Combining Pediocin (ALTA 2341) with Postpackaging Thermal Pasteurization for Control of *Listeria monocytogenes* on Frankfurters

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MS 03-571: Received 19 December 2003/Accepted 28 March 2004

ABSTRACT

Frankfurters packaged in 1-link, 5-link, or 10-link packages were surface-inoculated with a five-strain mixture of *Listeria monocytogenes* (3.40 or 5.20 log CFU/g) after treatments with 3,000 arbitrary units (AU) or 6,000 AU pediocin (in ALTA 2341) per link. The frankfurters were vacuum packaged, after which the packages were heated in hot water at 71, 81, or 96°C for 30, 60, or 120 s. *L. monocytogenes* was enumerated following the treatments. Selected treatments were subsequently evaluated during storage at 4, 10, and 25°C for up to 12 weeks. *L. monocytogenes* was reduced by all treatments, but 81°C or more for at least 60 s in combination with pediocin (Pdn-6000) was necessary to achieve a 50% reduction of initial inoculations. Heat treatments were most effective for 1-link packages and least effective for 10-link packages. Little or no growth of *L. monocytogenes* occurred on frankfurters for 12 weeks at 4 or 10°C, and for 12 days at 25°C. Generally, the treatments mentioned above did not significantly (*P* > 0.05) affect the sensory qualities of frankfurters. Therefore, pediocin (in ALTA 2341) in combination with postpackaging thermal treatment offers an effective treatment combination for improved control of *L. monocytogenes* on frankfurters.

Postprocessing contamination of ready-to-eat processed meat products by *L. monocytogenes* represents a serious health risk (6, 7) and has become a major concern for the meat processing industry (20). Because *L. monocytogenes* is widespread and difficult to avoid before or during packaging (4), additional processing treatments in the form of prepackaging or postpackaging technologies may be necessary to control the growth of this pathogen and to enhance the safety of these products during storage (3).

Samelis et al. (20) emphasized that *L. monocytogenes* can resist many food preservation approaches, colonize in meat plants, and survive under unfavorable conditions. *L. monocytogenes* has often been isolated from floors, drains, cleaning aids, walls, ceilings, and other critical control point areas in food processing facilities (20, 24). Unfortunately, sanitation strategies and practices applied in plants are often insufficient to prevent contamination and growth of *L. monocytogenes* on processed meat products (4, 20). Extensive efforts to control *L. monocytogenes* can reduce the level of contamination, but eradication from the processing environments or from all finished products is not considered possible (4). Several studies have reported that meat products that tested positive for *L. monocytogenes* at retail stores harbored the pathogen typically at levels of $10^3$ CFU/g or less (11). Thus, postpackaging hurdle technologies (13) that will reduce *L. monocytogenes* numbers by similar amounts are needed to control *L. monocytogenes* in meat products during storage.

The problem of *L. monocytogenes* contamination typically develops from postthermal process contamination because the pathogen is relatively heat susceptible and is normally killed by typical thermal processes used for ready-to-eat processed meats. Thus, this pathogen may be inactivated by relatively mild heat treatments without negatively impacting the product quality. This suggests that *L. monocytogenes* on ready-to-eat meats may be effectively reduced by postpackaging thermal pasteurization (PPTP). Roering et al. (18) concluded that thermal pasteurization, at temperatures of 77°C or greater for at least 60 s, was sufficient to eliminate appreciable numbers of *L. monocytogenes* in summer sausage, regardless of other factors. Because *L. monocytogenes* is more heat resistant than *Salmonella* spp. and *Escherichia coli* O157:H7 (10, 12), pasteurization treatments for *L. monocytogenes* may have additional benefits, for example, reducing levels of *E. coli* O157:H7 or *Salmonella* spp. on processed meat products.

Samelis et al. (21) suggested that PPTP also may increase the antilisterial effects of antimicrobial agents in cooked cured meats. Because initial contamination levels of *L. monocytogenes* are typically low, a 1- to 2-log reduction of the surface contaminants on commercially manufactured products may be adequate to completely inactivate or reduce the pathogen populations to undetectable levels. At low population levels, heat-stressed cells are less likely to recover and thus become more susceptible to other antimicrobial treatments (19).

Pediocin has been studied in a variety of meat applications (14, 16, 17), including frankfurters (5, 8, 9), to re-
duce or inhibit the growth of \textit{L. monocytogenes}. Spraying or dipping of products in antimicrobial (pediocin) solutions before packaging (21) combined with PPTP treatments (18) could enhance overall \textit{L. monocytogenes} control. The results from a study by Samelis et al. (19), for example, indicated a limited effectiveness of PPTP treatments against \textit{L. monocytogenes} in the absence of antimicrobials. This may have significant practical implications for processors, suggesting that processors should not rely solely on one method for \textit{L. monocytogenes} control without evaluation of its effectiveness for different products.

Postprocessing application of antimicrobials directly on the product surface where \textit{L. monocytogenes} cells may be present may be more advantageous than addition to the formulation (21). In addition to the potential for increased antilisterial effects, surface application of antimicrobials may have less potential for negative effects on the sensory quality of meat products when applied to the surface as opposed to the larger quantities generally required for formulation as part of the product.

The first objective of this study was to assess the inhibitory effectiveness of the commercially available ALTA 2341 in combination with PPTP treatments on \textit{L. monocytogenes} on the surface of frankfurters in 1-link, 5-link, and 10-link packages. ALTA 2341 is a fermentation product available from Quest International (Sarasota, Fla.) that has significant pediocin activity. The second objective was to assess inhibitory effects of selected antimicrobial treatments during storage at 4, 10, and 25°C for up to 12 weeks. Finally, physical, chemical, and sensory properties of frankfurters subjected to selected antimicrobial treatments were evaluated to determine the effects of the treatments on product quality.

