Center for Meat Process Validation

Annual Report on Research Supporting Critical Limit Choices

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University of Wisconsin-Madison

Dennis R. Buege and Steven C. Ingham

In 2003-2004, we completed several research projects providing scientific evidence for Critical Limit choices by meat and poultry processors. The results of these studies are summarized in the table below. The table also identifies the attached report containing detailed information on each study. In addition, several of the studies have been accepted for publication by a peer-reviewed scientific journal. The citation for each of these articles is also listed in the table. To obtain a copy of an article, please contact Steve Ingham at 608-265-4801 or scingham@wisc.edu. The research described in this report, along with information from ongoing research, will also be available on our website http://www.wisc.edu/foodsafety/meatresearch/

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SUMMARY OF STUDIES COMPLETED TO-DATE

Product/Step	Pathogens of Concern	Critical Limits Supported by Results	Report	Article Citation
Ham[a]/Cooking	*Staphylococcus aureus*	Internal temp. of 50-93°F for≤ 4 h, 93-115°F for ≤5 h, and 115 - 130°F for ≤5 h.	#1 (p.6)	Journal of Food Protection Volume 67, pages 1512-1516
Raw meat[b]/ Warmest Step	*Escherichia coli* O157:H7 *Salmonella* spp.	Exposure to 41- 50°F for ≤8 h or exposure to 41-72°F for ≤ 2 h; product chilled to ≤ 41°F by end of exposure time limit.	#2 (p. 29)	Journal of Food Safety In Press
Raw poultry[c] Warmest Step	*Escherichia coli* O157:H7 *Salmonella* spp.	Exposure to 41- 50°F for ≤8 h; product chilled to ≤ 41°F by end of exposure time limit.	#2 (p. 30)	Journal of Food Safety In Press
Pre-cooked Meats[d]/ Warmest Step	*Escherichia coli* O157:H7 *Salmonella* spp.	Exposure to 41- 50°F for ≤6 h or exposure to 41 - 70°F for ≤ 5 h; product chilled to ≤ 41°F by end of exposure time limit.	#3 (p. 54)	None
Head Cheese/ Formulation	*Listeria monocytogenes*	pH of 4.2 – 4.4 and refrigeration at ≤41°F	#4 (p. 68)	None

Snack Sticks/ Processing and Formulation	*Listeria monocytogenes*	various pH, % water-phase salt and water activity combinations (see Table below)	#5 (p. 75)	Journal of Food Protection In Press
Summer Sausage/Processing and Formulation	*Listeria monocytogenes*	various pH, % water-phase salt and water activity combinations (see Table below)	#5 (p. 75)	Journal of Food Protection In Press
Beef Jerky/ Processing and Formulation	*Listeria monocytogenes*	various pH, % water-phase salt and water activity combinations (see Table below)	#5 (p. 75)	Journal of Food Protection In Press
Pork Rind/ Cracklin Processing and Formulation	*Listeria monocytogenes*	various pH, % water-phase salt and water activity combinations (see Table below)	#5 (p. 75)	Journal of Food Protection In Press

[a]Ham must be pumped with brine to attain at least 2.3% sodium lactate, at least 0.8% sodium chloride (salt) and at least 200 ppm ingoing sodium nitrite.

[b]Ground beef, intact beef, intact pork, bratwurst, uncooked corned beef.

[c]Chicken white meat and chicken dark meat.

[d]Pre-cooked pork chops, turkey slices and ham slices in the “heat treated but not fully cooked, not shelf-stable” HACCP category.

Combinations of pH, % water-phase salt, and water activity that inhibited growth of *Listeria monocytogenes* on ready-to-eat meat products. For detailed information, please see report #5, starting on page 73.

Product Category	pH	% water-phase salt[a]	water activity
Beef/Venison Snack Sticks	4.8	7.6	0.91
	5.0	5.6	0.95
	5.0	5.9	0.93
Summer Sausage and	4.7	3.9	0.96
Related Sausage Products	4.9	5.2	0.95
	4.8	5.0	0.96
	5.2	6.5	0.95
	5.3	4.5	0.96
Beef Jerky	5.6	14.4	0.75
Pork Rinds/Cracklins	6.0	56.9	0.29
	6.1	60.7	0.27
	6.1	69.3	0.27
	6.7	69.2	0.28

[a] Also referred to as brine concentration. To calculate, divide the % salt by the sum of % salt and % water. Multiply the answer by 100.

Report #1: Evaluating *S. aureus* growth potential in slow-cooked hams

Modified version of article published in the Journal of Food Protection, Volume 67, pages 1512-1516

A Research Note

Evaluation of *Staphylococcus aureus* Growth Potential in Ham During a Slow-Cooking Process: Use of Predictions Derived From the USDA-PMP 6.1 Predictive Model and an Inoculation Study

Steven C. Ingham*[1], Jill A. Losinski[1], Brenda K. Dropp[1], Laura L. Vivio[1], and Dennis R. Buege[2]

