Hot Water Postprocess Pasteurization of Cook-in-Bag Turkey Breast Treated with and without Potassium Lactate and Sodium Diacetate and Acidified Sodium Chlorite for Control of *Listeria monocytogenes*

JOHN B. LUCHANSKY, GEORGE COCOMA, AND JEFFREY E. CALL

Abstract

Surface pasteurization and food-grade chemicals were evaluated for the ability to control listeriae postprocess on cook-in-bag turkey breasts (CIBTB). Individual CIBTB were obtained directly from a commercial manufacturer and surface inoculated (20 ml) with a five-strain cocktail (ca. 7.0 log) of *Listeria innocua*. In each of two trials, the product was showered or submerged for up to 9 min with water heated to 190, 197, or 205°F (ca. 87.8, 91.7, or 96.1°C) in a commercial pasteurization tunnel. Surviving listeriae were recovered from CIBTB by rinsing and were then enumerated on modified Oxford agar plates following incubation at 37°C for 48 h. As expected, higher water temperatures and longer residence times resulted in a greater reduction of *L. innocua*. A ca. 2.0-log reduction was achieved within 3 min at 205 and 197°F and within 7 min at 190°F. In related experiments, the following treatments were evaluated for control of *Listeria monocytogenes* on CIBTB: (i) a potassium lactate–sodium diacetate solution (1.54% potassium lactate and 0.11% sodium diacetate) added to the formulation in the mixer and 150 ppm of acidified sodium chlorite applied to the surface with a pipette, or (ii) a potassium lactate–sodium diacetate solution only, or (iii) no potassium lactate–sodium diacetate solution and no acidified sodium chlorite. Each CIBTB was inoculated (20 ml) with ca. 5 log CFU of a five-strain mixture of *L. monocytogenes* and then vacuum sealed. In each of two trials, half of the CIBTB were exposed to 203°F water for 3 min in a pasteurization tunnel, and the other half of the CIBTB were not; then, all CIBTB were stored at 4°C for up to 60 days, and *L. monocytogenes* was enumerated by direct plating onto modified Oxford agar. Heating resulted in an initial reduction of ca. 2 log CFU of *L. monocytogenes* per CIBTB. For heated CIBTB, *L. monocytogenes* increased by ca. 2 log CFU per CIBTB in 28 (treatment 1), 28 (treatment 2), and 14 (treatment 3) days. Thereafter, pathogen levels reached ca. 7 log CFU per CIBTB in 45, 45, and 21 days for treatments 1, 2, and 3, respectively. In contrast, for nonheated CIBTB, *L. monocytogenes* levels increased from ca. 5 log CFU per CIBTB to ca. 7 log CFU per CIBTB in 28, 21, and 14 days for treatments 1, 2, and 3, respectively. Lastly, in each of three trials, we tested the effect of hot water (203°F for 3 min) postprocess pasteurization of inoculated CIBTB on the lethality of *L. monocytogenes* and validated that it resulted in a 1.8-log reduction in pathogen levels. Collectively, these data establish that hot water postprocess pasteurization alone is effective in reducing *L. monocytogenes* on the surface of CIBTB. However, as used in this study, the potassium lactate–sodium diacetate solution and acidified sodium chlorite were only somewhat effective at controlling the subsequent outgrowth of this pathogen during refrigerated storage.

**Listeria monocytogenes** remains a significant foodborne pathogen, especially for ready-to-eat (RTE) meats. Evidence for this can be found in a risk assessment of 23 selected categories of RTE foods (http://www.foodsafety.gov/~dfs/llmr2-toc.html), wherein deli foods on a per annum basis and non-reheated frankfurters on a per serving basis were identified as the highest-risk foods. Further evidence is provided by the recovery of this pathogen from commercially prepared and retail RTE foods and by the continued documentation of product recalls and human illness. Surveys in the United States between 1990 and 2003 involving ca. 100,000 food samples provided estimations that the prevalence of *L. monocytogenes* was 1.6 to 7.6% in meat, fish, and vegetable products, most of which were RTE foods (15, 18, 38). Thomsen and McKenzie (37) reported that, between 1982 and 1998, there was an average of five recalls per year due to the contamination of foods with *L. monocytogenes*. In recent years, moreover, there have been several illnesses and deaths attributed to foodborne listeriosis (8–11, 23). Thus, further research is warranted to evaluate the ability of postprocess interventions to enhance the safety of high-volume RTE foods that may be associated with *L. monocytogenes* or that may support its growth.

