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Viability of a Five-Strain Mixture of *Listeria monocytogenes* in Vacuum-Sealed Packages of Frankfurters, Commercially Prepared with and without 2.0 or 3.0% Added Potassium Lactate, during Extended Storage at 4 and 10°C†‡

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ABSTRACT

The viability of *Listeria monocytogenes* was monitored on frankfurters containing added potassium lactate that were obtained directly from a commercial manufacturer. Eight links (ca. 56 g each) were transferred aseptically from the original vacuum-sealed bulk packages into nylon-polyethylene bags. Each bag then received a 4-ml portion of a five-strain mixture of the pathogen. Frankfurters containing 2.0 or 3.0% potassium lactate were evaluated using 20 CFU per package, and frankfurters containing 3.0% potassium lactate were evaluated using 500 CFU per package. The packages were vacuum-sealed and stored at 4 or 10°C for up to 90 or 60 days, respectively. During storage at 4°C, pathogen numbers remained at about 1.6 $\log_{10}$ CFU per package over 90 days in packages containing frankfurters with 2.0% potassium lactate that were inoculated with about 20 CFU. In packages containing frankfurters with 3.0% potassium lactate that were inoculated with about 20 CFU and stored at 4°C, pathogen numbers remained at about 1.4 $\log_{10}$ CFU per package over 90 days. In packages containing frankfurters with 3.0% potassium lactate that were inoculated with about 500 CFU and stored at 4°C, pathogen numbers remained at about 2.4 $\log_{10}$ CFU per package over 90 days. However, in the absence of any added potassium lactate, pathogen numbers increased to 4.6 and 5.0 $\log_{10}$ CFU per package after 90 days of storage at 4°C for starting levels of 20 and 500 CFU per package, respectively. During storage at 10°C, pathogen numbers remained at about 1.4 $\log_{10}$ CFU per package over 60 days in packages containing frankfurters with 2.0% potassium lactate that were inoculated with about 20 CFU. In packages containing frankfurters with 3.0% potassium lactate that were inoculated with about 20 CFU and stored at 10°C, pathogen numbers remained at about 1.1 $\log_{10}$ CFU per package over 60 days of storage. In the absence of any added potassium lactate, pathogen numbers increased to 6.5 $\log_{10}$ CFU per package after 28 days and then declined to 5.0 $\log_{10}$ CFU per package after 60 days of storage at 10°C. In packages containing frankfurters with 3.0% potassium lactate that were inoculated with about 500 CFU per package, pathogen numbers remained at about 2.4 $\log_{10}$ CFU per package over 60 days of storage at 10°C, whereas in the absence of any added potassium lactate, pathogen numbers increased to about 6.6 $\log_{10}$ CFU per package within 40 days and then declined to about 5.5 $\log_{10}$ CFU per package after 60 days of storage. The viability of *L. monocytogenes* in frankfurter packages stored at 4 and 10°C was influenced by the pH and the presence or levels of lactate but not by the presence or levels of indigenous lactic acid bacteria or by the proximate composition of the product. These data establish that the addition of 2.0% ($P < 0.0004$) or 3.0% ($P < 0.0001$) potassium lactate as an ingredient in frankfurters can appreciably enhance safety by inhibiting or delaying the growth of *L. monocytogenes* during storage at refrigeration and abuse temperatures.

Although most episodes of listeriosis in the United States are isolated cases *(34),* there have been at least four well-publicized outbreaks: (i) the California outbreak in 1985, linked to a serotype 4b isolate in Mexican-style cheese (142 cases, 48 deaths *(22)*); (ii) the Wisconsin, Illinois, and Michigan (7 invasive, typical cases, 0 deaths *(31)*) and Illinois (45 noninvasive, atypical cases, 0 deaths *(11)*) outbreak in 1994, linked to a serotype 1/2b isolate in chocolate milk; (iii) the multistate outbreak in 1998 to 1999, linked to a serotype 4b isolate in frankfurters and deli meats (101 cases, 21 deaths *(7)*); and (iv) the multistate outbreak in 2000, linked to a serotype 1/2a isolate in deli turkey meat (29 cases, 4 deaths, 3 miscarriages or stillbirths *(8)*). The latter two outbreaks in particular have fueled considerable debate on the current "zero tolerance" regulatory policy for *Listeria monocytogenes* in the United States, as well as considerable research on the development of (post-process) intervention strategies for cooked, ready-to-eat (RTE) foods. Recent data from the Centers for Disease Control and Prevention estimate the burden of illness at

