Research Note

Effect of Age of Cook-in-Bag Delicatessen Meats Formulated with Lactate-Diacetate on the Behavior of *Listeria monocytogenes* Contamination Introduced When Opening the Packages during Storage

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ABSTRACT

This study evaluated the potential effect of age of cook-in-bag ham and turkey breast delicatessen meats formulated with lactate-diacetate on survival and/or growth of *Listeria monocytogenes* introduced after opening of packages and slicing of product. Commercially prepared cured ham and turkey breast products formulated with potassium lactate and sodium diacetate were stored at 1.7°C unsliced, in their original cook-in-bags, and without postlethality exposure. On days 5, 90, 120, and 180 of storage, product slices (10.2 by 7.6 cm) were surface inoculated (1 to 2 log CFU/cm²) with a 10-strain mixture of *L. monocytogenes*, vacuum packaged (seven slices per bag), and stored at 4°C for up to 13 weeks. Inoculated levels of *L. monocytogenes* on both products were 1.4 to 1.5 log CFU/cm². Irrespective of product age at slicing and inoculation, after 13 weeks of vacuum-packaged storage (4°C), pathogen counts on product slices were 1.5 to 2.3 (ham) and 2.3 to 2.5 (turkey) log CFU/cm². Overall, the results of the study showed that the age of the cook-in-bag products prior to slicing and inoculation with the pathogen did not (P ≥ 0.05) affect the behavior of *L. monocytogenes* during vacuum-packaged storage (4°C, up to 13 weeks) of ham and turkey slices. Mean counts of lactic acid bacteria and yeasts and molds, when detected, did not exceed approximately 1 and 2 log CFU/cm², respectively, among all stored samples. Findings of the study will be useful to the meat industry and risk assessors in their efforts to control *L. monocytogenes* in ready-to-eat meat products.

Contamination of delicatessen meats with *Listeria monocytogenes* during processes that involve handling of the product after the lethality (cooking) step remains a concern due to the ready-to-eat (RTE) nature of these products and the potentially serious clinical outcomes associated with infection. Factors contributing to the difficulty in controlling the pathogen in deli meats (e.g., ham, turkey breast, bologna, and roast beef) and other RTE meat and poultry products include its ability to persist in refrigerated storage of various RTE meat products, especially when these antimicrobials are used in combination (1, 2, 4, 5, 10, 12, 13, 15). In the majority of published studies, antilisterial properties of lactate-diacetate combinations were evaluated by inoculating freshly prepared products with the pathogen followed by vacuum packaging, refrigerated storage, and periodic analysis of samples during storage to determine survival and/or growth of *L. monocytogenes*. These studies, therefore, simulated introduction of the pathogen during postlethality exposure of fresh product to the processing facility environment (e.g., during slicing and packaging). Furthermore, the refrigerated vacuum-packaged storage period represented the temperature-time conditions that the product would be exposed to during ideal or temperature abuse conditions during distribution and retail storage. Processors of cook-in-bag RTE deli meat products may, however, not immediately expose product to

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the environment of the establishment after the lethality treatment. Depending on potential factors such as processing facility scheduling or a change in demand of a specific product, freshly prepared cook-in-bag deli meat chubs may be stored under refrigeration conditions for a period of time before being exposed to a postlethality environment by slicing and packaging. Therefore, in such instances, there is potential for *L. monocytogenes* contamination of product of different ages and associated physicochemical and microbiological characteristics. As such, there is interest by the industry to determine whether the antilisterial effects of lactate-diacetate are influenced by the age of the product at contamination. Therefore, the objective of this study was to evaluate the potential effect of age of cook-in-bag delicatesen meats (ham and turkey breast) formulated with lactate-diacetate on survival and/or growth of *L. monocytogenes* introduced after opening of packages and slicing of product.

**MATERIALS AND METHODS**

**Bacterial strains.** The inoculum comprised 10 *L. monocytogenes* strains: NA-1 (serotype 3b, pork sausage isolate), 558 (serotype 1/2, pork meat isolate), N-7150 (serotype 3a, meat isolate), N1-225 and N1-227 (serotype 4b, clinical and food isolates, respectively, associated with the same 1998 through 1999 outbreak linked to RTE meat products), R2-500 and R2-501 (serotype 4b, food and clinical isolates, respectively, associated with the same 2000 outbreak linked to cheese), and R2-763, R2-764, and R2-765 (serotype 4b, clinical, food, and environmental isolates, respectively, associated with the same 2002 outbreak linked to sliced deli meats). All N1 and R2 strains were kindly provided by Dr. Martin Wiedmann (Department of Food Science, Cornell University, Ithaca, NY) (3).