Because of the severity of listeriosis and the number and magnitude of food recalls, regulatory agencies have...
established requirements for manufacturers to better control *L. monocytogenes* in meat and poultry. More specifically, the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) has established new rules and guidelines for manufacturers of RTE meat and poultry products (1). In simple terms, this ruling provides manufacturers with the following three alternatives for determining the degree to which regulatory testing would be implemented: alternative 1, whereby a manufacturer would use a postprocess lethality step and an antimicrobial to control outgrowth (lowest testing frequency); alternative 2, whereby a manufacturer would use either a postprocess lethality step or an antimicrobial to control outgrowth (moderate-to-increased testing relative to alternative 1); and alternative 3, whereby a manufacturer would rely on sanitation alone (most testing). Although further clarification is expected regarding how much lethality is required or how little outgrowth is allowed, there is an immediate and critical need to identify and implement postprocess interventions for killing or inhibiting *L. monocytogenes* in RTE meats.

Various interventions are effective against *L. monocytogenes*, including some that have proven useful in controlling this pathogen when RTE foods are contaminated postprocess. Heat is arguably the most effective and most well-studied method for postprocess pasteurization. Several investigators have validated the use of heat to control *L. monocytogenes* associated with frankfurters, summer sausage, ground beef, ground pork, deli-type meat, and poultry products (5, 16, 28, 29, 31, 39, 41). Depending on the product and on the times and temperatures used for heating, reductions of *L. monocytogenes* ranging from 2.0 to 7.0 log CFU have been reported. However, certain food-grade chemicals, including organic acids (e.g., potassium lactate, sodium diacetate) alone or in combination with other interventions, are also effective at controlling *L. monocytogenes* in RTE meats (3, 4, 6, 21, 30, 34–36, 40). In addition to the use of such chemicals as a flavorant, the USDA-FSIS has approved the use of “lactates” at levels up to 4.8% of the total formulation to slow the growth of pathogens in fully cooked products. pH-altering inorganic compounds, such as acidified sodium chloride (ASC), are also effective at controlling this pathogen, notably on fresh beef and on beef and broiler carcasses (7, 17). In the United States, ASC is approved at 500 to 1,200 ppm, pH 2.3 to 2.9, for various applications, including for poultry and red meats and processed, comminuted, or formed meat products. For example, Mullerat et al. (24) reported reductions of *L. monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* that ranged from <1.0 to 5.0 log after a 15-min exposure to a solution containing 10 mM sodium chlorite. Although heat and food-grade chemicals have antilisterial activity, a combination of interventions may be required to assist producers in satisfying current regulatory requirements for controlling *L. monocytogenes* in RTE meat products without adversely affecting product quality. Thus, the objective of the present study was to investigate the efficacy of a combination of potassium lactate and sodium diacetate as part of the ingredient formulation and ASC as a surface-applied agent, coupled with postpackaging hot water pasteurization, for controlling *L. monocytogenes* on the surface of cook-in-bag turkey breasts (CIBTB).

### MATERIALS AND METHODS

**Bacterial strains.** As described previously (30), approximately equal numbers of each of the following five strains of *L. monocytogenes* were used as a cocktail in this study: (i) Scott A (serotype 4b, clinical isolate), (ii) H7776 (serotype 4b, frankfurter isolate), (iii) LM-1011M (serotype 4b, beef and pork sausage isolate), (iv) F6854 (serotype 1/2a, turkey frankfurter isolate), and (v) MFS-2 (serotype 1/2a, environmental isolate from a pork processing plant). Likewise, approximately equal numbers of the following five strains of *Listeria innocua* were used as a cocktail in some experiments: (i) 2428 (raw egg), (ii) 2283 (turkey), (iii) LA-1 (cheese), (iv) SS-VJ-S (sausage), and (v) LG2 (ground lamb). Isolates were passed twice in brain heart infusion broth (Difco, Becton Dickinson, Sparks, Md.) at 37°C so that cells would be in the stationary phase for each experiment. Stock cultures were maintained by storage in brain heart infusion broth plus 10% (wt/vol) glycerol in 1.5-ml portions in cryovials and held at −80°C.

