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Postpackage Pasteurization of Ready-to-Eat Deli Meats by Submersion Heating for Reduction of *Listeria monocytogenes*†

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ABSTRACT

A mixed cocktail of four strains of *Listeria monocytogenes* was resuspended in product purge and added to a variety of ready-to-eat (RTE) meat products, including turkey, ham, and roast beef. All products were vacuum sealed in shrink-wrap packaging bags, massaged to ensure inoculum distribution, and processed by submersion heating in a precision-controlled steam-injected water bath. Products were run in pairs at various time-temperature combinations in either duplicate or triplicate replications. On various *L. monocytogenes*–inoculated RTE deli meats, we were able to achieve 2- to 4-log cycle reductions when processed at 195°F (90.6°C), 200°F (93.3°C), or 205°F (96.1°C) when heated from 2 to 10 min. High-level inoculation with *L. monocytogenes* ($\sim 10^7$ CFU/ml) ensured that cells infiltrated the least processed surface areas, such as surface cuts, folds, grooves, and skin. *D*- and *z*-value determinations were made for the *Listeria* cocktail resuspended in product purge of each of the three meat categories. However, reduction of *L. monocytogenes* in product challenge studies showed much less reduction than was observed during the decimal reduction assays and was attributed to a combination of surface phenomena, including surface imperfections, that may shield bacteria from the heat and the migration of chilled purge to the product surface. The current data indicate that minimal heating regimens of 2 min at 195 to 205°F can readily provide 2-log reductions in most RTE deli meats we processed and suggest that this process may be an effective microbial intervention against *L. monocytogenes* on RTE deli-style meats.

Listeria monocytogenes is a significant foodborne pathogen that is readily present on raw meat products used in the manufacture of processed meats *(5). L. monocytogenes* has several characteristics that make it a formidable pathogen and contaminant in food processing environments: relatively high heat and salt tolerance, ability to grow at refrigeration temperatures, and ability to form biofilms on all kinds of surfaces. Past and recent outbreaks associated with ready-to-eat (RTE) foods have shown it to be most problematic with processed meat products, presumably due to cross-contamination during packaging. Its persistence in food processing environments, the reduction of competing microflora on processed meats, and the potential for temperature abuse of these refrigerated RTE products contribute to problems experienced with *L. monocytogenes* on RTE meat products *(9).*

Outbreaks associated with *L. monocytogenes* have prompted governmental regulatory agencies to impose stricter regulations, especially in RTE foods. The U.S. Department of Agriculture (USDA) and the U.S. Food and Drug Administration have maintained a longstanding zero tolerance for *L. monocytogenes* (and *Salmonella*) in RTE foods. More recently, the USDA Food Safety and Inspection Service (FSIS) implemented the Pathogen Reduction Act/hazard analysis and critical control point (HACCP) plan to reduce meat- and poultry-related outbreaks due to *L. monocytogenes, Escherichia coli* O157:H7, *Campylobacter,* and *Salmonella (2).* Although RTE meat products are fully cooked, either in casings or cook-in bags, they are often reexposed to the processing environment to be packaged in a final retail packaging wrap. The reexposure of product to the processing environment after cooking could lead to possible contamination by bacteria that may be present on conveyors, on metal surfaces, in condensation drippage, in contaminated air filters, in splashed standing water, or on the workers themselves. With recent outbreaks of listeriosis attributed to RTE deli meat products, the USDA-FSIS has introduced additional directives and notices in regard to *Listeria* testing and verification of HACCP plans *(3, 4).*

Current efforts in food safety of meat and meat products have included both the reduction of microbes on raw meat at slaughter (carcass pasteurization, vacuum steaming, and acid or hot water rinses, etc.) and processes aimed at reduction of potential pathogens that may be acquired as incidental contaminants on fully cooked products (e.g., acidulants, antimicrobials, irradiation, heat treatments). RTE products are most often consumed without further cooking, and therefore, the presence of pathogens presents a considerable food safety threat. This concern has prompted interest in applying postpackage or postprocess heat treatments to reduce surface contaminants and increasing shelf life in packaged products by microwave *(14),* steam *(8),* and hot water *(6, 7).* Roering et al. *(13)* pasteurized chubs of sum-

