Evaluation of Small-Scale Hot-Water Post-Packaging Pasteurization Treatments for Destruction of *Listeria monocytogenes* on Ready-To-Eat Beef Snack Sticks and Natural Casing Wieners

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ABSTRACT

This study was conducted to evaluate small-scale hot-water post-packaging pasteurization (PPP) as a post-lethality (post-cooking) treatment against *Listeria monocytogenes* on Ready-to-Eat beef snack sticks and natural casing wieners. Using a commercially available plastic packaging film specifically designed for PPP applications and 2.8 liters of boiling water (100°C) in a sauce pan on a hot plate, an average reduction in *L. monocytogenes* numbers of $\geq$ 2 logs was obtained using heating times of 1.0 minute for individually packaged beef snack sticks (three brands), 4.0 minutes for four-per-package beef snack sticks (two brands) and seven-per-package beef snack sticks (three brands). Average product surface temperatures, measured as soon as possible after PPP and opening the package, were 47-51.5°C, 58-61.5°C, and 58.5 – 61°C for the beef snack sticks packaged one, four, and seven pieces per package, respectively. A treatment of 7.0 minutes for four-per-package natural casing wieners (three brands) achieved *L. monocytogenes* reductions of $\geq$ 1.0 log and average product surface temperature of 60.5 – 63.5°C. Cooked-out fat and moisture resulting from tested treatments ranged from 0.2 to 1.1% by weight for beef snack sticks and from 0.4 to 1.2% by weight for natural casing wieners. For natural casing wieners, PPP had no detrimental effect on overall product desirability to consumers; results suggested that PPP may significantly enhance appearance of this product. However, for beef snack sticks the cooked-out fat and moisture following PPP had a significant negative effect on consumer opinions of product appearance.

On June 6, 2003, the United States Department of Agriculture (USDA) published an interim final rule addressing the control of *Listeria monocytogenes* on ready-to-eat (RTE) meat and poultry products (*9*). This rule went into effect on October 6, 2003 and was intended to encourage processors of RTE products to take one or more specific steps to ensure the absence of *L. monocytogenes* on their products. Possible steps range from using focused sanitation procedures, to adding ingredients or using processing treatments designed to kill *L. monocytogenes* or inhibit its growth. Under the regulations, the processor is also required to perform testing for *L. monocytogenes* or *Listeria* spp. on food contact surfaces in the area of the plant in which RTE products are handled after cooking. The amount of testing is related to the types of RTE products made, product ingredients, and how the products are processed and handled. In particular, the rule requires processors of RTE meat and poultry products to adopt one of three designated "Alternatives" to control *L. monocytogenes* on their products. The Alternatives involve varying levels of control and microbiological testing. In Alternative 1, the processor uses a post-lethality treatment that reduces or eliminates *L. monocytogenes* and an antimicrobial agent or process that suppresses or limits *L. monocytogenes* growth throughout product shelf-life. In Alternative 2, the processor uses either a post-lethality treatment that reduces or eliminates *L. monocytogenes* or an antimicrobial agent or process that suppresses or limits *L. monocytogenes* growth throughout product shelf-life. Under Alternative 3, only sanitation measures are relied upon to control *L. monocytogenes*.

The objective of this work was to evaluate small-scale hot-water post-packaging pasteurization, for use as a post-lethality treatment by small and very small processors under Alternatives 1 or 2 of the USDA regulations. This post-lethality treatment was studied for use with beef snack sticks and natural casing wieners, two products commonly made by small and very small processors. In the processing of beef snack sticks, the addition of salt, along with cooking and smoking have already been shown to be effective antimicrobial processes by making the finished product unsuitable for *L. monocytogenes* growth (*4*). Some processors also acidify beef snack sticks, either through fermentation or through the addition of an acidulant. Either means of acidification would provide further inhibition of *L. monocytogenes* growth. For beef snack sticks then, the adoption of hot-water post-packaging pasteurization as an effective post-lethality treatment could allow processors to operate under Alternative 1. The second product studied, natural casing wieners (finely ground cured sausage contained inside an intestine casing, often used to make a “hot dog” sandwich), has traditionally been made without the addition of any ingredients that effectively inhibit *L. monocytogenes* growth. Thus, conducting hot-water post-packaging pasteurization of this product would allow processors to operate under Alternative 2. However, many processors have begun adding an anti-microbial agent such as sodium lactate to their natural casing wiener recipes. For these processors, the use of hot-water post-packaging pasteurization would allow operation under Alternative 1.

There are a large number of variables involved in hot-water post-packaging pasteurization. These include packaging film composition and thickness, mass of product, volume of water, proportions of water and treated product, water temperature, and treatment time. In order to simplify potential techniques that small and very small processors might adopt, we focused our efforts by using a single type of packaging film, typical masses and packaging configurations of products, a single amount of hot water, and a single initial water temperature. We varied the treatment time, keeping in mind that processors would prefer shorter treatments for reasons of economics and efficiency.