**Inoculum preparation.** The inoculum preparation procedure involved initially culturing the *L. monocytogenes* strains in a broth culture medium followed by habituation (4°C, 70 to 72 h) in deli meat (ham or turkey breast) homogenates to acclimate the cells to a low-temperature food environment prior to product inoculation (7, 8, 11, 14). More specifically, the strains were individually cultured and subcultured (30°C, 22 h) in 10 ml of tryptic soy broth (Difco, BD, Sparks, MD) supplemented with yeast extract (0.6%; Acumedia, Lansing, MI). Cell cultures were then individually harvested by centrifugation and cells were washed with phosphate-buffered saline (pH 7.4) as previously described (2). Washed cell pellets of each strain were then resuspended in 10 ml of sterile ham or turkey deli meat homogenate and held at 4°C for 70 to 72 h. The homogenates were prepared by pummeling (2 min) (Masticator, IUL Instruments, Barcelona, Spain) commercially available cured ham or turkey breast (formulated without antimicrobials) with distilled water to yield 10% (wt/wt) product suspensions. The suspensions were passed through cheesecloth, and the liquid portions were autoclaved and cooled (4°C) before use. Two sets of the 10 *L. monocytogenes* strains were habituated: one set in ham homogenate (served as the inoculum for ham slices) and the second in turkey homogenate (served as the inoculum for turkey slices). After the habituation period (i.e., on the day of product inoculation), the 10 strains of each set were combined and serially diluted in freshly prepared ham or turkey homogenates to approximately 4 log CFU/ml.

**Inoculation of deli meat slices.** Cured ham and cured turkey breast products formulated with a commercial blend of potassium lactate and sodium diacetate (PURASAL Opti.Form PD Plus, Purac, Lincolnshire, IL) were commercially manufactured (Bar-S Foods Co., Clinton, OK). The concentrations of potassium lactate and sodium diacetate were 2.07 and 0.148%, respectively, in the ham product and 2.24 and 0.16%, respectively, in the turkey product. The cook-in-bag products were stored unsliced without postlethality exposure at 1.7°C (35°F). On days 4, 89, 119, and 179 of storage, products were sliced (10.2 by 15.2 cm [4 by 6 in.]) by the manufacturer, vacuum packaged, and shipped overnight to Colorado State University (Fort Collins) for inoculation with *L. monocytogenes* on the day of arrival; therefore, the actual product ages at inoculation were 5, 90, 120, and 180 days.

**TABLE 1.** Populations of *L. monocytogenes* on vacuum-packaged ham slices stored at 4°C for up to 13 weeks

<table>
<thead>
<tr>
<th>Product age at inoculation (days)</th>
<th>0</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>11</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.5 ± 0.1 a A</td>
<td>1.3 ± 0.1 a A</td>
<td>1.3 ± 0.1 a A</td>
<td>1.4 ± 0.1 a A</td>
<td>1.7 ± 0.5 a A</td>
<td>1.5 ± 0.3 a A</td>
</tr>
<tr>
<td>90</td>
<td>1.5 ± 0.0 a A</td>
<td>1.5 ± 0.1 a A</td>
<td>1.4 ± 0.1 a A</td>
<td>1.5 ± 0.1 a A</td>
<td>1.6 ± 0.3 a A</td>
<td>1.6 ± 0.2 a A</td>
</tr>
<tr>
<td>120</td>
<td>1.5 ± 0.1 a A</td>
<td>1.4 ± 0.1 a A</td>
<td>1.4 ± 0.1 a A</td>
<td>1.4 ± 0.1 a A</td>
<td>1.8 ± 0.5 a A</td>
<td>2.2 ± 1.2 a A</td>
</tr>
<tr>
<td>180</td>
<td>1.5 ± 0.1 a A</td>
<td>1.3 ± 0.1 a A</td>
<td>1.4 ± 0.1 a A</td>
<td>1.7 ± 0.5 a A</td>
<td>2.0 ± 0.6 a AB</td>
<td>2.3 ± 1.0 a A</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations (n = 6). Within a column, means with a common lowercase letter are not different (P ≥ 0.05). Within a row, means with a common uppercase letter are not different (P ≥ 0.05).