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† Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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2,493 cases and 499 deaths in the United States due to listeriosis *(26).* Moreover, both the number and the magnitude of recalls of food due to contamination with *L. monocytogenes* have risen dramatically in the last few years *(1).* In 1999 alone, the USDA Food Safety and Inspection Service issued 62 recalls of cooked meats, and 31 of these recalls were due to *L. monocytogenes* (http://www.fsis.usda.gov/OA/recalls/rec;llintr.htm). Far less information, however, is available on the sources and levels of contamination in RTE foods, including frankfurters, and such foods, if contaminated, may be more likely to cause illness. Nevertheless, it is noteworthy that the relative frequencies of listeriosis are similar in countries such as Germany (ca. 3 cases per million inhabitants) that have established tolerance levels for *L. monocytogenes* and countries such as the United States (ca. 4 to 5 cases per million inhabitants) that maintain a zero tolerance policy for this pathogen *(3).*

L. monocytogenes is common in the environment, as well as in foods, in food processing plants, and at catering establishments *(15, 17, 33).* In meat products, the incidence of this bacterium can be as low as 3% and as high as 90% *(21),* with an estimated overall average of 16% *(20).* When present, the levels of the bacterium range from <10 to 1,000 CFU/g *(4, 21),* but levels of $\geq 10^2$ CFU/g have been recorded for pâté and some RTE sliced meats *(27, 28, 40).* Qvist and Liberski *(32)* found that 6% of the cooked frankfurters they examined were positive for *L. monocytogenes* at packaging and 13% were positive at the expiration of storage at 8 to 10°C. Wang and Muriana *(42)* reported that 7 of 93 (8%) packages from 19 different brands of retail franks tested positive for *L. monocytogenes.* In other studies, Tiwari and Aldenrath *(39)* reported a prevalence of 13 to 21% for *L. monocytogenes* in wieners and sliced meats, and Bersot et al. *(2)* reported that 8 of 30 (27%) retail samples of Brazilian mortadella tested positive for this pathogen. As a final example, Hudson et al. *(19)* conducted a retail survey of 203 RTE meat products from retail outlets in New Zealand. Their survey revealed a prevalence of 6% (9 of 149 samples) in cooked products, 10% (2 of 20 samples) in fermented meats, and 42% (13 of 31 samples) in smoked products. The pathogen was present at higher incidences in prepackaged (19%; 13 of 70 samples) than in delicatessen-type (8%; 11 of 133 samples) foods. These data substantiate the frequent association of *L. monocytogenes* with a variety of RTE meats.

Several investigators have published studies on the ability of selected meat products to support the growth or survival of *L. monocytogenes.* For example, McKellar et al. *(24)* reported that about 66% (40 of 61 samples) of frankfurters purchased at retail stores supported the growth of this bacterium. Other investigators have also reported that listeriae can survive or grow in a variety of RTE meats, including vacuum-sealed packages of frankfurters or exudates derived therefrom, under certain conditions *(6, 18, 40, 45).* However, most inoculated-package studies have typically used relatively high ($>10^3$ to 10^4 CFU/g) initial numbers and only a single strain of *L. monocytogenes.* Moreover, previous studies have paid little attention to the influence of formulation on pathogen viability. The purpose of the present study was to quantify the viability of relatively low levels of a five-strain mixture of *L. monocytogenes* in vacuum-sealed packages of commercially prepared frankfurters with and without added potassium lactate during extended storage at refrigeration and abuse temperatures.

MATERIALS AND METHODS

Bacterial strains. A five-strain mixture of *Listeria monocytogenes* isolates (Scott A [serotype 4b, clinical isolate *(22)*], H7776 [serotype 4b, frankfurter isolate *(7)*], LM-101M [serotype 4b, beef and pork sausage isolate *(18)*], F6854 [serotype 1/2a, turkey frankfurter isolate *(35)*], and MFS-2 [serotype 1/2c, environmental isolate from a pork processing plant; this study]) was used to inoculate packages of frankfurters, which were then vacuum-sealed. A nominal ($\leq$100 μl) portion of a frozen suspension of each isolate, maintained at −20°C in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Mich.) plus 10% glycerol (Sigma Chemical Co., St. Louis, Mo.), was streak plated onto a BHI agar (1.5%; Difco) plate to confirm its purity. A single colony of each isolate was then separately transferred into 50 ml of BHI broth and incubated at 37°C for 24 h with shaking (100 rpm). A 100-μl portion thereof was added to 50 ml of fresh BHI broth, and the cells were incubated at 37°C for an additional 18 h with shaking. The resulting stationary-phase cells of each of the five strains were diluted separately in 0.1% peptone water (Bacto Peptone; Difco). The five-strain mixture of *L. monocytogenes* isolates was prepared by combining equal volumes of the five individual cell suspensions. The levels of *L. monocytogenes* in the initial inoculum were determined by spread plating a 250-μl portion of the freshly prepared mixture onto duplicate modified Oxford *(10)* and BHI agar plates. After incubation for 48 h at 37°C, representative colonies were counted and confirmed. The confirmation tests for *L. monocytogenes* included Gram staining, as well as tests for motility in Bacto Motility Test Medium (Difco), catalase reaction on glass slides, hemolysis on BHI agar plates containing 5% sheep's blood, and reactivity on biochemical tests strips (API 10 300 Listeria Test, Bio Mérieux Laboratories, St. Louis, Mo.).