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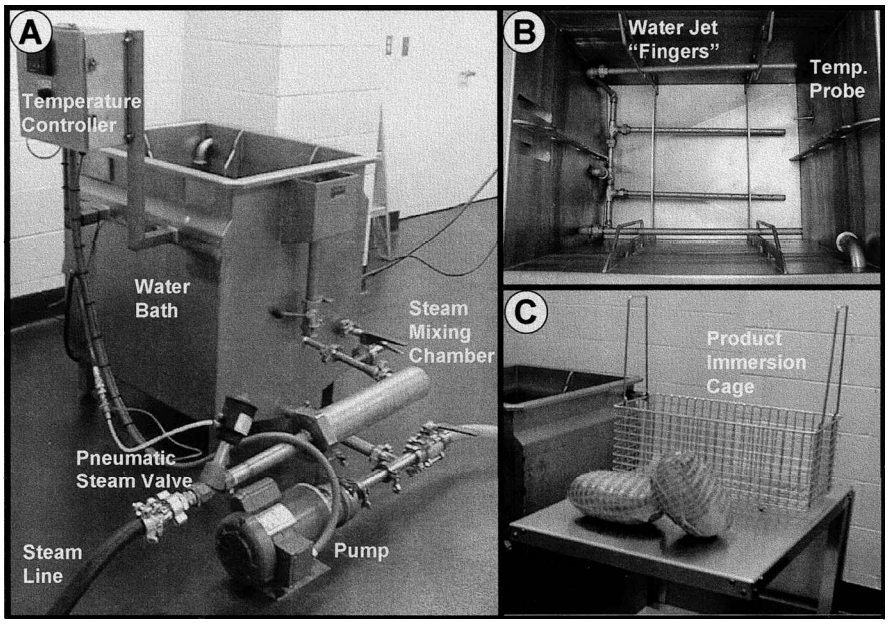

FIGURE 1. *(A) Water bath with digital temperature control, pneumatic valve controlling steam entry, and stainless steel steam injection tube. (B) Inside of water bath showing four sets of directional water jets and the tank temperature probe. (C) Stainless steel cage used to submerge products in the water bath.*

mer sausage inoculated with *L. monocytogenes* and sealed in vacuum packages, achieving as high as 3-log reductions at 210°F. However, little or no published literature is available that adequately addresses submerged water pasteurization of large-sized RTE deli meats (5 to 12 lb) as used herein. In this article, we have examined postpackage submersion heating as an effective means for reduction of *L. monocytogenes* surface contamination in RTE deli meats.

MATERIALS AND METHODS

Cultures. A four-strain cocktail of *L. monocytogenes* (Scott A-2, V7–2, PMM39–2, PMM383–2) was used in decimal reduction assays and postpackage pasteurization experiments. Strains Scott A and V7 are well-known strains; strains PMM383 and PMM39 were isolated from raw and RTE meat products, respectively. The strains have been made constitutively resistant to streptomycin (100 μg/ml) and rifamycin (10 μg/ml) (Sigma Chemical Co., St. Louis, Mo.) by passage on these antibiotics, thereby allowing recovery of viable and heat-injured cells on all-purpose plating media containing these antibiotics (e.g., tryptic soy agar) or recovery from mixtures containing other background microflora. Individual strains of *L. monocytogenes* were cultured overnight in brain heart infusion broth at 30°C (86°F) for use the next morning in inoculation trials.

Decimal reduction assays. Thermal death time curves were performed in thin-walled glass capillary tubes (100 mm, 1.1- to 1.2-mm inside diameter, 0.25-mm wall; VWR Scientific Products, South Plainfield, N.J.). Cultures were grown in brain heart infusion broth at 30°C (86°F), mixed in equal amounts, centrifuged, and resuspended in filter-sterilized purge obtained from smoked turkey, roast beef, or smoked ham RTE deli products. A temperature-controlled water bath was used to maintain specific temperatures of 145°F (62.8°C), 150°F (65.6°C), 155°F (68.3°C), or 160°F (71.1°C). Purge-suspended cells (50 μl) were introduced into glass capillary tubes with a gas-tight, 100-μl Hamilton syringe (Fisher Scientific Co., Pittsburgh, Pa.). Capillary tubes were sealed by flame and kept on ice before and after retrieval from the heated water bath. After cooling, capillary tubes were completely broken in sterile 50-ml Oak Ridge tubes containing 5 ml of sterile diluent. Appropriate dilutions were made, plated on tryptic soy agar, and then incubated at 30°C (86°F) for enumeration after 48 h. All assays were run in triplicate replications.

