Identifying Ingredients That Delay Outgrowth of *Listeria monocytogenes* in Natural, Organic, and Clean-Label Ready-to-Eat Meat and Poultry Products

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**ABSTRACT**

The objective of this study was to identify ingredients that inhibit *Listeria monocytogenes* in natural, organic, or clean-label ready-to-eat meat and poultry products. Fourteen ingredients were screened in uncured (no-nitrate-or-nitrite-added), traditionally-cured (156 ppm of purified sodium nitrite), cultured (alternative cured, natural nitrate source, and *Staphylococcus carnosus*), or preconverted (alternative cured, natural nitrite source) turkey slurries. Slurries were cooked, cooled, inoculated to yield 3 log CFU/ml *L. monocytogenes*, stored at 4°C, and tested weekly for 4 weeks. Three antimicrobial ingredients, 1.5% vinegar–lemon–cherry powder blend, 2.5% buffered vinegar, and 3.0% cultured sugar–vinegar blend, were incorporated into alternative-cured ham and uncured roast beef and deli-style turkey breast. Controls included all three meat products without antimicrobial ingredients and a traditional-cured ham with 2.8% sodium lactate–diacetate. Cooked, sliced products were inoculated with 2.0 log CFU/g *L. monocytogenes*, vacuum packed, and stored at 4 or 7°C for up to 12 weeks. For control products without antimicrobial agents stored at 4°C, a 2-log *L. monocytogenes* increase was observed at 2 weeks for ham and turkey and at 4 weeks for roast beef. Growth (>1-log increase) in the sodium lactate–diacetate was delayed until week 6. Compared with the control, the addition of either vinegar–lemon–cherry powder blend or buffered vinegar delayed *L. monocytogenes* growth for an additional 2 weeks, while the addition of cultured sugar–vinegar blend delayed growth for an additional 4 weeks for both ham and turkey. The greatest *L. monocytogenes* delay was observed in roast beef containing any of the three antimicrobial ingredients, with no growth detected through 12 weeks at 4°C for all the treatments. As expected, *L. monocytogenes* grew substantially faster in products stored at 7°C than at 4°C. These data suggest that antimicrobial ingredients from a natural source can enhance the safety of ready-to-eat meat and poultry products, but their efficacy is improved in products containing nitrite and with lower moisture and pH.

Currently, many U.S. meat and poultry processed product manufacturers utilize combinations of sodium nitrite (nitrite) and other effective antimicrobial agents such as sodium or potassium lactates–sodium diacetate to suppress the growth of *Listeria monocytogenes* should postlethality environmental contamination of the product occur. Ready-to-eat (RTE) meat and poultry products either uncured and traditionally cured with nitrite can support the growth of *L. monocytogenes* during storage at 4°C if they do not contain supportive ingredients for increasing antimicrobial effects, reducing pH, and/or lowering water activity (1, 11, 14). Nitrite itself has antilisterial properties, with the effect being concentration dependent (11, 43, 42). However, pathogen inhibition in cured products is significantly enhanced when combined with other antilisterial ingredients such as lactate and diacetate, offering additive or synergistic responses (12, 13, 18, 31).

Over the past decade, consumer interest and subsequent market growth for natural, organic, and more recently, clean-label foods have steadily increased by 20 to 22% of the annual market share from 1997 to 2007 (10, 22, 28). Demand for natural, organic, and clean-label RTE meat and poultry products is similarly increasing (22, 23). However, the microbial inhibition in these products is a concern due to limitations on allowable ingredients, some of which are critical for food safety (21, 36).

By U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS) labeling standards (39, 40), meat and poultry products labeled “natural” are not permitted to contain any artificial flavoring, coloring ingredient, or chemical preservative, as defined in 21 CFR 101.22. Further, organic food labeling follows requirements established by the 1990 Organic Food Production Act, which detail a list of allowed and prohibited ingredients (41). As such, approved and commonly used synthetic antimicrobial agents effective for inhibiting *L. monocytogenes* are not permitted in these products. Finally, both nitrate and nitrite are considered chemical preservatives under the definition of “natural” and are specifically listed as prohibited.

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ingredients for products following organic labeling criteria. “Clean-label” foods are not restricted to FSIS “natural” or “organic” definitions, but have simple ingredients that are recognized by consumers and perceived as being derived from a nonchemical source, such as vinegar, flavorings, cultured sugars or dairy ingredients, or ingredients derived from plant material.

Therefore, intervention strategies that meet clean-label, natural, or organic labeling criteria are needed to inhibit L. monocytogenes growth in RTE meats prior to consumption to reduce the hazard of foodborne illness. Currently available technologies and ingredients serving as effective alternative L. monocytogenes interventions are limited and often provide minimal effectiveness, with varying results (44). Some RTE meat and poultry product manufacturers have adopted postpackage heat treatments to eliminate pathogens, but these processes can cause negative sensory changes such as those resulting from separation of fat or increased package exudate(s). Others have adopted high pressure pasteurization, but this technology can be costly, is not widely used in the industry, and barotolerant strains of L. monocytogenes might not be eliminated (5, 29). Furthermore, reliance on postpackage treatments will not protect against listerial growth should the product be recontaminated after opening, such as in a retail deli. Many manufacturers have utilized “alternative curing” to include nitrite derived from a natural source in an effort to attain similar quality and safety properties found in traditionally cured (direct addition of purified sodium nitrite) meat and poultry products. However, lower ingoing concentrations of nitrite due to technology limitations has resulted in quality and safety shortcomings, with less inhibition of L. monocytogenes (29, 36).