Inoculation and vacuum-packaging of frankfurters. Freshly processed, peeled frankfurters (ca. 56 g per link) in vacuum-sealed bulk packages (18 to 30 lb) were obtained from a commercial manufacturer. The frankfurters contained pork, water, beef, salt, spices, paprika, sodium erythrobate, and sodium nitrite. Two different formulations of links were tested. For one formulation, potassium lactate (Purac America, Inc., Lincolnshire, Ill.) was added to the batter at a final concentration of ca. 2.0 or 3.0%, and the other formulation did not contain any added potassium lactate. Frankfurters were removed aseptically from the original bulk package and repackaged (8 links per bag) into nylon-polyethylene bags (3 mil standard barrier, 20.3 by 30.5 cm, $O_2 < 0.6$ $cm^3/100$ $in^2/24$ h at 0°C relative humidity with a moisture vapor transmission rate of 0.6 g of H_2O per 100 in^2 per 24 h at 38°C; Koch Industries, Kansas City, Mo.). Each package was inoculated with a 4-ml portion of the five-strain *L. monocytogenes* mixture diluted as necessary in 0.1% peptone water to achieve a target level of about 20 or 500 CFU per package. Control packages were inoculated with 4 ml of 0.1% peptone water. Each package was then massaged by hand for about 2 min to distribute the inoculum. The packages were vacuum-sealed to 95 kPa with a Multivac A300/16 vacuum-packaging unit (Sepp Haggemüller KG, Wolfertschwenden, Germany) and incubated at 4 and 10°C. The packages stored at 4°C were analyzed after 0, 7, 15, 21, 28, 60, and 90 days, and the packages stored at 10°C were analyzed after 0, 5, 8, 11, 21, 28, 40, and 60 days. The control samples stored at

TABLE 1. *Lactic acid bacteria (LAB) counts (log_{10} CFU per package) and pH values for vacuum-sealed packages of frankfurters stored at 4°C for 90 days*

	0.0% potassium lactate				2.0% potassium lactate		3.0% potassium lactate			
	20 CFU[a]		500 CFU[b]		20 CFU[c]		20 CFU[b]		500 CFU[b]	
Days	LAB	pH	LAB	pH	LAB	pH	LAB	pH	LAB	pH
0	2.56 ± 3.51	5.85 ± 0.49	3.91 ± 0.96	5.54 ± 0.70	2.28 ± 1.23	5.84 ± 0.24	0.65 ± 0.91[d]	6.11 ± 0.0	1.49 ± 2.11	6.11 ± 0.0
7	4.39 ± 3.66	5.97 ± 0.29	6.25 ± 3.45	5.42 ± 0.89	4.18 ± 2.19	5.92 ± 0.44	2.70 ± 0.54	6.15 ± 0.06	4.77 ± 6.74[d]	6.04 ± 0.01
15	4.23 ± 3.16	5.87 ± 0.45	10.09 ± 0.73	5.38 ± 0.88	2.97 ± 1.06	5.99 ± 0.09	1.93 ± 2.73	6.21 ± 0.01	5.86 ± 2.02	6.01 ± 0.0
21	5.96 ± 2.65	5.58 ± 0.44	11.10 ± 1.13	5.13 ± 0.20	5.51 ± 3.44	5.66 ± 0.53	3.64 ± 2.23	6.10 ± 0.11	6.27 ± 2.48	5.64 ± 0.53
28	7.40 ± 2.19	5.60 ± 0.40	11.15[e]	5.03 ± 0.0	6.21 ± 3.07	5.63 ± 0.36	4.73 ± 3.13	5.94 ± 0.24	2.92	6.02 ± 0.0
40	ND[f]	ND	12.71	5.30 ± 0.0	ND	ND	ND	ND	9.12	5.30 ± 0.0
60	7.83 ± 2.98	5.16 ± 0.59	11.80 ± 0.63	5.21 ± 0.01	6.18 ± 4.23	5.46 ± 0.33	5.31 ± 7.52[d]	5.82 ± 0.38	8.16 ± 5.27	5.49 ± 0.41
90	7.59 ± 3.37	5.22 ± 0.71	12.23	5.31 ± 0.00	7.00 ± 4.76	5.71 ± 0.37	10.63 ± 1.89	5.43 ± 0.04	11.88 ± 3.13	5.38 ± 0.16

[a] Mean of five trials ± standard deviation.
[b] Mean of two trials ± standard deviation.
[c] Mean of three trials ± standard deviation.
[d] Mean of two trials, with one of the trials being below the threshold of detection (recorded as 0).
[e] Value from one trial only for entries without a standard deviation listed.
[f] ND, not determined.