**TABLE 2.** Aerobic microbial populations on vacuum-packaged ham slices stored at 4°C for up to 13 weeks

<table>
<thead>
<tr>
<th>Product age at inoculation (days)</th>
<th>0</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>11</th>
<th>13</th>
</tr>
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<tbody>
<tr>
<td>5</td>
<td>1.5 ± 0.1 a A</td>
<td>1.4 ± 0.0 a A</td>
<td>1.3 ± 0.1 a A</td>
<td>1.4 ± 0.1 a A</td>
<td>1.7 ± 0.5 a A</td>
<td>1.5 ± 0.3 a A</td>
</tr>
<tr>
<td>90</td>
<td>1.5 ± 0.1 a A</td>
<td>2.4 ± 1.1 a A</td>
<td>2.4 ± 1.1 a A</td>
<td>2.5 ± 1.2 a A</td>
<td>2.8 ± 1.4 a A</td>
<td>2.8 ± 1.4 a A</td>
</tr>
<tr>
<td>120</td>
<td>1.5 ± 0.1 a A</td>
<td>1.4 ± 0.2 a A</td>
<td>1.6 ± 0.2 a A</td>
<td>1.5 ± 0.4 a A</td>
<td>2.2 ± 0.5 a A</td>
<td>2.2 ± 1.1 a A</td>
</tr>
<tr>
<td>180</td>
<td>1.6 ± 0.2 a A</td>
<td>2.2 ± 0.5 a A</td>
<td>2.2 ± 0.7 a A</td>
<td>2.3 ± 0.5 a A</td>
<td>2.5 ± 0.4 a A</td>
<td>2.9 ± 0.5 a A</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations (n = 6). Within a column, means with a common lowercase letter are not different (P ≥ 0.05). Within a row, means with a common uppercase letter are not different (P ≥ 0.05).
TABLE 3. Populations of *L. monocytogenes* on vacuum-packed turkey breast slices stored at 4°C for up to 13 weeks

| Product age at inoculation (days) | *L. monocytogenes* (log CFU/cm²) at storage week
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.4 ± 0.1 a A 1.5 ± 0.5 a A 1.7 ± 0.7 a A 1.9 ± 0.7 a A 2.3 ± 1.2 a A 2.4 ± 1.1 a A</td>
</tr>
<tr>
<td>90</td>
<td>1.5 ± 0.1 a A 1.9 ± 0.4 a A 2.1 ± 0.6 a A 2.2 ± 0.5 a A 2.6 ± 0.8 a A 2.4 ± 0.8 a A</td>
</tr>
<tr>
<td>120</td>
<td>1.4 ± 0.1 a A 1.5 ± 0.1 a A 1.7 ± 0.3 a A 2.0 ± 0.4 a A 2.3 ± 0.5 a A 2.5 ± 0.6 a A</td>
</tr>
<tr>
<td>180</td>
<td>1.4 ± 0.1 a A 1.6 ± 0.2 a A 1.8 ± 0.3 a A 1.9 ± 0.4 a A 2.0 ± 0.2 a A 2.3 ± 0.4 a A</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations (n = 6). Within a column, means with a common lowercase letter are not different (P ≥ 0.05).

Microbiological and pH analyses. Samples were microbiologically analyzed on the day of inoculation (week 0) and after 4, 6, 8, 11, and 13 weeks of vacuum-packaged storage. The seven slices per vacuum pouch were aseptically transferred to a Whirl-Pak bag (17.8 by 33.0 cm; Nasco, Modesto, CA) containing 400 ml of diluent (0.85% NaCl and 0.1% peptone). An aliquot (25 ml) of the diluent was pipetted out of the Whirl-Pak bag containing the product slices and used to rinse the inside of the vacuum pouch that had previously contained the slices. The rinseate was then transferred back into the sample bag containing the deli meat slices. Samples were vertically shaken 30 times within approximately 30 s to detach cells from the slices. Tenfold serial dilutions were prepared on 0.1% buffered peptone water (Difco, BD), and aliquots of appropriate dilutions were surface plated in duplicate onto PALCAM agar (Difco, BD) for enumeration of *L. monocytogenes*, onto tryptic soy agar (Difco, BD) supplemented with 0.6% yeast extract (TSAYE) for enumeration of aerobic microbial populations, and onto rose bengal chloramphenicol (RBC) agar (Difco, BD) for enumeration of yeasts and molds. Lactic acid bacteria counts were also determined by pour plating 1-ml aliquots of appropriate dilutions in 10 ml of molten (45°C) lactobacilli de Man Rogosa Sharpe (MRS) agar (Difco, BD) (acidified to pH 5.5 with 5 N HCl). After the MRS agar had set, a 10-ml overlay of molten medium was added to each plate. PALCAM agar plates were incubated at 30°C (48 h), and TSAYE (72 h), MRS agar (5 days), and RBC agar (7 days) plates were incubated at 25°C before counting of colonies. The detection limit of the microbiological analysis was 0.4 CFU/cm².