**Inoculation of commercially prepared CIBTB with *L. innocua* for use in hot water postprocess pasteurization studies.** Whole turkey breasts (ca. 8 to 9 lb) were de-boned, hand formed, vacuum sealed, cooked, and cooled to 4°C by a cooperating commercial processor. After processing, the uncurled CIBTB were boxed, transported back to the laboratory, and stored at 4°C for up to 7 days before use. Each CIBTB was aseptically removed from its original packaging and repackaged in a postpasteurization bag (12 by 18 in, CNP310T, Cryovac, Saddlebrook, N.J.). Twenty milliliters of the *L. innocua* cocktail was added to each package to achieve a target level of ca. 7 log CFU per CIBTB. Each package was then massaged by hand for ca. 2 min to distribute the inoculum and vacuum sealed to 95 kPa with a Multivac A300/16 vacuum-packaging unit (Sepp Haggemüller KG, Wolfertschwenden, Germany). Next, the CIBTB were exposed to 205°F (96.1°C) water for residence times of 0 to 3, 5, and 7 min; or 190°F (87.8°C) water for 0, 5, 7, and 9 min in a pilot-plant-scale pasteurization tunnel (model PT1030, Gal-Esh Stainless Steel Products Ltd., Holon, Israel). In brief, the CIBTB were transported through the pasteurization tunnel on a conveyor belt adjusted by the variable speed variator of the tunnel to achieve the targeted residence times (i.e., 1 to 9 min, depending on the experiment). While in the tunnel, approximately half of each CIBTB was submerged in water heated to the target temperature, and the other half was continuously showered with hot water heated to the target temperature. The temperatures of the top and bottom surfaces of selected CIBTB were monitored during runs at all three temperatures with a stainless steel DeltaTRAK (model EBI-125A [48 mm in diameter by 28 mm thick], Delta Trak, Inc., Pleasanton, Calif.) data logger. For each CIBTB, a portion of the meat approximately the same diameter and thickness of the data logger was removed from the product and replaced with the data logger. For each temperature and trial, a data logger was placed on the top and bottom surfaces of a single CIBTB to monitor the temperature during transit through the pasteurization tunnel. After heating, the CIBTB were quickly cooled by submersion in an ice water bath. For each of two trials, three CIBTB were analyzed per residence time at each temperature.

**Inoculation of commercially prepared CIBTB with *L. monocytogenes* for use in postprocess validation studies.** A sin-
TABLE I. Proximate composition analyses of cook-in-bag turkey breasts (CIBTB) treated with and without K-lactate—Na-diacetate and acidified sodium chloride (ASC)\(^a\)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>NaCl (g/100 g)(^b)</th>
<th>pH</th>
<th>Moisture (g/100 g)</th>
<th>Protein (g/100 g)(^b)</th>
<th>Fat (g/100 g)(^b)</th>
<th>CHOs (g/100 g)(^b)</th>
<th>Phenolics (mg/g)</th>
<th>Lactic acid (%)</th>
<th>Nitrite (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIBTB prepared without</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-lactate—Na-diacetate or ASC</td>
<td>0.87</td>
<td>6.38</td>
<td>71.88</td>
<td>12.11</td>
<td>10.28</td>
<td>3.61</td>
<td>757</td>
<td>0.615</td>
<td>9.98</td>
</tr>
<tr>
<td>CIBTB prepared with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-lactate—Na-diacetate and ASC</td>
<td>1.58</td>
<td>6.51</td>
<td>68.92</td>
<td>13.82</td>
<td>5.31</td>
<td>8.3</td>
<td>895</td>
<td>2.95</td>
<td>7.09</td>
</tr>
</tbody>
</table>

\(^a\) Two CIBTB were analyzed per treatment. All CIBTB were obtained on day 0 of the shelf life study experiments.

\(^b\) Values and levels were significantly different (\(P < 0.05\)) between CIBTB treatments.

