Evaluation of Hot-Water Post-Packaging Pasteurization as a Post-Lethality Treatment against *Listeria monocytogenes* on Ready-to-Eat Beef Sticks and Natural Casing Wieners

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ABSTRACT

This study was conducted to evaluate hot-water post-packaging pasteurization (PPP) as a post-lethality 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 in a sauce pan on a hot plate, an average reduction in *L. monocytogenes* numbers of at least 2 logs was obtained for three brands of beef snack sticks and three brands of natural casing wieners using heating times of 1.0 minute for individually packaged beef snack sticks and 4.0 minutes for seven-per-package beef snack sticks. A treatment of 7.0 minutes for four-per-package natural casing wieners was also judged to be of potential commercial use. Cooked-out fat and moisture resulting from selected treatments ranged from 0.4 to 1.1% by weight for beef snack sticks and from 0.6 to 1.2% by weight for natural casing wieners. For all products tested in consumer sensory evaluation panels, the products treated by hot-water PPP (with cooked-out fat and moisture removed) were rated equal to or significantly better than corresponding untreated products.

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 (USDA, 2003). This rule went into effect 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 a potential post-lethality treatment, hot-water post-packaging pasteurization, for use 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 made by a large number of small and very small

processors. In the processing of beef snack sticks, the addition of salt, cooking, and smoking have already been shown to be effective antimicrobial processes by making the finished product unsuitable for *L. monocytogenes* growth (Ingham et al., 2004). 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, 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°C± 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 (AOAC International, 1998). 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.

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. 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. Initial inoculum levels were typically 4.5 – 5.0 log CFU per sample (see below for description of sampling procedure) for beef snack sticks (range was 3.1 – 5.3) and 5.5 – 6.0 for natural casing wieners (range of 3.0 – 6.3).

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 113 and 118 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. A trial consisted of three packages of product, all inoculated with an inoculum cocktail from the same broth cultures, for each of several treatment times. Untreated control packages were handled identically to treated packages other than during submersion in the boiling water.

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. A composite sample, consisting of a 2.5 cm segment from the center of each beef snack stick or natural casing wiener in the package, was obtained from the package and transferred to a stomacher filter bag. 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, further dilutions were made, as appropriate, using BPD. 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 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 each trial, the difference in log CFU between treated and untreated product was considered the treatment lethality. The lethality of each trial for each treatment is shown in Table 2, along with mean lethality (and standard deviation when n = 3) for each treatment.

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 and 4 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 heated 5, 6, and 7 minutes. Results of these trials are shown in Table 3. Unscreened panelists (n from 178-192) 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, which were presented in random order in cups coded with a three-digit random number. The treated and control natural casing wieners were separately warmed by being placed under boiling water for 4 minutes and then held warm in an insulated container prior to serving. 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 (Steel and Torrie, 1960) was conducted.

Results of sensory evaluation and subsequent statistical analysis of the sensory data are shown in Table 4 and 5.

Results and Discussion

Compliance guidelines from USDA state that an effective post-lethality treatment must reduce numbers of *L. monocytogenes* by at least 1.0 log (USDA, 2004). 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 had some lethality against *L. monocytogenes* on vacuum-packaged beef snack sticks and natural casing wieners (Table 2). With the compliance guidelines in mind, we can recommend 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 as having potential commercial utility (Table 2).

Treating individual beef snack sticks for 1.0 minute often eliminated all inoculum *L. monocytogenes* cells. Results from these trials would support validation of the 1.0 minute treatment for commercial use. Treating one-per-package beef snack sticks for 1.5 minutes and seven-per-package beef snack sticks for at least 4.0 minutes also resulted in complete elimination of inoculum *L. monocytogenes*. The seemingly high variability in some of the latter trials was solely the result of trial-to-trial differences in the number of cells in the inoculum. Thus, these results would support validation of a 1.5 minute treatment time for

individually packaged beef snack sticks and a 4.0 minute treatment time for seven-per-package beef snack sticks. Trials with four-per-package beef snack sticks were less extensive, but the results showed that a treatment time of 4.0 minutes would consistently come close to or exceed a 2.0 log reduction. Although a treatment time of 5.0 minutes was only tested on four-per-package beef snack sticks from one processor, it is likely that this treatment time could be validated for commercial use because this treatment time was consistently effective on seven-per-package beef snack sticks.