\section*{MATERIALS AND METHODS}

\textbf{The experimental protocol.} The experimental protocol was designed to first compare the effects of ALTA 2341 concentration and PPTP treatments on inhibition of \textit{L. monocytogenes} on frankfurters in different packaging arrangements. The most effective combinations were then evaluated for inhibitory effects during storage at 4, 10, and 25°C and for product quality changes.

\textbf{Frankfurter preparation.} Frankfurters were manufactured from frozen beef trim (~80% lean) and fresh pork trim (~50% lean) purchased from commercial suppliers. Frozen beef (~20°C) was tempered at 2 to 4°C for 24 h before processing. All trimmings were coarse ground (0.95-cm plate) and fat content measured by Amyl-Ray (Kartridg-Pak Co., Davenport, Iowa). Frankfurter batches were formulated using a 45.4-kg meat block, including 22.7 kg of beef trim and 22.7 kg of pork trim, plus 9.1 kg of ice water, 1.49 kg of a commercial spice (A.C. Legg Packing Co., Inc., Birmingham, Ala.), 908 g of salt, and 112 g of curing salt (sodium nitrite and sodium chloride) (A.C. Legg Packing Co.).

The lean beef trim was chopped first with ice water, salt, spices, and cure to 4.5°C, after which the fat pork trim was added and chopping continued to 13.9°C to form the batter. The frankfurter batter was vacuum stuffed (Risco Model RS 4003-165; Stoughton, Mass.) into 24-mm cellulose casings and linked at 12 to 14 cm in length. Stuffed frankfurters were smoked, cooked to internal temperature of 71.1°C, and showered in an Alkar oven (Alkar, Lodi, Wis.). Chilling was achieved at 2°C overnight before peeling. Finished, peeled frankfurters were stored at 2°C for 12 to 18 h while packaging and inoculation preparations were done and before random assignment to different experimental treatments. Finished weight of frankfurters was 45 ± 1 g per link. Fat, moisture (1), and protein contents (2) of finished, chilled products were measured.

\textbf{Inoculation and packaging.} Frankfurters were packaged in vacuum bags using three different packaging arrangements as follows: (i) 1 link per package, (ii) 5 links per package in a single row, and (iii) 10 links per package in a double row of 5 each. Each packaging group was divided into two subgroups for treatment with one of two levels of ALTA 2341 (Quest Int.) before inoculation with \textit{L. monocytogenes}. ALTA 2341 was formulated at 40% (w/v) in sterile, distilled water to achieve 3,000 arbitrary units (AU) of pediocin per ml. The AU was determined by placing 5 μl each of a series of dilutions on indicator plates inoculated with \textit{L. monocytogenes}, Scott A. Briefly, Trypticase soy agar (TSA) plates were surface inoculated with \textit{L. monocytogenes}. The pediocin solution was sequentially diluted and “spotted” on to the inoculated plates. After the solution was absorbed by the agar, a second layer of TSA was layered over the original agar. Plates were then incubated at 37°C. One AU was defined as the inverse of the highest dilution that produced an inhibition zone of greater than 2 mm on the indicator plates (23).

To apply the ALTA 2341, frankfurters were placed in bags, then sprayed with either 1 or 2 ml of ALTA 2341 suspension per link. This was equivalent to 3,000 AU or 6,000 AU pediocin per link (Pdn-3000 or Pdn-6000). The vacuum bags (Cryovac B-2540, Cryovac Sealed Air Corp., Duncan, S.C.; water vapor transmission = 0.5 to 0.6 g/100 in² at 100°F; 100% relative humidity [RH] in 24 h; oxygen transmission rate = 3 to 6 cm³/m² at 40°F; 0% RH in 24 h) with frankfurters and ALTA 2341 were hand massaged for 5 to 10 s for even distribution of the solution before vacuum sealing (Multivac A 300/52, Multivac Sepp Haggenmüller GmbH & Co., Wolfertschwenden, Germany).

For inoculation of the frankfurters, a five-strain cocktail mixture of \textit{L. monocytogenes} cultures including \textit{L. monocytogenes} Scott A, H7764 1/2a, H7969 4b, H7962 4b, and H7762 4b was used. With the exception of the Scott A strain, all strains were obtained as clinical isolates from the Bil Mar Foods outbreak of 1998 and 1999 (CDC, Atlanta, Ga.). The cultures were individually grown in Trypticase soy broth plus 0.6% yeast extract (Difco, Becton Dickinson and Co., Sparks, Md.) at 35°C for 24 h. Then, 1 ml of culture from each individual strain was combined to give 5 ml of mixed culture of \textit{L. monocytogenes}. The mixed culture was transferred to 500-ml Trypticase soy plus 0.6% yeast extract broth and incubated at 35°C for 24 h to reach the stationary phase.

Inoculation of frankfurters was done by adding 1 ml per link from the stationary-phase five-strain mixture of \textit{L. monocytogenes} (9.30 log CFU/ml) or of a 1:100 (7.30 log CFU/ml) dilution of the five-strain mixture to result in a high (5.2 log CFU/g) or low (3.4 log CFU/g) inoculation level. The inoculum was added to the frankfurters after they were placed in the appropriate package, after addition of ALTA 2341, and before the package was sealed. The actual product inoculation level was determined by counting the cells recovered from control packages immediately after inoculation. Packages of inoculated frankfurters without addition of ALTA 2341 served as controls.