University of Wisconsin-Madison

[1] Department of Food Science

[2]Department of Animal Sciences

*Corresponding author

University of Wisconsin-Madison, Department of Food Science

1605 Linden Drive, Madison, WI 53706-1565

Phone 608-265-4801 Fax 608-262-6872

e-mail scingham@wisc.edu

ABSTRACT

The United States Department of Agriculture (USDA) has cautioned against slow-cooking of meat such that the interior temperature increases from 50°F (10°C to 130°F (54.4°C) in ≥ 6 h. During a commercial ham-smoking process, the ham cold-point is typically between 50 and 130°F (10 and 54.4°C) for 13 h, but is later heated enough to kill vegetative pathogenic bacteria. Thus, production of heat-stable staphylococcal enterotoxin is the primary biological hazard. For this study, uncooked surface and uncooked ground interior ham were inoculated with a three-strain *Staphylococcus aureus* mixture, exposed to simulated surface and interior slow-cook conditions, respectively, and analyzed periodically using the Baird-Parker agar (B-P) and 3M™ Petrifilm™ Staph Express (PF-SE) count plate methods. For the surface and interior conditions, respectively, *S. aureus* numbers increased by no more than 0.1 and 0.7 log units. Predictions derived from actual time/temperature data and *S. aureus* growth values from a computer-generated model (PMP 6.1, USDA) were for 2.7 (ham surface) and 9.9 – 10.5 (ham interior) generations of *S. aureus* growth, indicating that use of model-derived growth values would not falsely indicate safe slow-cooking of ham. The B-P method recovered significantly ($P < 0.05$) greater numbers of *S. aureus* than the PF-SE method. For hams pumped with brine to initially attain: 1) 18% (w/w) weight gain, 2) ≥ 2.3% sodium lactate, 3) ≥ 0.8% sodium chloride, and 4) 200 ppm sodium nitrite, slow-cooking critical limits of ≤ 4 h between 50 and 93°F (10°C and 34°C), ≤5 h between 93 and 115°F (34°C

and 46°C), and ≤ 5 h between 115 and 130°F (46 and 54.4°C) could be considered adequate to ensure safety.

INTRODUCTION In 1999, the United States Department of Agriculture (USDA) issued microbiological performance standards to be followed in the cooking of beef and poultry products (*3*). USDA has also published a compilation of product interior time/temperature combinations considered validated to meet the performance standards (*4).* This compilation, known in the meat and poultry industry as Appendix A, is legally applicable to certain beef and poultry products, but has also been used as a guide for cooking pork products. Appendix A cautions processors against slow-cooking processes in which the interior of the product increases from 50°F (10°C) to 130°F (54.4°C) in greater than six hours. Although slow-cooking procedures are not expressly forbidden, the onus for safety validation is on processors using them. Under the mandatory Hazard Analysis Critical Control Point (HACCP) system, validated time/temperature parameters would likely serve as critical limits for the Critical Control Point of cooking in a slow-cooking process. Thus, studies are needed to examine the safety of slow-cooking procedures and possibly validate critical limits for their use under the HACCP system.

One common industry slow-cooking procedure is the smoking of large bone-in and boneless hams. During this process, the ham interior is eventually exposed to lethal temperatures ($\geq$ 130°F or 54.4°C) for times far longer than necessary to meet the performance standards for destruction of *Salmonella* spp. (*4*). There is little historical evidence to suggest that growth of spore-forming pathogenic bacteria during slow-cooking of ham is a significant hazard. For example, out of

680 reported foodborne illness outbreaks caused by pathogenic bacteria in 1999-2001, only 19 and 76 were caused by *Bacillus cereus* and *Clostridium perfringens*, respectively (*8*). Of these few outbreaks, pork was a potential vehicle in only two *C. perfringens* outbreaks and ham was never a potential vehicle. Furthermore, the presence of added sodium chloride and relatively high concentrations of sodium nitrite appears likely to minimize the risk of germination, growth, and toxigenesis by *Bacillus cereus* (*6,16*) and germination, growth, and sporulation-linked toxigenesis by *Clostridium perfringens* (*13,15,17*). In contrast, there is an association between ham and outbreaks of *Staphylococcus aureus* intoxication. During the same 3-year period cited above, 63 of the 680 outbreaks were caused by *S. aureus,* with 11 involving ham *(8*). Thus, the most significant biological hazard in the slow-cooking of hams is production of heat-stable enterotoxin by *Staphylococcus aureus*. Intoxication resulting from growth and enterotoxin production by *S. aureus* in foods is estimated to account for 1.3% of United States foodborne illness cases, but a high proportion of cases are believed to be sporadic and unreported (*14*). The intoxication rarely results in hospitalization, and is even less likely to cause death.

There are two plausible ways in which hams could be contaminated with *S. aureus* before cooking. First the surface of the ham could be contaminated as a result of slaughter, fabrication, or handling. A USDA baseline microbiological survey of 2,112 market hogs in 1995-1996 found that 16.0% of the carcasses were contaminated with *S. aureus*, with a mean level of 1.92 log CFU per square

cm for samples testing positive (*2*). Humans, including food handlers (*1,10,12*), have been identified as frequent carriers of *S. aureus,* and the ham surface could become contaminated with this organism during processing and handling (*7*). The second possible route of contamination is via the brine solution that is pumped throughout the ham using long needles. If the brine solution or pumping apparatus is contaminated with *S. aureus*, the organism could be introduced into the ham interior. Likewise, *S. aureus* cells on the ham surface could be carried into the muscle interior by the needles during pumping. In the process examined in the present study, the cure solution is prepared and refrigerated prior to pumping the ham. The present study considered potential *S. aureus* contamination of both the ham surface and interior.

