Effect of Reheating on Viability of a Five-Strain Mixture of *Listeria monocytogenes* in Vacuum-Sealed Packages of Frankfurters following Refrigerated or Frozen Storage†‡

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ABSTRACT

The purpose of this study was to assess consumer preferences for storing and reheating frankfurters and to use this information to assess the effect of product formulation and storage times and temperatures on the viability of *Listeria monocytogenes* after reheating of frankfurters. Individual links were inoculated with about 8.0 log CFU per package of a five-strain mixture of the pathogen, vacuum sealed, and stored at 4–8°C for 3 and 15 days and at –18°C for 30 days. Frankfurters formulated with and without 2% added potassium lactate were heated to a surface temperature of 60, 70, 80, or 90°C for up to 8 min by submersing the packages in a thermostatically controlled circulating water bath. Surviving bacteria were recovered and counted by rinsing the contents of each package with sterile phosphate water and plating this solution directly onto modified Oxford selective agar plates. In general, the results revealed that about a 5-log unit reduction was achieved by reheating to a surface temperature of 70°C for about 2 min or 80 or 90°C for about 0.6 min regardless of storage conditions or formulation. Product formulation did not appreciably affect the viability of the pathogen after heating; there was no appreciable difference in the number of cells surviving the heat treatment in product prepared with or without potassium lactate. These findings can be used to establish reheating guidelines for consumers to ensure that frankfurters, which may become contaminated with low levels of *L. monocytogenes* prior to packaging and after unpackaging, are adequately reheated prior to consumption.

Listeria monocytogenes is an established foodborne pathogen of considerable public health concern worldwide (22). It is able to grow at refrigeration temperatures and is one of the most thermotolerant, non–spore-forming pathogens known (9). The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) identified *L. monocytogenes* as responsible for about 50% (31 of 62) of recalls of cooked meat in 1999 and identified ready-to-eat (RTE) meats as a primary vehicle for listeriosis (www.fsis.usda.gov/OA/recalls/rec_intr.htm). Although adequate cooking during manufacture eliminates any *L. monocytogenes* that may be naturally present in raw frankfurter emulsions (28), the bacterium may be found on occasion in the finished product because of a failure in the heating process or postprocess contamination (12, 24, 27). Postprocess contamination also may occur in the consumer’s refrigerator, even on foods that may not be reheated before consumption (23). Because about 7 billion frankfurters are consumed during the summer months and about 20 billion frankfurters are consumed per year in the United States (www.PreparedFoods.com, August 1999) and because the bacterium has been associated with disease cases from consumption of contaminated frankfurters, there are numerous investigations underway to develop strategies to decrease the risk of listeriosis associated with such products.

The addition of antimicrobial compounds to the frankfurter formulation and low-temperature storage are effective in slowing *L. monocytogenes* growth (1, 15, 16); however, neither of these barriers alone are effective for eliminating the pathogen from the product in the event of postprocess contamination. One of the most common and effective methods for reducing the levels of *L. monocytogenes* in foods is the application of heat. Although frankfurters are considered an RTE food, a survey of consumers conducted by the American Meat Institute Foundation revealed that only 72% of 1,000 individuals surveyed reheated frankfurters before eating them (www.meatami.com). However, 15% of these 1,000 people admitted eating frankfurters directly from the package and 11% admitted that someone in their household ate frankfurters without reheating them. During the multistate outbreak of listeriosis involving frankfurters in 1998, 89% of the cases (16 of 18) and 32% of controls (6 of 19) stated that they ate reheated product during the month prior to the onset of illness (3). Assuming that 1 to 14% of frankfurters are not reheated before consumption, frankfurters rated a high predicted relative risk of causing listeriosis based on total overall consumption in the recent risk assessment conducted by the U.S. Department of Health and Human Services/Food and Drug Administration/Center for Food Safety and Applied Nutrition.
in collaboration with the USDA/FSIS in consultation with the Centers for Disease Control and Prevention, hereinafter referred to as the L. monocytogenes risk assessment for RTE foods (www.foodsafety.gov/~dms/lmrisk.html).