Lethality of hot-water PPP against *L. monocytogenes* on natural casing wieners was lower than that seen for beef snack sticks (Table 2). This difference is likely caused by the wieners having a larger mass than the beef snack sticks and thus taking longer to heat. 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.

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.

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 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. Particularly for beef snack sticks, cooked-out fat will be visible in packaged product and may be unacceptable to consumers. 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 4). 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 5).

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 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.

References

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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

Table 2. Lethality of 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 CUF/piece) in *Listeria monocytogenes*[a] Trial Values			Mean	Std. Deviation (if n = 3)
Beef Snack Sticks							
1 per pkg.	0.5	A	0.7	2.3[b]	0.3	1.1	1.0
		B	2.4	1.7	1.3	1.8	0.6
		C	2.5	3.5[b]	2.9	3.0	0.5
	1.0	A	1.0	2.3[b]	2.7[b]	2.0	0.9
		B	3.7[b]	2.5	2.9[b]	3.0	0.6
		C	3.6[b]	3.2	3.6	3.5	0.2
	1.5	A	4.4[b]	2.3[b]	2.7[b]	3.1	1.1
		B	3.7[b]	3.5[b]	2.9[b]	3.4	0.4
		C	3.6[b]	3.5[b]	3.9[b]	3.7	0.2
Beef snack sticks							
4 per pkg.	3	B	1.4	1.5	0.2	1.0	0.7
		C	1.4	1.4	0.9	1.2	0.3
	4	A	3.9	3.9[b]	4.0[b]	3.9	0.1
		B	2.7	3.9[b]	1.4[b]	2.7	1.3
		C	2.1	1.9	2.5	2.2	0.3
	5	A	4.0[b]	3.9[b]	4.0[b]	4.0	0.1

Beef snack sticks, 7 per pkg.	3	B	2.5	2.7	1.5	2.2	0.6
		C	2.1	1.8	0.4	1.4	0.9
	4	B	4.0 [b]	2.8 [b]	4.3 [b]	3.7	0.8
		C	2.2 [b]	2.8 [b]	2.8 [b]	2.6	0.3
	5	A	2.3 [b]	2.1 [b]	4.1 [b]	2.8	1.1
		C	2.2 [b]	2.8 [b]	2.8 [b]	2.6	0.3
	6	A	2.3 [b]	2.1 [b]	4.1 [b]	2.8	1.1
Natural Casing Wieners 4 per pkg.							
	5	A	1.3	0.9			
		B	1.0	1.0	1.1	1.0	0.1
		D	1.3				
	6	A	2.0	1.2	1.5	1.6	0.4
		B	1.1	1.1	1.4	1.2	0.2
		D	1.1	1.4			
	7	A	4.3	1.1	1.9	2.4	1.7
		B	1.4	3.4			
		D	1.6	1.8			

[a] Each value is the result from an individual trial. Three samples were analyzed for each trial.
[b] Indicates that no surviving cells were detected. For such a trial, a value of [Log (zero time) - Log (Detection Limit)] + 0.1 was used for the decrease in cells.

Table 3. 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	
		B	0.2
		C	0.8
			0.9
			0.9
			0.6
			0.6
	4	A	
7 per package	4	B	
		C	0.4
			0.7
			1.1
			0.8
			0.7
			0.7
	5	A	
		B	
		C	
Natural Casing Wieners			
4 per package	5	A	0.4
		D	0.4
	6	A	0.8
		D	0.5
	7		1.2
		B	1.0
		D	0.6

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

Table 4. Consumer acceptance of beef snack sticks (seven per package) treated for 4.0 minutes with hot-water post-packaging pasteurization. "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 5. Consumer acceptance of natural casing wieners (four per package) treated for 7 minutes with hot-water post-packaging pasteurization. "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		NO	