After microbial analysis, samples were homogenized for 2 min (Masticator, IUL Instruments), and the pH of the homogenates was measured with a pH meter fitted with a glass electrode (Denver Instruments, Arvada, CO).

Statistical analysis. The study was performed twice using different production lots (manufactured on two separate days) of ham and turkey breast. For each replicate, three samples per product were analyzed on each analysis day. Microbial counts were converted to log CFU per square centimeter before statistical analysis. Data (microbial counts and pH) were analyzed as a randomized complete block design using the GLIMMIX procedure of SAS (version 9.2., SAS Institute, Cary, NC), considering replicate as a block. Independent variables included product age before slicing and inoculation (5, 90, 120, and 180 days), storage time of sliced inoculated products (0, 4, 6, 8, 11, and 13 weeks), and their interaction. Means were separated using Tukey’s honestly significant difference test, and means were considered significantly different when *P* values were less than 0.05.

RESULTS AND DISCUSSION

The effectiveness of sodium or potassium lactate and sodium diacetate as growth inhibitors of *L. monocytogenes* on RTE meat products regarded as high risk has been established (1, 2, 4, 5, 10, 12, 13, 15); therefore, this issue was not the objective of this study. There is limited information, however, on the potential impact of RTE meat product age on pathogen survival and/or growth. Possible differences in levels of natural microflora and product characteristics associated with freshly prepared products versus those products stored for a period of time may influence the behavior of the pathogen. Lianou et al. (7, 8) and Zhang et al. (19) inoculated *L. monocytogenes* onto deli meat slices derived from product chubs of different ages and

TABLE 4. Aerobic microbial populations on vacuum-packaged turkey breast slices stored at 4°C for up to 13 weeks

| Product age at inoculation (days) | Aerobic bacteria (log CFU/cm²) at storage week
<table>
<thead>
<tr>
<th></th>
<th></th>
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<tbody>
<tr>
<td>5</td>
<td>1.5 ± 0.1 a A 1.6 ± 0.4 a A 1.8 ± 0.6 a A 2.0 ± 0.7 a A 2.3 ± 1.1 a A 2.4 ± 1.1 a A</td>
</tr>
<tr>
<td>90</td>
<td>1.7 ± 0.1 a A 2.7 ± 1.2 a A 2.6 ± 1.2 a A 2.9 ± 1.3 a A 3.1 ± 1.4 a A 3.0 ± 1.4 a A</td>
</tr>
<tr>
<td>120</td>
<td>1.4 ± 0.1 a A 1.5 ± 0.1 a A 2.2 ± 0.9 a A 2.8 ± 0.9 a A 2.5 ± 0.7 a A 2.9 ± 1.0 a A</td>
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<td>180</td>
<td>1.6 ± 0.2 a A 1.8 ± 0.3 a A 1.9 ± 0.4 a A 2.6 ± 1.0 a A 2.5 ± 0.5 a A 2.7 ± 0.8 a A</td>
</tr>
</tbody>
</table>

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examined the fate of the pathogen during aerobic storage; therefore, these investigators simulated contamination scenarios that could occur during slicing and/or handling of deli meats at retail or at home, followed by consumer storage of the contaminated product. In the present study we examined the behavior of *L. monocytogenes* potentially introduced during slicing and handling of product that had been held at the processing facility for up to 180 days postproduction. Inoculated slices were then vacuum packaged and periodically analyzed for microbial counts during simulated retail storage (4°C for up to 13 weeks).

Initial *L. monocytogenes* levels and aerobic microbial populations on inoculated ham slices were 1.4 to 1.5 and 1.5 to 1.6 log CFU/cm², respectively, regardless of product age before slicing and inoculation (Tables 1 and 2). Initial *L. monocytogenes* levels and aerobic microbial populations on inoculated turkey slices were 1.4 to 1.5 and 1.4 to 1.7 log CFU/cm², respectively (Tables 3 and 4). For all tested product ages, *L. monocytogenes* counts on ham samples after 13 weeks of vacuum-packaged storage were either similar to (*P* ≥ 0.05) or not more than 0.9 log CFU/cm² higher than (*P* < 0.05) initial (week 0) counts (Table 1). Pathogen counts on turkey slices of all product ages increased by 0.9 to 1.1 log CFU/cm² by the end of storage; however, these increases were not significant (*P* ≥ 0.05) (Table 3). These findings are in agreement with those from previous studies (1, 5, 7, 8, 15), i.e., inclusion of lactic-diacetate in the formulation of deli meats effectively suppresses the growth of *L. monocytogenes* during refrigerated storage.