There have been relatively few controlled studies on the effects of product formulation or storage time and temperature on the subsequent reheating times and temperatures on the survival of L. monocytogenes on frankfurters. Such data may prove useful for establishing reheating guidelines for consumers to ensure the microbiological safety of frankfurters at the point of consumption. One objective of the present study was to assess consumer preferences for storing and reheating frankfurters. Another objective was to use this information to investigate the effect of heating times and temperatures on the viability of a five-strain mixture of L. monocytogenes in vacuum-sealed packages of commercially prepared frankfurters, formulated with and without added potassium lactate, following storage at refrigeration and freezing temperatures.

MATERIALS AND METHODS

Bacterial strains. As previously described (15), stationary-phase cells of a five-strain mixture of L. monocytogenes (Scott A [serotype 4b, clinical isolate], LM-101M [serotype 4b, beef and pork sausage isolate], H7776 [serotype 4b, food isolate], F6854 [serotype 1/2a, turkey frankfurter isolate], and MFS-2 [serotype 1/2a, environmental isolate from a pork processing plant]) were used to inoculate packages of frankfurters that were then vacuum sealed. Stock cultures were grown in brain heart infusion (Difco Laboratories, Detroit, Mich.) broth plus 20% (wt/vol) glycerol and frozen in 1.5-ml portions in cryovials held at −80°C (15).

Inoculation and vacuum packaging of frankfurters. Freshly processed and peeled frankfurters (ca. 56 g per link) in vacuum-sealed bulk packages were obtained from a commercial processor. Two different formulations of pork/beef frankfurters were tested: one formulation contained potassium lactate added to the batter at a final concentration of ca. 2.0%, and the other formulation did not contain any added potassium lactate. Individual frankfurters were transferred aseptically from the original bulk package and repackaged into separate nylon/polyethylene bags (3 mil standard barrier; 20.3 by 30.5 cm; O2 < 0.6 ml/100 in2/h at 0°C [32°F]) relative humidity with moisture vapor transmission rate of 0.6 g H2O/100 in2/h at 38°C [100°F]; Koch Industries, Kansas City, Mo.). Each package was inoculated with a 1-ml portion of the five-strain mixture of L. monocytogenes, and with without prior dilution in 0.1% peptone water, to achieve a target level of 8.0 log CFU per package that was counted manually and their identity confirmed using approved USDA/FSIS procedures (11). Bacterial numbers were expressed as log CFU per package. In addition to direct plating, surviving bacteria were recovered by enrichment by adding 10 ml of the rinsate or dilutions thereof onto modified Oxford agar plates using an Autoplate 4000 spiral plater (Spiral Biotech, Gaithersburg, Md.). The plates were incubated at 37°C for 48 h, and the colonies typical of Listeria were counted manually and their identity confirmed using approved USDA/FSIS procedures (4). Bacterial numbers were expressed as log CFU per package. In addition to direct plating, surviving bacteria were recovered by enrichment by adding 10 ml of the rinsate to 90 ml of UVM broth for processing according to the standard USDA/FSIS protocol (4).

Thermal treatment. After removal from storage, each package was opened aseptically, and a thermocouple (type J, Omega Engineering Inc., Stamford, Conn.) was embedded just under the surface (ca. 1 to 2 mm) of each end of the frankfurter. The packages were again heat sealed to 100 kPa as described above. Packages stored at −18°C were thawed overnight at 4°C before testing. Three bags were tested at each sampling interval by complete submersion in a thermostatically controlled water bath (Exacal-251HT, Neslab Instruments Inc., Newington, N.H.) and heated to a surface temperature of 60°C (140°F) for 2, 4, 6, and 8 min, 70°C (158°F) for 0.75, 1.5, 2, and 3 min, or 80°C (176°F) or 90°C (194°F) for 0.25, 0.5, 0.75, and 1 min. Bags were moved backward and forward by hand in the water bath during the first 30 s to ensure a more rapid come-up time and more homogeneous temperature distribution. The temperature of the samples was monitored and recorded using a data logger software system (National Instruments, Austin, Tex.). At the appropriate intervals, the samples were removed from the heated water bath, cooled immediately in an ice-water bath, and then stored at 4°C for up to 3 h until analyzed.