Additional antimicrobial ingredients need to be identified to ensure the safety of high-moisture RTE products during extended refrigerated storage. Numerous studies have reported the antilisterial activity of plant extracts, essential oils, and microbial fermentation by-products in a variety of food and nonfood applications (2, 4, 7, 8, 17). These ingredients can contain phytophenols, flavonoids, bacteriocins, or blends of organic acids, all of which can exhibit antimicrobial activity (6, 20). In contrast, relatively few studies have demonstrated the antilisterial activity of any single “natural antimicrobial ingredient” when used in the formulations of uncured or “alternative-cured” meat systems (9, 15, 16, 24, 34, 38). However, a thorough assessment of ingredients possessing antimicrobial activity with potential to serve as a feasible approach for improving the safety of natural, organic, and clean-label RTE meat and poultry products has not taken place. Therefore, the objectives of this study were to (i) assess the antilisterial activity of a variety of ingredients from natural sources in both uncured and alternative-cured model meat systems, and (ii) validate the most effective ingredients in bonelless ham, roast beef, and deli-style turkey breast.

MATERIALS AND METHODS

The study was divided into two phases focusing on low-fat products that typically have less antagonistic effect on fat-soluble antimicrobial agents. The purpose of phase 1 was to screen a variety of ingredients derived from natural sources for antilisterial activity; ingredients with the most consistent antimicrobial activity were further tested in a meat system to identify which had minimal effect on quality and sensory properties by using objective and subjective criteria for taste, color, aroma, and texture. The purpose of phase 2 was to validate the antilisterial activity of three selected ingredients identified from phase 1 in alternative-cured ham, uncured roast beef, and uncured deli-style turkey breast.

Phase 1a: antimicrobial ingredient screening in model meat system. Fourteen ingredients that met the 2006 FSIS definition for “natural” (39), with potential for use in organic products, and/or are considered “clean label” were evaluated in four different cure systems. The ingredients included commercially available natural flavorings, organic acid blends, plant extracts, and microbial fermentation by-products. Ingredients for this study were selected based on a comprehensive literature review and manufacturer reports on antimicrobial activity (Table 1). Each ingredient was evaluated for L. monocytogenes inhibition in ground turkey slurries in four different curing systems including (i) uncured (no nitrate-nitrite inclusion), (ii) traditional cured (0.0156% purified sodium nitrite), (iii) cultured (alternative cured with a natural, plant-based nitrate source and a nitrate-reducing bacterial starter culture Staphylococcus carnosus), and (iv) preconverted (alternative cured with a natural, plant-based nitrate source). Usage levels for each ingredient were determined from published literature or manufacturer recommendations. Slurries were prepared with 25% raw ground turkey breast, 2.0% sodium chloride (NaCl), the target concentration of test antimicrobial ingredient, and the balance as distilled, deionized water (26).

The uncured meat system included no additional nitrate- or nitrite-containing ingredients. The traditional-cured meat system included 0.0156% sodium nitrite and 0.0547% sodium erythorbate (cure accelerator). The cultured meat system contained 0.4% celery powder (35,000 ppm of nitrate, reported as sodium nitrate equivalence and providing an ingoing concentration of 140 ppm or 0.014%; VegStable 502, Florida Food Products, Eustis, FL) serving as a natural source of nitrate, 0.2% cherry powder (18% ascorbic acid; VegStable 515, Florida Food Products) as a natural source of ascorbic acid (cure accelerator), and 0.18% S. carnosus bacterial starter culture (CS 299 Bactoferm, Chr. Hansen, Inc., Milwaukee, WI) for nitrate-to-nitrite reduction. Prior to antimicrobial addition to the cultured system, the slurries were held for 120 min at 35 to 38°C to allow for the microbial reduction of nitrate to nitrite before cooking, following previously established methods of Sindelar et al. (32, 33). Because of reaction limitations as discussed by Sindelar et al. (32, 33) and Sebranek et al. (29), a 50 to 70% reduction of nitrate to nitrite was expected to likely generate between 70 and 100 ppm of nitrate (0.007 and 0.010%, respectively) in our experiments. The preconverted system contained 0.3% celery powder (15,000 ppm of nitrate, reported as sodium nitrate equivalence providing an ingoing concentration of 60 ppm or 0.0060%; VegStable 504, Florida Food Products) serving as a natural source of nitrate and 0.2% cherry powder (VegStable 515) as a cure accelerator. In addition, baseline data were collected for the behavior of L. monocytogenes in three control treatment slurries. The first, a positive control containing no antimicrobial agents, was included for each curing system (n = 4). A second control, representing a meat industry standard, contained traditional cure at 0.0156% sodium nitrite, 1.68% sodium lactate solids (2.8% of 60% syrup; PURASAL S, Purac, Inc., Lincolnshire, IL), 0.112% sodium diacetate (Sigma-Aldrich Corp., St. Louis, MO), 2.0% NaCl, 0.0547% sodium erythorbate,
TABLE 1. Phase 1: Inhibition of *Listeria monocytogenes* in turkey slurries combined with uncured, alternative, and traditional-curing systems supplemented with antimicrobial ingredients, and stored at 4°C for 4 weeks$^{a,b}$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Usage level (%)$^c$</th>
<th>Description</th>
<th>Uncured</th>
<th>Preconverted</th>
<th>Cultured</th>
<th>Traditional</th>
<th>pH$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSV</td>
<td>3.00</td>
<td>Cultured sugar-vinegar blend</td>
<td>−0.38</td>
<td>0.02</td>
<td>−0.27</td>
<td>−0.87</td>
<td>5.78</td>
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<tr>
<td>VL</td>
<td>2.50</td>
<td>Vinegar–lemon juice blend</td>
<td>−0.50</td>
<td>−0.34</td>
<td>−0.40</td>
<td>0.41</td>
<td>5.68</td>
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<tr>
<td>RT</td>
<td>0.08</td>
<td>Rosemary–tocopherol blend</td>
<td>5.52</td>
<td>4.80</td>
<td>3.23</td>
<td>3.05</td>
<td>5.98</td>
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<tr>
<td>GT</td>
<td>0.10</td>
<td>Green tea extract</td>
<td>5.59</td>
<td>4.77</td>
<td>3.34</td>
<td>2.73</td>
<td>6.07</td>
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<tr>
<td>HAE</td>
<td>0.05</td>
<td>Herbal active emulsion</td>
<td>4.75</td>
<td>4.47</td>
<td>3.48</td>
<td>0.20</td>
<td>6.00</td>
</tr>
<tr>
<td>CC</td>
<td>2.00</td>
<td>Cranberry concentrate</td>
<td>5.19</td>
<td>4.40</td>
<td>2.52</td>
<td>0.40</td>
<td>5.91</td>
</tr>
<tr>
<td>TTO</td>
<td>0.05</td>
<td>Tea tree oil</td>
<td>−0.88</td>
<td>−0.50</td>
<td>−0.11</td>
<td>0.06</td>
<td>6.29</td>
</tr>
<tr>
<td>RN</td>
<td>0.20</td>
<td>Rosemary extract plus nisin</td>
<td>3.42</td>
<td>0.92</td>
<td>2.78</td>
<td>0.05</td>
<td>6.22</td>
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<tr>
<td>CP</td>
<td>0.50</td>
<td>Cherry powder</td>
<td>5.19</td>
<td>1.46</td>
<td>0.89</td>
<td>0.10</td>
<td>5.84</td>
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<tr>
<td>VLC</td>
<td>1.50</td>
<td>Vinegar–lemon–cherry powder blend</td>
<td>−0.12</td>
<td>0.08</td>
<td>0.15</td>
<td>−0.20</td>
<td>5.98</td>
</tr>
<tr>
<td>GSE</td>
<td>0.03</td>
<td>Grape seed extract</td>
<td>4.55</td>
<td>0.84</td>
<td>−0.21</td>
<td>−0.42</td>
<td>6.03</td>
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<tr>
<td>BV</td>
<td>2.00</td>
<td>Buffered vinegar</td>
<td>−0.18</td>
<td>−0.27</td>
<td>−0.24</td>
<td>−0.15</td>
<td>5.73</td>
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<tr>
<td>LSE1</td>
<td>1.00</td>
<td>Liquid smoke extract 1</td>
<td>3.76</td>
<td>4.06</td>
<td>2.33</td>
<td>1.67</td>
<td>6.00</td>
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<tr>
<td>LSE2</td>
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<td>Liquid smoke extract 2</td>
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<td>1.37</td>
<td>0.83</td>
<td>−0.55</td>
<td>5.82</td>
</tr>
</tbody>
</table>