A single batch of chopped or formed turkey breasts was prepared by the same commercial manufacturer as listed above. For these studies, 2.75% of a 60% solution of potassium lactate and sodium diacetate (K-lactate—Na-diacetate, a 56% potassium lactate plus 4% sodium diacetate solution in dH\(_2\)O; Opti-Form 4, Purac America, Inc., Lincolnshire, Ill.) was added, as needed, to a ribbon mixer. The K-lactate—Na-diacetate solution was added to achieve a target concentration of potassium lactate and sodium diacetate of ca. 1.54 and 0.11%, respectively. After processing, the unincubated CIBTB were boxed, transported back to the laboratory, and stored at 4°C for up to 7 days before use. Each CIBTB was aseptically removed from the original vacuum packaging and repackaged as described. As needed, a stock solution of ASC (a 3.35% solution of sodium chloride; Bio-Cide International, Norman, Okla.) was prepared. This solution was then activated as follows: 2.84 g of citric acid (Sigma, St. Louis, Mo.) was added to 7.1 ml of the 3.35% sodium chloride solution and allowed to “activate” for 10 min at room temperature. The activated or acidified sodium chloride was then diluted in 947 ml of sterile distilled water to achieve a final concentration of 250 ppm. The pH of the ASC stock solution was ca. pH 2.5. Next, 15 ml of the 250-ppm stock solution was applied to the surface of the CIBTB treated with the K-lactate—Na-diacetate solution, with the aid of a pipette, to achieve a final concentration of ca. 150 ppm when combined with the \(L.~monecytogenes\) inoculum. The supplier of ASC suggested this level to complement the types and levels of the other ingredients. Twenty milliliters of the \(L.~monecytogenes\) cocktail was added to each package to achieve a target level of ca. 7 log CFU per CIBTB. Each package was then massaged by hand for ca. 2 min to distribute the ASC and the inoculum before being vacuum sealed as described. The CIBTB were exposed to 203°F water for residence times of 0, 1, 3, 5, and 7 min in the above-mentioned pasteurization tunnel, and the temperatures of the top and bottom surfaces of selected CIBTB were monitored as described. After heating, the CIBTB were quickly cooled by submersion in an ice water bath. For each of three trials, three CIBTB were analyzed per residence time at 203°F.

Inoculation of commercially prepared CIBTB with \(L.~monecytogenes\) for use in hot water postprocess pasteurization and shelf life studies. A single batch of CIBTB, with and without K-lactate—Na-diacetate, was obtained from the above-mentioned commercial processor. The CIBTB were treated as follows: (i) K-lactate—Na-diacetate plus ASC; (ii) K-lactate—Na-diacetate only; and (iii) no K-lactate—Na-diacetate and no ASC. Each CIBTB was repackaged in a pasteurization bag, and 20 ml of the \(L.~monecytogenes\) cocktail was added to each package to achieve a target level of ca. 5 log CFU per CIBTB. For each of the three treatments, one portion of the inoculated CIBTB was not heated, whereas another portion of the CIBTB was exposed to 203°F (95°C) water for 3 min in the pasteurization tunnel. All CIBTB were stored at 4°C and analyzed 0, 7, 14, 21, 28, 45, and 60 days postinoculation. Control samples that were not inoculated with the pathogen were analyzed on days 1, 45, and 60 postinoculation. For both trials, three CIBTB, heated and nonheated, were analyzed per sampling time point for each of the three treatments.

Microbiological analyses. Surviving listeriae were enumerated by the USDA Agricultural Research Service package rinse method (20). Next, 250 μl of the resulting rinse fluid or dilutions thereof was spread plated onto duplicate modified Oxford (12) agar plates with a spiral plater (Autoplate 4000, Spiral Biotech, Gaithersburg, Md.) and incubated for 48 h at 37°C. Bacterial numbers were expressed as log CFU per package, with each package containing a single CIBTB. Colonies were randomly selected and confirmed as \(L.~monecytogenes\) following standard USDA-FSIS methods (12).

Chemical analyses. The proximate composition of CIBTB was determined by methods approved and described by the Association of Official Analytical Chemists (22) as conducted by a commercial testing laboratory. For day 0 for one trial of the shelf life study, proximate analyses were performed on two CIBTB treated with K-lactate—Na-diacetate and ASC and on two CIBTB not treated with either of these food-grade chemicals.

Statistical analyses. Data were analyzed by version 8.0 of the SAS statistical package (SAS Institute, Inc., Cary, N.C.). Data were compared by analysis of covariance and \(F\) tests to evaluate the effects of time, temperature, and food-grade antimicrobials on the viability of listeriae in packages of CIBTB during extended storage at 4°C for the shelf life component of this study and to evaluate the effect of chemicals on the thermal stability of the pathogen after its passage through the pasteurization tunnel in the validation component of this study.