We also paid considerable attention to whether effective hot-water post-packaging pasteurization treatments affected the sensory properties of beef snack sticks and natural casing wieners. Because these products would be subjected to a thermal process in a confining package, cook-out (almost entirely fat) was determined to be a potential drawback of this procedure. Therefore, we measured the amounts of cook-out and also conducted consumer sensory panels of treated and untreated products.

MATERIALS AND METHODS

Meat products. Three brands each of beef snack sticks and natural casing wieners were purchased at local grocery stores and transported within 30 minutes to the laboratory. Samples were refrigerated (5 $\pm$ 1°C) until use. A sample from a randomly chosen lot of each product was analyzed in the laboratory for water activity using a Decagon water activity meter (AquaLab

Series 3 TE, Pullman, WA) and pH (sample homogenized in distilled water) using an Accumet AB15 pH meter (Fisher Scientific, Itasca, IL) and probe. Samples were also sent to a commercial laboratory to be analyzed for % moisture, % salt, and % fat using forced air oven determination of moisture – AOAC method 950.46Bb, potentiometric method for salt – AOAC method 980.25, and Soxhelet method for fat – AOAC method 960.39 (*2*). The Moisture: Protein ratio (MPR) and % water-phase salt (WPS) were calculated from these analytical results. Representative products were measured to determine average length and diameter. Physical and chemical characteristics of the products are shown in Table 1.

Bacterial cultures and preparation of inoculum. The *L. monocytogenes* strains used in this study were obtained from the laboratory of Dr. Eric Johnson at the Food Research Institute, University of Wisconsin-Madison. Strain Scott A was a clinical isolate, strains LM 101 and LM 108 were isolated from hard salami, strain LM 310 was isolated from goat cheese, and strain V7 was isolated from raw milk. Stock cultures were maintained at -20°C in Brain Heart Infusion broth (BHIB; Difco, Becton-Dickinson, Sparks, MD) with 10% (w/v) glycerol (Fisher) added. Working cultures, maintained at 4°C on Brain Heart Infusion agar (BHIA; Difco), were prepared monthly from frozen stock cultures. To obtain a working culture, a strain was cultured twice successively at 35°C for 18-24 h in BHIB, streaked to a BHIA plate, incubated at 35°C for 18-24 h and examined for purity, and then stored at 4°C. Inoculation cultures were

prepared for each strain by transferring a loopful of growth from the working culture plate to 9 ml of BHIB and incubating at 35°C for 20-24 h. To prepare the 5-strain inoculum cocktail, the BHIB cultures were combined into a 50-ml sterile plastic centrifuge tube, and centrifuged for 10 minutes at 5,000 x g. The supernatant in the tube was decanted, and the pellet was resuspended to the original volume in Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, WI) to make an inoculum cocktail. The inoculum cocktail was serially diluted in BPD and plated to determine cell concentration (ca. 8 log CFU/ml).

Inoculation of meat products. Two different methods were used to inoculate products. Natural casing wieners, and beef snack sticks to be packaged four-per-package or seven-per-package, were inoculated by placing 110 ml of a 1:10 dilution (in BPD) of inoculum cocktail in a large glass Petri dish and rolling an individual product piece through the inoculum for two complete revolutions. This procedure was used to ensure that in situations where product piece-to-piece contact would occur, all product surfaces were inoculated, thus maximizing the opportunity for protection of cells via piece-to-piece contact. Individually packaged beef sticks do not experience piece-to-piece contact within the package, so each beef snack stick to be packaged individually was inoculated by placing 25 µl of the 1:10 dilution (BPD) of inoculum cocktail on each of 5 sites along the beef snack stick. The inoculum was then spread across the beef snack stick surface using a sterile plastic spreader. After inoculation, products were allowed to dry at room temperature (20-22°C) for 90 minutes in a

biosafety level 2 hood before packaging. The mean starting inoculum levels in initial trials were 4.2 log CFU per sample (see below for description of sampling procedure) for beef snack sticks (range of 3.1 – 5.3, standard deviation = 0.7) and 5.9 log CFU per sample for natural casing wieners (range of 5.7 – 6.3, standard deviation = 0.2). In confirmatory trials, mean starting inoculum levels were 3.9 log CFU per sample for beef sticks (range of 2.7 – 5.5, standard deviation = 0.7) and 5.7 log CFU per sample for natural casing wieners (range of 4.7 – 6.1, standard deviation = 0.4).

Packaging of meat products. A commercial plastic film designed for use in hot-water/steam post-packaging pasteurization was obtained from Curwood, Inc. (Product SPP94, Material No. CPS302976; New London, WI). Bags were custom-sized for products using scissors and a heat-sealer on a commercial-type vacuum packaging machine. Dry, inoculated products were placed in appropriate bags and vacuum-packaged (approx. 1 atm). Trials were done with one, four, and seven beef snack sticks per package and with four natural casing wieners per package.