TABLE 2. *Lactic acid bacteria (LAB) counts (log_{10} CFU per package) and pH values for vacuum-sealed packages of frankfurters stored at 10°C for 60 days*

	0.0% potassium lactate				2.0% potassium lactate		3.0% potassium lactate			
	20 CFU[a]		500 CFU[b]		20 CFU[c]		20 CFU[b]		500 CFU[b]	
Days	LAB	pH	LAB	pH	LAB	pH	LAB	pH	LAB	pH
0	1.70 ± 0.61	5.99 ± 0.28	3.69 ± 1.26	6.10 ± 0.10	1.18 ± 0.18	5.78 ± 0.28	1.63 ± 2.31	6.11 ± 0.11	1.49 ± 2.11	6.11 ± 0.11
4	6.57 ± 3.23	5.51 ± 0.58	8.45[c]	5.45 ± 0.59	3.34 ± 0.48	5.87 ± 0.13	4.04 ± 0.88	6.03 ± 0.01	5.30 ± 0.85	6.03 ± 0.04
8	7.70 ± 3.68	5.49 ± 0.33	10.24 ± 0.95	5.13 ± 0.19	4.68 ± 2.79	5.44 ± 0.17	5.30 ± 2.11	6.07 ± 0.02	5.29 ± 0.43	6.03 ± 0.08
11	8.01 ± 2.63	5.08 ± 0.12	10.25 ± 0.92	5.03 ± 0.0	5.25 ± 5.68[d]	5.51 ± 0.05	7.36 ± 2.60	5.34 ± 0.04	7.15 ± 0.20	5.52 ± 0.31
21	10.52 ± 1.83	5.01 ± 0.50	11.03 ± 0.87	5.28 ± 0.20	9.45 ± 2.71	5.21 ± 0.08	9.75 ± 1.70	5.52 ± 0.20	7.18 ± 4.69	5.73 ± 0.70
28	9.85 ± 1.20	4.96 ± 0.67	ND[e]	ND	10.87 ± 0.49	5.39 ± 0.10	10.62	5.30	7.84	5.51
40	11.24 ± 0.81	5.01 ± 0.77	13.08	5.28	10.95 ± 0.78	5.23 ± 0.25	11.95	5.41	10.95	5.36
60	10.62 ± 1.31	4.94 ± 0.64	13.48	5.37	11.68 ± 0.98	5.55 ± 0.21	11.00 ± 0.36	5.58 ± 0.23	10.93 ± 0.35	5.60 ± 0.05

[a] Mean of four trials ± standard deviation.
[b] Mean of two trials ± standard deviation.
[c] Value from one trial only for entries without a standard deviation listed.
[d] Mean of two trials, with one of the trials being below the threshold of detection (recorded as 0).
[e] ND, not determined.

TABLE 3. *Chemical composition of vacuum-sealed packages of frankfurters prepared with 3% and without potassium lactate*

Analysis	With potassium lactate	Without potassium lactate
NaCl (g/100 g)	2.00[a]	1.73
Protein (g/100 g)	9.05	11.26
Fat (ether extraction) (g/100 g)	29.89	28.96
Lactic acid (g/100 g)	3.27	0.957
pH	5.68	6.18
Nitrite (μg/g)	4.79	4.17
Phenolics (as catechin) (μg/g)	811	803

[a] Proximate analyses were performed on two samples from one trial from day 0.

4°C were analyzed after 0, 28, and 90 days, and those stored at 10°C were analyzed after 0, 28, and 60 days. There were at least two trials conducted for each treatment. For each trial, three packages per sampling interval were analyzed for each treatment.

Microbiological analyses. The outside surface of the package was wiped with an ethanol-soaked (70%, vol/vol) paper towel, and the package was opened with alcohol-sterilized scissors. Sixteen milliliters of sterile 0.1% peptone water was added, and the mouth of the package was folded shut. The packages were massaged and shaken by hand for 2 min, and the resulting fluid (ca. 20 ml) was transferred to a sterile 50-ml screw-cap conical centrifuge tube using a pipet.

The pathogen was enumerated by spread plating 250 μl of the resulting fluid (i.e., rinsate) or dilutions thereof onto duplicate modified Oxford agar plates and incubating for 48 h at 37°C. Colonies typical of listeriae, 1- to 2-mm round isolates surrounded by black zones due to esculin hydrolysis, were counted, and representative colonies were confirmed to be *L. monocytogenes* as described above and were retained for further analyses. Lactic acid bacteria (LAB) were enumerated by spread plating 250 μl of the original rinsate onto Rogosa SL (Sigma) agar plates and incubating anaerobically (10.1% carbon dioxide, 4.38% hydrogen and balance nitrogen; Bactron IV Anaerobic/Environmental Chamber, Sheldon Manufacturing Inc., Cornelius, Oreg.) for 48 h at 37°C. Colonies on Rogosa SL agar plates were counted, and several were selected at random and retained for further analyses. Bacterial numbers were expressed as $\log_{10}$ CFU per package.