An initial evaluation of the potential for *S. aureus* growth during slow-cooking is possible using actual product time/temperature data and *S. aureus* growth values from a computer-generated predictive model. The USDA Agricultural Research Service has developed such models, collectively known as PMP 6.1 (USDA-ARS, Eastern Regional Research Center, Wyndmoor, PA; note that a more recent version of the models is now available via the internet), for several food-borne pathogens. Although the models are based on data from studies using isothermal conditions in a laboratory medium, the senior authors have frequently used these models as a primary tool in evaluating meat/poultry processing critical limits and deviations. However, computer-generated predictive models are not acceptable as the sole means of validating critical

limits. Other methods, such as inoculation studies, are needed to fully validate critical limits.

The objectives of the present study were 1) to evaluate a typical ham slow-cooking procedure for *S. aureus* growth potential using *S. aureus* growth values from the PMP 6.1 model and an inoculation study, and 2) if the slow-cooking procedure was determined to be safe, to recommend potential critical limits for validation.

MATERIALS AND METHODS

Ham Processing Parameters. At a commercial meat processing facility, hams were pumped at a target level of 18% using (for 1565 lb. or 709 kg of pumped hams) a mix of 208.8 lb. (94.6 kg) water, 44.8 lb. (20.3 kg) sodium lactate, 16.3 lb. (7.4 kg) sodium chloride, 16.8 lb (7.6 kg) sucrose, 5.3 lb. (2.4 kg) of a commercial curing salt containing 6.25% sodium nitrite, 14.1 oz. (0.4 kg) sodium ascorbate, and proprietary amounts of phosphates and seasonings. Three pumped bone-in hams, weighing between 13.2 and 16.5 lb (6 and 7.5 kg), were shipped in refrigerated insulated containers to the laboratory immediately after pumping.

Preparation of the Ham for Laboratory Studies. Each ham was stored at 41°F (5°C) until prepared for laboratory studies. To prepare samples, ham was placed on a surface previously treated with 70% ethanol and a flame-

sterilized knife was used to slice off a 0.5 in (1.2 cm) exterior layer. The exterior layer was cut into 2 in x 2 in x 0.5 in (5 cm x 5 cm x 1.2 cm) standardized pieces, and placed in sterile petri plates for the exterior inoculation study. Next, sections of ham muscle were aseptically removed to reach the center. Interior muscle was aseptically removed and ground using a meat grinder with a plate having 0.016 in / 4.0 mm holes (Univex, Model MG8912, Salem, NH) and previously treated with 70% ethanol. Ground interior meat (0.18 ounce / 5 g) was transferred to each of several sterile 0.85 oz (25ml) plastic tubes (Falcon brand, Fisher Scientific, Itasca, IL). Tubes were manually shaken to force meat to the bottom of the tube and minimize air pockets. All samples were stored at 41°F (5°C) until used.

Inoculum Preparation. A cocktail was made using three strains of *Staphylococcus aureus*. Strains ATCC 25923 and ATCC 12600 were obtained from the American Type Culture Collection (Manassas, VA), and FRI 1007 was provided by Dr. Amy Wong (Food Research Institute, University of Wisconsin-Madison). Stock cultures were maintained at -4°F (-20°C) in Brain Heart Infusion Broth (BHIB; Difco, Becton-Dickinson, Mansfield, MA) containing 10% (w/v) glycerol (Fisher). Working cultures, maintained at 40°F (4°C) on Brain Heart Infusion Agar (BHIA; Difco) plates, were prepared monthly from frozen stock. To obtain working cultures, each strain was cultured twice at 95°F (35°C) in BHIB, streaked to a BHIA plate, incubated for 18-24 h at 95°F (35°C), and observed for culture purity. For each experiment, fresh cultures were prepared by transferring

a loopful of growth from the working culture plate to 0.3 oz (9 ml) BHIB and the inoculated tubes were incubated at 95°F (35°C) for 20-24 h. Cocktails were prepared by vortex-mixing each culture, combining the cultures into a 1.7 oz (50 ml) conical tube (Falcon brand, Fisher) and centrifuging at 5,000 x g for 10 minutes. The supernatant was decanted and cells were re-suspended to the original volume in Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, WI). The cocktail was then serially diluted in BPD, and plated on Baird Parker Agar (B-P; Difco) and 3M™ Petrifilm™ Staph Express (PF-SE; 3M Microbiology, St. Paul, MN) for initial enumeration. The PF-SE plates were incubated at 95°F (35°C) for 24 h and the B-P plates were incubated for 48 h at the same temperature. If only red-to-purple colonies were observed on a PF-SE plate after 24 h, they were counted as presumptive *S. aureus*. If more than one type of colony was observed, a thermonuclease disk was applied to the PF-SE plate, and plates were re-incubated at 95°F (35°C) for 1 – 3 h. Colonies displaying thermonuclease activity, as indicated by a pink halo on the thermonuclease disk after the second incubation period, were counted as presumptive *S. aureus*. Circular, smooth, convex, and gray to jet-black colonies surrounded by an opaque zone on B-P were counted as presumptive *S. aureus*.