RESULTS

Proximate composition and temperature profile of CIBTB. A representative sample, consisting of two CIBTB treated with and two CIBTB treated without K-lactate—Na-diacetate and ASC that had been collected from a single trial of the shelf life study, was forwarded to a private testing laboratory for proximate composition analyses (Table I). Chemical analyses revealed significant differences (\(P < 0.05\)) in the NaCl, fat, carbohydrate (CHO), and lactic acid levels between the CIBTB treated with and without K-lactate—Na-diacetate and ASC, but these analyses did not reveal appreciable differences in the levels of the other chemicals assayed. Although there were significant differences in the levels of some of the chemicals tested, empirical analyses revealed that these differences did not have an
appreciable effect on the outgrowth of *L. monocytogenes* in CIBTB during storage. Results also showed no untoward effects on the texture or appearance of the CIBTB under such conditions (data not shown). The sampling of noninoculated packages of CIBTB showed the absence (i.e., <20 CFU per package) of indigenous *L. monocytogenes* by direct plating (data not shown).

To assess heat transfer and delivery, we measured the temperature achieved for both the top and bottom surfaces of CIBTB during exposure to water heated to 190, 197, 203, and 205°F. The maximum top surface temperatures obtained for the hot water postprocess pasteurization studies with *L. innocua* were 194, 191, and 176°F (ca. 90, 88.3, and 80°C) when the water in the pasteurization tunnel was heated to 205, 197, and 190°F, respectively. The maximum bottom surface temperatures obtained for these studies were 195, 185, and 186°F (ca. 90.5, 85, and 85.5°C) when the water in the pasteurization tunnel was heated to 205, 197, and 190°F, respectively. During postprocessing pasteurization validation studies at 203°F for 3 min with CIBTB containing K-lactate–Na-diacetate and ASC and inoculated with *L. monocytogenes*, the maximum top and bottom surface temperatures achieved were 182 and 192°F (ca. 83.3 and 88.8°C), respectively. In comparison, the maximum top and bottom surface temperatures achieved in CIBTB not treated with food-grade chemicals were 183 and 192°F (ca. 83.9 and 88.8°C), respectively, following treatment at 203°F for 3 min. These data indicate that the temperature of the water was higher than the temperature of the product and that, in general, the temperatures at the bottom were higher than at the top, probably because the bottom surface was submerged, which facilitated better heat transfer.

**Postpackaging surface pasteurization of CIBTB inoculated with *L. innocua***. We used an *L. innocua* cocktail as a surrogate for *L. monocytogenes* to gain insight on the fate of the listeriae on CIBTB following transit through the pasteurization tunnel. These data were used in subsequent inoculated package experiments to validate the lethality of the hot water pasteurization step on *L. monocytogenes*. As expected, the higher the temperature and the longer the residence time, the greater the reduction in the population of *L. innocua* (Table 2). For each of the three temperatures tested, we observed at least a 2.0-log reduction within 7 min at 190°F, 3 min at 197°F, and 3 min at 205°F. The greatest reductions, that being 2.1 and 2.55 log, were achieved within 3 min at 205°F and within 7 min at 197°F. Visual inspection confirmed that these time and temperature combinations had little or no effect on the texture or appearance of the CIBTB (data not shown).

**Validation of a defined time and temperature combination for postpackaging pasteurization of CIBTB to control *L. monocytogenes***. On the basis of the results we obtained from the hot water surface pasteurization of *L. innocua* on CIBTB and on input from an industry partner, we validated the lethality toward *L. monocytogenes* of processing CIBTB in a pasteurization tunnel with water heated to 203°F (Table 3). One portion of the CIBTB was formulated with K-lactate–Na-diacetate and surface treated with ASC (treatment 1); another portion was formulated without K-lactate–Na-diacetate and ASC (treatment 2). On the basis of the average of three independent trials, with the possible exception of heating for 1 min (wherein a 1.5-log reduction was achieved for CIBTB without antimicrobials [i.e., treatment 2] and a 0.92-log reduction was achieved for CIBTB containing antimicrobials [i.e., treatment 1]), there was essentially no difference in lethality relative to the ingredient formulation for CIBTB processed in the pasteurization tunnel with water heated to 203°F for 3, 5, or 7 min. These data validate that processing for 3 min with water heated to 203°F would result in a ca. 1.8-log reduction in the levels of *L. monocytogenes* when the