Chemical analyses. The pH of the rinsate obtained from washing the contents of representative packages was determined with a Corning model 3-in-1 combination electrode and a model 340 meter (Corning Inc., Corning, N.Y.). The proximate composition of frankfurters was determined by methods approved and described by the Association of Official Analytical Chemists *(25)* as conducted by a commercial testing laboratory. Proximate analyses were performed on two samples from one trial on day 0.

Statistical analyses. Data were analyzed with version 8.0 of the SAS statistical package (SAS Institute, Inc., Cary, N.C.). Analyses of covariance were performed to evaluate the effects and interactions of formulation, temperature, and inoculum level on the regression of *L. monocytogenes* over time. The results presented in Tables 1 and 2 are the arithmetic means ± standard deviations. Some standard deviations were larger than the means because bacterial counts in one of the trials were below the threshold of detection. In these cases, a value of 0 was used for the determination of the arithmetic mean.

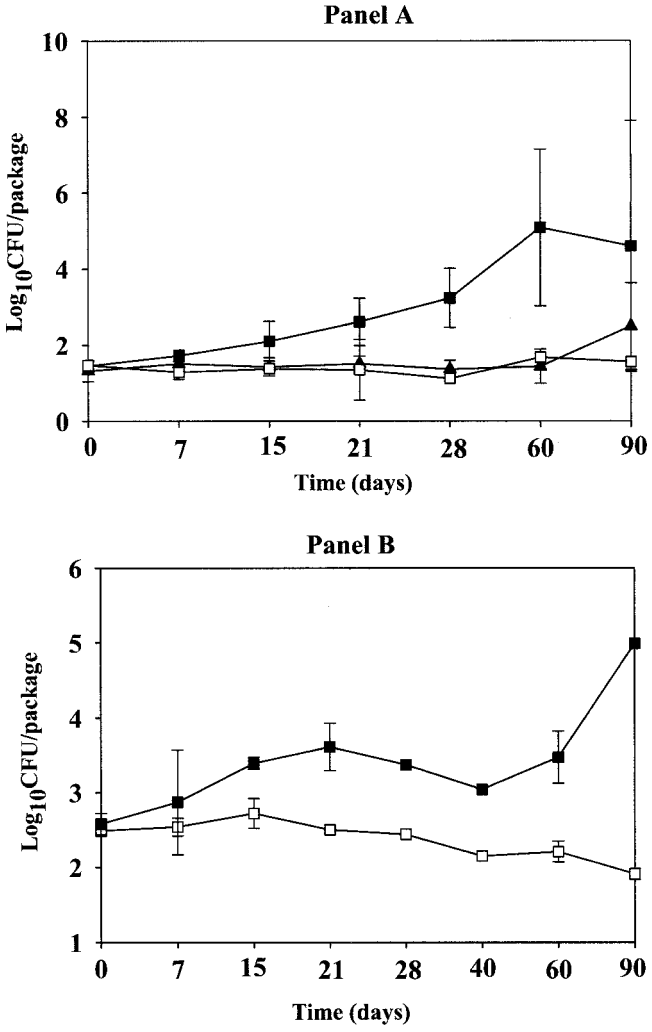

FIGURE 1. *Viability of* L. monocytogenes *at 4°C on frankfurters inoculated with (A) 20 and (B) 500 CFU per package containing 0.0% (■; n = 5), 2.0% (▲; n = 3), and 3.0% (□; n = 2) potassium lactate. Packages were inoculated with a five-strain mixture (Scott A, H7776, LM-101M, F6854, and MFS-2). Vertical bars represent the standard deviation of the mean.*

RESULTS

Recovery of bacteria from packages. Preliminary studies were conducted to compare different sampling methods for recovery of relatively low (ca. 20 CFU per package) levels of *L. monocytogenes* from vacuum-sealed packages of frankfurters. We compared the approved USDA Food Safety and Inspection Service product compositing enrichment method, whereby a 25-g composite sample is first enriched and then plated onto selective agar plates *(10),* with the USDA Agricultural Research Service package rinsing method, whereby a 25-ml rinsate of the entire package and its contents is directly plated onto selective agar plates *(41).* By these methods, the pathogen was recovered at frequencies (i.e., percentages of packages positive for *L. monocytogenes*) of 17% and 100% for the USDA Food Safety and Inspection Service product compositing method and the USDA Agricultural Research Service package rinsing method, respectively. Moreover, the USDA Agricultural Research Service package rinsing