Microbiological analyses. Packages were sampled using the USDA/Agricultural Research Service package rinse method (11), which is about six times more effective at recovering L. monocytogenes from frankfurter packages than the approved USDA/FSIS product composite enrichment method because it concomitantly samples the purge, product surface, and packaging materials. The outside of each package was disinfected with 70% (vol/vol) ethanol, air dried, and then aseptically opened using alcohol-sterilized scissors. Nineteen milliliters of sterile 0.1% peptone water was added, and the packages were massaged by hand for about 2 min. The resulting fluid, about 20 ml, was transferred by pipette to a 50-ml conical centrifuge tube. The bacteria were counted by spread plating 250 μl of the rinsate or dilutions thereof onto modified Oxford agar plates using an Autoplate 4000 spiral plater (Spiral Biotech, Gaithersburg, Md.). The plates were incubated at 37°C for 48 h, and then colonies typical of Listeria were counted manually and their identity confirmed using approved USDA/FSIS procedures (4). Bacterial numbers were expressed as log CFU per package. In addition to direct plating, surviving bacteria were recovered by enrichment by adding 10 ml of the rinsate to 90 ml of UVM broth for processing according to the standard USDA/FSIS protocol (4).

Statistical analyses. Data were analyzed using version 8.0 of the SAS Statistical Program (SAS Institute, Cary, N.C.). Each replicate experiment included samples reheated at 60, 70, 80, or 90°C. The survivor curve was obtained by plotting recovered log CFU per package versus reheating temperatures. Means and standard deviations of L. monocytogenes survivors at each of the four reheating temperatures were determined from the average of three replicate experiments.

RESULTS

Survey of consumers about storing and preparing frankfurters. An informal, nonrandomized survey was prepared to gain insight into consumer preferences for storing and reheating frankfurters. A total of 182 respondents answered at least one of the nine questions asked in this survey (Table 1). Many of the survey questions were multiple-choice; thus, the number of responses for each varied depending on the question. Although all of the answers were interesting and potentially useful, for the purposes of this study, the most useful responses were (i) only 47 of...
TABLE 1. Informal consumer survey on preparation and storage practices of frankfurters in the home

<table>
<thead>
<tr>
<th>Question</th>
<th>No. of respondents</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Do you consider frankfurters as ready to eat or as requiring cooking?</td>
<td>162</td>
<td>Require cooking: 115 (71%); ready to eat: 47 (29%); significant difference</td>
</tr>
<tr>
<td>2. How often do you eat frankfurters?</td>
<td>181</td>
<td>Monthly: 97 (53.6%); weekly: 50 (27.6%); daily: 4 (2.2%); small numbers in other categories</td>
</tr>
<tr>
<td>3. How many frankfurters do you eat per sitting?</td>
<td>174</td>
<td>1 or 2: 160 (92%); 3 or 4: 14 (8%); &gt;4: 0</td>
</tr>
<tr>
<td>4. What type of frankfurters do you eat?</td>
<td>174</td>
<td>Beef: 61 (30%); pork: 30 (15%); beef and pork: 83 (41%); turkey: 10 (5%); chicken: 6 (3%); other: small percentages</td>
</tr>
<tr>
<td>5. Do you look at the label on the packages?</td>
<td>106</td>
<td>Looked at the label: 106 (61%); did not look at label: 68 (39%); significant difference</td>
</tr>
</tbody>
</table>
| If so, what do you look for? | 106 | Fat content: 22 (21%); ingredients: 15 (14%); meat source: 15 (14%); brand: 14 (12.7%); salt content: 12 (11.5%); expiration date: 10 (9.7%); other: (<3%)
| 6. How do you store your frankfurters? | 238 | Refrigerator: 127 (53%); freezer: 111 (47%)
| 7. How long do you store your frankfurters? | 238 | Refrigerator: 127 (53%); freezer: 111 (47%)
| Refrigerator | 108 | 1 to 2 weeks: 40 (37%); 3 to 7 days: 37 (34%); >45 days: 42 (33%); 30 to 45 days: 30 (24%); 21 to 30 days: 27 (21%); 15 to 20 days: 14 (11%); 7 to 14 days: 13 (10%)
| Freezer | 126 | Thawed: 63 (43%); did not thaw: 87 (57%)
| 8. In case of freezing, do you thaw before cooking? | 150 | Refrigerator: 26 (41%); microwave: 23 (37%); sink: 10 (16%); Heating: 175 (97.2%); no heating: 5 (2.8%); significant difference |
| If thawed, what method? | 63 | Grill: 85 (30.6%); microwave: 53 (19%); add to water and then boil: 47 (17%); boil water then add frankfurters: 46 (16%); fry: 37 (13%)