Hot-water post-packaging pasteurization treatments. Prior to pasteurization, 2.8 liters of tap water were placed in an aluminum sauce pan. Pan dimensions were 21 cm diameter and 12 cm height. A plastic test tube rack was submerged in the pan using a lead “donut” weight. This rack was used to hold the packaged product off of the pan bottom during heating and thus allow

maximum water contact with the product. Either of two hot plates was used to heat the water to boiling, whereupon pasteurization trials were conducted. The hot plates had maximum power of 1113 and 1118 watts. A single package of inoculated product was placed between levels of the test tube rack in the boiling water. Although the water stopped boiling when packaged product was first added, it resumed boiling within a short time (< 30 seconds for the largest packages). After the prescribed time had elapsed, the package was immediately removed from the water and placed in ice-water slush. The next sample was not heated until after the water had again boiled. Additional water was added to the pan as needed to maintain water volume. An initial trial consisted of three packages of product, all inoculated with an inoculum cocktail from the same broth cultures, for each of several treatment times. A confirmatory trial control consisted of one package of product per treatment. Untreated control packages were handled identically to treated packages other than during submersion in the boiling water. Initial trials for one-per-package, four-per-package, and seven-per-package beef snack sticks were done for 0.5, 1.0, and 1.5; 3.0, 4.0, and 5.0; and 3.0, 4.0, 5.0, and 6.0 minutes, respectively, while initial trials with four-per-package natural casing wieners were for 5.0, 6.0, and 7.0 minutes. Confirmatory trials were done with treatments of 1.0 and 4.0 minutes for one-per-package and four- or seven-per-package beef snack sticks, respectively, and 7.0 minutes for four-per-package natural casing wieners.

Enumeration of inoculum bacteria. Each chilled package of product was removed from the ice-water slush, after which the package surface was patted dry with a paper towel and then sprayed with 70% ethanol. Following a 20-minute drying period, each package was aseptically opened. The ends of each beef snack stick or natural casing wiener in the package were removed, leaving 2.5 cm pieces. These pieces comprised a sample and were transferred to a stomacher filter bag (typical sample weights are shown in Table 1). The package, minus remaining product, was also transferred to the stomacher filter bag. A standard 198 ml volume of BPD was added to the stomacher bag and the contents were then manually massaged for 1 minute and manually shaken for 1 minute. This procedure ensured thorough contact of BPD with product and package interior surfaces. From the initial sample dilution(arbitrarily denoted as 10^{-2}), further dilutions were made, as appropriate, using BPD. For initial trials, no effort was made to allow repair and subsequent enumeration of injured cells. From the initial dilution, 1.0 ml was distributed for spread-plating among three plates (0.3, 0.3, and 0.4 ml) of Listeria Selective Agar (LSA; Oxoid, Ogdensburg, NY) with Listeria Selective Supplements (Oxford formulation; Oxoid). From the original dilution and each subsequent dilution, 0.1 ml was spread on one LSA plate per dilution. Plates were incubated at 35°C for 48 h, according to the manufacturer's instructions, and then examined for typical *L. monocytogenes* colonies (small-medium, brown-to-black colonies surrounded by a black precipitate zone), which were counted. For each product tested in each trial, one presumptive *L. monocytogenes* colony was selected for confirmation testing.

The colony was transferred to BHIA and cultured, and then tested for Gram stain reaction, cellular morphology, oxidase activity, and biochemical characteristics (API Listeria kit, bioMerieux, Hazelwood, MO). Throughout the study, all presumptive isolates were confirmed as *L. monocytogenes*. For confirmatory trials, spread-plating was done on BHIA, and a one-hour 35°C incubation was employed in an attempt (unpublished method) to allow repair by injured cells. Following this incubation period, each BHIA plate was then overlaid with about 10 ml of tempered (46°C) LSA + supplements and then incubated 48 h at 35°C. For each trial, the difference in log CFU between treated and untreated product was considered the treatment lethality. When no colonies resulted from the least dilute plates (arbitrary 10^{-2} dilution), a value of 9 colonies on a hypothetical 10^{-1} dilution plate (effectively one colony less than the detection limit) was assigned. The log of 90 CFU, rounded to 1.9, was then used in calculating treatment lethality.

Statistical analysis of microbiological data. The two-sample t-test (Minitab, Release 14; Minitab, Inc., State College, PA) with a 5% significance level was used to make the following comparisons for log CFU reduction in initial trials: 1) processor vs. processor for a given combination of product, number of pieces per package, and processing time; 2) heating time vs. heating time for a given combination of product, processor, and number of pieces per package; and 3) heating time vs. heating time for a given combination of product and number of pieces per package (data for three processors combined). For confirmatory

trials, the same analysis was done to compare processor vs. processor for a given combination of product, number of pieces per package, and heating time.

Determination of product surface temperature. Using conditions otherwise identical to those in the initial and confirmatory trials, additional experiments were done with uninoculated products to determine product surface temperature at various times during hot-water post-packaging pasteurization. At the designated time, the package of product was rapidly removed from the hot water, opened with a knife, and three product suface temperatures were rapidly measured at mid-piece (same location from which samples were obtained for microbiological analysis) using an infrared non-contact thermometer (Mini Temp FS, Raytek; Santa Cruz, CA). Particularly for multi-piece packages, the product size and shape, along with piece-to-piece contact, resulted in a range of surface temperatures. Therefore, the temperature was determined at three different mid-piece locations and the three temperatures were averaged for that package as follows. For individually packaged beef snack sticks, the termperature was measured at three places evenly around the beef snack stick circumfrence at mid-piece. For four-pieces-per-package beef snack sticks and natural casing wieners, the temperature was measured on the upper surface mid-piece for pieces 1 and 2 (counting from left to right), and piece 2 was rotated to obtain the surface temperature mid-piece where piece 2 had contacted piece 3. For seven-pieces-per-package beef snack sticks, surface temperatures were obtained on the upper surface mid-piece for pieces 1 and 3, and piece 3 was rotated to obtain

the surface temperature mid-piece where piece 3 had contacted piece 4. For each combination of product, processor, number of pieces per package, and heating time, duplicate three-temperature averages were obtained and a mean of the duplicates was calculated.

