Inactivation of *Listeria monocytogenes* during Reheating of Frankfurters with Hot Water before Consumption

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**ABSTRACT**

Hot water may be used to kill *Listeria monocytogenes* on frankfurters immediately before consumption. This study evaluated the effectiveness of different time and water temperature combinations in destroying *L. monocytogenes* on frankfurters formulated with or without potassium lactate and sodium diacetate (PL/SD). Frankfurters were inoculated (1–2 log CFU/cm²), vacuum-packaged and stored at 4°C (manufacturer/retail conditions). On days 18, 40 and 60, packages were opened, reclosed and stored at 7°C (household conditions). At 0, 7 and 14 days of simulated household storage, frankfurters were exposed to hot water (80 or 94°C) that was either maintained at constant temperature or removed from the heat source. The 80°C (60, 120 s) and 94°C (30, 60 s) treatments reduced pathogen counts on frankfurters with PL/SD to the detection limit (-0.4 log CFU/cm²) or below from initial levels of 0.6–0.9 log CFU/cm². For frankfurters without PL/SD, where pathogen numbers on the control reached 5.3 log CFU/cm², hot water treatments reduced counts by 0.3 (80°C, 30 s) to > 5.7 (94°C, 300 s) log CFU/cm². No survivors were detected in the heated water after any treatment. Findings of this study may be useful for the development of science-based recommendations for reheating of frankfurters by consumers in their homes.

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**INTRODUCTION**

*Listeria monocytogenes* is the causative agent of listeriosis, a disease that produces an estimated 2,500 cases in the United States every year (99% of them foodborne), with a hospitalization rate of 92% and a case fatality rate of 20% (11). It mostly affects susceptible individuals such as pregnant women and their fetuses, the elderly and the immunocompromised (12, 18). *L. monocytogenes* is a ubiquitous organism that can be found in different foods such as salads, cheeses and ready-to-eat (RTE) meat and poultry products (9, 18, 24). In the case of RTE meat and poultry products, cross-contamination and/or recontamination with *L. monocytogenes* can occur after the product has undergone the lethality (i.e., cooking) treatment (14, 19), for example, during slicing of deli meats or peeling of frankfurters (24, 25, 26). Frankfurters, among other RTE meat products, can support growth of the pathogen to high numbers and, according to the 2003 *L. monocytogenes* risk assessment (22), non-reheated frankfurters are considered high risk, both on a per-serving and per-annum basis. Therefore, without...
Further treatment before consumption, frankfurters contaminated with this pathogen represent a risk for consumers, especially to those with a compromised immune system.

The role of consumers in food safety is important, since they are responsible for the last treatments (i.e., cooking and/or reheating) of food products immediately before consumption (17). In a survey by Porto et al. (13), it was reported that 72% of the participants reheated frankfurters before eating, and 33% of these individuals preferred boiling over other methods (such as grilling, microwaving and freezing). However, most brands of frankfurters do not offer instructions on their labels about reheating. Only a few brands provide consumers with reheating directions, but no information is available on the effectiveness of such recommendations on the inactivation of *L. monocytogenes*. Appropriate reheating instructions for this type of product are especially important for the population groups at particularly high risk for foodborne listeriosis infection. This study evaluated the efficacy of combinations of time and water temperature for destruction of *L. monocytogenes* contamination on frankfurters formulated with or without potassium lactate and sodium diacetate, during storage under simulated manufacturer/retail and household conditions.

**MATERIALS AND METHODS**

**Preparation of frankfurters**

Frankfurter emulsions were formulated with or without 1.5% potassium lactate (PL, Purac Purasal® HiPure P, Lincolnshire, IL) and 0.1% sodium diacetate (SD, Niacet Corporation, Niagara Falls, NY) as antimicrobials. The meat mixture consisted of 40% beef (beef chuck, 76–78% lean) and 60% pork (pork shoulder, 70–72% lean). Water, as ice, and seasonings and salts (dextrose, sodium chloride, corn syrup solids, dry mustard, polyphosphate, sodium nitrite, sodium erythorbate, paprika, onion powder, garlic powder, coriander and white pepper) were added according to the formulation of Samelis et al. (15). After emulsification in a vacuum bowl chopper (RMF, Kansas City, MO) the batter was stuffed into cellulose casings, linked at approximately 9 cm lengths, cooked and cooled (4°C) overnight, as described by Byelashov et al. (5). Frankfurters (65 cm³) were then manually peeled and moved to the microbiology laboratory for inoculation, packaging, storage, treatment and testing.