After inoculation, the packages were hand massaged for 5 to 10 s to evenly distribute the inoculum on the surface of each frankfurter, then vacuum sealed. After vacuum packaging, all frankfurters were stored at 2 to 4°C for 14 to 18 h before sampling.
**PPTP treatments.** Packages of inoculated frankfurters were heated by immersion in water at 71°C (±1°C), 81°C (±1°C), or 96°C (±2°C), each for 30, 60, or 120 s. A water bath (Fisher Isotemp-220, Fisher Scientific, Pittsburgh, Pa.) and a Fisher immersion circulator (model 730, Fisher Scientific) were used to heat the water prior to immersion and to maintain water temperature. Twelve packages were immersed as a group for each heating treatment. Preliminary experiments determined that up to 12 packages could be immersed for a treatment without affecting water temperature by more than 2°C. Water temperature was monitored throughout the process. Packages were held in heated water for the prescribed time, then immersed in 15°C water for 5 to 10 min to chill before placement in refrigerated storage at 2 to 4°C for 14 to 18 h.

**Initial microbiological evaluation of treatments.** Packages of frankfurters were aseptically opened, using sterile scissors, 18 h after treatments. Except for 1-link packages from which one frankfurter was used, samples were collected by taking two frankfurters, one from the center of the package row and one from the outside. For 10-link packages, these two frankfurters were taken from different rows. Frankfurters were aseptically cut in half (ca. 20- to 23-g portions) with sterile scissors and tweezers, combined and homogenized (Seward Stomacher blender, model 4000, Tekmar Co., Cincinnati, Ohio) at normal speed for 2 min in sterile stomacher bags (Whirl-Pak Filter Bag B01318, A Nasco, Ft. Atkinson, Wis.) with sufficient 0.1% sterile peptone water to give a 1:5 dilution of the sample. Three samples (triplicates) were prepared from 1-link and multiple-link packages for each analysis. *L. monocytogenes* cells were enumerated by serially diluting 1 ml of the blended sample in 9 ml of 0.1% peptone water, then plating 0.1 ml of the dilution on modified Oxford (MOX) (Difco) agar plus 0.1% Oxford antimicrobic supplement. Samples were also plated on TSA (Difco). Incubation of all plates was at 35°C for 48 h. Typical *L. monocytogenes* colonies were enumerated, identified by Gram stain, and confirmed using Analytical Profile Index *Listeria* kits (BioMérieux, Inc., Hazelwood, Mo.). The lowest detection limit with these procedures was 0.3 CFU/g.

**Evaluations during storage.** Based on the initial evaluation of treatments, those treatments that showed the greatest immediate inhibitory effectiveness were selected for subsequent evaluation of long-term effectiveness during storage. Treatments that were selected included ALTA 2341 alone at 6,000 AU (Pdn-6000), in combination with PPTP treatments of 81°C for 60 s, 96°C for 60 s, or 96°C for 120 s. The inoculation level used for storage evaluations was 3.40 log CFU/g of the same five-strain mixture of *L. monocytogenes* used for the initial evaluation. Samples were stored at 4, 10, and 25°C for up to 12 weeks. Untreated samples surface-inoculated with 3.40 log CFU/g served as control samples. All package sizes (1-link, 5-link, and 10-link packages) were included in the storage time evaluation. Comparison of package size effects on the inhibition of *L. monocytogenes* was studied using the 96°C for 120-s PPTP treatment.

**Microbiological evaluations during storage.** For enumeration of samples held in storage, packages were opened aseptically and whole frankfurters were mixed for 1 min in a Seward Stomacher blender with an equal amount of 0.1% peptone water (50% dilution) to rinse them thoroughly. *L. monocytogenes* cells were enumerated as previously described except that diluted samples were plated on both MOX agar and TSA plus 0.6% yeast extract (TSAYE; Difco). When increased sensitivity was required, 1.0-ml samples of the rinse were plated directly onto MOX or TSAYE. When using 1.0-ml samples, 0.33 ml was plated onto each of three plates.

**Physical and chemical analyses of frankfurters.** Un inoculated frankfurters were used for measurement of treatment effects on purge, color, texture, odor quality, pH, and thiobarbituric acid reactive substances (TBARS). For purge accumulation, two packages were each weighed and opened and the frankfurters removed. The packages and links were wiped dry and reweighed. The weight difference was calculated as purge and expressed as a percentage of unpackaged product weight.

Color measurements, using Commissie Internationale d’Eclairage (CIE) L*a*b* designations for lightness, redness, and yellowness, respectively, were made with a Hunter Labscan spectrophotometer (Hunter Associates Laboratory, Inc., Reston, Va.), with illuminant A and 10° observer (incandescent light) with a 0.635-cm port insert. Samples were overwrapped with clear film, and surface color was measured at two locations (center and end) of each frankfurter. Five links from each treatment were measured for color. For texture measurement, a texture analyzer (Stable Micro Systems, Model TA.XT2i, Godalming, U.K.) was used for assessment of skin toughness and interior firmness. Puncture resistance and interior texture was measured with a 3-mm puncture probe. Five frankfurters from each treatment were measured in the center and the end of each link. The probe was programmed to penetrate 12 mm into the samples following measurement of the surface skin resistance. Penetration speed was 1.5 mm/s. All samples were measured at room temperature 3 h after removal from the refrigerator.