Prediction of process lethality using surface temperature data. The lethality of the hot-water post-packaging pasteurization process was estimated by inputting average surface temperature and time data into the American Meat Institute Foundation process lethality spread sheet (*1*). Along with the time and temperature data, a reference temperature of 62.8°C (145°F) and a Z-value of 7.9°C (14.2°F), determined by Muriana et al (*5*) for *L. monocytogenes* in purge from smoked ham, were entered into the spreadsheet. These values were chosen because smoked ham, of the three products studied by Muriana et al (*5*), was closest in composition to the beef snack sticks and natural casing wieners. The F_o values determined using the spreadsheet were divided by the previously determined $D_{62.8}$-value of 67.6 seconds (*5*) for *L. monocytogenes* in purge from smoked ham to obtain a predicted log reduction.

Sensory quality of treated products. The percent of product weight lost as cooked-out fat and water was determined for single trials of uninoculated beef snack sticks heated for 3.0 and 4.0 minutes (four beef snack sticks per package) and for 4 and 5 minutes (seven beef snack sticks per package), and for single trials of uninoculated natural casing wieners (four per package) heated 5.0, 6.0, and 7.0 minutes. Unscreened, untrained panelists (n ranging from 178 to192)

also evaluated treated (4 minutes for beef snack sticks packaged seven-per-package and 7 minutes for natural casing wieners packaged four-per-package) and untreated beef snack sticks or natural casing wieners. The beef snack sticks were presented to panelists with cooked-out fat and moisture removed. The treated and control natural casing wieners were separately warmed after being removed from the packaging by being placed under boiling water for 4.0 minutes and then held warm in an insulated container prior to serving. Panelists were self-selected from consumers entering a campus facility serving lunch and ice cream. Samples were presented in random order in cups coded with a three-digit random number. The panelist ballot contained two structured seven-point hedonic scales with possible scores ranging from 1 = dislike very much to 7 = like very much. Mean scores for treated and untreated products were calculated and an analysis of variance appropriate for a randomized complete block design (*8*) was conducted. Follow-up sensory evaluation panels were done with beef snack sticks and natural casing wieners from processor B (rated highest by panelists in the initial sensory panels). For the follow-up sensory evaluation panels, the packages of treated and untreated products were opened, and the packaging film lifted from the product surface so that cooked-out fat and moisture were visible. The film surface was then loosely placed back over the product surface and the bag re-sealed by stapling. Panelists (n = 198) at the same location were asked to evaluate the product appearance of control and treated products on a ballot with two structured seven-point hedonic scales with possible scores ranging from 1 = extremely unappealing to 7 = extremely appealing. Mean scores for treated

and untreated products were calculated and an analysis of variance appropriate for a randomized complete block design (*8*) was conducted.

RESULTS AND DISCUSSION

In initial trials with beef snack sticks packaged one or four pieces per package, the average lethality increased with heating time (Table 2), with some statistically significant differences between heating treatments. Specifically, increasing the heating time from 0.5 to 1.5 minutes for individually packaged beef snack sticks and from 3.0 to 4.0 or 5.0 minutes for four-per-package beef snack sticks resulted in significantly greater lethality. Lethality differences between treatment times for beef snack sticks produced by a single processor were seldom significant for individually packaged and four-per-package product. Statistically significant differences in lethality for beef snack sticks from different processors were also rare. For seven-per-package beef snack sticks, a statistically significant increase in lethality was achieved when the heating time was increased from 3.0 to 4.0 minutes (Table 2). Further increases in heating time did not result in a corresponding increase in lethality because of the plating detection limit being reached for PPP treatments of $\geq$ 4.0 minutes. For seven-per-package beef snack sticks from a single processor, there were no significant differences in lethality between heating times, probably because of low trial numbers and relatively high inter-trial variation. In initial trials with natural casing wieners, increasing the heating time from 5.0 to 7.0 minutes resulted in significantly greater lethality (Table 2), while significant differences between

wieners from different processors occurred with heating times of 6.0 and 7.0 minutes. In confirmatory trials (Table 3), there were no significant differences in lethality between beef snack sticks or natural casing wieners from different processors. Throughout the study, there was noticeable trial-to-trial variation. Potential causes of this variability include lot-to-lot variation in product composition, surface smoothness, and shape; variation in inoculum level; and variation in how closely the packaging film contacted the product surface.