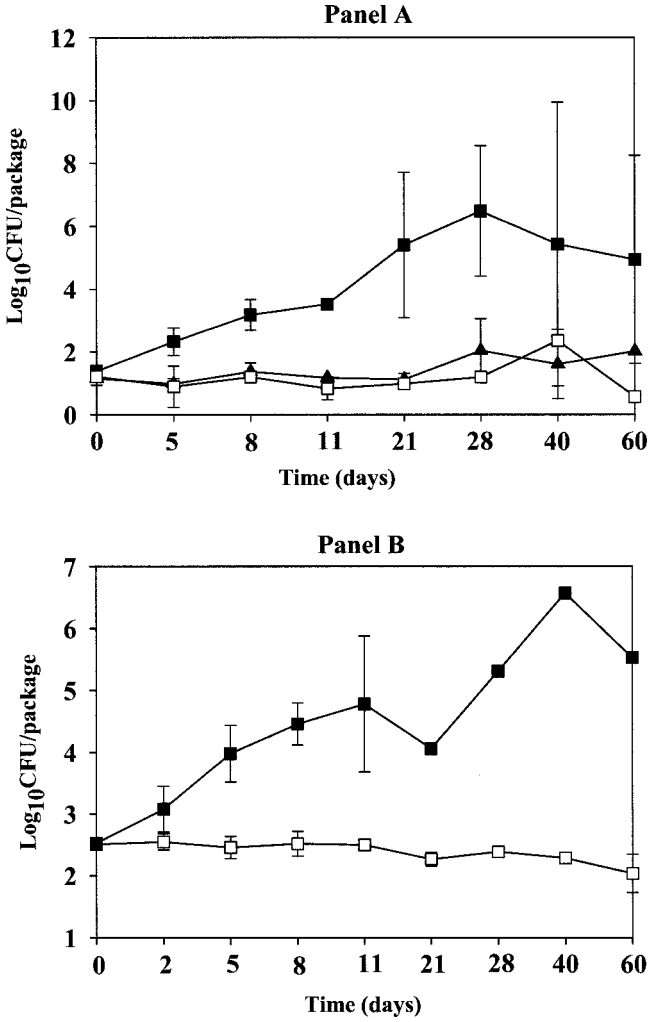

FIGURE 2. *Viability of* L. monocytogenes *at 10°C on frankfurters inoculated with (A) 20 and (B) 500 CFU per package containing 0.0% (■; n = 4), 2.0% (▲; n = 3), and 3.0% (□; n = 2) potassium lactate. Packages were inoculated with a five-strain mixture (Scott A, H7776, LM-101M, F6854, and MFS-2). Vertical bars represent the standard deviation of the mean.*

method displayed an efficiency (i.e., percentage of recovery of initial levels of *L. monocytogenes*) of 95%. The USDA Food Safety and Inspection Service product compositing method is qualitative only. Based on these data, the USDA Agricultural Research Service package rinsing method was used for the remainder of this study to recover both *L. monocytogenes* and LAB from vacuum-sealed packages of frankfurters.

Levels of indigenous flora and pH values and proximate compositions of rinsates and/or links. Direct plating of samples of representative packages and links from the manufacturer that were not inoculated with the pathogen revealed the absence of any indigenous *L. monocytogenes* (≤5 CFU per package). Sampling of representative packages revealed that the initial levels of LAB and the pHs varied among formulations and batches (Tables 1 and 2). In general, the levels of LAB increased and the pHs decreased during storage at both temperatures tested. For frankfurters formulated with 2.0% potassium lactate that were inoculated with about 20 CFU per package, levels of LAB increased by 4.7 $\log_{10}$ CFU per package over 90 days at 4°C (Table 1), whereas levels of LAB increased by 10.5 $\log_{10}$ CFU per package over 60 days at 10°C (Table 2). For frankfurters formulated with 3.0% potassium lactate that were inoculated with about 20 CFU per package, levels of LAB increased by 10.0 $\log_{10}$ CFU per package over 90 days at 4°C, whereas levels of LAB increased by 9.4 $\log_{10}$ CFU per package over 60 days at 10°C. In the absence of added potassium lactate, levels of LAB increased by 5.0 and 8.9 $\log_{10}$ CFU per package during storage at 4 and 10°C, respectively. For frankfurters formulated with 3.0% potassium lactate that were inoculated with about 500 CFU per package, levels of LAB increased by 10.4 $\log_{10}$ CFU per package over 90 days at 4°C, whereas levels of LAB increased by 9.4 $\log_{10}$ CFU per package over 60 days at 10°C. In the absence of added potassium lactate, levels of LAB increased by 8.3 and 9.8 $\log_{10}$ CFU per package during storage at 4 and 10°C, respectively. The addition of potassium lactate to the batter may have caused a decrease in the levels and/or specific types of competitive flora, and this in turn may have allowed the LAB to achieve higher levels in packages containing frankfurters formulated with added potassium lactate than in packages without any added potassium lactate. Although the initial pH values were somewhat different among the various treatments and batches, the final pH values did not vary appreciably among the different treatments and batches following storage at either temperature tested. These data indicate that potassium lactate had some effect on the levels of LAB, but not pH, in vacuum-sealed packages of frankfurters. The results of the chemical analyses of frankfurters are presented in Table 3. Only subtle differences were observed in the levels of sodium chloride, protein, fat, nitrite, and phenolics in vacuum-sealed packages between these two formulations of frankfurters. However, a threefold difference in the initial levels of lactic acid and a 0.5-U difference in pH were observed between formulations. It remains unclear why frankfurters formulated without added potassium lactate contained 0.957% lactic acid. However, it is possible that some of the lactic acid present in frankfurters prepared without potassium lactate was due to the postmortem production of lactic acid in muscle tissue.