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TABLE 2. Effect of postprocess hot water pasteurization treatments on CIBTB inoculated with *Listeria innocua*<sup>a</sup>

<table>
<thead>
<tr>
<th>Residence times (min)</th>
<th>Pasteurization temperatures&lt;sup&gt;b&lt;/sup&gt;:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>205°F</td>
</tr>
<tr>
<td>0</td>
<td>7.05 (6.6)</td>
</tr>
<tr>
<td>1</td>
<td>5.53 (5.4)</td>
</tr>
<tr>
<td>2</td>
<td>5.19 (5.3)</td>
</tr>
<tr>
<td>3</td>
<td>4.95 (4.8)</td>
</tr>
<tr>
<td>5</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> *n* = 2 trials, *n* = 3 CIBTB per sampling point. CIBTB were not treated with K-lactate–Na-diacetate or ASC for these experiments.

<sup>b</sup> Values are log CFU per package (standard deviations [log]).

<sup>c</sup> ND, not determined.

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TABLE 3. Validation of postprocess hot water pasteurization (203°F for 3 min) treatment of CIBTB inoculated with *Listeria monocytogenes*<sup>a</sup>

<table>
<thead>
<tr>
<th>Samples</th>
<th>Residence times (min)&lt;sup&gt;b&lt;/sup&gt;:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Treatment 1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.07 (6.6)</td>
</tr>
<tr>
<td>Treatment 2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.78 (6.4)</td>
</tr>
</tbody>
</table>

<sup>a</sup> *n* = 3 trials, *n* = 3 CIBTB per sampling point.

<sup>b</sup> Values are log CFU per package (standard deviations [log]).

<sup>c</sup> CIBTB treated with K-lactate–Na-diacetate and ASC.

<sup>d</sup> CIBTB not treated with K-lactate–Na-diacetate or ASC.
TABLE 4. Effect of postprocess hot water pasteurization treatments, K-lactate–Na-diacetate, and acidified sodium chlorite (ASC) on the viability of L. monocytogenes inoculated onto the surface of CIBTB during storage at 4°C

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage times (days):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Treatment 1 + heat</td>
<td>3.14 B (2.79)</td>
</tr>
<tr>
<td>Treatment 2 + heat</td>
<td>3.15 B (3.25)</td>
</tr>
<tr>
<td>Treatment 3 + heat</td>
<td>3.15 B (2.82)</td>
</tr>
<tr>
<td>Treatment 1, no heat</td>
<td>5.35 B (4.70)</td>
</tr>
<tr>
<td>Treatment 2, no heat</td>
<td>5.25 B (4.72)</td>
</tr>
<tr>
<td>Treatment 3, no heat</td>
<td>5.37 B (4.85)</td>
</tr>
<tr>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

^a n = 2 trials, n = 3 CIBTB per sampling point.
^b Treatment 1, CIBTB treated with K-lactate–Na-diacetate and ASC; treatment 2, CIBTB treated with K-lactate–Na-diacetate only; treatment 3, CIBTB not treated with K-lactate–Na-diacetate or ASC. Initial levels of L. monocytogenes were lower for treatments that were heated than for treatments that were not heated due to the exposure of CIBTB of water heated at 203°F for 3 min prior to storage.
^c Values are log CFU per package (standard deviations [log]). Log CFU per package values with the same letter were statistically similar.
^d ND, not determined.

Effect of postprocessing surface pasteurization and ingredient formulation of CIBTB for control of L. monocytogenes during refrigerated storage. In addition to verifying the initial or direct reduction achieved with postprocessing surface pasteurization at 203°F, we evaluated the effect of heat and food-grade chemicals on precluding the outgrowth of L. monocytogenes during the expected refrigerated shelf life of the product. The CIBTB were formulated as follows: K-lactate–Na-diacetate as an ingredient (treatment 1); K-lactate–Na-diacetate as an ingredient, along with surface-applied ASC (treatment 2); and no K-lactate–Na-diacetate and no ASC (treatment 3). In general, the levels of L. monocytogenes were significantly reduced (P < 0.05) on the surface of the CIBTB that were subjected to 203°F water for 3 min, regardless of treatment type, compared to the CIBTB that were not heated (Table 4). Specifically, pathogen numbers were reduced 2.2, 2.4, and 2.2 log CFU for treatments 1, 2, and 3, respectively, for heated CIBTB. These results compare favorably with the results detailed in the previous section on processing CIBTB for 3 min at 203°F for postprocess pasteurization, wherein we observed a 1.8-log reduction in the levels of L. monocytogenes.