Products and inoculation procedures. RTE deli-style whole or formed turkey (cured, smoked, pastrami-style, sodium lactate-injected versus noninjected formed turkey), ham (cured, smoked, roasted, peppered), and roast beef products were provided by commercial processors. The products were received in commercial vacuum packages in either their original cook-in bag or retail-ready packages. Products were removed and placed into new shrink-wrap vacuum-packaging bags (Cryovac 12 by 17 in., no. 02398–10203 or Viskase equivalent). The product-specific purge was recovered and saved as diluent for resuspending the *Listeria* inoculum. The *Listeria* cultures were equally mixed and diluted 1:10 with purge obtained from specific products (i.e., 5 ml of mixed culture + 45 ml of purge), and then 50 ml of this purge-cell suspension was pipetted over the surface of each product in the packaging bags. The purpose of resuspending the inoculum in product purge was to provide the same liquid suspension chemistry as would be found by contaminating cells in actual product (i.e., as opposed to buffer or media). The products were then vacuum-sealed, massaged to distribute the cell inoculum, and maintained at 4.4°C (40°F) for 30 to 60 min before heat processing.

Submersion heating process. A stainless steel water bath (2 ft 6 in. length by 2 ft wide by 2 ft 6 in. deep) was modified by Unitherm Food Systems, Inc. (Bristow, Okla.) for use in our postpackage heating trials (the method of heating and control is similar to Unitherm's commercial Aquaflow food processor). The water bath included continuous pumping of water from the bottom of the bath through a pump and into a mixing chamber. Steam is injected into the chamber and continues to be pumped into the water bath through four "fingers" with exit holes directed toward the geometric center of the water bath. Injection of steam is controlled by a digital controller connected to a temperature sensor probe that activates a pneumatic valve to inject steam into the external chamber if the temperature is below the set point (Fig. 1). A stainless steel cage was used to manually dip two products (i.e., 5 to 12 lb each) per heating regimen (2 to 10 min). After heating, the products were quickly removed from the water bath and submersed completely in ice water to mimic a brine chill cooling procedure. Products were jostled in the ice water to hasten

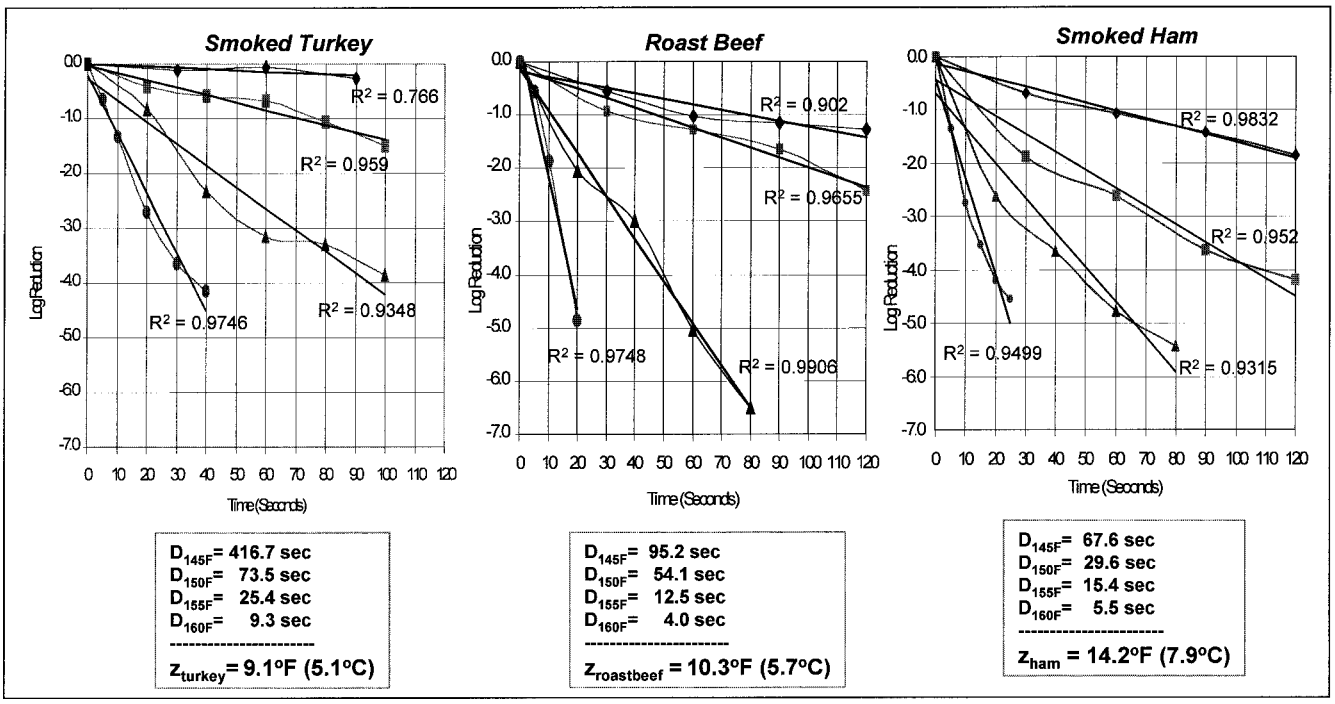

FIGURE 2. *Decimal reduction curves, regression trend lines (straight lines), and associated* D- *and* z*-values for* L. monocytogenes *heated in purge from RTE smoked turkey, roast beef, and smoked ham deli products. Heating regimens were performed at 145°F (62.8°C; ♦), 150°F (65.6°C; ■), 155°F (68.3°C; ▲), and 160°F (71°F; ●).*