Compliance guidelines from USDA state that an effective post-lethality treatment must reduce numbers of *L. monocytogenes* by at least 1.0 log (*10*). However, a higher standard of lethality must be met in order to reduce regulatory sampling frequency. In the latter case, *L. monocytogenes* numbers must be reduced by at least 2.0 logs. All of the PPP treatments tested in initial trials had some lethality against *L. monocytogenes* on vacuum-packaged beef snack sticks and natural casing wieners (Table 2). With the compliance guidelines in mind, hot-water PPP treatments of at least 1.0 minute for individually packaged beef snack sticks, at least 4.0 minutes for four-per-package and seven-per-package beef snack sticks, and at least 7.0 minutes for four-per-package natural casing wieners were viewed as having potential commercial utility. Some beef snack stick products have already been shown not to support *L. monocytogenes* growth during storage (*4*). Applying validated hot-water post-packaging pasteurization treatments to such products would allow the processor to operate under Alternative 1 of the USDA regulations.

Treating individual beef snack sticks for 1.0 minute often eliminated all inoculum *L. monocytogenes* cells. Results from these initial trials would support validation of the 1.0 minute treatment for commercial use. Results of confirmatory trials using this treatment also support commercialization of this treatment (Table 3). Treating four-per-package and seven-per-package beef snack sticks for at least 4.0 minutes also resulted in reductions in numbers of inoculum *L. monocytogenes* that met or exceeded the 2.0 log standard (Tables 2 and 3). Heating times needed to achieve a 2.0 log reduction increased with the number of pieces per package. The *L. monocytogenes* cells located at piece-to-piece contact surfaces could have been protected from the heating treatment. Results of trials to determine average surface temperature (Table 4), support this hypothesis.

Lethality of hot-water PPP against *L. monocytogenes* on natural casing wieners was lower than that seen for beef snack sticks (Tables 2 and 3). This difference is likely caused by the wieners having a larger mass than the beef snack sticks and thus taking longer to heat (Table 4). As with the beef snack sticks, it is likely that piece-to-piece contact protected *L. monocytogenes* on the contact surfaces. A treatment time of 7.0 minutes appeared to result in sufficient lethality to meet the guideline for effectiveness, but did not reach the lethality level needed to reduce regulatory sampling frequency.

Comparison of Tables 2 and 3 would suggest that greater reductions in numbers of *L. monocytogenes* occurred in confirmatory trials than in initial trials, and that the overlay plating procedure, intended to allow enumeration of injured

cells, failed to achieve its objective. However, the overlay method always resulted in greater time-zero values that direct plating did in confirmatory trials. This finding indicates that some *L. monocytogenes* cells were initially injured upon exposure to the product surface. Numbers of cells recovered after the hot-water post-packaging pasteurization treatments were similar for overlay and direct plating methods, suggesting that few injured cells survived heating. The combination of greater time-zero cell numbers and comparable post-heating cell numbers was responsible for the apparent difference in lethality between the initial and confirmatory trials.

Variability in results for wieners and beef snack sticks was probably due to slight differences in product size, product surface topography, heat transfer through adjoining pieces, and tightness of vacuum-packaging. Increasing the PPP treatment time beyond those times studied here would undoubtedly decrease the variability of results and increase lethality, but would probably not be economically feasible for processors.

The process lethality values calculated using surface temperature data are shown in Table 5. Calculated process lethality values were generally much lower than what was observed in initial (Table 2) and confirmatory (Table 3) trials, with the exception of seven-per-package beef snack sticks heated for 6.0 minutes and four-per-packaged natural casing wieners heated 7.0 minutes. For these product/treatment combination, calculated lethality was relatively closer to observed lethality. Possible reasons for the difference between observed and calculated lethality include compositional characteristics of beef snack sticks and

natural casing wieners enhancing thermal destruction of *L. monocytogenes* relative to that occurring in the purge from smoked ham (the medium in which *L. monocytogenes* D- and Z-values were determined), differences in thermotolerance between the strains used in the present study and those used by Muriana et al (*5*), and lower-than-actual surface temperature measurements resulting from product cooling during the 5 – 10 seconds elapsing while the package was opened and the surface temperature measured.

Several related studies on post-packaging pasteurization of ready-to-eat meat products have already been conducted. Collectively, these studies differ from the present one in three important ways: 1) the desired target for reducing numbers of *L. monocytogenes* cells was often greater than that required in the USDA compliance guideline, 2) different products were tested, 3) the volume of hot water used for treatments, along with the degree of circulation and source of heat, ensured that addition of cold packaged products did not cause any reduction in water temperature. Thus, direct comparisons between the present study and earlier work are difficult. Chen et al (*3*) studied post-packaging pasteurization of peeled (no casing) frankfurters, packaged five per package, using 96°C water. The frankfurters also contained pediocin, which may have enhanced thermal lethality relative to that observed in our study. Reported reductions in *L. monocytogenes* were approximately 2.5 – 3.5 log CFU in 30-120 seconds. Murphy et al (*7*) reported that a 5-minute exposure of 4 kg inoculated turkey breast to 96°C water in a cooker with a heat transfer coefficient of 800 W/m^2k resulted in a 2.0 log reduction in *L. monocytogenes* numbers. Similarly,

Muriana et al (*6*) found that a 2-minute exposure of 1.8 – 5.0 kg deli turkey product in 189 l of 93.3°C water always resulted in a $\geq$ 2.0 log CFU reduction in *L. monocytogenes* numbers.