During subsequent storage at 4°C (Table 4), L. monocytogenes levels in heated CIBTB increased by ca. 2 log CFU per CIBTB in 28 (treatment 1), 28 (treatment 2), and 14 (treatment 3) days. Thereafter, pathogen levels reached ca. 7 log CFU per CIBTB in 45, 45, and 21 days for treatments 1, 2, and 3, respectively. In contrast, in nonheated CIBTB, the pathogen numbers increased from ca. 5 log CFU per CIBTB to ca. 7 log CFU per CIBTB in 28, 21, and 14 days for treatments 1, 2, and 3, respectively. Thereafter, in all treatments, pathogen levels continued to increase to ca. 10^8 to 10^10 CFU per CIBTB. In general, the inclusion of K-lactate–Na-diacetate, ASC, or both had no apparent effect on controlling the subsequent outgrowth of the pathogen during refrigerated storage. These data indicate that the use of K-lactate–Na-diacetate as an ingredient alone or in combination with surface-applied ASC is not totally effective at controlling the outgrowth of L. monocytogenes during extended refrigerated storage.

**DISCUSSION**

The number, as well as the magnitude, of meat and poultry recalls in recent years due to the contamination of RTE foods with L. monocytogenes is staggering. Between 1999 and 2002, there were five major recalls that, alone, resulted in the voluntary recall of some 130 million lb of RTE foods. Of particular relevance to the present study was a recall in 2002 of some 30 million lb of deli turkey products linked to 43 illnesses and 13 deaths (11). The various recalls and epidemiologically linked illnesses associated with RTE foods have most likely been a major factor in the implementation of a recent USDA-FSIS ruling that requires manufacturers of postlethality-exposed RTE foods to control L. monocytogenes contamination of such products by means of a hazard analysis critical control point plan or sanitation standard operating procedure. Manufacturers are also required to formulate or process RTE meat and poultry products to achieve some level of postprocess pathogen lethality and/or to prevent the subsequent outgrowth of the pathogen during the shelf life of the product. Thus, along with several other investigators, we evaluated processes aimed at eliminating any cells of L. monocytogenes that might be acquired postprocess as incidental contaminants of RTE meat and poultry products. In the present study, we tested different temperatures and residence times for processing CIBTB in a commercial hot water pasteurization tunnel for postprocess lethality against L. monocytogenes. We also evaluated food-grade chemicals for the ability to inhibit L. monocytogenes during extended refrigerated storage. Several investigators have confirmed that these treatments are effective when used singly, but far fewer investigators have evaluated the efficacy in controlling L. monocytogenes when used in combination for RTE poultry products during refrigerated storage. Because heat is the most common and probably the most effective intervention, experiments were conducted to evaluate a range of time and temperature combinations like-
ly to be used by manufacturers as a postprocessing pathogen lethality treatment for RTE poultry products. In these experiments, a five-strain cocktail of L. innocua was used as a surrogate for L. monocytogenes and CIBTB that did not contain any lactates. The times and temperatures that we evaluated in this portion of the study resulted in an appreciable reduction in pathogen levels (ca. 2.5 log per CIBTB) but did not result in any undesirable effects on the product per se (e.g., discoloration, foul odors). These results compare favorably with those already published.