cooling and were removed after 3 to 5 min to refrigerated containers where they remained until sampled (usually within 5 to 30 min).

Sample plating. After thermal processing and chilling, the purge was aseptically collected and its total volume recorded. Sample dilutions were pour plated with tryptic soy agar containing streptomycin (100 μg/ml) and rifamycin (10 μg/ml) and enumerated for our *L. monocytogenes* after 48 h at 30°C (86°F). Samples of unfiltered purge recovered from original product bags were also plated on the same media containing antibiotics to ensure that the indigenous flora from the original product (i.e., in the purge) did not come up against the antibiotic-containing media. For thermal death time assays, purge samples were clarified (i.e., filter sterilized) before resuspending the *Listeria* cells. For postpackage pasteurization assays, baseline levels of inoculated *Listeria* were obtained from inoculated but nonheated product samples for comparison with reductions obtained with inoculated products that were heated. Samples for each trial replication were run in pairs and recorded as the average of the paired samples and each trial was run either in triplicate (manufacturer A's products) or duplicate (manufacturers B, C, and D's products) replications. Product samples were run in pairs for each replication, which was done in either triplicate (manufacturer A's products) or duplicate (manufacturers B, C, and D's products) replications. Each replication was run on products obtained from different lots, and multiple replications were performed on different days.

Temperature profiling. Temperature profiles were obtained using a Nomadics TC6 Thermocouple laptop computer card with data logging software (Nomadics Inc., Stillwater, Okla.). T-type probes and thermocouple wires were connected to the card and extended into packages of uninoculated product that were subsequently sealed. Temperatures were obtained for (i) water bath, (ii) product surface, and (iii) under skin or in netting grooves when present.

RESULTS AND DISCUSSION

Recent outbreaks and recalls of RTE meat products due to *L. monocytogenes* have indicated the potential for *Listeria* surface contamination and a need for antilisterial interventions and/or increased environmental or product sampling in facilities that manufacture RTE products (*Listeria* risk assessment and action plan, FSIS docket 00–048N). In this study, we have examined a postpackage submerged water pasteurization process as a means of reducing incidental surface contamination of *L. monocytogenes* on three types of RTE deli meat products.

To complement our product challenge studies, we obtained precise thermal death time curves for our *L. monocytogenes* four-strain cocktail in purge collected from the various product categories that were tested in postpackage pasteurization trials. These data give an indication of the lethality that might be expected when *L. monocytogenes,* in each of the specific purge fluids, is heated at specific times and temperatures under ideal conditions (i.e., homogeneous heating). The use of purge as a suspension medium was most appropriate since any contaminating, or inoculated, *Listeria* cells on these products would be immersed and heated in these purge fluids during any form of postpackage heating. Using the traditional glass capillary tube method, we obtained *D*-values at 145°F (62.8°C), 150°F (65.6°C), 155°F (68.3°C), and 160°F (71.1°C) in purge from deli roast beef, smoked turkey, and ham, respectively, along with appropriate *z*-values (Fig. 2). At 160°F (71.1°C), we obtained D_{160}-values of D_{RBeef} = 4.0 s, D_{Ham} = 5.5 s, and D_{Turkey} = 9.3 s. If extrapolated to temperatures used in our postpackage pasteurization trials, one would presume the pos-