Although process conditions in the present study were fairly specific, certain adjustments could be legitimately made to increase processing versatility without losing lethality. For example, use of larger volumes of boiling water, adding salt to the water to elevate boiling point temperature, and/or increasing the heat source power could be done with the same product and packaging material. However, changing the product composition, number of product pieces per package, or type of packaging film would invalidate application of the present study results.

In addition to the challenges inherent in achieving consistently adequate lethality in an economical manner, processors adopting PPP would face the challenge of maintaining desirable sensory properties in the treated product. The cook-out loss in beef snack sticks treated by PPP ranged from 0.2 to 1.1% by weight for beef snack sticks and from 0.4 to 1.2% by weight for natural casing wieners (Table 6). Cook-out loss could potentially be reduced by the addition of phosphates to the products. Processors seeking to use hot-water post-packaging pasteurization treatments may wish to investigate such formulation changes. When PPP-treated beef snack sticks (with cooked-out fat and moisture removed) were served alongside untreated beef snack sticks to untrained consumer panelists, no negative effect of the PPP treatment was detected (Table 7). The hot-water PPP-treated beef snack sticks from processor B were actually

evaluated as significantly better than the untreated control. Similar results were obtained for the natural casing wieners (Table 8). However, when cooked-out fat and moisture from beef snack sticks were clearly visible to panelists in follow-up sensory evaluation trials, the PPP-treated product was rated significantly less desirable in appearance than untreated beef snack sticks (Table 9). In contrast, the appearance of PPP-treated natural casing wieners (with no product heating after the package was opened) was rated significantly better than for untreated wieners. Panelist comments suggested that product color and shape (resulting from compression as the packaging film shrank during PPP) were important factors resulting in higher appearance scores. None of the comments indicated a problem with the amount of cooked-out fat and moisture.

In summary, we believe that it is possible to meet USDA compliance guidelines for an effective post-lethality treatment by treating beef snack sticks and natural casing wieners with the relatively simple technique of small-scale hot-water PPP. Before PPP can be widely adopted by small and very small processors of these products, however, further validation studies are necessary. In addition, processors must determine whether hot-water PPP treatments would result in product characteristics that are acceptable to their customers.

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Table 1. Physical and chemical characteristics of commercial beef snack sticks and natural casing wieners prior to hot-water post-packaging pasteurization.

Product	Beef Snack Sticks			Natural Casing Wieners		
Processor	A	B	C	A	B	D
Average Pre-heat Length (cm)	20	12.6	15.1	12	13.2	13.2
Average pre-heat Diameter (cm)	1.3	1.6	1.4	2.1	2.4	2.5
pH	4.5	5.0	5.0	5.9	6.1	6.3
Water activity (a_w)	0.91	0.86	0.89	0.95	0.97	0.97
Moisture (%)	36.0	29.1	34.6	53.6	52.3	58.5
Protein (%)	20.6	17.2	20.0	12.3	12.8	12.3
Moisture:Protein	1.7	1.7	1.7	4.3	4.1	4.7
Water-phase Salt (%)	9.6	8.8	10.1	3.6	3.8	3.1
Fat (%)	35.6	46.4	36.6	27.1	31.6	25.7
Typical sample wt[a](g)						

1 per package	4.5	6.0	4.5	----	---	---
4 per package	18.0	23.0	18.0	44.0	43.0	41.0
7 per package	31.0	40.0	31.0	---	----	---

[a]Sample consisted of one 2.5 cm longitudinal segment from the middle of each piece of product, obtained by making two cuts in the plane of the product diameter.

Table 2. Initial trials (no attempt to allow repair and enumeration of injured cells) to determine lethality of small-scale hot-water post-packaging pasteurization treatments against *Listeria monocytogenes* on commercial beef snack sticks and natural casing wieners. A single package of product was submerged in 2.8 liters of boiling water for the time indicated, then chilled immediately thereafter in ice-water slush.

Product	Treatment Time (min.)	Processor	Decrease (log CFU/sample) in *Listeria monocytogenes*[a] Trial Values			Mean	Group Mean
Beef Snack Sticks							
1 per pkg.	0.5	A	0.7	2.3[b]	0.3	1.1	
		B	2.4	1.7	1.3	1.8[C,1]	1.9[10]
		C	2.5	3.5[b]	2.9	3.0	
	1.0	A	1.0	2.3[b]	2.7[b]	2.0	
		B	3.7[b]	2.5	2.9[b]	3.0	2.8
		C	3.6[b]	3.2	3.6	3.5	
	1.5	A	4.4[b]	2.3[b]	2.7[b]	3.1	
		B	3.7[b]	3.5[b]	2.9[b]	3.4[1]	3.4[10]
		C	3.6[b]	3.5[b]	3.9[b]	3.7	
Beef snack sticks							
4 per pkg.	3	B	1.4	1.5	0.2	1.0	