**Preparation of inoculum and inoculation of frankfurters**

The inoculum consisted of a mixture of 10 *L. monocytogenes* strains, including 558 (serotype 1/2, pork meat isolate), NA-1 (serotype 3b, pork sausage isolate), N-7150 (serotype 3a, meat isolate), N1-225 and N1-227 (serotype 4b, clinical and food isolates, respectively, associated with the same outbreak), R2-500 and R2-501 (serotype 4b, food and clinical isolates, respectively, associated with the same outbreak), and R2-763, R2-764 and R2-765 (serotype 4b, clinical, food, and environmental isolates, respectively, associated with the same outbreak). Strains N1-225, N1-227, R2-500, R2-501, R2-763, R2-764, and R2-765 (7) were kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, NY). Each strain was individually activated and suspended (30°C, 20–24 h) in tryptic soy broth (Difco, Becton Dickinson, Sparks, MD) supplemented with 0.6% yeast extract (Acumedia, Lansing, MI), and then harvested and washed as previously described (5, 8). Culture pellets of each strain were resuspended separately in 10 ml of autoclave-sterilized frankfurter extract and were stored at 7°C for 72 h, to acclimate the cells to a low temperature food environment (10). To prepare the extract, frankfurters formulated without PL/SD were homogenized (2 min; Masticator, IUL Instruments, Barcelona, Spain) with distilled water to yield a 10% (wt/wt) product suspension. The suspension was passed twice through cheesecloth, and the liquid portion was autoclaved and cooled to ambient (25°C) temperature before use (10).

Following the acclimatization period (7°C, 72 h), the 10 strains were mixed, and serially diluted in freshly prepared frankfurter extract; 0.2 ml of the diluted mixture (approximately 4 log CFU/ml) was used to inoculate the surface of each frankfurter, using a sterile glass spreader (5). The target inoculation level on each frankfurter link was 1–2 log CFU/cm². Inoculated frankfurters were placed at 4°C for 15 min to allow for cell attachment. Samples (six frankfurters per bag) were placed in zip-top vacuum bags (Zip Vac 15.2 × 20.3 cm, nylon/EVA copolymer, Winpak, Winnipeg, MB, Canada), and were vacuum-packaged (LVII Super, Hollymatic Corp., Countryside, IL) and stored at 4°C for up to 60 days (simulating manufacturing and/or retail storage conditions). On days 18, 40 and 60, the zip-lock of each bag was opened to release the vacuum seal and the bag was then resealed and stored at 7°C for up to 14 days (simulating aerobic, home storage conditions).

**Hot water treatments**

Hot water treatments were applied to frankfurters on days 0, 7 and 14 of aerobic storage (7°C). For selection of the treatments (Fig. 1), recommendations found on some commercial packages of frankfurters from certain manufacturers were considered. Such recommendations included “Boil in water for 5 min,” “Place in boiling water, cover and remove from heat, let stand 5–7 min,” and “Heat 2/3 cup of water in skillet, add franks, cover and simmer 7–9 min.” Treatments in this study were applied by placing two frankfurters (approx. 28 g each) in a stainless steel bowl (22.5 cm diameter, 10 cm deep, 2.84 liter capacity) containing sterile distilled water (350 ml) preheated to 80°C or 94°C on a hot plate (Corning Hot Plate Model PC-101, Corning Incorporated, New York, NY) (Fig. 1). For the 80°C treatments, the bowl containing the frankfurters and water was left on the hot plate for 0, 30, 60, or 120 s. For the 94°C treatments, the bowl containing the frankfurters and water was either left on the heat source (0, 30, 60, 120, or 300 s) or removed and left to stand for 180, 300 or 420 s. An untreated control (dry control, no water treatment) was either left on the heat source (0, 30, 60, 120, or 300 s) or removed and left to stand for 180, 300 or 420 s. An untreated control (dry control, no water treatment) and two ambient temperature water controls (two frankfurters submerged in 25°C water for 300 or 420 s) were also included (Fig. 1).