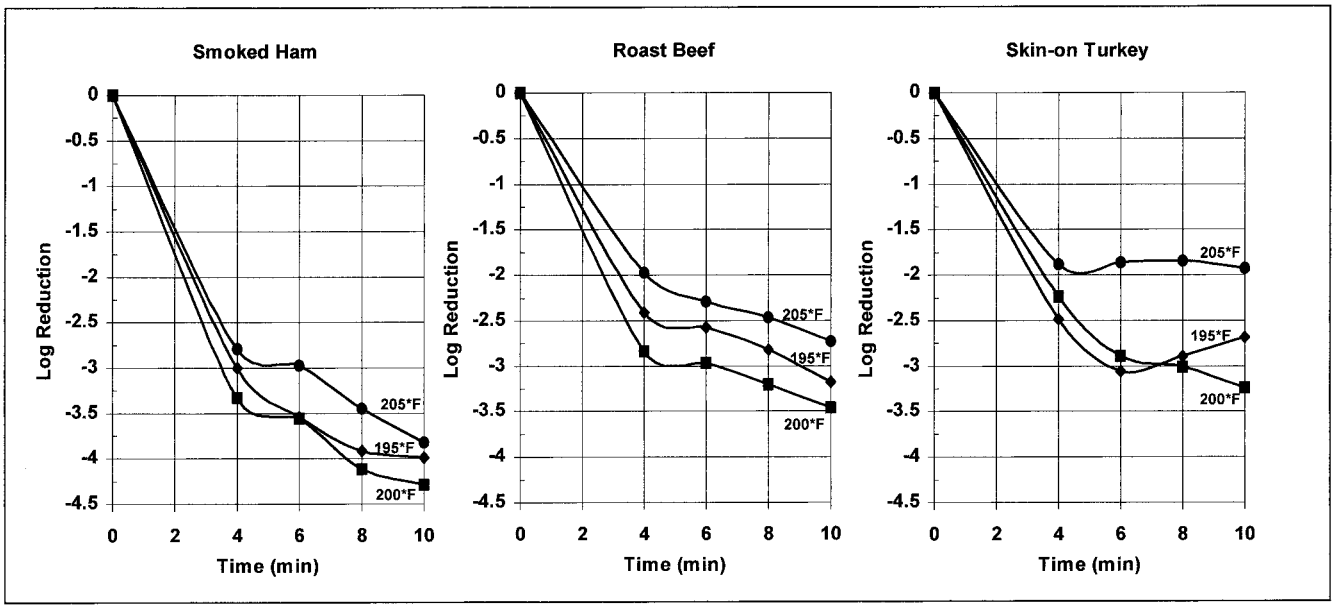

FIGURE 3. *Reduction of* L. monocytogenes *on RTE deli-style smoked ham, roast beef, and turkey from manufacturer A at 195°F (90.6°C), 200°F (93.3°C), and 205°F (96.1°C).*

sibility of high-level reductions at those temperatures. The procedure to use cells resuspended in specific product purge fluids was also extended to the *L. monocytogenes* inoculum for our product pasteurization experiments.

Several studies have looked at surface pasteurization of RTE products. Cygnarowicz-Provost et al. *(8)* tested frankfurters for reduction of *Listeria* using flash steam pasteurization, whereas Kozempel et al. *(11)* examined multiple cycles of vacuum and steam, both of which were applied *prepackage*. Cooksey et al. *(6)* obtained approximately a 4-$\log_{10}$ reduction using *postpackage* pasteurization of fully cooked 50-g beef loin chunks inoculated with *L. monocytogenes* and heated at 180°F (82.2°C) to an internal temperature of 140°F (60°C). Even more relevant is the work of Hardin et al. *(10),* who examined postprocess pasteurization of precooked beef roasts (<2 lb) that were surface inoculated with *L. monocytogenes* before packaging. The beef roasts and *L. monocytogenes* inoculum were sealed in cook-in bags and then pasteurized at 196°F (91°C) or 205°F (96°C) for either 3 or 5 min by submersion heating in circulating hot water. Hardin et al. *(10)* indicated that they achieved as high as a 4.5-$\log_{10}$ reduction for a 5-min process; however, several differences may prevent direct comparison of our data with the reduction obtained in their study. These include the following: (i) the recovery of *L. monocytogenes* on selective media after thermal processing is likely to prevent the recovery of heat-injured, yet viable, *Listeria*; (ii) the use of only a 24-h period before counting selective plates rather than 48 h; (iii) no mention of dilution of the original inoculum concentration by purge generated during product heating; and (iv) the use of *Listeria* cells resuspended in phosphate buffer rather than in product purge.

In our study, we took into account various considerations that might affect evaluation of postpackage pasteurization of RTE deli meats, including application of inoculum, time-temperature heating regimen, microbial recovery, plating, and assessment of reduction level. The processing of up to two 12-lb (approximate) products previously refrigerated at 40°F (4.4°C) did not affect the heat capacity of the water bath, which maintained constant temperatures during processing as monitored externally using thermocouple temperature sensors and internally via the water bath's digital control system. In addition, four water "fingers" (Fig. 1B) pumped water continuously toward product that was suspended in the middle of the tank using a wire basket (Fig. 1C). Although the water bath's digital control system monitored water temperature continuously during processing, product temperature profiles were only obtained on select uninoculated product samples during each pasteurization trial. A minimum of two thermocouple sensors were often placed on uninoculated products to obtain temperature profiles. Usually one was placed along the outermost product surface (i.e., inside the packaging) and another in a depression or subsurface anomaly (netting groove, cut, below skin). Subsequently, DataTrace probes have been used that can be sealed within the packaging film along with product.