Control + LD$^f$ | NT$^g$ | NT | NT | 0.25 | 5.90
Control + LD + STPP$^f$ | NT | NT | NT | 0.06 | 6.19
Positive control$^f$ | 6.10 | 4.02 | 3.39 | 2.91 | 6.05

$^a$ Turkey slurries containing 25% raw ground turkey breast, 2.0% sodium chloride (NaCl), antimicrobial ingredient, and the balance as deionized water.

$^b$ Uncured system contained no antimicrobial agent; preconverted system contained 0.3% celery powder and 0.2% cherry powder; cultured system contained 0.4% celery powder, 0.2% cherry powder, and 0.18% *Staphylococcus carnosus* bacterial starter culture; traditional-cure system included 0.0156% purified sodium nitrite and 0.0547% sodium erythorbate.

$^c$ Usage level inclusion based upon total slurry weight.

$^d$ Log CFU per milliliter population changes for *L. monocytogenes* over a 4-week sampling period.

$^e$ pH of turkey slurry treatment combination prior to heating.

$^f$ Control + LD: 0.0156% sodium nitrite, 1.68% sodium lactate solids (2.8% of 60% syrup), 0.112% sodium diacetate, 2.0% NaCl, and 0.0547% sodium erythorbate. Control + LD + STPP: Control + LD without 0.4% sodium tripolyphosphates (STPP). Positive control: 2.0% NaCl and no added nitrate, nitrates, or antimicrobial ingredient.

$^g$ NT, not tested in this curing system, since control contained purified sodium nitrate.

and 0.4% sodium tripolyphosphates. A third control contained the same ingredients as the second control, except for the exclusion of sodium tripolyphosphates to mimic a product labeled “natural,” where phosphates would not be allowed.

For all treatments, slurries were cooked with agitation to 74°C and held at that temperature for 30 min to kill background microflora, then rapidly chilled on ice to 4°C. *Listeria monocytogenes* strains Scott A (clinical isolate, serotype 4b), 101 (hard salami isolate, serotype 4b), 108 (hard salami isolate, serotype 1/2a), 310 (goat’s milk cheese isolate, 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 × g, 20 min) and suspended in 4.5 ml 0.1% 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 3 log CFU/ml slurry and dispersed in 3-ml portions to sterile tubes for incubation. Purity and populations of each strain and the mixture were verified by plating on Trypticase soy agar and modified Oxford agar (*Listeria Selective Agar base, Difco, BD, Sparks, MD*). Duplicate tubes per variable were assayed at 0 time and 1, 2, 3, and 4 weeks of storage at 4°C for changes in listerial populations after mixing the sample and removing 0.5 ml for serial dilutions and surface plating on modified Oxford agar (35°C for 48 h). Residual nitrite was determined (Association of Official Analytical Chemists [AOAC] colorimetric method 973.31 (3)) as was pH (Accumet Basic pH meter and Orion 8104 combination electrode, Thermo Fisher Scientific, Waltham, MA (30)) for uninoculated, cooked samples.

**Phase 1b: intermediate qualitative acceptability screening.** A qualitative confirmation–screening experiment was conducted to ensure the inclusion of ingredients identified as possessing the most consistent antimicrobial activity would not negatively affect product quality attributes. Seven ingredients (cultured sugar–vinegar blend [CSV], vinegar–lemon juice blend, cranberry concentrate, vinegar–lemon–cherry powder blend [VLC], cherry powder, grape seed extract, and buffered vinegar [BV]), identified as having the most consistent inhibitory properties for cured and uncured meat systems, were selected and included in the formulation of an uncured deli-style chicken breast at the same concentrations used for the microbiological screening study. A deli-style chicken breast product having a neutral flavor profile was chosen as a conservative test product, since any off-colors, aromas, or flavors would be pronounced and readily detectable.