Several studies have evaluated the use of both pre- and postprocess surface pasteurization of RTE meat and poultry products for controlling L. monocytogenes. For example, Gande and Muriana (14) and Muriana et al. (25, 26) reported significant reductions in L. monocytogenes on delitype turkey following postprocess submersion in hot water and a radiant oven. Similar results were obtained by Murphy et al. (27) on turkey breasts by means of submersion heating to reduce the levels of L. monocytogenes. Conditions for heating should also be adjusted to accommodate differences in the lethality of L. monocytogenes due to ingredient formulation or type of CIBTB and manufacturing conditions (25). Regardless, our findings validated that hot water postprocess pasteurization delivered at least a 1.8-log reduction of L. monocytogenes per CIBTB after passage for 3 min in a pasteurization tunnel while being showered or submerged with water heated to 203°F. In practice, it would be desirable in terms of cost, product quality, and pathogen lethality to process CIBTB in ≤3 min through the pasteurization tunnel with water heated to 203°F. The challenge is to apply a sufficient amount of heat for a sufficient time to achieve the desired reduction in the target pathogen without causing undesirable effects on the product.

The efficacy of potassium lactate and sodium diacetate for controlling L. monocytogenes in RTE meats has been demonstrated by a number of researchers (3, 4, 30, 32, 33, 40). In a previous study, we also showed that the inclusion of 2% potassium lactate as an ingredient in frankfurters was quite effective at preventing the outgrowth of this pathogen during refrigerated storage (i.e., a <1.0-log increase over 90 days) (30). However, we did report that reheat at near-boiling temperatures for ca. 36 s was sufficient to achieve a 5-log reduction in pathogen numbers (29). The results of our previous studies are in agreement with the present study, relative to the thermal inactivation of the pathogen, but are not in agreement relative to the outgrowth of the pathogen during refrigerated storage. This may be partly due to the lower levels of potassium lactate used in the present study (ca. 1.5%), compared to our previous study (ca. 2.0%), or it may be due to the differing formulation, shape, surface area, or topography of frankfurters and CIBTB. However, in the present study, we used sodium diacetate (ca. 0.11%) as an ingredient in combination with potassium lactate, and we were anticipating a synergistic antilisterial effect. Further experiments must be conducted to optimize the levels and types of antimicrobials for controlling listeriae on CIBTB and to evaluate the most effective method for delivering antimicrobials to RTE products.

Although the effectiveness of ASC has not been thoroughly demonstrated for RTE meats, a recent study showed that 0.12% ASC reduced levels of L. monocytogenes on fresh beef by ca. 1.8 log within 2 weeks at 4°C (although the color of the beef was negatively affected) (19). Frank et al. (13) reported that 10-min exposures of a biofilm of L. monocytogenes on stainless steel to a combination of alkali cleaning and ASC were effective at achieving a ca. 6.0-log reduction of the pathogen. Previous studies have also evaluated higher levels (500 to 1,200 ppm) of ASC than those used in the present study (150 ppm). Regardless, in the present study, ASC did not have an appreciable effect on inhibiting the outgrowth of L. monocytogenes, presumably because far less ASC was applied than what is recommended or typical. Further work is warranted to determine if higher levels and different points of delivery or different delivery methods would yield a greater antilisterial response with ASC or other food-grade chemicals.

We initiated this study in response to the voluntary recall of some 30 million lb of poultry products by two processors in the northeastern portion of the United States due to contamination with L. monocytogenes (11). It is critical to optimize and implement interventions to eliminate any L. monocytogenes cells that may become associated with CIBTB after cooking, but before final packaging, and then to preclude any residual cells from growing to potentially dangerous levels during refrigerated storage. In addition to demonstrating the effectiveness of hot water postprocess pasteurization of CIBTB for achieving “alternative 2” status, the optimization of food-grade chemicals for preventing the outgrowth of L. monocytogenes may allow manufacturers of such products to reformulate and achieve “alternative 1” status. This may also be significant if regulatory agencies soften the current “zero-tolerance” policy and allow a certain level, e.g., 100 CFU per g, of L. monocytogenes in products that do not support its growth (2). Our findings establish that hot water postprocess pasteurization is effective in reducing initial levels of L. monocytogenes by ca. 2.0 log CFU on CIBTB and that K-lactate–Na-diacetate added as an ingredient and ASC applied to the surface of CIBTB are not effective at controlling the subsequent outgrowth of L. monocytogenes during refrigerated storage. Studies are ongoing to investigate in more detail the optimal time and temperature parameters required to enhance the thermal inactivation of L. monocytogenes in CIBTB, as well as to identify effective food-grade chemicals and delivery methods that act as antimicrobials, to prevent the outgrowth of this pathogen in RTE meat and poultry products.

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