Preliminary product postpackage pasteurization trials with *L. monocytogenes* indicated that our initial temperature preferences of 185 to 190°F (85 to 88°C) would be too low to provide desirable reduction levels (i.e., ≥2-log reduction) on deli meats during short-term heating (data not shown), although these same temperatures provided excellent log reductions in our thermal death time curve experiments (Fig. 2). Using higher temperature regimens of 195 to 205°F (90.6 to 96.1°C), we were able to obtain significant reductions of *L. monocytogenes* (≥2 log) within 2 to 4 min with most products provided by four different RTE deli meat manufacturers (Figs. 3 through 5). However, variability in reduction efficiency was observed between products

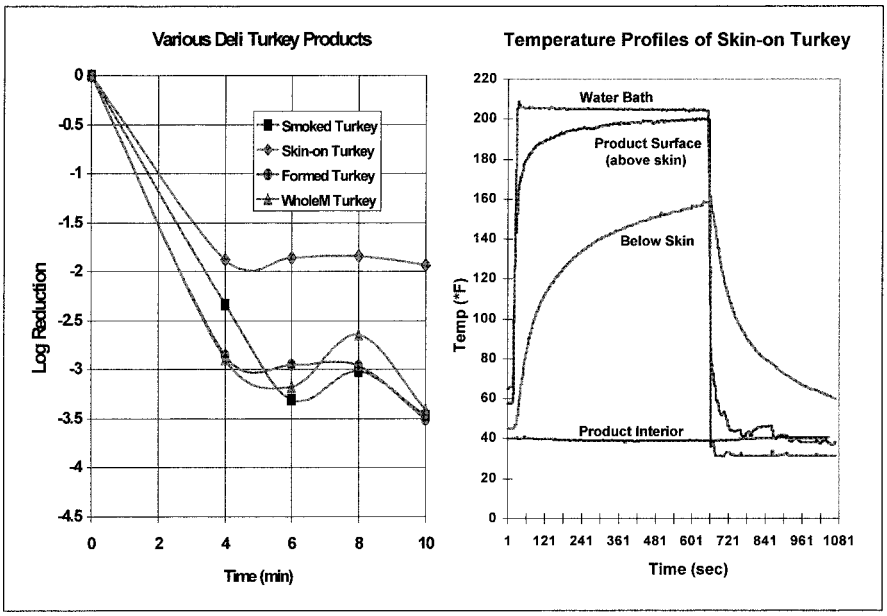

FIGURE 4. *(Left) Reduction of* L. monocytogenes *on various types of RTE deli-style turkey products from manufacturer A at 205°F (96.1°C). (Right) Temperature profiles of water bath temperature, product surface, and below the skin of skin-on deli turkey processed at 205°F (96.1°C).*