Small (6.9 kg) batches of RTE, uncured deli-style chicken breast were manufactured, following a typical formulation and manufacturing protocols in the University of Wisconsin–Madison Meat Laboratory. Boneless, skinless chicken breasts (48.3 kg) were ground through a 9.5-mm plate, mixed with water (2.42 kg), salt
(0.73 kg), dextrose (0.73 kg), and modified food starch (0.49 kg) for 3 min, and then ground through a 4.76-mm plate. The meat mixture was then separated into eight 6.59-kg batches, and the antimicrobial ingredient \((n = 7)\) was added, after first being mixed in 0.30 kg of water, followed by mixing for 4 min to achieve uniform ingredient distribution and adequate protein extraction. A control containing no added antimicrobial ingredient was also included in this experiment. Treatment batches were stuffed into 5.72-cm-diameter fibrous casings (Vista International Packaging, L.L.C., Kenosha, WI) and cooked with a single-truck thermal processing oven (model 450 MiniSmoker, Alkar Engineering Corp., Lodi, WI), employing a common meat industry steam-cook smokehouse schedule, reaching an internal temperature of 71.1°C. After chilling to colder than 4.4°C, the chubs were utilized for objective and subjective evaluations.

Each treatment was evaluated for color, aroma, flavor, and texture characteristics by using an experienced sensory panel consisting of University of Wisconsin faculty, staff, and students, with processed meat product and sensory evaluation expertise. Refrigerated (3.3 to 5.6°C), sliced chicken samples were evaluated by the panelists, without reheating the samples, which would be characteristic for this product. Panelists \((n = 7)\) were presented two 4.76-mm slices of each treatment in a covered Styrofoam plate, one at a time. Panelists were asked to rate the desirability of surface color, aroma, flavor, and overall acceptance of the deli-style chicken breast samples. Panelists used a 9-point hedonic scale where 1 = “dislike extremely”’’ to 9 = “like extremely” for recording their responses.

Instrumental color (CIE \(L^*a^*b^*\) values [lightness, redness, yellowness, respectively]) was measured on the cut surface of each treatment with a colorimeter (model CR-310, 50-mm aperture, illuminant C, 2° standard observer, Minolta Corp., Ltd., Osaka, Japan) calibrated with a white plate \((L^* 97.74, a^* –0.06, b^* 2.55)\). The pH of each treatment was measured by blending 10 g of sample with 90 ml of distilled, deionized water, filtering through a Whatman no. 1 filter paper \((30)\), and with the pH measured on the fat-free solution (Accumet Basic AB15 Plus pH Meter). Measurements were made in duplicate for each treatment.

Phase 2: ingredient validation in deli-style meat and poultry products. Based on the microbiological ingredient efficacy from the meat slurry study and qualitative acceptability screening, three antimicrobial ingredients from natural sources (CSV, VLC, and BV) were chosen for further evaluation in each of three low-fat (<5% fat) RTE products: deli-style turkey breast, whole-muscle roast beef, and boneless ham. All products were manufactured using typical commercial product formulations and processing practices in the University of Wisconsin–Madison Meat Laboratory and transported to the Food Research Institute BSL-2 laboratories for inoculation, incubation, and testing. A total of 13 formulations (3 antimicrobial ingredient treatments × 3 products + 4 controls) were tested, and each formulation was replicated twice.

Uncured, deli-style turkey breast was manufactured with fresh (2°C), boneless, skinless turkey breast ground (model 4732, Hobart Corporation, Troy, OH) through a 2.54-cm plate and separated into four batches (11.34 kg). Brines were formulated to provide a 20% total ingredient addition including 1.5% salt, 1.5% dextrose, the antimicrobial ingredient (CSV, VLC, BV) or no antimicrobial ingredient (control), and the balance added as water, all calculated on a raw meat weight basis. All antimicrobial agents for this and the other two products (roast beef, boneless ham) replaced a portion of the ingoing water and were added at the same level used in the previous phase (Table 1). Both meat and brine were added to a vacuum tumbler (model LT-40, Lyco, Janesville, WI) and were tumbled under vacuum continuously for 1 h to achieve adequate protein extraction, free-brine pickup, and ingredient distribution. After tumbling was completed, the turkey breast mixtures were transferred to a rotary-vane vacuum-filling machine (VF608 Plus Vacuum Filler, Handtmann CNC Technologies, Inc., Buffalo Grove, IL) and stuffed into 101.6-mm fibrous casings (Vista International Packaging, L.L.C.).

Roast beef was manufactured with closely trimmed, cap-off, semi-membranous beef muscles. Brines were prepared with going levels 0.50% salt and 0.40% dextrose, calculated from the raw meat weight. As with the deli-style turkey breast, the antimicrobial ingredients (CSV, VLC, BV) were added at the same levels previously identified with their formulation inclusion weight subtracted from the water portion of the brine. A control was prepared with no antimicrobial ingredient in this experiment. Beef muscles were injected with a multi-needle injector (Formaco model FGM 20/20S, Food Machinery Company A/S, Copenhagen, Denmark) to 20% over the initial meat weight, followed by 20 min of vacuum tumbling to ensure uniform ingredient distribution and facilitate free-brine pickup. After tumbling was completed, the injected beef muscles were separated into four equal pieces (approximately 2 kg) and placed in cook-in barrier bags (model BS40, Cryovac Sealed Air Corp., Duncan, SC).