Viability of listeriae in packages held at 4 and 10°C. Regardless of the inoculation level or the incubation temperature, pathogen numbers were appreciably lower in packages containing frankfurters formulated with 2.0% ($P < 0.0004$) or 3.0% ($P < 0.0001$) potassium lactate than in packages containing frankfurters formulated without potassium lactate. However, statistical analysis did not identify appreciable differences in the antilisterial effects of the frankfurter formulation with 2% potassium lactate and the formulation with 3% potassium lactate. More specifically, pathogen numbers for frankfurters formulated with 2.0% potassium lactate that were inoculated with about 20 CFU per package remained at about 1.6 $\log_{10}$ CFU per package over 90 days at 4°C (Fig. 1). When such frankfurters were formulated with 3.0% potassium lactate and stored at 4°C

for 90 days, pathogen numbers remained at about 1.4 $\log_{10}$ CFU per package. For frankfurters with 3.0% potassium lactate that were inoculated with about 500 CFU per package and stored at 4°C, pathogen numbers remained at about 2.4 $\log_{10}$ CFU per package over 90 days. In contrast, pathogen numbers increased to 4.6 and 5.0 $\log_{10}$ CFU per package for initial levels of 20 and 500 CFU per package, respectively, when frankfurters were not formulated with added potassium lactate and were stored at 4°C for 90 days.

During storage at 10°C (Fig. 2), for frankfurters formulated with 2.0% potassium lactate and inoculated with about 20 CFU per package, pathogen numbers remained at about 1.4 $\log_{10}$ CFU per package after 60 days of storage. For frankfurters formulated with 3.0% potassium lactate, pathogen numbers remained at about 1.1 and 2.4 $\log_{10}$ CFU per package after 60 days of storage for initial levels of about 20 and 500 CFU per package, respectively. For frankfurters formulated without added potassium lactate and inoculated with 20 CFU, pathogen numbers increased to 6.5 $\log_{10}$ CFU per package after 28 days and then declined to 4.9 $\log_{10}$ CFU per package after 60 days of storage. Pathogen numbers increased to 6.6 $\log_{10}$ CFU per package after 40 days and then declined to 5.5 $\log_{10}$ CFU per package after 60 days of storage for frankfurters formulated without added potassium lactate that were inoculated with about 500 CFU of the pathogen. These data establish the antilisterial potential of potassium lactate in frankfurters stored at low temperatures.

DISCUSSION

In the past 20 years, *Listeria monocytogenes* has been associated with illnesses linked to a variety of foods. However, until the frankfurter-linked 1998 to 1999 outbreak and the deli-meat-linked 2000 outbreak, RTE meats had not been a vehicle for a major outbreak of listeriosis in North America. The association of *L. monocytogenes* with such high-volume RTE products poses an appreciable threat to consumer safety. These outbreaks have been the catalyst for additional research to develop and optimize processes and/or products to eliminate or better control *L. monocytogenes* in cooked RTE meats. In addition to physical (i.e., steam, X rays, high pressure) and biological (i.e., LAB and associated antimicrobial agents) interventions, food-grade chemicals (i.e., benzoate, lactate, diacetate, sorbate) have also been extensively studied. With regard to the latter, there have been a few reports that quantified the effects of proximate composition on the viability of *L. monocytogenes* during extended and/or refrigerated storage of vacuum-sealed meats *(12, 14, 45).* As an extension of our previous research, here we evaluated the effect of the formulation of commercial frankfurters with and without added potassium lactate on the viability of relatively low numbers of *L. monocytogenes.* In addition to use as flavoring agents, the USDA Food Safety and Inspection Service has approved sodium or potassium lactate at levels up to 4.8% of the total formulation to slow the growth of pathogens in fully cooked products *(16).*