**Microbiological analyses**

Immediately after each treatment, frankfurters (two frankfurters per sample) were transferred to a Whirl-Pak® bag (15 × 23 cm, Nasco, Modesto, CA) containing 50 ml of maximum recovery diluent.
and vertically shaken 30 times to release cells from the surface of the samples (20). The rinsate was serially diluted with 0.1% buffered peptone water (Difco) and plated on PALCAM agar (Difco) and tryptic soy agar (Difco) supplemented with 0.6% yeast extract (TSAYE) for enumeration of \textit{L. monocytogenes} survivors and total microbial populations, respectively. PALCAM agar plates were incubated at 30°C for 48 h and TSAYE plates were incubated at 25 ± 2°C for 72 h. The detection limit for the microbiological analysis of frankfurters was -0.4 log CFU/cm², which was calculated by taking into consideration the surface area of the frankfurters and the volume of MRD added to each sample. The heated water in which frankfurters were immersed was also serially diluted and plated on PALCAM agar for enumeration of possible \textit{L. monocytogenes} survivors. The detection limit for the analysis of water samples was -2.4 log CFU/ml.

Frankfurter and water samples were kept at 4°C after microbiological analysis (and product pH measurements; described below), for possible enrichment in the event that no \textit{L. monocytogenes} survivors would be recovered by direct plating. In such cases, the US Department of Agriculture Food Safety and Inspection Service method (21) was followed with some modifications. Briefly, 100 ml of University of Vermont broth (UVM, Difco) was added to each sample and incubated for 24 ± 2 h at 30°C. After incubation, 1 ml of the UVM enrichment was transferred to 9 ml of Fraser broth (Difco) for secondary enrichment at 35°C. Fraser broth tubes were checked for darkening after 24 and 48 h of incubation. If no darkening appeared, the sample was recorded as negative for \textit{L. monocytogenes} by enrichment. If darkening of the medium occurred, a loopful was streaked onto PALCAM agar plates and incubated at 30°C for 48 ± 2 h. Samples with PALCAM agar plates having typical \textit{Listeria} colonies were recorded as positive for the pathogen by enrichment.

**Physicochemical analyses**

All frankfurter samples analyzed for microbial counts were homogenized (2 min; Masticator) after plating, and pH measurements were taken from a 5 ml aliquot of the homogenate, using a Denver Instruments (Arvada, CO) pH meter and glass electrode. Water activities (a_w) of the two frankfurter formulations (i.e., with or without PL/SD) were measured (AquaLab model series 3, Decagon Devices, Pullman, WA) on day 0 of vacuum-packaged storage. Fat and moisture content analyses were conducted following AOAC International methods 960.39 and 950.46B, respectively (1).

**Statistical analysis**

Two complete replications were conducted, in a randomized block design. For each replication, three samples received the same treatment on each sampling day. Data were analyzed with storage time (days) under vacuum-packaged conditions, storage time (days) under aerobic conditions, hot water treatments, and the interactions of storage time under vacuum-packaged conditions × hot water treatments, and storage time under aerobic conditions × hot water treatments as independent variables, using the Glimmix Procedure of SAS/STAT® (16). Least-squares means were calculated, and mean separation was performed with Tukey's Honestly Significant Differences method, using a level of significance of 0.05.
RESULTS AND DISCUSSION

Physicochemical properties of frankfurters

Values of $a_w$, fat content and moisture content were similar between frankfurters with and without PL/SD. The fat content was $15.37 \pm 0.97\%$ and $15.43 \pm 0.5\%$ for product with and without PL/SD, respectively. As expected, $a_w$ and moisture content were slightly lower in the product formulated with PL/SD ($0.964 \pm 0.005$ and $59.22 \pm 0.59\%$, respectively), compared with the product without PL/SD ($0.970 \pm 0.008$ and $61.09 \pm 0.51\%$, respectively). The pH values of the frankfurters with and without PL/SD on the day of inoculation were $5.92 \pm 0.07$ and $5.93 \pm 0.10$, respectively. As expected, there was no effect ($P \geq 0.05$) of hot water treatments on pH values of the product (data not shown). For frankfurters with PL/SD, pH remained constant ($P \geq 0.05$) throughout storage (Table 1). However, there was an effect of storage time (both in vacuum and aerobic packages) on the pH of frankfurters without PL/SD, most likely due to growth of $L.\ monocytogenes$ and other background flora to high levels in these products (Fig. 2 and 3). For this formulation, 60-day-old vacuum-packaged samples had a lower ($P < 0.05$) pH than corresponding samples stored for 18 days.

In general, during each aerobic storage cycle, the pH of 0- and 7-day samples were not different ($P \geq 0.05$), but decreased ($P < 0.05$) in samples stored for 14 days (Table 1).