For pH measurement, 10 g of sample was blended with 90 ml of distilled water in a Waring blender and the slurry measured with a pH meter (Fisher Accumet Model 925, Fisher Scientific) using a sealed combination electrode (Omega Engineering, Inc., Stamford, Conn.). TBARS values were measured using the modified method for cured meats (25) and expressed as milligrams of malonaldehyde per kilogram of sample. Duplicate measurements of pH and TBARS values were recorded for each sample.

**Sensory evaluation.** Sensory evaluations were conducted using a panel of 16 trained panelists, all being students, staff, or faculty in the Department of Food Science and Human Nutrition at Iowa State University. All panelists were volunteers and were trained by using commercial frankfurters for the odor notes and terminology to be measured and the scale used. Only uninoculated samples were used for sensory evaluation. Panelists evaluated samples for purge, color, texture, and odor using 15-cm unstructured line scale. Fluorescent-lighted booths were used for sample presentation to panelists.

The amount of purge and external color was scored using intact, unopened packages of frankfurters. For the texture assessment, the panelists used the edge of a dinner fork to cut a cross-section thorough the center of a frankfurter. For odor evaluation, frankfurters were heated in boiling water for 2 min, cut into sections, and placed in 150-ml covered containers before presentation to the panel. Panelists evaluated odor for smoky, burnt, and acidic traits, all of which were established during the training session. The numeric scales used for sensory intensity of purge, color, texture, and odor were described as 0 = none, extremely light, extremely soft, or none, respectively; while 15 = extremely abundant, dark, firm, or intense, respectively. All sensory evaluations were conducted within 2 weeks of the product manufacturing date and were repeated three times.

**Statistical analyses.** Microbiological data were transformed into logarithms of the number of CFU (log CFU/g). The statistical
FIGURE 1. Survival of *Listeria monocytogenes* and aerobic bacteria on the surface of frankfurters treated with pediocin (in ALTA 2341) and postpackaging thermal pasteurization (PPTP). (A) *L. monocytogenes* counts on MOX agar (3.40 log CFU/g inoculation), (B) aerobic plate counts on TSA (3.40 log CFU/g inoculation), (C) *L. monocytogenes* counts on MOX agar (5.20 log CFU/g inoculation), (D) aerobic plate counts on TSA (5.20 log CFU/g inoculation). Control (untreated) frankfurters resulted in 3.60 log CFU/g and 5.50 log CFU/g after 24 h at 4°C, respectively, for the two inoculation levels. Pdn-3000: Frankfurters (1, 5, and 10 link[s] per package) treated with 3,000 AU pediocin per link. Pdn-6000: Frankfurters (1, 5, and 10 link[s] per package) treated with 6,000 AU pediocin per link. 0.00 log CFU/g represents an undetectable (lowest detection limit of 0.3 CFU/g) number of *L. monocytogenes*.

Statistical analyses for the initial evaluation of treatments. Data from the initial evaluation of treatments were treated as a split, split-plot design with *L. monocytogenes* inoculation levels and packaging types as the main plot, ALTA 2341 concentrations as the split plot, and combinations of pasteurization temperature and heating times as the split-split plot. Duplicate samples were analyzed for each treatment in the three replications. All data were analyzed using SAS with the general linear model procedure. Comparisons of means were based on Tukey’s range test for least significant differences.

Statistical analyses for evaluations during storage. The data from the evaluations during storage were analyzed as a split plot with eight treatments in the main plot and 11 to 13 sampling dates in the subplot. The sampling dates were either day 0 to day 10 or week 0 to week 12 depending on storage temperature. For the storage study, one sample was analyzed for each treatment combination in each of the three replications of the experiment. All data were analyzed using SAS with the general linear model procedure. Comparisons of means were based on Tukey’s range test for least significant differences.

RESULTS AND DISCUSSION

Composition. The results from composition analyses showed that the frankfurters contained 28.25% crude fat, 57.66% moisture, and 13.44% protein.

Initial inhibitory effects of treatments. No *L. monocytogenes* and very few (less than 1 log CFU/g) aerobic bacteria were detected by culture enrichment on uninoculated frankfurters. Addition of ALTA 2341 (Pdn-3000 or Pdn-6000) reduced counts on samples inoculated with 3.40 log CFU/g by ca. 1.5 to 1.8 log, and the thermal pasteurization resulted in further reductions of 1.5 to 3.0 log and 1.6 to 3.4 log, respectively. For the high inoculum (5.20 log CFU/g), addition of ALTA 2341 (Pdn-3000 or Pdn-6000) reduced counts by ca. 1.6 to 2.1 log. In this case, the thermal pasteurization delivered additional effects to reach ca. 1.9 to 5.1 log and 2.3- to 5.2-log reduction in numbers, respectively, at the two ALTA 2341 concentrations. Survival of *L. monocytogenes* on inoculated frankfurters, treated with ALTA 2341 and PPTP, is shown in Figure 1. For frankfurters with the lower initial inoculation level (3.40 log CFU/g), shown in Figure 1A, *L. monocytogenes* populations were reduced by 1.5 to 3.4 log CFU/g depending on the type of package. The inhibitory effect of ALTA 2341 was significant (*P* < 0.05), regardless of the other treatments. Treatment combinations (ALTA 2341 plus PPTP)
resulted in greater reduction of \textit{L. monocytogenes} counts than for ALTA 2341 only treatments, regardless of package size (8).

Under the same conditions, frankfurters that were surface inoculated with the higher initial level of \textit{L. monocytogenes} (5.20 log CFU/g) showed reduction of \textit{L. monocytogenes} (Fig. 1C) of 1.9 to 5.2 log CFU/g. The reduction achieved by the combined treatments was again greater than that for samples treated with ALTA 2341 only (8).