Inoculation and Simulation of Ham Slow-Cooking Conditions.

Surface ham pieces were inoculated with 0.003 oz (0.1ml) of a 1:1000 dilution (BPD) of the *S. aureus* cocktail. The inoculum was spread evenly over the meat surface using a sterile bent plastic spreader (Daigger, Vernon Hills, IL). Each

petri plate (no lid) containing the inoculated ham exterior piece was placed in a 72°F (22°C) incubator. To simulate time/temperature data obtained for the commercial process of interest, the incubator temperature was increased after 30 minutes to 99°F (37°C), followed by another increase to 117°F (47°C) after 1.5 h. This temperature was maintained for an additional 2.0 h. The ground interior ham in each tube was inoculated with 0.003 oz (0.1ml) of *S. aureus* cocktail (diluted 1:1000 in BPD) by using a pipette tip to insert inoculum in the center of the ground meat. To simulate time/temperature data obtained for the commercial process of interest, the samples remained at room temperature for 2 h before being placed in a 80°F (26.7°C) water bath (Precision Reciprocal Shaking Bath; Precision Scientific, Chicago, IL). Subsequent increases in temperature occurred hourly. To monitor temperature, a calibrated thermometer was placed in the center of 0.18 oz (5 g) of uninoculated ground ham in the same type of tube used for the inoculated ham. This tube was placed in the water bath amongst the other tubes. Both experiments were done in triplicate (one trial per ham) with duplicate samples analyzed at each sampling time in each trial.

Enumeration of Inoculum Organisms Before and During Slow-Cooking. To evaluate survival of *S. aureus* during the slow-cooking ham procedure, samples were analyzed at periodic intervals. Each ham surface piece was placed in a sample bag, and 3.35 oz (99ml) BPD was added before stomaching for 2 minutes on medium using a Stomacher 400 lab blender (Fisher). To analyze each ham interior sample, ground ham was aseptically

removed from the Falcon tube to a sample bag, weighed, and BPD was added to make a 1:10 dilution. Samples were stomached for 2 minutes on medium using a Stomacher 400 lab blender. For both interior and exterior samples, subsequent dilutions were made in BPD and plated to B-P and PF-SE. The initial dilution was transferred to three B-P plates (0.01, 0.01 and 0.013 oz / 0.3, 0.3, and 0.4 ml) and one PF-SE plate. All subsequent dilutions were transferred to one plate each of B-P and PF-SE. Plates were incubated as previously described, and typical colonies were counted and log CFU was calculated for each sample. To confirm that colonies counted were *S. aureus*, one presumptive colony from each plating method from each trial was further tested for cell morphology, gram reaction, and coagulase activity. Throughout the study, all presumptive colonies were confirmed as *S. aureus*.

Deriving Predictions from *S. aureus* Growth Values from the PMP 6.1 Model. For each 0.5 – 2 h interval of the ham cooking simulation, the *S. aureus* growth values from the PMP 6.1 predictive growth model were used to predict the proportion of *S. aureus* lag phase that would elapse or the number of generations of *S. aureus* growth that would occur. To obtain the growth values, the following environmental conditions were used in the model: aerobic growth (chosen because aerobic growth is more rapid than anaerobic), pH 6.5 (typical pre-pumping value for hams; curing causes a drop of about 0.2 pH units), 2.5% water-phase sodium chloride (calculated from “worst case” values of 1.8% sodium chloride and 68% moisture), and 150 ppm added sodium nitrite (highest

value allowed in the model). The model covers the temperature range of 50 to 108°F (10°C to 42°C). For each time interval with temperatures below the optimum for *S. aureus* growth (93°F / 34°C), the maximum temperature for the interval was assumed to have occurred for the entire interval. For time intervals between 93°F (34°C) and the maximum temperature for *S. aureus* enterotoxin production (115°F / 46°C; *12*) the lowest growth temperature in the interval was assumed to have occurred for the entire interval. It was assumed that *S. aureus* was initially in lag phase (plausible since both the ham and the cure solution are refrigerated),and that the lag time at each given temperature during heating was the same as for the corresponding temperature during an isothermal experiment. Based on these assumptions, the proportion of lag phase elapsing during each time interval was obtained from the model and the cumulative proportion of lag phase was calculated (see Table 1). Similarly, when the entire lag phase had elapsed, the number of growth generations predicted during each time interval was determined from the corresponding isothermal model information and the cumulative number of growth generations was calculated.

Statistical Analyses. For the ham exterior data, mean log CFU (CFU) per piece of meat was calculated for each sampling time, along with standard deviation. The mean, and standard deviation of log CFU/g, were calculated for the ground interior ham for each sampling time. For each experiment, the paired t-test (release 12, Minitab, Inc., State College, PA) was done to determine if a

significant ($P < 0.05$) difference existed between the B-P and PF-SE enumeration methods.

RESULTS AND DISCUSSION

In both the ham surface and ham interior experiments, there was a small, but statistically significant ($P < 0.05$) difference between the two methods used for *S. aureus* enumeration. On average, the B-P method recovered slightly more cells than the PF-SE method. A previous study of inoculated smoked fish, Mozzarella cheese, and Parmesan cheese found a similar difference between the two methods for Mozzarella cheese samples stored at 40°F (4°C) for 28 and 42 days (11). However, there was no difference between the B-P and PF-SE methods for Mozzarella cheese samples stored 0 or 14 days, or for the Parmesan cheese and smoked fish. It is possible that some environmentally stressed *S. aureus* cells are unable to grow on the PF-SE plate, causing the difference observed in the present study.