162 (29%) individuals considered frankfurters an RTE food, (ii) individuals were just about as likely to refrigerate (47%, 111 of 238 responses) as they were to freeze (53%, 127 of 238 responses) their frankfurters in the home, and (iii) more individuals preferred boiling (33%, 93 of 278) to grilling (31%, 85 of 278), microwaving (19%, 53 of 278), and frying (13%, 37 of 278). Based on these responses, experiments were designed to store vacuum-sealed packages of frankfurters that were contaminated with L. monocytogenes at refrigeration or freezing conditions and then to reheat these frankfurters at near-boiling temperatures.

Effect of freezing or refrigeration on survival of L. monocytogenes in vacuum-sealed packages of frankfurters. The viability of L. monocytogenes was determined after storage for 3 or 15 days at 4°C or after storage for 30 days at −18°C. In general, pathogen numbers remained relatively constant after 1 month of frozen storage regardless of the formulation. At 4°C, bacterial numbers remained relatively unchanged in product formulated with potassium lactate, whereas numbers increased slightly, to about 0.58 log CFU per package, in the absence of potassium lactate. Non-inoculated control packages were negative for the pathogen by direct plating (<20 CFU per package) and by enrichment.

Effect of reheating on survival of L. monocytogenes in vacuum-sealed packages of frankfurters previously stored at 4 or −18°C. At select intervals following frozen or refrigerated storage, vacuum-sealed packages containing the pathogen were completely submerged in a thermostatically controlled water bath maintained at 60, 70, 80, or 90°C for up to 8 min. The level of L. monocytogenes survivors for each time interval at each of the four reheating temperatures is presented in Figure 1. The data are the average number of survivors recovered for each of the two formulations and three storage conditions at each of the four reheating temperatures. Reheating to a surface temperature of 70°C for at least 2 min or to 80 or 90°C for at least 0.6 min is sufficient to achieve about a 5-log unit reduction of the pathogen on frankfurters formulated with and without potassium lactate and stored at either 4 or −18°C. These data also show quite clearly that pathogen numbers decreased as the temperature increased regardless of frankfurter formulation or prior storage temperature.

DISCUSSION

Infections with L. monocytogenes result in higher rates of hospitalization than do infections from any other foodborne pathogen and cause nearly half of all deaths reported from foodborne infections (5, 22). Despite extensive prevention efforts by the food industry and a zero-tolerance regulatory posture for RTE meats by government agencies, more than 500,000 lb (227 × 10³ kg) of frankfurters and luncheon meats were recalled in 1999 alone because of possible contamination with L. monocytogenes (www.fsis.usda.gov/OA/recalls/rec-intr.htm). The addition of certain
FIGURE 1. Thermal inactivation of a five-strain mixture of *Listeria monocytogenes* in vacuum-sealed packages of frankfurters. The data are the composite of three trials of the number of survivors recovered following heating at 60, 70, 80, and 90°C for frankfurters prepared with and without 2% potassium lactate and stored at 4 and -18°C. Error bars represent ± 1 standard error.

antimicrobial compounds (1, 15, 16, 20) to RTE meat formulations can inhibit growth of this pathogen during extended refrigerated storage. The addition of preservatives or other compounds to processed meats may affect the thermal resistance of *L. monocytogenes* (6). However, our data revealed that the thermal inactivation observed for a five-strain mixture of this pathogen in frankfurters formulated with potassium lactate was not significantly different (P > 0.05) from the inactivation observed in frankfurters formulated without added potassium lactate. Thus, the addition of 2% potassium lactate did not inhibit *L. monocytogenes* on frankfurters during reheating. Yen et al. (25) did not observe any protective effect of sodium nitrite or sodium erythrobate against *L. monocytogenes* in ground pork but did observe a protective effect of sodium chloride, dextrose, and a phosphate mixture. Samelis et al. (19) observed that postprocess pasteurization did not inhibit the pathogen but did extend the recovery time and retard the growth of heat-treated *L. monocytogenes* in the presence of the lowest sodium lactate concentration tested, which was 1.8%. Contrasting results such as these suggest that the only reliable and quantitative way to establish the heat resistance or viability of a pathogen in a product is to conduct a challenge or inoculated package study (14).