\textbf{Inhibitory effects of PPTP on \textit{L. monocytogenes}.} The exposure of inoculated packages to hot water at 71, 81, or 96°C for 30, 60, or 120 s resulted in a wide range of inactivation effectiveness, regardless of other factors (Fig. 1). With an inoculation of 3.40 log CFU/g (Fig. 1A), all treatments resulted in at least a 1.4-log reduction of \textit{L. monocytogenes}. The 1-link packages showed approximately a 3.0-log reduction except for the 30-s treatments at 71 and 81°C. Twelve of the eighteen treatment combinations shown in Figure 1A for 1-link packages reduced \textit{L. monocytogenes} to undetectable levels.

The 5-link packages showed a \textit{L. monocytogenes} reduction of 1.5 to 3.0 log with increased effectiveness when pasteurization temperature or heating time was increased. While the effects of increased ALTA 2341 concentration were significant ($P < 0.05$), the difference between 3,000 AU and 6,000 AU was not large. For 10-link packages, the population reduction by PPTP of \textit{L. monocytogenes} was 1.4 to 2.2 log. There was less impact of increasing pasteurization temperature or heating time for packages with 10 links than observed for the other package sizes.

For packages inoculated with 5.20 log CFU/g (Fig. 1C), results were similar to those observed for the 3.40 log CFU/g inoculation. All treatments resulted in at least a 1.9-log reduction of \textit{L. monocytogenes}. The 1-link packages showed nearly a 5.0-log reduction of \textit{L. monocytogenes} at the higher temperature (81°C or greater), and the effects were more complete with the greater concentration of ALTA 2341. Samples immersed in hot water at 96°C for at least 60 s or at 81°C for 120 s in combination with Pdn-6000 resulted in an undetectable level of \textit{L. monocytogenes}. The 5-link packages showed ca. a 3.0-log reduction of \textit{L. monocytogenes} at the most effective temperature (96°C). The effects of heating time were significant for 5-link packages but less marked than in the case of 1-link packages. The effect of heating time and ALTA 2341 concentration was again significant ($P < 0.05$), but none of the treatments achieved a complete (5.2-log) reduction of \textit{L. monocytogenes}. For 10-link packages, the population reduction of \textit{L. monocytogenes} was 2.9 log, at best. The effectiveness of increased pasteurization temperature and increased heating time was lessened by the larger package.

The impact of increased pasteurization temperature was generally greater than the impact of increased heating time within the limits of this study. Regardless of other factors, there was a significant ($P < 0.05$) difference between 71 and 96°C pasteurization temperatures. The 81°C pasteurization temperature did not differ ($P > 0.05$) from either 71 or 96°C. It was necessary to hold samples at 81 or 96°C for at least 60 s to achieve a 50% reduction of initially inoculated \textit{L. monocytogenes} numbers, while at 71°C at least 120 s was required to reach a 50% reduction of \textit{L. monocytogenes}. At the high inoculation level of \textit{L. monocytogenes} (5.20 log CFU/g), most of the treatments resulted in some survival. However, samples treated with Pdn-6000 in 1-link packages and immersed in hot water at 81°C for at least 120 s or at 96°C for at least 60 s (Fig. 1C) reduced the high inoculum to an undetectable level.

\textbf{Effect of package sizes on \textit{L. monocytogenes}.} PPTP treatments were clearly most effective ($P < 0.001$) in 1-link packages (Fig. 1A and 1C), followed by 5-link packages. The 10-link packages resulted in the greatest ($P < 0.001$) \textit{L. monocytogenes} survival. For frankfurters in 1-link packages, the pathogen numbers were reduced by 3.0 log or more for all but the two least severe time–temperature combinations. Frankfurters in 5-link packages, exposed to 81°C for at least 60 s or 96°C for at least 30 s, achieved at least a 2.0-log reduction in pathogen populations. The numbers of \textit{L. monocytogenes} on frankfurters in 10-link packages were reduced by about 1.5 to 2.5 log CFU/g, at best, even when a high ALTA 2341 concentration (Pdn-6000) was combined with pasteurization at 96°C for at least 60 s. The population reduction reported by Muriana et al. (15) for PPTP was slightly less than that for frankfurters in 10-link packages from our study. Muriana et al. (15) suggested that \textit{L. monocytogenes} cells (~10^7 CFU/g) infiltrated irregular areas on product surfaces, such as surface cuts, folds, and grooves, and were protected as a result. The package size is an important consideration for PPTP inactivation of \textit{L. monocytogenes} on frankfurters. Samelis et al. (19) reported that 1-link frankfurters in packages heated at 75 or 80°C for 90 s provided immediate reduction of the pathogen. Counts remained lower (2.1 log) than the initial inoculation level (3.9 log) of the unheated controls throughout 50 days of storage at 4°C. Our results were similar for 1-link packages. However, in our study, reduced effectiveness was observed for treatments of the larger multiple-link packages when interfacial surface areas shielded cells from the heat process. The results from our study indicated that there were significant ($P < 0.05$) differences between 1-link packages and 10-link packages. This difference was more evident with increased pasteurization temperature, heating time, and ALTA 2341 concentration. Most sample packages showed more survivors in samples at the center area of the package compared with those from the outside area (data not shown). It is very clear that the greater interfacial areas in 10-link packages decreased the heat exposure for the organism and created more space to shield or protect the pathogen from effects of heating.