Alternative-cured, boneless ham was manufactured with closely trimmed fresh biceps femoris and semimembranous with attached semitendinosus muscles. The preconverted alternative-curing system was utilized for all treatments (CSV, VLC, BV) and a positive control (no antimicrobial ingredient) formulations. Because ham is a product that routinely contains purified sodium nitrite, an additional negative-growth control was manufactured for testing to compare results from alternate cured ham with traditional-cured products supplemented with sodium lactate–diacetate as a typical industry standard. Salt and sugar were included at 2.35 and 1.65%, respectively, based on a raw meat weight basis. Ham brines were formulated for injected levels of preconverted celery powder (VegStable 504) and cherry powder (VegStable 515) at 0.30 and 0.20%, respectively. The negative-growth control contained 0.0156% purified sodium nitrite, 0.0547% sodium erythorbate, and 2.8% sodium lactate–sodium diacetate blend (equivalent to 1.568% sodium lactate solids and 0.112% sodium diacetate; OptiForm SD4, Purac America, Lincolnshire, IL), all calculated on a raw meat weight basis. No phosphates were added to any ham formulations, because boneless hams were intended to be similar to natural or organic product, the processing of which restricts phosphate usage.

For boneless ham manufacture, fresh ham batches \((n = 5, 11.34 \text{ kg each})\) were injected with the multi-needle injector to 125% of the initial meat weight. The injected ham muscles were ground with a 2.54-cm plate, transferred to a vacuum tumbler for 1 h of continuous tumbling to achieve adequate protein extraction, uniform ingredient dispersion and free-brine pickup, transferred to a rotary-vane vacuum-filling machine, and stuffed into the 101.6-mm fibrous casings.

All three products were thermal processed separately the a single-truck thermal processing oven by using steam-cook thermal processes. The deli-style turkey breast and boneless ham followed a steam-cook ramp schedule until an internal temperature of 70.0°C was achieved. The whole-muscle roast beef used an industry-common reverse-AT steam-cook schedule to achieve an internal temperature of 54.4°C, held for 121 min. After thermal processing, the finished products were chilled at 0 to 2°C for 10 to 15 h and held at 4°C prior to inoculation within 72 h.

Cooked products were aseptically removed from the casing and sliced on a sanitized hand slicer to a thickness of 3 mm,
vacuum packaged in barrier bags, and stored at 3 to 4 ℃ until use within 3 days. Sliced products were inoculated with 0.5 ml of a five-strain mixture of L. monocytogenes, as described previously to yield approximately 5 log CFU/100-g package (3 log CFU/g). Inoculated products were vacuum packaged in gas-impermeable pouches (3-mil-high barrier EVOH pouches, Deli 1 material, oxygen transmission of 2.3 cm³ per cm², 24 h at 24 ℃, water transmission 7.8 g per cm², 24 h at 37.8 ℃, and 90% relative humidity; Doug Care Equipment, Inc., Springville, CA) and stored at 4 or 7 ℃ for up to 12 weeks.

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. Triplicate samples for each variable were assayed at 0 time and after 2, 4, 6, 8, 10, and 12 weeks of storage for L. monocytogenes populations by plating serial dilutions on modified Oxford agar (35 ℃, 48 h). In addition, duplicate uninoculated samples were assayed for changes in pH and populations of lactic acid bacteria (APT with 0.2% bromocresol purple, 25 ℃, 48 to 72 h; BD, Franklin Lakes, NJ). Testing of a variable was discontinued if listerial growth was confirmed (defined as >1-log increase for two consecutive sampling intervals or >2-log average increase at any time interval).

Moisture (5 h, 100 ℃, AOAC vacuum-oven method 950.46 (2)), pH (10-g homogenized portion diluted with 90 ml of distilled, deionized water, pH of slurry measured with the Accumet Basic pH meter and Orion 8104 combination electrode (30)), NaCl (measured as percent Cl⁻, AgNO₃ potentiometric titration, Mettler DL22 food and beverage analyzer), residual nitrite (AOAC colorimetric method 973.31 (2)), and water activity (AquaLab 4TE water activity meter, Decagon, Pullman, WA) were assayed with triplicate samples of each treatment.

**Data analysis.** Fourteen ingredients were evaluated in the phase 1 antimicrobial ingredient model meat system screening study. The changes in L. monocytogenes population are reported as average log CFU per milliliter from duplicate slurries for each treatment. In the phase 1 qualitative acceptability screening study, seven treatments and a control were investigated for subjective (sensory) and objective (color, pH) attributes. In phase 2, three ingredients were investigated for L. monocytogenes growth and analytical attributes. The experimental design for both the phase 1 qualitative acceptability screening study and phase 2 was a randomized complete block performed by using a mixed-effects model. Statistical analysis was performed for all measurements with the Statistical Analysis System (version 9.2, SAS Institute Inc., Cary, NC) mixed-model procedure. The model included the fixed main effects of treatment (CSV, VL, CC, VLC, CP, GSE, BV, and control) and replication (n = 2), resulting in 16 observations for phase 1 quality screening and 8 observations (CSV, VLC, BV, and control) each for deli-style turkey and roast beef and 10 observations (CSV, VLC, BV, control, and negative-growth control) for boneless ham. The random effect was the interaction of treatment × replication. All least significant differences were found with the Tukey-Kramer pairwise comparison method. Significance levels were determined at P < 0.05.

**RESULTS AND DISCUSSION**

**Phase 1: antimicrobial ingredient screening in model meat system.** Data from the baseline slurry experiment show the control of listerial growth in each curing system (n = 4) without added antimicrobial ingredients (Fig. 1). Populations of L. monocytogenes increased 1 log at 1 week in the uncured control without antimicrobial agents stored at 4 ℃, but no growth was observed for the nitrite-containing treatments at the same sampling interval, regardless of nitrite source. L. monocytogenes increased by 1.0, 1.0, and 1.6 log CFU/ml at 2 weeks for the traditional, cultured, and preconverted nitrite systems, respectively. Final L. monocytogenes populations after 4 weeks of storage increased 6.0, 2.9, 3.4, and 4.0 log CFU/ml for uncured, traditional, cultured, and preconverted systems, respectively. In contrast, screening in the model turkey system confirmed no growth of L. monocytogenes in the sodium lactate–diacetate–nitrite controls with or without phosphates during the 4 weeks of storage at 4 ℃. In addition, it is reasonable to conclude concentrations of nitrite were lower in the preconverted than in the cultured system due to limitations of ingredients and more favorable in situ nitrate-to-nitrite conversion (29), with both being lower than the traditional-cured system. The control slurry experiment confirmed previous reports that nitrite by itself inhibits L. monocytogenes, but the degree of inhibition is greater at a higher nitrite concentration (20).