Contamination of the ham interior with *S. aureus* is less likely to occur than surface contamination. However, interior contamination poses a greater potential food safety hazard because the ham interior heats much more slowly than the surface. Predictions made from *S. aureus* growth values from the PMP 6.1 computer-generated model were for 9.9 to 10.5 generations, roughly 3 log units, of growth for *S. aureus* exposed to the temperature conditions in each of the three trials. This level of growth would result in enterotoxin production sufficient to cause illness, about 10^6 cells, if *S. aureus* numbers prior to growth were in the

3 log range (*5).* The inoculation studies showed that the predictions based on *S. aureus* growth values from the PMP 6.1 model, as applied to this situation, were extremely conservative. Observed increases in *S. aureus* numbers did not exceed 0.7 log units (Table 2), between two and three generations of growth, for the period during which the ham was within the temperature range supporting *S. aureus* growth. It is likely that the ham composition was not as suitable for growth as the broth-based media used in obtaining data for the computer-generated model. Interestingly, during two of the three trials, *S. aureus* numbers began to decline during hour 14 of the experiment, suggesting that death of the organism begins between 127 and 130°F (52.5 and 54°C). This decrease accounted for the noticeably higher standard deviation observed for hour 14 data (Table 2).

Surface contamination of ham with *S. aureus* is more likely to occur than interior contamination, but the ham surface is exposed to suitable growth temperatures for a much shorter time than the interior. Predictions based on *S. aureus* growth values from the PMP 6.1 model were for 2.7 generations of *S. aureus* growth under temperature conditions to which the ham surface was exposed. This level of growth would only result in production of illness-causing amounts of enterotoxin if initial numbers of *S. aureus* were extremely high. The inoculation studies showed no significant increase in *S. aureus* numbers during the heating period (Table 3).

For hams having a composition similar to those used in this study, the inoculation study results clearly showed that slow-cooking critical limits for

product internal temperature of ≤ 4 h between 50 and 93°F (10°C and 34°C), ≤5 h between 93 and 115°F (34°C and 46°C), and ≤ 5 h between 115 and 130°F (46 and 54.4°C) will not allow growth of *S. aureus* to levels where enough toxin is produced to cause illness. Thus, the critical limits are safe and could be validated for use in HACCP plans. Key compositional levels that should be met for pumped hams cooked according to these critical limits would be a weight gain of 18% (w/w), ≥ 2.3% sodium lactate, ≥ 0.8% sodium chloride and 200 ppm sodium nitrite (the legal maximum). However, since brine has been shown to be a source of bacterial contamination (*9*), processors should frequently change the brine and clean and sanitize the brine container and pumping needles. Periodic microbiological testing is recommended to verify that the cure solution and pumping needles are not contaminated with high numbers of *S. aureus*. It is clear from our results that the *S. aureus* growth values from the PMP 6.1 model can be safely used to evaluate the safety of ham slow-cooking procedures, although such use of the values will greatly overestimate *S. aureus* growth.

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Table 1. Use of *Staphylococcus aureus* growth values from the PMP 6.1 predictive model to estimate growth of *S. aureus* growth during slow-cooking of ham. Growth parameters obtained for the following environmental conditions: aerobic growth, pH 6.5, 2.5% water-phase sodium chloride, and 150 ppm added sodium nitrite. Time/temperature data used was based on actual commercial conditions.

Time Elapsed	Length of Interval	Actual Temp. [a]	Assigned Temp.	Lag Time From Model	% Lag Phase Calculated[b]	Generation Time From Model	Generations Calculated
Surface Runs 1-3							
0.5 h	0.5 h	72 – 99	99	1.8 h	27.8 (27.8)	0.6 h	-----[c]
1.0 h	0.5 h	99	99	1.8 h	27.8 (55.6)	0.6 h	-----
1.5 h	0.5 h	99 - 117	99	1.8 h	27.8 (83.4)	0.6 h	-----
3.5 h	2.0 h	117	108	2.3 h	16.6 (100)	0.6 h	2.7
Interior Run 1							
2.0 h	2.0 h	48 – 73	73	5.8 h	34.5 (34.5)	1.8 h	-----
3.0 h	1.0 h	73 – 81	81	3.1 h	32.2 (66.8)	1.1 h	-----

4.0 h	1.0 h	81 – 93	93	1.9 h	33.2 (100)	0.7 h	0.4 (0.4)
5.0 h	1.0 h	93 – 97	97	1.8 h	-----[d]	0.6 h	1.7 (2.1)
7.0 h	2.0 h	97 – 107	99	1.8 h	-----	0.6 h	3.4 (5.5)
8.0 h	1.0 h	107 – 111	107	2.2 h	-----	0.6 h	1.6 (7.1)
10.0 h	2.0 h	111 – 118	108	2.3 h	-----	0.6 h	3.3 (10.4)