\textbf{Effect of treatment combinations on \textit{L. monocytogenes}.} The results indicated greater reduction of \textit{L. monocytogenes} with the higher pasteurization temperatures, longer heating time, and fewer frankfurters in the packages. With greater initial numbers of cells, longer heating time was necessary to cause complete destruction, as might be expected. Pasteurization temperatures and package size had greater effects than heating time.
Aerobic plate counts for aerobic bacteria on TSA (Fig. 1B and 1D), resulted in similar survival patterns as observed with MOX agar. Most of the TSA counts were slightly higher than those on MOX agar. The colonies grown on TSA plates were predominantly *L. monocytogenes*, as suggested by uniform appearance on both agars, typical of *Listeria* spp., though other organisms are included on TSA agar. However, some counts on TSA in these initial experiments were slightly lower than those on MOX agar. Therefore, we decided to use TSA plus 0.6% yeast extract for the remaining experiments, instead of TSA alone to improve the recovery of *L. monocytogenes*, particularly injured cells, during product storage.

**Comparison of growth curves during storage at 4°C.** The data in Figure 2 show survival and growth of *L. monocytogenes* on treated frankfurters stored at 4°C. In Figure 2A, it is clear that ALTA 2341 alone, at either level, achieved an initial reduction of *L. monocytogenes* counts as noted previously. The reduction was greater when ALTA 2341 was combined with heating treatments. No marked increases in populations of the control or the treated samples occurred for at least 7 weeks at 4°C. After week 7, the counts for most of the samples appear to slowly increase. However, only the control samples resulted in a significant (P < 0.05) increase after 7 weeks.

Figure 2C shows results for 5-link packages only, treated with 6,000 AU of ALTA 2341 and selected PPTP treatments, relative to the control. The selected treatments were more (P < 0.05) effective than ALTA 2341 alone (Fig. 2A) during storage at 4°C. The growth curves for the populations following these three treatment combinations did not differ greatly (P > 0.05). At week 0, the selected treatment combinations of ALTA 2341 and PPTP provided an immediate reduction of the *L. monocytogenes* numbers to 0.46 to 1.11 log CFU/g, compared to the control at 3.47 log CFU/g.

The pasteurization temperature at 96°C was significantly (P < 0.05) more effective than 81°C. However, there...
FIGURE 3. Survival and growth of *Listeria monocytogenes* (3.40 log CFU/g inoculation) and aerobic bacteria on the surface of frankfurters treated with pediocin (in ALTA 2341) combined with postpackaging thermal pasteurization (PPTP) and stored at 10°C. (A) All selected treatments listed in Figure 2 legend (*L. monocytogenes* on MOX agar), (B) all selected treatments listed in Figure 2 legend (aerobic plate counts on TSAYE), (C) treatments for frankfurters heated at 81 or 96°C for 60 or 120 s in 5-link packages (*L. monocytogenes* on MOX agar), (D) frankfurters in 1-link, 5-link, and 10-link packages heated at 96°C for 120 s (*L. monocytogenes* on MOX agar). For identification of treatments see key with Figure 2.

was no significant (*P* > 0.05) difference between 60 s and 120 s at 96°C. In general, pasteurization temperatures had a greater effect than heating times for PPTP treatments, as noted previously, and this effect continued during storage.

Comparison of the results from the different package sizes (1-link, 5-link, and 10-link packages) stored at 4°C is shown in Figure 2D. At week 0, there was an immediate reduction of *L. monocytogenes* counts to 0.46 to 0.80 log CFU/g, regardless of package size. Frankfurters in 1-link packages treated with the 96°C for 120-s treatment showed little or no subsequent growth throughout the 12-week storage period. The *L. monocytogenes* populations for all of the other treatments were able to recover and grow after 7 to 8 weeks. However, populations remained lower (maximum of 2.10 log CFU/g) throughout storage than the original inoculation level (3.47 log CFU/g) of the control.

Plating on either MOX agar or TSAYE produced comparable results leading to similar conclusions (Fig. 2A and 2B).

Comparison of growth curves during storage at 10°C. The data from Figure 3 show survival and growth of *L. monocytogenes* on treated frankfurters stored at 10°C. In Figure 3A, the selected treatments fall into two categories similar to results at 4°C. The combinations of ALTA 2341 and PPTP, in general, achieved greater (*P* < 0.05) initial reduction of *L. monocytogenes* counts than ALTA 2341 alone or the control. After delayed growth of about 2 weeks, most of the treated samples showed recovery and relatively rapid growth. Only the most severe PPTP treatment (96°C for 120 s) combined with ALTA 2341 showed growth suppression during 12 weeks of storage at 10°C.

Figure 3C again shows the results for 5-link packages. Growth appears to be delayed for about 2 weeks and populations steadily increased thereafter. There were significant (*P* < 0.05) differences at specific sampling times among these three treatments. The 5-link, 96°C, 120-s treatments had significantly (*P* < 0.05) lower counts than the 5-link, 81°C, 60-s treatment during the first 4 weeks. However,
after storage for 4 weeks at 10°C, there was no significant (P > 0.05) difference among these three treatments. There was no significant (P > 0.05) difference between the heating times (60 and 120 s) at 96°C throughout storage for 12 weeks.