Overall, the results of the study indicated that the behavior of *L. monocytogenes* on ham and turkey slices was not influenced (*P* ≥ 0.05) by the age of the products at inoculation. More specifically, statistical comparison of the data revealed that the interaction of product age before slicing and inoculation (5, 90, 120, and 180 days) and storage time of sliced inoculated products (0, 4, 6, 8, 11, and 13 weeks) was not significant (*P* ≥ 0.05) for both deli meat types (data not shown). Therefore, the antilisterial effect of the potassium lactate and sodium diacetate combination added to the formulation of each deli meat product was similar (*P* ≥ 0.05) regardless of product age at the time of inoculation. In the study by Zhang et al. (19), product age for five brands each of cured and uncured turkey, ham, and roast beef (formulated with or without antimicrobials) was reported not to influence the growth of *L. monocytogenes* in aerobically stored samples. By contrast, Lianou et al. (8) found that the growth rates of the pathogen during aerobic storage of uncured turkey formulated without lactate-diacetate decreased (*P* < 0.05) with product age. Pathogen growth was slower on older product (deli meat chops sliced and inoculated after 25 and 50 days of storage) than on fresher product (chubs sliced and inoculated after 5 and 15 days of storage). The presence of higher levels of spoilage microorganisms associated with the older product was likely responsible for the slower pathogen growth rates on 25- and 50-day-old product (8). In the same study, however, product age did not affect the growth rates of *L. monocytogenes* on uncured turkey formulated with lactate-diacetate (8).

In the present study, aerobic plate counts after 13 weeks of storage were 1.5 to 2.9 log CFU/cm² for inoculated ham samples and 2.4 to 3.0 log CFU/cm² for inoculated turkey samples, irrespective of initial product age (Tables 2 and 4). In most cases, aerobic plate counts were similar to corresponding *L. monocytogenes* counts (Tables 1 through 4), indicating that the microbial populations recovered on TSAYE were predominantly those of the inoculated pathogen. When aerobic plate counts were higher than the pathogen counts, the difference in mean log values did not exceed 1.2 and 0.8 log CFU/cm² for ham and turkey samples, respectively. Therefore, low levels of spoilage microflora were associated with the analyzed samples. This finding is supported by low counts, when detected, of lactic acid bacteria and yeasts and molds associated with the products (data not shown). Mean lactic acid bacteria counts for stored slices ranged from nondetectable (–0.4 log CFU/cm²) for both products to not more than 0.3 ± 1.9 (ham) and 1.1 ± 1.9 (turkey) log CFU/cm². Colonies growing on RBC agar were almost exclusively yeasts, and counts ranged from nondetectable (–0.4 log CFU/cm²) to not more than 1.8 ± 2.5 (ham) and 1.9 ± 2.6 (turkey) log CFU/cm² (data not shown). As indicated by the high standard deviations, the presence of lactic acid bacteria and yeasts was highly variable among samples. The overall absence of significant levels of spoilage microflora associated with the stored ham and turkey slices is supported by the relatively unchanged (*P* ≥ 0.05) pH of samples throughout storage (data not shown). The pH of ham, irrespective of product age at inoculation, ranged from 6.28 to 6.33 on the day of inoculation and 6.24 to 6.34 after 13 weeks of vacuum-packaged storage. Similarly, initial and final pH values for turkey were 6.29 to 6.34 and 6.26 to 6.36, respectively.

In summary, under the conditions of this study, the age of the cook-in-bag ham and turkey deli meats did not affect the antilisterial activity of the potassium lactate and sodium diacetate combination added to the formulation of each product. These findings will be useful to the meat industry in their efforts to control *L. monocytogenes* in high-risk RTE meat and poultry products, and the data generated may be used to supplement existing information in future risk assessments.

**ACKNOWLEDGMENTS**

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**REFERENCES**


monocytogenes strain sets for research and validation studies. J. Food Prot. 69:2929–2938.