Interior Run 2

2.0 h	2.0 h	43 – 70	70	8.2 h	24.3 (24.3)	2.3 h	-----
3.0 h	1.0 h	70 – 82	82	2.8 h	35.7 (70.0)	1.0 h	-----
4.0 h	1.0 h	82 – 91	91	1.9 h	30.0 (100)	0.7 h	0.8 (0.8)
5.0 h	1.0 h	91 – 96	96	1.8 h	-----	0.6 h	1.4 (2.2)
7.0 h	2.0 h	96 – 106	99	1.8 h	-----	0.6 h	3.4 (5.6)
8.0 h	1.0 h	106 – 111	106	2.1 h	-----	0.6 h	1.6 (7.2)
10.0 h	2.0 h	111 – 117	108	2.3 h	-----	0.6 h	3.3 (10.5)

Interior Run 3

2.0 h	2.0 h	50 – 69	69	9.1 h	22.0 (22.0)	2.5 h	-----
3.0 h	1.0 h	69 – 81	81	3.0 h	33.3 (55.3)	1.1 h	-----
4.0 h	1.0 h	81 – 90	90	2.0 h	44.7 (100)	0.7 h	0.2 (0.2)
5.0 h	1.0 h	90 – 95	95	1.8 h	-----	0.6 h	1.4 (1.6)
7.0 h	2.0 h	95 – 104	99	1.8 h	-----	0.6 h	3.4 (5.0)
8.0 h	1.0 h	104 – 109	104	2.0 h	-----	0.6 h	1.6 (6.6)
10.0 h	2.0 h	109 – 115	108	2.3 h	-----	0.6 h	3.3 (9.9)

[a] Air temperature for surface studies; temperature of interior ground ham for interior studies. Value is °F.

[b]Value is % of lag time for the given time interval with cumulative % of lag time in parentheses.

[c]Absence of value indicates that the calculated lag phase had not ended. When value is provided it is number of generations for given time interval with cumulative number of generations in parentheses.

[d]No value provided because lag phase was completed in preceding time interval.

Table 2: Log of S*taphylococcus aureus* CFU/g in ground interior ham meat during simulation of a commercial slow-cooking process. Values are mean (n=3) and standard deviation (SD). *S. aureus* was enumerated by plating on Baird-Parker agar (B-P) and the 3M™ Petrifilm™ Staph Express Count plate (PF-SE).

Time	Target Temperature[a]	Actual Temperature	Log CFU/g B-P	Log CFU/g PF-SE
T(h)	(°C)	Mean (SD)	Mean (SD)	Mean (SD)
0	5.0	47 (3.8)	3.0 (0.1)	2.9 (0.1)
2	21.1	71 (2.3)	3.0 (0)	2.8 (0)
3	26.7	82 (0.5)	2.9 (0.1)	2.9 (0.1)
4	32.2	91 (1.6)	3.0 (0.1)	2.9 (0.1)
5	35.0	96 (1.1)	3.2 (0.5)	2.9 (0.3)
6	37.8	101 (0.9)	2.9 (0.1)	3.1 (0.5)
7	40.6	105 (1.4)	2.9 (0.1)	3.0 (0.2)
8	43.3	111 (1.1)	2.9 (0.1)	2.9 (0.1)
9	45.6	115 (0.9)	3.0 (0.2)	2.9 (0.2)
10	46.7	116 (1.4)	3.4 (0.4)	3.4 (0.5)
11	47.8	118 (1.1)	3.7 (0.7)	3.4 (0.7)
12	49.4	120 (2.0)	3.4 (0.8)	3.0 (0.8)
13	52.8	127 (0.5)	3.7 (1.1)	3.0 (1.1)
14	54.4	131 (1.4)	2.8 (1.5)	2.0 (1.2)

[a] Based on typical ham interior temperature during a commercial slow-cooking process.

Table 3: Log of S*taphylococcus aureus* CFU per exterior piece of ham during simulation of a commercial slow-cooking process. Values are mean (n=3) and standard deviation (SD). *S. aureus* was enumerated by plating on Baird-Parker agar (B-P) and the 3M™ Petrifilm™ Staph Express Count plate (PF-SE).

Time	**Incubator Temperature[a]**	**Log CFU/piece B-P**	**Log CFU/piece PF-SE**
(h)	**°F**	**Mean (SD)**	**Mean (SD)**
0	41	2.6 (0.1)	2.5 (0.1)
0.5	99	2.7 (0.1)	2.6 (0.1)
1	99	2.7 (0.1)	2.6 (0.1)
1.5	117	2.7 (0.1)	2.6 (0.1)
2 .0	117	2.4 (0.2)	2.3 (0.1)
2.5	117	2.4 (0.1)	2.3 (0.2)
3 (n=2)	117	2.3 (0.4)	2.2 (0.3)
3.5[b]	117	2.5 (0.1	2.5 (0.3)

[a] Based on typical smokehouse temperature in a commercial ham slow-cooking process.

[b] Experiment ended after 3.5 h because smokehouse temperature exceeded 130°F (54.4°C).