Figure 3D compares the antilisterial effect of different package sizes stored at 10°C. The antilisterial effects observed for the 1-link packages were significantly (P < 0.05) greater than for 5-link and 10-link packages, which had significantly (P < 0.05) lower counts than the control. There was no significant (P > 0.05) difference between the 5-link and 10-link packages. Delayed growth of the pathogen on samples in the 1-link, 96°C, 120-s treatment is evident. There was a delay in growth of 3 to 4 weeks for 1-link packages compared to 1 to 2 weeks for the 5-link and 10-link packages. After storage for 3.5 weeks, the pathogen in 1-link packages still did not show a significant (P > 0.05) increase, though some survivors recovered resulting in highly variable subsequent counts. The variable counts probably indicate recovery of injured cells. The population did not exceed 2.31 log CFU/g throughout storage for 12 weeks and remained significantly (P < 0.05) lower than the other treatments. Samples in 5-link packages showed significantly (P < 0.05) fewer survivors than those in 10-link packages during the 1- to 2-week lag phase. Further, the antilisterial effects of treatments in both the 5-link and 10-link packages were evident (P < 0.05) compared to the growth of L. monocytogenes in control samples. The population in the treated 5-link and 10-link packages remained significantly (P < 0.05) lower than controls for up to 6 weeks.

The populations of L. monocytogenes on TSAYE (Fig. 3B) were again generally similar or slightly higher than those on MOX agar (Fig. 3A).

**Comparison of growth curves during storage at 25°C.** Survival and growth of L. monocytogenes on treated frankfurters stored at 25°C is shown in Figure 4. With storage at 25°C, L. monocytogenes grew within 2 to 3 days for all but one treatment (Fig. 4). In Figure 4A, the treatments that combined ALTA 2341 with PPTP resulted in greatest effectiveness relative to the control. The growth profile and treatment effects were generally similar at 25°C to those observed with storage at 4 and 10°C.

Figure 4C shows the antilisterial effectiveness in 5-link packages of the Pdn-6000 treatment and selected PPTP treatments during storage at 25°C. Only a slight delay in growth of the pathogen occurred on frankfurters during the first 1 or 2 days, and populations steadily increased thereafter. Throughout storage for 10 days, the populations of L.
Table 1. Comparison of physical and chemical analyses of frankfurters following selected treatments with pediocin (in ALTA 2341) and postpackaging thermal pasteurization (PPTP)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Purge (%)</th>
<th>Color (CIE)</th>
<th>Firmness (kg)</th>
<th>pH</th>
<th>TBA (mg MDA/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L*</td>
<td>a*</td>
<td>b*</td>
<td>Skin</td>
</tr>
<tr>
<td>Control</td>
<td>0.61 ± 0.25</td>
<td>44.98 ± 0.80</td>
<td>0.61 ± 0.03</td>
<td>0.64 ± 0.11</td>
<td>13.67 ± 0.98</td>
</tr>
<tr>
<td>Pdn-3000</td>
<td>3.33 ± 0.25</td>
<td>44.38 ± 0.80</td>
<td>0.37 ± 0.03</td>
<td>0.67 ± 0.11</td>
<td>13.35 ± 0.98</td>
</tr>
<tr>
<td>Pdn-6000</td>
<td>5.13 ± 0.25</td>
<td>42.93 ± 1.50</td>
<td>0.92 ± 0.03</td>
<td>0.67 ± 0.11</td>
<td>13.19 ± 0.98</td>
</tr>
<tr>
<td>PH-81-60</td>
<td>5.76 ± 0.25</td>
<td>42.85 ± 1.50</td>
<td>0.96 ± 0.03</td>
<td>0.67 ± 0.11</td>
<td>13.10 ± 0.98</td>
</tr>
<tr>
<td>PH-96-60</td>
<td>5.35 ± 0.25</td>
<td>42.72 ± 1.50</td>
<td>0.96 ± 0.03</td>
<td>0.67 ± 0.11</td>
<td>13.10 ± 0.98</td>
</tr>
</tbody>
</table>

a Means with different letters in the same column are significantly different (P < 0.05).

Monocytogenes on samples heated at 81°C appeared to be greater than those of samples heated at 96°C, but these differences were not significant (P < 0.05). Again, there was no significant (P > 0.05) difference at 96°C between 60- and 120-s treatments.

Figure 4D shows the comparison for antilisterial effectiveness of the different package sizes at 25°C. The 1-link package again clearly resulted in greater effectiveness for the PPTP treatment. Interestingly, in this case, the populations on samples in 5-link packages were somewhat greater than those in 10-link packages after day 0 and during storage.

As expected, growth of *L. monocytogenes* in 5-link and 10-link packages was much more rapid on frankfurters during storage at 25°C than at 4°C or 10°C. After storage for 3 to 5 days, the counts increased significantly (P < 0.05) to 3.02 to 4.01 log CFU/g, and within 6 to 10 days the populations reached a maximum level of 5.22 to 5.77 log CFU/g, respectively. Frankfurters in the 1-link packages remained below 2.75 log CFU/g throughout storage for 12 days.

The populations of *L. monocytogenes* measured on TSAYE (Fig. 4B) were again similar or slightly higher than those on MOX agar (Fig. 4A).

Storage temperature effects. As expected, growth of *L. monocytogenes* was more rapid on treated frankfurters stored at 10°C (Fig. 3A) for 12 weeks than on those stored at 4°C (Fig. 2A) and was most rapid at 25°C (Fig. 4A). The population reduction of the pathogen on treated frankfurters was similar following the heat treatments, but growth during storage was largely dependent on storage temperatures used in this study.

Product quality analyses. Based on the microbiological results, the most effective treatment combinations were evaluated for potential product quality changes that might be introduced by the treatments. The treatments selected for sensory evaluations included the frankfurters treated with pediocin alone and 6,000 AU of ALTA 2341 combined with heat at 81 or 96°C, each for 60 s. The 96°C treatment for 60 s was chosen because it provided equivalent antilisterial effectiveness as 96°C for 120 s and would be more practical to implement.