**Effect of storage time on microbial populations of frankfurters**

A dry control was used to evaluate changes in $L.\ monocytogenes$ and total microbial populations on frankfurters during storage under vacuum-packaged and aerobic conditions. On day 0 (day of inoculation), $L.\ monocytogenes$ counts on inoculated frankfurters with and without PL/SD in the formulation were $1.8 \pm 0.0$ and $1.7 \pm 0.1$ log CFU/cm$^2$, respectively. During vacuum-packaged storage ($4^\circ C$), these initial numbers remained unchanged ($P \geq 0.05$) for up to 18 days on frankfurters without PL/SD in the formulation and then increased to $2.7 \pm 1.5$ and $4.5 \pm 2.1$ log CFU/cm$^2$ after 40 and 60 days, respectively (Fig. 2). Once the packages were opened and stored at $7^\circ C$, $L.\ monocytogenes$ counts increased by $0.6$ to $1.6$ log CFU/cm$^2$ for every 7 days of storage (Fig. 2). Total microbial counts also increased during storage, and were comparable to those of $L.\ monocytogenes$ (Fig. 3).

Growth of $L.\ monocytogenes$ was inhibited on frankfurters formulated with PL/SD, under both vacuum-packaged and aerobic storage conditions (Fig. 4). Pathogen numbers on product stored for 60 days under vacuum-packaged conditions followed by 14 days under aerobic conditions were $1.2 \pm 0.2$ log CFU/cm$^2$ (Fig. 4); growth of total microbial populations was also inhibited (Fig. 5). These results highlight the importance of including antimicrobials in the formulation of frankfurters that inhibit growth of $L.\ monocytogenes$ during refrigerated storage ($2, 3, 8, 15$), since it has been reported that consumers may store this type of product for periods of time exceeding recommendations ($6$), a practice that may allow for growth of $L.\ monocytogenes$ to high numbers in the absence of inhibitors.

**Effect of hot water treatments on microbial populations of frankfurters**

To determine more accurately the effect of the hot water treatments on $L.\ monocytogenes$ and total microbial populations, the rinsing effect of the water in which samples were immersed

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Vacuum-packaged ($4^\circ C$)</th>
<th>Aerobic ($7^\circ C$)</th>
<th>With PL/SD</th>
<th>Without PL/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>0</td>
<td>5.94 ± 0.13 A</td>
<td>6.09 ± 0.16 A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5.97 ± 0.11 A</td>
<td>6.01 ± 0.08 AB</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>5.96 ± 0.11 A</td>
<td>5.98 ± 0.19 BC</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>5.97 ± 0.10 A</td>
<td>6.02 ± 0.20 AB</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5.97 ± 0.12 A</td>
<td>5.99 ± 0.23 BC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>5.94 ± 0.20 A</td>
<td>5.88 ± 0.33 D</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>6.00 ± 0.12 A</td>
<td>5.96 ± 0.38 BC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5.97 ± 0.13 A</td>
<td>5.91 ± 0.36 DC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>5.94 ± 0.22 A</td>
<td>5.70 ± 0.39 E</td>
<td></td>
</tr>
</tbody>
</table>

A-E Means with the same letter within a column are not significantly different ($P \geq 0.05$)

PL/SD: Potassium lactate (1.5%) and sodium diacetate (0.1%)
was taken into consideration by including two ambient temperature water controls: two frankfurters immersed in water at 25°C for 300 or 420 s. There was no significant difference \((P \geq 0.05)\) between the counts found on frankfurters after these two control treatments; therefore, the results and discussion presented in the following sections are based on the ambient temperature control treatment applied for 300 s, which is referred to as “control” and which is common to both product formulations (with and without PL/SD, Fig. 1).

As expected, the effectiveness of the hot water treatments applied at a constant temperature (80 or 94°C) on frankfurters formulated without PL/SD was influenced by initial counts on frankfurters, which depended on the storage conditions (vacuum vs. aerobic; 4°C vs. 7°C) and age of the product (Fig. 2). Longer storage times allowed for an increase in \(L.\) monocytogenes counts up to 5.3 ± 2.7 log CFU/cm² on the control (ambient temperature control, 300 s; Fig. 2). Naturally, these high numbers required longer times and/or higher temperatures to be reduced to below the detection limit (< -0.4 log CFU/cm²). Initial counts on the control of less than 3 log CFU/cm² were reduced to below the detection limit when treated for 120 s at 80°C or ≥ 60 s at 94°C. As counts on the control increased to 3–4 log CFU/cm², no treatments at 80°C were effective in reducing counts to below the detection limit, and the most effective treatments were ≥ 120 s at 94°C, with reductions of ≥ 4.2 log CFU/cm². The only treatment applied at constant temperature that reduced initial counts of > 4 log CFU/cm² to below the detection limit was 300 s at 94°C, but the pathogen was detected by enrichment in some samples (enrichment data not shown). Treatments that involved removal of frankfurters from the heat source (180, 300 and 420 s) consistently resulted in product with counts below the detection limit, regardless of initial levels, and accounted for reductions of up to ≥ 5.7 log CFU/cm²; however, some samples were positive by enrichment (enrichment data not shown). At a water temperature of 94°C, reductions achieved at 300 s were similar when the temperature was kept constant or when bowls were removed from the heat source (Fig. 2).