Report #2 Short-term temperature abuse of raw meats

Modified version of article accepted for publication by the Journal of Food Safety

Growth of *Escherichia coli* O157:H7 and *Salmonella* Serovars on Raw Beef, Pork, Chicken, Bratwurst, and Cured Corned Beef: Implications for HACCP Plan Critical limits

Steven C. Ingham[1*], Jill A. Losinski[1], Katie L. Becker[1], and Dennis R. Buege[2]

University of Wisconsin-Madison

[1]Department of Food Science

[2] Department of Animal Sciences

* Corresponding author:

1605 Linden Drive, Madison, WI 53706-1565

Phone 608-265-4801, fax 608-262-6872, e-mail scingham@wisc.edu

ABSTRACT Small amounts (0.35 – 0.88 oz / 10 – 25 g; 0.98 – 3.22 in^2 / 6.3 – 20.8 cm^2 inoculated area) of raw ground beef; intact beef, pork, and chicken (dark and white meat); bratwurst, and cured corned beef were inoculated with *Salmonella* serovars and *E. coli* O157:H7, refrigerated 24 h at 41°F (5°C), and then held either at 48 - 52°F / 9 - 11°C for up to 8 h or at room temperature (68 - 76°F / 20 – 24°C) for up to 2h. Except for a 0.2 log CFU increase in *Salmonella* spp. in ground beef during 2 h at room temperature, pathogens did not grow. Results of trials with commercial amounts of beef, pork, chicken, ground beef, and bratwurst exposed to 50°F (10° C) for 8 h or 72°F (22°C) for 2 h also showed no pathogen growth. Potential critical limits for processing of previously refrigerated raw meat products are exposure temperatures between 41 and 50°F (5 and 10 °C) for not more than 8 hours or between 41 and 72°F (5°C and 22°C) for not more than 2 hours.

INTRODUCTION Under the United States Department of Agriculture (USDA) regulations mandating the Hazard Analysis Critical Control Point (HACCP) system for ensuring safe meat and poultry products (USDA, 1996), a Critical Control Point (CCP) is defined as “a point, step, or procedure in a food process at which control can be applied and, as a result, a food safety hazard can be prevented, eliminated, or reduced to acceptable levels”. The major CCP in HACCP plans for raw meat and poultry products is often the step in the process at which the product is warmest. For example, the stuffing step may be the CCP in a process for making uncooked bratwurst. At such a step, certain critical limits must be met to ensure control of significant hazards previously identified by the processor when doing a hazard analysis. Two significant microbiological hazards associated with raw meat and poultry products are *Salmonella* serovars and *Escherichia coli* O157:H7. Processors of raw products may have limited control over whether these pathogens are present in the raw meat they receive for processing. Therefore, processors must select a CCP and associated critical limits that will minimize the risk associated with contaminated raw ingredients. Regulatory officials expect processors of raw products to use CCP critical limits (time and temperature) that have been scientifically validated to not allow growth of *Salmonella* serovars and *E. coli* O157:H7. For processors of raw poultry products, the existing regulatory limit of 55°F (13°C) for maximum product temperature during processing (United States Department of Agriculture, 2003) can be used as a critical limit, or a more restrictive critical limit may be chosen. Although validation of critical limits ideally should involve in-plant microbiological

testing (Brashears, *et al*., 2002), wide-scale microbiological testing is not feasible for most very small processors. The objective of this study was to provide initial, laboratory-based evidence supporting simple critical limits that could be validated for use in raw meat and poultry HACCP plans.

MATERIALS AND METHODS

Preparation of Inoculum Although contamination of raw meat or poultry by a single pathogen strain is possible in a plant setting, the present study used multi-strain "cocktails" of *Escherichia coli* O157:H7 and *Salmonella* serovars to account for potential variation. The following *Escherichia coli* O157:H7 strains were used: ATCC 43894, 51657, 51658, and 43895 (obtained from American Type Culture Collection, Manassas, VA; the first three strains were originally from infected patients and the fourth was from ground beef implicated in an outbreak), and USDA-FSIS-380-94 (obtained from Dr. John Luchansky, Food Research Institute, University of Wisconsin-Madison; originally from salami implicated in an outbreak). *Salmonella* serovars used were *S.* Hadar S21, *S.* Typhimurium S9, *S.* Infantis S20, *S.* Enteritidis E40, *S.* Anatum S14, and *S.* Heidelberg S13. All of the salmonellae were obtained from Dr. Eric Johnson, Food Research Institute, University of Wisconsin-Madison and had been used in previous challenge studies. The original source was unknown for strains S21 and S20, while strains S9, S13, and S14 were originally isolated from samples submitted to the Wisconsin State Laboratory of Hygiene. Strain E40 was a chicken ovary isolate. Frozen stock cultures were maintained in Brain Heart Infusion broth (BHIB; Difco,

Becton Dickinson, Sparks, MD) with 10% (v/v) added glycerol (Fisher Scientific, Itasca, IL). Working cultures were prepared by growing each strain for two passages in BHIB and then streaking on Brain Heart Infusion Agar (BHIA; Difco). Following growth on BHIA for 24 h at 95°F (35°C), the working cultures were stored at 41°F (5°C). For each trial, a colony of each culture was transferred separately to 0.3 oz (9 ml) of BHIB and incubated for 24 h at 95°F (35°C), and then centrifuged at 8,000 x g for 10 minutes. Each culture was then re-suspended in 0.3 oz (9 ml) of Butterfield's Phosphate Diluent (BPD, Nelson-Jameson, Marshfield, WI) and all of the cultures for each genus were combined. For bratwurst and corned beef experiments, and trials with commercial amounts of products, the cultures for each genus were combined in a sterile 1.7 oz (50-ml) plastic tube, centrifuged, decanted, re-suspended to the original volume in BPD and then the resulting cocktails for the two genera were combined, with an overall population for each genus of 9.6 – 10.3 log CFU/oz (8.1 – 8.8 log CFU/ml).