		C	1.4	1.4	0.9	1.2 [2]	1.1 [11,12]
	4	A	3.9	3.9 [b]	4.0 [b]	3.9 [3]	
		B	2.7	3.9 [b]	1.4 [b]	2.7	2.9 [11,13]
		C	2.1	1.9	2.5	2.2 [2,3]	
	5	A	4.0 [b]	3.9 [b]	4.0 [b]	4.0	4.0 [12,13]
Beef snack sticks, 7 per pkg.	3	B	2.5	2.7	1.5	2.2	1.8 [14]
		C	2.1	1.8	0.4	1.4	
	4	B	4.0 [b]	2.8 [b]	4.3 [b]	3.7	3.1 [14]
		C	2.2 [b]	2.8 [b]	2.8 [b]	2.6	
	5	A	2.3 [b]	2.1 [b]	4.1 [b]	2.8	2.7
		C	2.2 [b]	2.8 [b]	2.8 [b]	2.6	
	6	A	2.3 [b]	2.1 [b]	4.1 [b]	2.8	2.8
Natural Casing Wieners 4 per pkg.							
	5	A	1.3	0.9		1.1	
		B	1.0	1.0	1.1	1.0	1.1 [15]
		D	1.3			1.3	

6	A	2.0	1.2	1.5	1.6 [4, 5]	
	B	1.1	1.1	1.4	1.2 [4, 6]	1.3
	D	1.1	1.4		1.3 [5, 6]	
7	A	4.3	1.1	1.9	2.4 [7, 8]	
	B	1.4	3.4		2.4 [7, 9]	2.2 [15]
	D	1.6	1.8		1.7 [8, 9]	

[a] Each value is the result from an individual trial. Three treated samples were analyzed for each trial.
[b] Indicates that no surviving cells were detected. For such a trial, a value of [Log (zero time) - 1.9 was used for the decrease in cells (see Materials and Methods for details).
[c]Pairs of means in a column with the same superscript number are significantly ($P < 0.05$) different.

Table 3. Confirmatory trials (procedure involved attempt to allow repair and enumeration of injured cells) to determine lethality of small-scale hot-water post-packaging pasteurization treatments against *Listeria monocytogenes* on commercial beef snack sticks and natural casing wieners. A single package of product was submerged in 2.8 liters of boiling water for the time indicated, then chilled immediately thereafter in ice-water slush.

Product	Treatment Time (min.)	Processor	Decrease (log CFU/sample) in *Listeria monocytogenes*[a] Trial Values			Mean	Std. Deviation (if n = 3)
Beef Snack Sticks 1 per pkg.							
	1.0	A	3.6[b]	3.5[b]	3.4[b]	3.5	0.1
		B	3.6[b]	3.0[b]	3.1[b]	3.2	0.3
		C	2.7[b]	4.4[b]	4.5[b]	3.9	1.0
Beef snack sticks 4 per pkg.							
	4	A	3.6[b]	3.8[b]		3.7	---
		C	3.4[b]	3.1[b]	3.0	3.2	0.2
Beef snack sticks, 7 per pkg.							
	4	A	2.9[b]	3.2[b]		3.1	---

		B	3.3	1.9	3.1 [b]	2.8	0.7
		C	2.0	2.2 [b]	2.1	2.1	0.1
Natural Casing Wieners 4 per pkg.							
	7	A	2.0	1.6	1.8	1.8	0.2
		B	3.4	2.9	2.1	2.8	0.7
		D	1.8	2.0		1.9	---

[a] Each value is the result from an individual trial. One treated sample was analyzed for each trial.
[b] Indicates that no surviving cells were detected. For such a trial, a value of [Log (zero time) - 1.9 was used for the decrease in cells (see Materials and Methods for details).

Table 4. Surface temperature of beef snack sticks and natural casing wieners during hot-water post-packaging pasteurization. Values are mean (n=2) for average of three surface mid-piece temperatures.

Product		Surface temperature (°C) after hot-water post-packaging pasteurization for given time (min)									
	Processor	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0	7.0
Beef Snack Sticks 1 per pkg.											
	A	5	43	47	56.5	---	---	---	---	---	---
	B	5	42	51.5	56	---	---	---	---	---	---
	C	5	43	49.5	51.5	---	---	---	---	---	---
Beef snack sticks 4 per pkg.											
	A	5	---	43.5	---	51	56.5	61	65	---	---
	B	5	---	39.5	---	51.5	55.5	58	61	---	---
	C	5	---	44	---	52	59.5	61.5	66	---	---
Beef snack sticks, 7 per pkg.											
	A	5	---	39	---	51	59	61	62.5	67	---
	B	5	---	40.5	---	46.5	52.5	58.5	62.5	66	---
	C	5	---	42	---	49	55	62	66	67.5	---

Natural Casing Wieners
4 per pkg.

A	5	---	42	---	45.5	53.5	58	58.5	62.5	62.5
B	5	---	40	---	44	51.5	53	56.5	60	60.5
D	5	---	39.5	---	45	47.5	54	58	60.5	63.5

Table 5. Calculated reduction in log CFU of *Listeria monocytogenes* during hot-water post-packaging pasteurization of beef snack sticks and natural casing wieners. Reductions based on measured surface temperatures (Table 4), and the American Meat Institute Foundation process lethality spreadsheet using $D_{62.8}$ = 67.6 seconds and Z = 7.9°C.