Treatments associated with manufacturers’ recommendations (“boil for 5 min” and “place frankfurters in boiling water, remove from heat and let stand for 5–7 min”; Fig. 1) were effective in reducing \(L.\) monocytogenes initial counts to below the detection limit, with reductions of up to 5.7 log CFU/cm² on frankfurters without PL/SD. However, the pathogen was detected in some frankfurter samples by enrichment, indicating that these directions for reheating may potentially allow for survival of small numbers of the pathogen on product formulated without PL/SD that had been stored. 

**FIGURE 2.** Listeria monocytogenes counts on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate, after treatment with hot water at 18 (A), 40 (B) and 60 (C) days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.
FIGURE 3. Total microbial counts on frankfurters formulated without 1.5% potassium lactate and 0.1% sodium diacetate, after treatment with hot water at 18 (A), 40 (B) and 60 (C) days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

under conditions that permitted growth to high levels (> 5.3 log CFU/cm²). Treatments of ≥ 60 s at 80°C and ≥ 30 s at 94°C applied to frankfurters formulated with PL/SD consistently reduced initial counts of the pathogen (0.6 ± 0.7 to 0.9 ± 0.7 log CFU/cm²) to levels at/below the detection limit (but sometimes detectable by enrichment), regardless of storage conditions (Fig. 4).

L. monocytogenes survivors in water

L. monocytogenes was detected (-0.7 ± 1.7 to 5.2 ± 1.4 log CFU/ml) in the water used for the ambient (25°C) temperature water control treatments (Fig. 6), indicating that cells were transferred from the frankfurters into the water. However, no survivors were found remaining, by direct plating or enrichment, in any of the heated water samples, regardless of frankfurrer formulation. It is thus important to devise treatments that destroy L. monocytogenes, not only on frankfurters but also in the water used for reheating, to avoid cross-contamination of the environment and other foods through the water (23).

Under the conditions of this study, results showed that L. monocytogenes contamination levels of ≤ 2 log CFU/cm² on frankfurters were reduced to below the level of detection (< -0.4 log CFU/cm²) with short-time exposure to hot water (at least 60 s at 94°C). However, when pathogen numbers on frankfurters increased to above 4 log CFU/cm² because of storage conditions, longer times (at least 300 s at 94°C) were needed. Treatments based on manufacturers’ recommendations tested in this study ("boiling for 5 min" and "placing frankfurters in boiling water, remove from heat and let stand for 5–7 min") allowed for survival of L. monocytogenes detectable only by enrichment, even with initial numbers of up to 5.3 log CFU/cm². Boiling rendered water used for frankfurter reheating (at either 80 or 94°C) safe for discarding without risk of cross-contamination of other kitchen surfaces with L. monocytogenes.

It has been suggested that food labels are an important tool for providing consumers with critical information (4), such as reheating instructions and safe handling of the product. However, in order to provide consumers with reliable directions, cooking and reheating instructions on labels should be validated and based on scientific data. The data provided here may be useful to the industry in the development of science-based recommendations for reheating of frankfurters by consumers in their homes.
FIGURE 4. *Listeria monocytogenes* counts on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate, after treatment with hot water at 18 (A), 40 (B) and 60 (C) days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.

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FIGURE 5. Total microbial counts on frankfurters formulated with 1.5% potassium lactate and 0.1% sodium diacetate, after treatment with hot water at 18 (A), 40 (B) and 60 (C) days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.


FIGURE 6. *Listeria monocytogenes* counts in water used for ambient temperature (25°C) control treatments of frankfurters formulated with and without 1.5% potassium lactate and 0.1% sodium diacetate (PL/SD), at 18 (A), 40 (B) and 60 (C) days of storage (4°C) in vacuum packages followed by aerobic storage (7°C) for 14 days.