Fourteen ingredients derived from natural sources were evaluated in each of the four curing systems previously described (Table 1). Five ingredients inhibited growth (<0.4-log increase) of L. monocytogenes during the 4-week study at 4 ℃ in uncured as well as traditional- and alternative-cured treatments. No growth of L. monocytogenes was observed in turkey slurries with or without nitrite supplemented with 1.5% VLC, 2.0% BV, 2.5% vinegar–lemon juice blend, 0.05% tea tree oil, or 3.0% CSV. Additional ingredients inhibited growth of the pathogen in the presence of nitrite regardless of source, but no inhibition was seen in uncured treatments. No growth was observed during the 4-week testing interval in the three nitrite-containing treatments supplemented with 0.03% grape seed extract powder, 1.0% liquid smoke extract 2, or 0.5% cherry powder, but growth of the pathogen in the uncured treatment for each was similar to the positive growth control without antimicrobial agents.

A rosemary-tocopherol blend and the green tea extract, tested at 0.08 and 0.10%, respectively, did not inhibit L. monocytogenes growth, compared with the controls without the antimicrobial ingredients, in any of the four curing treatments over 4 weeks of storage at 4 ℃. Higher concentrations could be effective, but they were not further explored in this study. Liquid smoke extract 1, rosemary extract plus nisin, cranberry concentrate, and herbal active emulsion had variable effects among the four curing systems. Addition of these ingredients had low or no inhibitory effect in the uncured treatments, and slightly greater effect in traditional-cured treatments that contained higher residual nitrate than in alternative-cured treatments with reported lower nitrite levels (nitrite data not shown). It is notable that with the exception of the high concentration of tea tree oil, all other ingredients that demonstrated antilisterial activity in the uncured treatment included vinegar (acetic acid) at varying levels. Acetic acid and other weak organic acids have shown antimicrobial activity and acted to destabilize microbial cell walls, causing injury.
or cell death (6, 20). In general, fat-soluble ingredients demonstrate greater antimicrobial activity than water-soluble varieties because of their ability to integrate with and disrupt the bacterial cell membrane (26) However, this activity is significantly reduced if oil-miscible antimicrobial agents preferentially bind to fats and hydrophobic proteins in foods (27).

Residual nitrite levels tested postcook for the cure system treatments containing nitrite were 20, 5, and 45 ppm for cultured, preconverted and traditional cured, respectively, and were similar to published data for comparable curing systems in a variety of meat types (35, 37, 43). Residual nitrite levels in some of the antimicrobial ingredient–containing turkey slurries in the nitrite-containing curing system treatments were less consistent than the no-antimicrobial agent–containing control treatments (data not shown), as pigmentation of some ingredients is believed to have interfered with the colorimetric nitrite assay. Regardless, this experiment confirmed that although nitrite by itself will not prevent growth, the degree of inhibition was increased at higher nitrite levels (20) and enhanced the antimicrobial activity of certain ingredients.

Phase 1: intermediate qualitative acceptability screening. Seven ingredients were further tested for their impact on color, aroma, flavor, and overall acceptance in uncured deli-style chicken breast (data not shown). Ingredients tested included a CSV, vinegar–lemon juice blend, cranberry concentrate, VLC, cherry powder, grape seed extract, BV, and a control with no antimicrobial agents. Two treatments (tea tree oil and liquid smoke extract 2) were excluded from sensory testing in this phase due to the strong organoleptic odors experienced. Herbal active emulsion and rosemary extract plus nisin treatments were not tested because of solubility issues and their limited ability to inhibit L. monocytogenes in the phase 1 growth-inhibition screening study. A cranberry concentrate treatment was included in this evaluation, because relevant research demonstrated antilisterial activity existed in several meat systems (1, 19, 25). Uncured deli-style chicken breast pH values ranged from 5.9 to 6.1. Objective L* color measurements ranged between 75.76 and 82.02, with cherry powder, VLC, and grape seed extract treatments appearing darker (P < 0.05) than the control. CSV and cranberry concentrate treatments were also found redder (P < 0.05) than the control, as indicated by higher a* values. For b*, the cranberry concentrate and grape seed extract treatments were more blue (P < 0.05), while the VLC and cherry powder treatments were more yellowish when compared with the control. A more bluish color imparted from an ingredient could result in a less consumer-desirable visual appearance. Sensory attributes, as assessed by the experienced panel, for the cranberry concentrate treatment were found less desirable (P < 0.05) for color, flavor, and overall acceptance than all other treatments and the control.

Formulations chosen for phase 2 were based on a combination of antilisterial activity and quality (subjective sensory and objective color) attributes. The treatment supplemented with 3.0% CSV had the highest overall acceptance and inhibited L. monocytogenes in all four slurry curing systems. The treatment with 1.5% VLC also had higher acceptance than the control and was chosen for further evaluation, because it not only inhibited L. monocytogenes in all four slurry curing systems, but it also represented the single ingredients vinegar–lemon juice and cherry powder in combination, which were also found to be as nearly effective. The 2.0% BV was chosen as the remaining treatment for further evaluation due to strong antilisterial activity and minimal negative-quality effects.