Product	Pieces per Package	Heating Time (min)	Processor	F_o (min)	Reduction in *L. monocytogenes* (log CFU)
Beef Snack Sticks	1	0.5	A	0	0
			B	0	0
			C	0	0
		1.0	A	0	0
			B	0.01	0.01
			C	0.01	0.01
		1.5	A	0.05	0.04
			B	0.05	0.04
			C	0.02	0.02
	4	3.0	A	0.12	0.11
			B	0.10	0.09

		C	0.24	0.21
	4.0	A	0.49	0.43
		B	0.28	0.25
		C	0.78	0.69
	5.0	A	1.75	1.55
		B	0.70	0.62
		C	2.40	2.13
7	3.0	A	0.20	0.18
		B	0.04	0.03
		C	0.07	0.06
	4.0	A	0.66	0.59
		B	0.20	0.18
		C	0.52	0.46
	5.0	A	1.42	1.26
		B	0.81	0.72
		C	2.20	1.95

		6.0	A	5.74	5.09
			B	2.55	2.26
			C	5.47	4.85
Natural	4	5.0	A	0.47	0.42
Casing			B	0.18	0.16
Wieners			C	0.22	0.19
		6.0	A	1.07	0.95
			B	0.48	0.43
			C	0.60	0.53
		7.0	A	1.99	1.77
			B	0.96	0.85
			C	1.48	1.31

Table 6. Cook-out loss during hot-water post-packaging pasteurization of beef snack sticks and natural casing wieners.

Product	Treatment Time (min.)	Processor	% weight lost in cook-out[a]
Beef Snack Sticks			
4 per package	3	A	0.9
		B	0.2
		C	0.8
	4	A	0.9
		B	0.6
		C	0.6
7 per package	4	A	1.1
		B	0.4
		C	0.7
	5	A	0.8
		B	0.7
		C	0.7
Natural Casing Wieners			
4 per package	5	A	0.4
		D	0.4
	6	A	0.8
		D	0.5

7	A	1.2
	B	1.0
	D	0.6

[a]Each value is for a single package of product.

Table 7. Consumer overall acceptance of beef snack sticks (seven per package) treated for 4.0 minutes with hot-water post-packaging pasteurization. Cooked-out fat and moisture was removed before presentation. "Trtmt" = treated product, "Ctrl" = untreated product.

Processor	A		B		C	
	Trtmt.	Ctrl	Trtmt	Ctrl	Trtmt	Ctrl
Assigned Descriptor						
Numerical score	Number of responses					
Like very much = 7	64	65	52	49	76	66
Like moderately = 6	71	69	84	66	61	89
Like slightly = 5	33	35	32	33	41	23
Neither like nor dislike = 4	6	7	8	11	7	7
Dislike slightly = 3	5	4	7	18	4	6
Dislike moderately = 2	2	2	2	7	2	1
Dislike very much = 1	1	0	1	2	1	0
Mean score	5.95	5.98	5.84	5.47	5.98	6.04
Trtmt significantly different?	NO		YES		NO	

Table 8. Consumer overall acceptance of natural casing wieners (four per package) treated for 7.0 minutes with hot-water post-packaging pasteurization. Product cooked in boiling water before presentation. "Trtmt" = treated product, "Ctrl" = untreated product.

Processor	A		B		D	
	Trtmt.	Ctrl	Trtmt	Ctrl	Trtmt	Ctrl
Assigned Descriptor						
Numerical score	Number of responses					
Like very much = 7	53	60	66	45	56	45
Like moderately = 6	79	81	68	79	71	76
Like slightly = 5	35	24	29	26	33	35
Neither like nor dislike = 4	5	9	12	14	7	12
Dislike slightly = 3	6	6	6	10	9	7
Dislike moderately = 2	3	3	2	6	2	3
Dislike very much = 1	2	0	1	4	0	0
Mean score	5.83	5.93	5.90	5.55	5.85	5.74

Trtmt significantly different? NO YES NOTable 9. Consumer acceptance of the in-package appearance of beef snack sticks (seven per package) treated for 4.0 minutes and natural casing wieners (four per package) treated for 7.0 minutes with hot-water post-packaging pasteurization.. “Trtmt” = treated product, “Ctrl” = untreated product.

Product	Beef Snack Sticks		Natural Casing Wieners	
Processor	B		B	
	Trtmt.	Ctrl	Trtmt	Ctrl
Assigned Descriptor				
Numerical score	Number of responses			
Extremely appealing = 7	13	36	34	23
Moderately appealing = 6	36	79	92	63
Slightly appealing = 5	39	44	39	45
Neither appealing nor unappealing = 4	31	16	15	24
Slightly unappealing = 3	51	12	15	33
Moderately unappealing = 2	19	7	1	6
Extremely unappealing = 1	9	4	2	4

Hot-water pasteurization of packaged RTE meats

Mean score	4.17	5.37	5.52	4.92
Trtmt significantly different?		YES		YES