Naturally contaminated frankfurters probably harbor <1,000 CFU/g (7); thus, a 5-log unit reduction as a consequence of reheating would deliver an adequate margin of safety. Our results established that a 5-log unit reduction could be achieved by heating to a surface temperature of 70, 80, or 90°C for as little as 120, 36, and, 36 s, respectively. These data are in general agreement with thermal inactivation data previously reported for *L. monocytogenes* by other investigators. For example, Boyle et al. (2) reported a 2.1- to 2.3-log unit and a 4.6- to 5.5-log unit reduction in *L. monocytogenes* numbers in ground beef that was inoculated, stored for 48 h at 4°C, and then heated to an internal temperature of 60°C for 8.4 min and 65°C for 10.6 min, respectively. Roering et al. (18) reported a 4.73-, 5.08-, and 6.26-log unit reduction in *L. monocytogenes* numbers in vacuum-sealed summer sausage heated after packaging at 77.6, 87.8, and 98.9°C for at least 4, 2, and 1.5 min, respectively. To address the question of whether or not the pathogen becomes embedded into the flesh of the sausage as a consequence of reheating, Roering et al. (18) also reported that there was little difference in counts of *L. monocytogenes* between the casing or skin and the liquid or rinsate following postprocess pasteurization of vacuum-sealed summer sausage. As another example, Yen et al. (25) reported a 7.1-, 5.01-, and 3.29-log unit reduction of listeriae after heating ground pork containing no additives, 2% sodium chloride, or cure salts, respectively, to an internal temperature of 60°C. In another study, Yen et al. (26) reported that heating ground pork, with and without 0.6% added (encapsulated) lactic acid–calcium lactate, to
an internal temperature of 62°C resulted in a 7.20- and 6.63-log unit reduction in *L. monocytogenes* numbers, respectively. Likewise, Harmayani et al. (9) reported that heating ground beef, with and without 3% added sodium lactate, at 65°C reduced initial numbers of *L. monocytogenes* by 4.41 and 4.24 log, respectively. Muriana et al. (13) evaluated the effect of postpackage pasteurization by submersion heating of a variety of RTE deli meats and reported a 2- to 4-log unit reduction in listeriae when the meat products were heated at 90.6 to 96.1°C for 2 to 10 min.

Differences in the heat resistance of *L. monocytogenes* observed in the present study compared with that in other studies may be explained by differences in the strains used or their physiological state and whether the inoculum was applied onto the frankfurter surface or distributed within the emulsion or comminuted within the product. Differences in thermal inactivation among the various studies could also be attributed to differences in the proximate composition of the products tested, in the come-up times needed for the product to reach the target temperatures, in prior exposure of the cells to heat or acid shock and other stresses, and in the microbiological media and incubation conditions used (6, 8, 10, 17, 18). The nature and number of links within packages may also influence the response of *L. monocytogenes* to reheating because of the existence of cold points. As illustrated in Figure 1, in a biological system there may be considerable variability among results of different trials and there may be spurious survivors within a population of cells even though the population in general has been significantly reduced.

Several researchers have determined that *L. monocytogenes* can survive and grow within vacuum-sealed packages of frankfurters during extended refrigeration or frozen storage. In the present study, we observed the viability of a five-strain mixture of the pathogen at 4 and −18°C for 15 and 30 days, respectively. These data are consistent with the results of Taormina and Beuchat (21), who found that pathogen numbers did not change significantly on frankfurters during storage at −20°C for 12 weeks. Although pathogen numbers do not increase during frozen storage, their numbers can increase dramatically upon subsequent thawing and refrigerated storage.

Our informal survey of consumers revealed, somewhat surprisingly, that most respondents considered frankfurters as requiring “cooking” prior to being eaten and that most consumers cooked their frankfurters by boiling them. Thus, it is important that a 5-log reduction in pathogen numbers can be achieved within about 36 s by reheating individually packaged links of frankfurters to near-boiling temperatures. Our findings revealed that product formulation, storage times and temperatures, and combinations thereof did not significantly influence the viability or thermal inactivation of *L. monocytogenes* on frankfurters at the reheating temperatures tested. These findings may be useful for establishing consumer guidelines for reheating frankfurters and, thus, for decreasing the risk of listeriosis.

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