Phase 2: ingredient validation in commercial products. Three ingredients that were found to prevent growth of L. monocytogenes in turkey slurries and provided minimal quality impact in phase 1 were chosen for further testing in three meat product systems (uncured turkey, uncured beef, and alternative-cured ham). Antimicrobial
agents tested in each meat system included 1.5% VLC, 2.0% BV, and 3.0% CSV. Controls included uncured deli-style turkey breast, uncured roast beef, and alternative-cured, boneless ham without antimicrobial ingredient addition. A traditional nitrite-cured ham with lactate-diacetate (LD) but no sodium phosphates was also included to serve as a negative control. Proximate analysis results for moisture, salt, pH, water activity, and residual nitrite are summarized in Table 2. Proximate moisture, pH, and water activity for the turkey breast and boneless ham treatments were as expected; however, proximate moisture and pH for the roast beef treatments were lower than expected, possibly due to lower water-holding capacities, likely attributed to a lower meat pH and formulation NaCl content. While the water activities for roast beef treatments were numerically higher, but not significant, compared with the turkey breast or ham treatments, the results suggest similar levels of free water existed for all three products.

Growth of L. monocytogenes in the turkey breast control containing no antimicrobial ingredients was significantly higher than treatments supplemented with CSV, BV, or VLC at 2 and 4 weeks of testing (Fig. 2). Turkey breast

FIGURE 2. Inhibition of Listeria monocytogenes from antimicrobial ingredients on uncured, sliced deli-style turkey breast stored at 4°C up to 6 weeks (duplicate trials, n = 6; error bars denote standard deviation).

TABLE 2. Least-squares means and standard deviations for moisture, salt (NaCl), pH, a_w, and residual nitrite in deli-style turkey breast, roast beef, and ham formulated with antimicrobial ingredients

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% moisture</th>
<th>% NaCl</th>
<th>pH</th>
<th>a_w</th>
<th>Residual nitrite (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey breast—control</td>
<td>74.10 ± 0.36</td>
<td>1.19 ± 0.03</td>
<td>6.16 ± 0.02</td>
<td>0.98 ± 0.002</td>
<td>NT*</td>
</tr>
<tr>
<td>Turkey breast—3.0% CSV</td>
<td>71.35 ± 0.29</td>
<td>1.06 ± 0.01</td>
<td>6.04 ± 0.05</td>
<td>0.97 ± 0.001</td>
<td>NT</td>
</tr>
<tr>
<td>Turkey breast—2.0% BV</td>
<td>73.41 ± 1.20</td>
<td>1.01 ± 0.11</td>
<td>6.07 ± 0.07</td>
<td>0.978 ± 0.002</td>
<td>NT</td>
</tr>
<tr>
<td>Turkey breast—1.5% VLC</td>
<td>73.85 ± 0.46</td>
<td>1.10 ± 0.01</td>
<td>6.33 ± 0.07</td>
<td>0.977 ± 0.001</td>
<td>NT</td>
</tr>
<tr>
<td>Roast beef—control</td>
<td>65.12 ± 0.98</td>
<td>0.46 ± 0.05</td>
<td>5.74 ± 0.11</td>
<td>0.985 ± 0.001</td>
<td>NT</td>
</tr>
<tr>
<td>Roast beef—3.0% CSV</td>
<td>65.75 ± 0.82</td>
<td>0.46 ± 0.01</td>
<td>5.69 ± 0.06</td>
<td>0.981 ± 0.001</td>
<td>NT</td>
</tr>
<tr>
<td>Roast beef—2.0% BV</td>
<td>66.36 ± 0.92</td>
<td>0.50 ± 0.02</td>
<td>5.64 ± 0.06</td>
<td>0.982 ± 0.001</td>
<td>NT</td>
</tr>
<tr>
<td>Roast beef—1.5% VLC</td>
<td>67.18 ± 0.24</td>
<td>0.45 ± 0.02</td>
<td>5.93 ± 0.08</td>
<td>0.982 ± 0.001</td>
<td>NT</td>
</tr>
<tr>
<td>Boneless ham—positive control</td>
<td>70.76 ± 1.40</td>
<td>1.57 ± 0.04</td>
<td>6.44 ± 0.04</td>
<td>0.979 ± 0.001</td>
<td>12 ± 1 G</td>
</tr>
<tr>
<td>Boneless ham—3.0% CSV</td>
<td>71.08 ± 0.70</td>
<td>1.57 ± 0.02</td>
<td>6.31 ± 0.09</td>
<td>0.974 ± 0.001</td>
<td>9 ± 1 GH</td>
</tr>
<tr>
<td>Boneless ham—2.0% BV</td>
<td>70.13 ± 0.88</td>
<td>1.64 ± 0.08</td>
<td>6.22 ± 0.10</td>
<td>0.974 ± 0.001</td>
<td>5 ± 0 H</td>
</tr>
<tr>
<td>Boneless ham—1.5% VLC</td>
<td>70.15 ± 1.53</td>
<td>1.64 ± 0.12</td>
<td>6.43 ± 0.02</td>
<td>0.975 ± 0.002</td>
<td>5 ± 0 G</td>
</tr>
<tr>
<td>Boneless ham—negative control</td>
<td>70.08 ± 0.56</td>
<td>1.57 ± 0.09</td>
<td>6.27 ± 0.03</td>
<td>0.974 ± 0.002</td>
<td>27 ± 2 H</td>
</tr>
</tbody>
</table>

* Treatments were turkey breast and roast beef control, no antimicrobial ingredient addition; boneless ham positive control, 0.3% preconverted celery powder and 0.2% cherry powder; CSV, 3.0% cultured sugar–vinegar blend; BV, 2.0% buffered vinegar; VLC, 1.5% vinegar–lemon–cherry powder blend; negative control, 0.0156% purified sodium nitrite, 0.0547% sodium erythorbate, and 2.8% sodium lactate–sodium diacetate.

Means in the same column within a product group (e.g., roast beef) with different letters are different (P < 0.05).