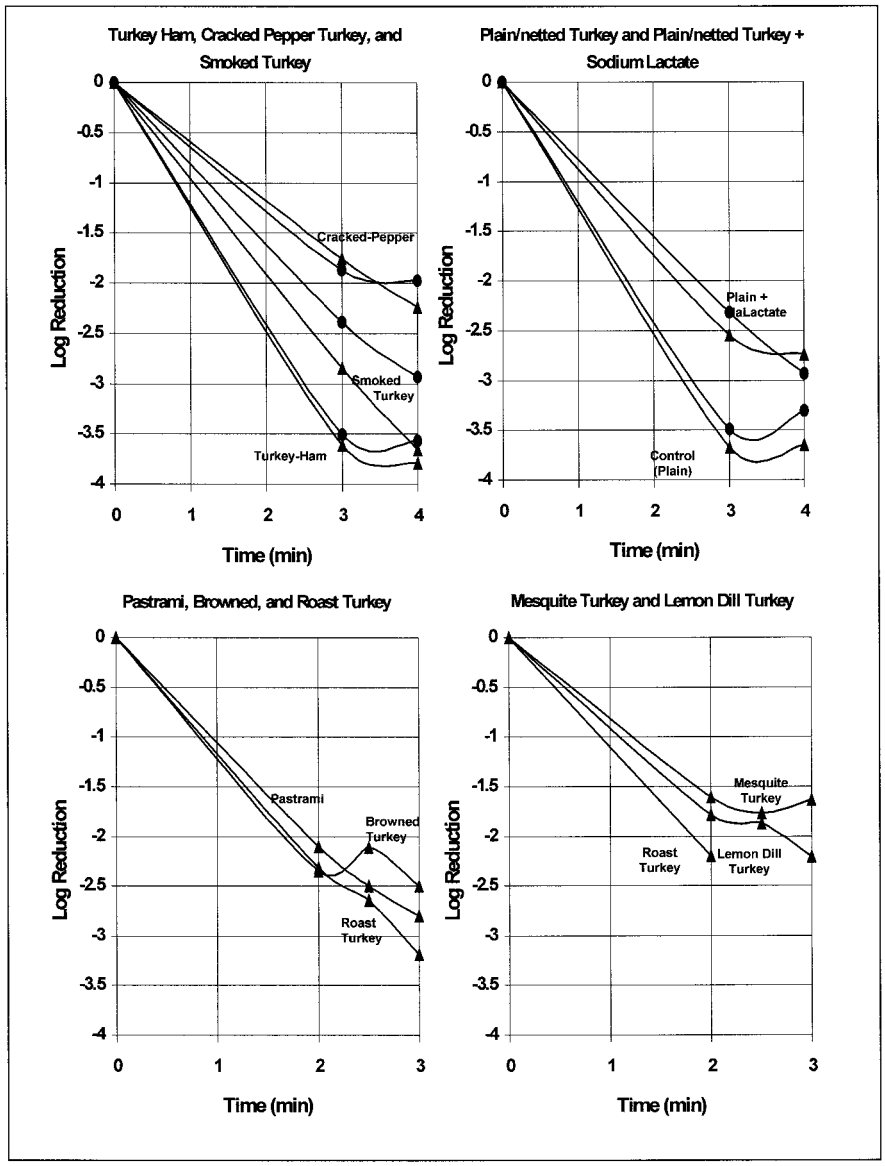

FIGURE 5. *Reduction of* L. monocytogenes *on various specialty deli turkey products from manufacturers B (top), C (bottom left), and D (bottom right) at 200°F (93.3°C; ●) and 205°F (96.1°C; ▲).*

from different manufacturers and even between different products from the same manufacturer (Figs. 3 through 5).

We used a high cell inoculum (i.e., ~10^7 to 10^8 CFU/ml) so that we would be able to enumerate surviving cells to establish the microbial reduction range for various time-temperature combinations. Although such high *Listeria* levels would not likely be encountered as cross-contamination in packaging room environments, they were able to act as conservative sensors by penetrating cracks and crevices in the product surface. The use of very low-level cell inoculations (e.g., ~100 CFU per entire product) as a possible alternative would not likely have addressed the issue of surface cuts and crevices over the entire surface of such large products (5 to 12 lb) that may otherwise hide and protect *Listeria* cells from subsequent heat treatment. Furthermore, a low-level inoculum protocol would have required an enrichment procedure for yes/no detection rather than a quantitative enumeration of the residual population and would have required a greater number of products to statistically prove any given reduction level. We also plated our postprocess heat-treated cells on tryptic soy agar containing the antibiotics for which our strains were constitutively resistant as opposed to plating on harsh *Listeria*-selective media that could otherwise inhibit heat-injured cells from recovery.

One product type that suggested that the irregular surface features may affect overall microbial reduction efficiencies was most exemplified by skin-on turkey products (Fig. 4). Skin-on turkey gave significantly lower microbial reductions than three other turkey products from the same company. When we examined the temperature profile above and below the skin layer, it was evident that any cells that happened to get caught below the skin would not receive anywhere near as lethal a process as those above the skin surface (Fig. 4). Similar protection could be imagined for other imperfections of product surfaces, such as folds from whole-breast turkeys, cuts, cracked pepper surfaces, and deep grooves from netted products. A cracked pepper product did not allow the shrink wrap to completely touch the product surface and may have reduced heat penetration (Fig. 5). The high cell populations used in our studies allowed us to empirically account for these surface anomalies in our process that would otherwise go undetected if very low-level inoculum levels were used.

A phenomenon that was observed in one series of postpackage pasteurization trials was a trend of lower reduction obtained at 205°F (96.1°C) compared with that obtained at 195°F (90.6°C) or 200°F (93.3°C) for the same product lines (Fig. 3). Since these particular trials were done in triplicate (products were run in pairs for each replication; each replication was performed in triplicate and on a different day with product obtained from a different lot for each replicate), it is unlikely that they were an aberration in our process. One explanation we may provide is that the shrinkage of the shrink wrap may be so severe at 205°F (96.1°C) that it is literally squeezing purge from the chilled 40°F (4.4°C) interior toward the surface at a faster rate than at 195°F (90.6°C) or 200°F (93.3°C) and, therefore, reducing the overall heating and concomitant microbial reduction.

