</td>
<td>6.11 ± 0.12</td>
</tr>
<tr>
<td>C</td>
<td>2.44 ± 0.24</td>
<td>6.11 ± 0.16</td>
<td>1.41 ± 0.12</td>
<td>6.21 ± 0.12</td>
<td>6.61 ± 0.12</td>
<td>6.11 ± 0.12</td>
</tr>
<tr>
<td>D</td>
<td>0.9 ± 0.3</td>
<td>6.09 ± 0.16</td>
<td>1.43 ± 0.12</td>
<td>6.21 ± 0.12</td>
<td>6.61 ± 0.12</td>
<td>6.11 ± 0.12</td>
</tr>
<tr>
<td>E</td>
<td>0.7 ± 0.3</td>
<td>6.09 ± 0.16</td>
<td>1.51 ± 0.12</td>
<td>6.21 ± 0.12</td>
<td>6.61 ± 0.12</td>
<td>6.11 ± 0.12</td>
</tr>
<tr>
<td>F</td>
<td>0.8 ± 0.3</td>
<td>6.09 ± 0.16</td>
<td>1.65 ± 0.12</td>
<td>6.21 ± 0.12</td>
<td>6.61 ± 0.12</td>
<td>6.11 ± 0.12</td>
</tr>
<tr>
<td>G</td>
<td>0.8 ± 0.3</td>
<td>6.09 ± 0.16</td>
<td>1.59 ± 0.12</td>
<td>6.21 ± 0.12</td>
<td>6.61 ± 0.12</td>
<td>6.11 ± 0.12</td>
</tr>
<tr>
<td>H</td>
<td>6.61 ± 0.18</td>
<td>6.09 ± 0.16</td>
<td>3.23 ± 0.12</td>
<td>6.61 ± 0.12</td>
<td>6.61 ± 0.12</td>
<td>6.11 ± 0.12</td>
</tr>
</tbody>
</table>

a) A, uncured control; B, natural nitrate, no antimicrobial; C, natural nitrate, antimicrobial A; D, natural nitrate, antimicrobial B; G, antimicrobial A, vinegar, lemon, and cherry powder blend; antimicrobial B, cultured sugar and vinegar blend. —, only natural nitrate sources required fermentation. SE, standard error.

b) Means with a common letter (a through e) within the same column do not differ significantly (P > 0.05).

c) Means with a common letter (a through e) within the same column do not differ significantly (P > 0.05).

d) Means with a common letter (a through e) within the same column do not differ significantly (P > 0.05).

Effect of curing treatment and antimicrobial on means of physicochemical properties of ham products during production and storage. Samples containing antimicrobial A had higher pH values (P < 0.05) than samples containing antimicrobial B when comparing treatments with the same nitrate or nitrite source. Samples with antimicrobial A had the lowest L* (darkest), which could be related to the higher ham pH (6). L* declined (P < 0.05) with time (days 0 and 35, 67.2 to 65.8, respectively). Although means separation occurred among cured samples for a* (redness), values ranged from 14.1 to 15.2; therefore, this would likely have little impact on visual appearance. Naturally cured samples with no antimicrobials had the lowest (P < 0.05) b* (yellowness) values, but again, little numerical difference existed. No differences were found for water activity, fat, moisture, or protein, but ash content differed (P < 0.05) among treatments, based on amount of ingoing ingredients. Schrader (16) found similar results for these traits in emulsified sausage, and Sindelar et al. (19) found no differences in color or pH in naturally and traditionally cured hams.

Consequently, the use of natural curing ingredients in combination with selected antimicrobials will result in hams that possess L. monocytogenes inhibitory properties that are similar to those of traditionally cured controls throughout 35 days of storage. Over the same storage period, the treatment cured with preconverted celery juice powder and no antimicrobial provided growth similar to the uncured control. The method of natural curing impacted the amount of ingoing nitrate, resulting in differences in pathogen growth. Increased risk of pathogen growth in naturally cured meats could be overcome with use of these commercially available antimicrobials. The natural antimicrobials and natural curing systems may have small impacts on physicochemical traits of naturally cured ham, but these are likely to be of little or no practical importance to consumers of these products.

REFERENCES