A cursory inspection of the available literature revealed that additional research was warranted to better quantify the effect of formulation on the fate of relatively low levels of *L. monocytogenes* in a cooked, small-diameter sausage, namely, the antilisterial contributions of lactates in all-beef frankfurters. Most organic acids, such as acetic and lactic acids, that are permitted in foods are typically used as acidulants, whereas the salts of organic acids such as potassium sorbate and sodium benzoate are typically used as preservatives *(5, 13, 29, 36, 44).* As summarized by Lou and Yousef *(23),* although the mechanism(s) of action is not precisely known, lactates at levels of 2 to 4% are generally more effective in foods than in synthetic media. A comparison of the calcium, potassium, and sodium salts of lactate by Chen and Shelef *(9)* revealed that all three were equally effective in inhibiting *L. monocytogenes* in cooked strained beef during storage at 20°C. Shelef and Yang *(38)* also reported that sodium lactate and potassium lactate at levels of 4% were equally effective in slowing the growth of *L. monocytogenes* during refrigerated storage of sterile comminuted chicken. In a related study, however, Weaver and Shelef *(43)* reported that calcium lactate was more effective than sodium lactate or potassium lactate in inhibiting the pathogen in pork liver sausage during storage at 5 and 20°C. The results of the present study are in accord with those previously published. In the absence of potassium lactate, pathogen numbers increased appreciably over time.

There have been several reports detailing the growth or survival of *L. monocytogenes* in vacuum-packaged RTE meats, including frankfurter-type products *(2, 6, 19, 24, 40).* Differences in the rate and magnitude of growth of the pathogen among studies can be readily explained by differences in the types, levels, and physiological states of strains, as well as differences in the formulations, techniques of processing, and ages of the frankfurters. Our data also revealed that potassium lactate as a frankfurter ingredient had a listeriostatic effect in vacuum-sealed packages of frankfurters during storage at both 4 and 10°C. The antilisterial activity was not due solely to the effect of pH, since the pH values within packages containing frankfurters formulated with potassium lactate were similar to the pH levels of those that did not contain added potassium lactate. Other investigators have also reported that calcium lactate and potassium lactate do not typically cause an appreciable decrease in pH in foods, whereas sodium lactate often does *(37).*

In summary, our data contribute to the growing body of evidence that vacuum-sealed frankfurters can provide a suitable environment for the growth and survival of *L. monocytogenes.* The extent of viability may depend, at least in part, on the initial pH, on the levels and types of LAB, and possibly to a more limited extent on the proximate composition of the product. As expected, pathogen numbers increased at a faster rate and to a higher level during storage at 10°C than during storage at 4°C, and this may or may not have been a consequence of a similar effect on the indigenous and competing population of LAB. More important, the results of this study validate the antilisterial contributions of potassium lactate as an ingredient in an RTE meat. Given the heat resistance of lactates in general,

this may be a facile and cost-effective strategy, particularly for relatively small manufacturers, for enhancing the safety of small-diameter sausages. Future studies will focus on optimization of the levels of potassium lactate required to yield both a desirable flavor and an appreciable antilisterial effect. We will also chronicle the clonality and succession of LAB within vacuum-sealed packages in addition to screening such isolates for direct antagonism toward *L. monocytogenes.*

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ERRATA

In the article "A Multiplex Polymerase Chain Reaction for the Differentiation of *Campylobacter jejuni* and *Campylobacter coli* from a Swine Processing Facility and Characterization of Isolates by Pulsed-Field Gel Electrophoresis and Antibiotic Resistance Profiles," *Journal of Food Protection* 65(2):266–273, for the third primer set in Table 1, C-4 needs to be added to the primer name so it reads C-1 and C-4 and the product size should read 159 instead of 160 base pairs. Also, on page 267 under "Multiplex PCR analysis," the primer C-4 should be substituted for C-2 so that the sentence reads ". . . primers cadF2B, cadR1B, COL1, COL2, C-1, and C-4 (Table 1). . . ."

In the article "Viability of a Five-Strain Mixture of *Listeria monocytogenes* in Vacuum-Sealed Packages of Frankfurters, Commercially Prepared with and without 2.0 or 3.0% Added Potassium Lactate, during Extended Storage at 4 and 10°C," *Journal of Food Protection* 65(2):308–315, in the "Materials and Methods" section, strain MFS-2 of *Listeria monocytogenes* is listed as being a serotype 1/2c strain. This determination was made by a private testing facility that subsequently informed us that they made a mistake and that MFS-2 is actually a serotype 1/2a strain. Since we have continued to use/publish results with strain MFS-2, we feel it is appropriate and essential to print an erratum so that anyone requesting this strain or relying on our results for their own purposes will have the most current and correct information.