Another consideration that we addressed in this study was the dilution of the initial inoculum cell concentration by purge generated during heating. This was addressed by (i) switching from a low-volume (2 ml) to high-volume (50 ml) inoculum and (ii) retrieving and measuring total purge fluids for standardizing microbial counts to a fixed purge volume. Most deli RTE products that we processed produced various amounts of purge during the heating process, often as much as 100 ml during extended heating regimens. With a 2-ml inoculum, direct plating of purge liquid would (falsely) show a 50-fold dilution and thus microbial reduction even if there was no thermal kill. To overcome this purge dilution effect, we initiated recovery of total purge volume after processing and implemented a normalization factor based on the original inoculum volume. In addition to identification of the amount of final purge or inoculum recovered, the switch to a 50-ml inoculum also ensured that the additional purge generated during processing did not drastically affect the initial inoculum concentration as it would if we only used a 1- to 2-ml inoculum.

Product purge is undesirable both aesthetically and because of lost yield. In our study, products producing excess purge (e.g., roast beef, formed turkey) were observed to have less microbial reduction than those that produced less purge and were considered the driest products (e.g., hams, smoked turkey; Figs. 3 through 5). One reason for limited microbial inactivation of products producing excess purge may be due to the migration of chilled purge from a chilled product interior to product surface, counteracting the heat gradient.

Although the heating regimens we used were as long as 10 min at 205°F, the interior temperature of the products never changed (Fig. 4). Heat penetration affected only the outer 1 cm of product, and surface temperatures were equilibrated back to below 50°F within a few minutes after submersion in a chill tank (Fig. 4). Trials with extended heating times (Figs. 3 and 4) showed that most of the inactivation of *L. monocytogenes* was usually obtained within 4 to 5 min, obviating the need for further heating only to obtain minimal gains. It was most significant, however, that the inoculated postpackage pasteurization data obtained herein was not reflective of the *D*-value data obtained with *L. monocytogenes* thermal death time curve assays (Fig. 2), which would have predicted a larger reduction for the time and temperature regimen of the current process. This underscores the importance of inoculated challenge studies with actual product as crucial confirmatory data to a model in which thermal inactivation data are obtained in one venue and extrapolated to a related product in a different format. For instance, Mazzotta *(12)* generated *D*- and *z*-value data with *L. monocytogenes* mixed into 5-g samples of crab meat and then extrapolated these data to suggest a process to inactivate *L. monocytogenes* on the surface of surimi products without conducting surface-inoculated pasteurization trials with surimi. In our study, the *D*-value data for *L. monocytogenes* resuspended in product purge (Fig. 2) showed that for roast beef at 160°F (71.1°C), we obtained nearly a 5-log reduction in 20 s. However, actual data obtained with inoculated roast beef product in postpackage

pasteurization experiments (Fig. 3) showed that even with prolonged heating for up to 10 min in which surface temperatures (data not shown) reached 160°F within 1.5 min and climbed to over 180°F by the end of the heat treatment, we obtained only a 4-$\log_{10}$ reduction. We would therefore suggest caution to those that would be tempted to overlay *D*-value data onto temperature profiles in an attempt to circumvent challenge studies with predictive pathogen reduction models for surface pasteurization of chilled RTE meat products.

The current work provides a framework for postpackage pasteurization to help reduce or eliminate incidental surface contamination of RTE deli meat products. The process only heats the outer 1 cm of surface and causes minimal effect on product appearance or quality (except for extended heating of roast beef). Recent outbreaks and recalls resulting in extensive liabilities have been attributed to contamination of these types of products, presumably occurring during or before packaging *(9).* This type of process could prove to be a significant and additional hurdle to providing safer RTE deli products and already has been integrated into various manufacturers' processing lines. In a recent recall of approximately 17 million lb of RTE turkey products, a portion of the plants' production that was processed by submerged water postpackage pasteurization was not subject to the recall by USDA-FSIS and provides a proof of concept for the proposed process *(1).* The data presented herein have already been accepted as confirmatory data for several companies who have included the postpackage pasteurization process in their HACCP plans. We believe that postpackage pasteurization is one of several technologies that could be implemented within the hurdle concept to reduce incidental surface contamination of *L. monocytogenes* on RTE deli meat products.

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