Effects of selected treatments on quality of frankfurters. The results for purge, color, texture, pH, and oxidative change (TBARS values) are presented in Table 1. Color values of the frankfurters were modified by the PPTP treatments (Table 1). In this study, the pasteurization treatments resulted in color that was darker (L* value) and redder (a* value) than controls. These differences were not large but were statistically significant. Texture characteristics, either exterior skin toughness or interior firmness, were unaffected by any of the treatments. There was no significant (P > 0.05) difference for Hunter b* values or TBARS values. The results showed that pH values were also significantly influenced by addition of ALTA 2341, although the decrease in pH was small.
TABLE 2. Sensory evaluation of frankfurters following selected treatments with pediocin (in ALTA 2341) and postpackaging thermal pasteurization (PPTP)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Purge</th>
<th>Color</th>
<th>Texture</th>
<th>Aroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.29 ± 0.17 x</td>
<td>5.98 ± 0.55 x</td>
<td>7.10 ± 0.95 x</td>
<td>7.04 ± 0.50 x</td>
</tr>
<tr>
<td>Pdn-3000</td>
<td>5.41 ± 1.73 y</td>
<td>6.99 ± 1.67 x</td>
<td>8.12 ± 1.03 xy</td>
<td>7.46 ± 0.41 x</td>
</tr>
<tr>
<td>Pdn-6000</td>
<td>6.19 ± 0.39 x</td>
<td>8.10 ± 1.42 x</td>
<td>9.50 ± 1.14 x</td>
<td>7.41 ± 0.53 x</td>
</tr>
<tr>
<td>PH-81-60*</td>
<td>8.54 ± 0.63 x</td>
<td>8.06 ± 0.54 x</td>
<td>9.68 ± 0.51 y</td>
<td>6.60 ± 0.64 x</td>
</tr>
<tr>
<td>PH-96-60*</td>
<td>8.64 ± 0.36 x</td>
<td>7.77 ± 0.39 x</td>
<td>9.50 ± 0.69 y</td>
<td>7.36 ± 0.64 x</td>
</tr>
</tbody>
</table>

* The numerical scales of sensory intensity use for purge, color, texture, aroma of smoky, burnt, and acidic were 0 = none, extremely light, extremely soft, and none, respectively; and 15 = extremely abundant, dark, firm, and intense, respectively.

Effects of pasteurization temperatures and times on purge accumulation of frankfurters. There was no effect of treatment on package purge except for the volumes of solutions added to packages (Table 1). The addition of ALTA 2341, for example, at 1 or 2 ml per frankfurter resulted in a significant increase in the water measured as purge. The purge accumulation for samples treated with 6,000 AU of ALTA 2341 and PPTP was 5.35% to 5.76% compared to control samples at 0.61%. However, the PPTP treatments did not increase the amount of measured purge over that of packages with ALTA 2341 (Pdn-6000) that did not receive a postpackaging heat treatment. On the other hand, it was clear that the larger packages accumulated greater (P < 0.05) purge (data not shown). The 10-link packages had the greatest purge, followed by the 5-link packages. The singly packaged frankfurters provided the least purge. These results indicate that the purge accumulation observed in inoculated samples after the treatments was primarily from addition of ALTA 2341 and L. monocytogenes inoculum, not from the frankfurters themselves.

Effects of selected treatments on sensory quality of frankfurters. Sensory panelists evaluated frankfurters for visual purge, color, texture, and specific preselected aroma notes. The results are shown in Table 2. The purge assessment again reflects the volume of added solutions. The control was significantly (P < 0.05) lower for apparent purge than the treatments because there was no added solution. There was no significant (P < 0.05) difference in purge as a result of the PPTP treatments.

Color scores (Table 2) seemed lower for the control group compared with all treatments, but this was not significant. Odor scores, evaluated as smoky, burnt, and acidic aroma, did not differ. The texture assessments by the sensory panel indicated increased force was necessary to cut the frankfurters with the edge of a fork in the case of the PPTP treatments (Table 2). The addition of ALTA 2341 also increased the product resistance to cutting and was significant at the higher level of addition (Pdn-6000). These textural effects were somewhat surprising, and a potential explanation is not clear. Instrumental evaluations of texture, however, showed no significant (P < 0.05) differences among the treatments or between the treatments and the control for either skin toughness or interior firmness of the frankfurters (Table 1). Except for the purge accumulation, panelists could not detect any other significant (P > 0.05) differences between samples after treatments. The results showed that the PPTP treatments evaluated did not appreciably alter the scores for color (7.77 to 8.06), texture (9.50 to 9.68), or aroma, which included smoky (6.60 to 7.36), burnt (1.65 to 2.30), and acidic (4.78 to 5.21) notes compared to control samples.

This study has effectively demonstrated that using PPTP and pediocin in ALTA 2341 provides a means to control L. monocytogenes on ready-to-eat processed meat products, such as frankfurters. Overall effects of the antilisterial treatments subjected to the three holding temperatures can be summarized as follows: while the initial reduction in L. monocytogenes numbers depended on the addition of ALTA 2341 and the thermal treatment, the length of the subsequent lag phase was dependent on the storage temperatures. Thermal pasteurization effects were, as noted earlier, very dependent upon package size with the order of effectiveness being 1-link > 5-link > 10-link packages. Temperature of the PPTP, particularly at 96°C, was more important than time for the pasteurization conditions studied.

ACKNOWLEDGMENTS

Support for this project from the American Meat Institute Foundation and Quest International (ALTA 2341) is gratefully acknowledged. This journal paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Projects 3594 and 3700, was supported by Hatch Act and State of Iowa funds.

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