NT, not tested.
with VLC supported 3-log growth of *L. monocytogenes* within 4 weeks of incubation, whereas turkey breast with CSV supported a 1-log increase of *L. monocytogenes* in a similar amount of time. Turkey breast with BV delayed growth of *L. monocytogenes* until 6 weeks of storage at 4°C. Significant differences in *L. monocytogenes* inhibition were not observed for turkey breast with CSV and BV throughout testing, despite the shorter time to an average 1-log *L. monocytogenes* increase for turkey breast with CSV. However, testing of formulations was discontinued at 6 weeks, since *L. monocytogenes* growth was observed. Glass and Doyle found that sliced, uncured turkey products without antimicrobial agents supported a $10^{3}$- to $10^{4}$-log CFU/g increase in *L. monocytogenes* populations when stored at 4.4°C for 6 weeks, and >1-log growth was observed within 2 weeks of storage, regardless of inoculum size (11).

The roast beef control supported growth of *L. monocytogenes* at 4 weeks of storage while the CSV, BV, and VLC treatments did not support growth throughout 12 weeks of 4°C storage (Fig. 3). Rapid growth of *L. monocytogenes* took place in the control compared with the antimicrobial ingredient treatment formulations, which resulted in significant differences between the control and...
all three antimicrobial ingredient formulations as early as 2 and 4 weeks (P = 0.0012 and P < 0.0001, respectively). Interestingly, similarly effective listerial control has been observed in roast beef containing LD, inoculated and stored at 4°C (45). However, the greater overall L. monocytogenes inhibition in roast beef over the boneless ham and turkey breast observed in both our study and the aforementioned study by Zhang et al. (45) might be due to lower compositional moisture, pH, and different meat species.

Alternative-cured, boneless ham with preconverted nitrite (approximately 12 ppm of residual nitrite) but no antimicrobial ingredients, serving as a positive control, supported the >2-log increase of L. monocytogenes within 2 weeks of storage at 4°C (Fig. 4). Growth of L. monocytogenes on the positive control was significantly higher than boneless ham containing CSV, BV, and VLC at 2 and 4 weeks (P < 0.0001). Boneless ham with preconverted nitrite and VLC or CSV supported L. monocytogenes growth after 4 weeks, while BV supported growth at 6 weeks of storage at 4°C. No statistical differences for growth of L. monocytogenes on any boneless hams containing any antimicrobial ingredient were noted until the 6-week testing time point. Boneless ham traditionally cured with nitrite and containing LD delayed growth at similar rates as did BV and CSV through 8 weeks of storage at 4°C, yet supported L. monocytogenes growth earlier than predicted when using a predictive modeling program (OptiForm Listeria Control Model, Purac, Lincolnshire, IL). Cooking losses for boneless ham treatments were higher than normal, ranging between 13.25 and 15.68%. Higher cook losses can be partially explained by the lack of phosphates. The greater cooking losses might also correlate with lower salt and LD in the final product. The final measured salt content was approximately 1.6% compared with the expected 2.0%, based on an injection rate of 2.35% ingoing. If LD levels were similarly decreased from the 2.80% addition, one would expect the L. monocytogenes growth rate to be increased.

Listerial growth in the boneless ham treatments we investigated had growth trends similar to those previously reported by Sullivan et al. (35) for VLC and CSV no-nitrate- or-nitrite-added hams, as well as preconverted and traditional-cured nitrite controls. However, total log increases varied between the studies, as the current study revealed larger overall L. monocytogenes increases but lower residual nitrite than the study performed by Sullivan et al. (35). It is unclear why discrepancies between these studies exist, but they can be possibly explained by nitrite concentration variation from different ingredient lots, differences in intrinsic ham properties, or microbiological testing differences such as the pathogen strains utilized or sampling procedures followed. Further growth occurred quicker than expected in the 12-week storage period; however, differences between the positive control and all treatments suggest ingredient efficacy.

The significant inhibition of listerial growth in roast beef compared with the boneless ham and turkey breast could be attributed to differences in product moisture and pH, as previously introduced. Boneless ham averaged 70% moisture and a pH of 6.3, turkey breast averaged 73% moisture and a pH of 6.2, while roast beef averaged 66% moisture and a pH of 5.8 (Table 2). Further, as expected, microbial growth was more rapid when products were stored at 7 than at 4°C. At 7°C, controls without antimicrobial ingredients supported a 5.5-, 3.7-, and 2.5-log increase at 2 weeks for turkey breast, boneless ham, and roast beef, respectively. All boneless ham and turkey breast treatments supported >2-log increase at 4 weeks, regardless of antimicrobial ingredient addition. In contrast, roast beef supplemented with CSV and BV delayed growth through 6 weeks, whereas VLC supported a 1-log increase of L. monocytogenes at 4 weeks.

This study evaluated numerous ingredients for their effectiveness in providing an antimicrobial agent contribution to uncured and alternative-cured RTE processed meat and poultry products. Because the ingredients assayed in this study were proprietary mixtures, the exact composition is unknown, and mode of action can only be speculated. The labeling suggested provided by the manufacturer suggests that each of the three ingredients tested in meats contained acetic acid, the acid from which sodium diacetate, a commonly used antilisterial agent, is derived (12, 18, 31). The antioxidant ascorbate found in cherry powder could have little antimicrobial activity by itself but enhance activity of the other ingredients. By-products from the cultured sugar could contain other organic acids and/or bacteriocins. The relatively short shelf life of the products made with natural ingredients compared with contemporary products supplemented with synthetic antimicrobial agents is likely to be associated with the low concentration of antimicrobial compounds found in ingredients derived from natural sources.

The results confirm the impact that nitrite concentration (regardless of source), compositional differences (pH, moisture, etc.) of the meat system, the type and concentration of antimicrobial ingredient, and storage temperature have on contributing to the overall safety of a product. Therefore, careful consideration of these factors should be taken when evaluating the use of ingredients possessing antimicrobial efficacy for use in natural, organic, or clean-label meat and poultry products.

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