Meat and poultry products For trials with small amounts (0.35 – 0.88 oz / 10 – 25 g; 0.98 – 3.22 in^2 / 6.3 – 20.8 cm^2 inoculated area) of meat, ground beef (20% fat), boneless beef round steak, boneless center cut pork chops, chicken leg quarters (connected drumstick and thigh, up to 6% retained water), and boneless skinless chicken breasts were purchased at a local grocery store (separate purchases for each trial), transported to the laboratory within 15 minutes, and refrigerated $\leq$ 24 h until used. Uncooked bratwurst, from three

different manufacturers, was purchased frozen from the same local grocery store, transported to the laboratory and thawed under refrigeration. None of these products contained ingredients added for the purpose of inhibiting microbial growth. Uncooked, cured, corned beef briskets with garnish spices added (proprietary mix) were shipped overnight from a local processor in cooler boxes with ice packs. The corned beef represented three production lots, with each lot being studied in an independent trial. The corned beef was vacuum-packaged and had been cured with up to 30% of a mixture of water, salt, sodium phosphate, sugar, hydrolyzed corn protein, monosodium glutamate, sodium nitrite, sodium erythorbate, sodium nitrate, flavoring, and dextrose. Although some of these ingredients could potentially inhibit *Salmonella* and *E. coli* O157:H7, their addition was done primarily to obtain desired color and flavor, and to inhibit endospore germination and outgrowth. The corned beef was refrigerated 4 d before use. To prepare samples, products were divided or cut into smaller portions (0.35 – 0.88 oz / 10 – 25 g; 0.98 – 3.22 in^2 / 6.3 – 20.8 cm^2 inoculation area) and stored at 41°F (5°C) until inoculation. The ground beef was subdivided and shaped into small “mini-patties” (0.9 oz / 25 g), and the beef and pork were cut into small pieces (1 in x 1 in / 2.5 cm x 2.5 cm surface area, 0.5 in / 1.2 cm thick). For chicken, the skin was removed from the chicken parts and small pieces (1 in x 1 in / 2.5 cm x 2.5 cm surface area, 0.5 in / 1.2 cm thick) were cut from the surface muscle. Bratwursts (4-5 links per lot) were cut into 16 equal-sized pieces. The pieces were left intact for external (casing surface) inoculation. For internal inoculation, each link was cut in half longitudinally, and

then the resulting half-links were subdivided into 16 equal-sized pieces. The outer layer of each corned beef brisket was aseptically removed and cut into 3 in x 3 in x 0.5 in thick (6.3 cm x 6.3 cm x 0.6 cm thick) pieces.

For trials with commercial amounts of meat products, two beef rounds (average weight 58.3 lb / 26.4 kg), two beef briskets (average weight 8. 8 lb / 4.0 kg), two boneless pork loins (average weight 9.3 lb / 4.2 kg), twelve boneless skinless chicken breasts (average weight 1.3 lb / 615 g), six 3.1 lb / 1.4 kg chubs of ground beef round, and 18 fresh pork bratwursts with natural casing (average weight 0.2 lb / 100 g) were purchased from local grocery stores and transported within 15 minutes to a controlled environment chamber at the University of Wisconsin-Madison where the products were stored at 41°F (5°C).

Inoculation of Meat Pieces Each mini-patty or piece of meat was inoculated with 0.003 oz (0.1 ml) of a 1:1,000 dilution (BPD) of the *E. coli* O157:H7 cocktail and 0.003 oz (0.1 ml) of a 1:1,000 dilution of the *Salmonella* spp. cocktail. For bratwurst and corned beef pieces, the inoculation was done with a 1:10 dilution of the combined two-genus cocktail, 0.003 oz (0.1 ml) for bratwurst and 0.01 oz (0.3 ml) for corned beef. Each inoculated piece was aseptically transferred to a sterile sample bag and refrigerated for 24 h at 41°F (5°C) to ensure that inoculum cells were not actively reproducing, thus simulating the situation on meat shipped and stored under refrigeration. After this 24 h refrigeration period, enumeration of inoculum organisms was done for three

patties/pieces per storage treatment for each trial of each product type, as described below.

In trials with commercial amounts of products, the package of product was aseptically opened, and the packaging material either folded back (beef, pork, ground beef) or removed (chicken, bratwurst). Products were inoculated with a 1:10 dilution of the combined two-species cocktail as follows: each of two beef rounds was inoculated at four sites with 0.017 oz (0.5 ml) spread over each 4 in x 4 in (10 x 10 cm) site, each of two beef briskets was inoculated at four sites with 0.017 oz (0.5 ml) spread over each 3 in x 3 in (7.5 x 7.5 cm) site, each of two boneless pork loins was inoculated at four sites with 0.017 oz (0.5 ml) spread over each 2.5 in x 3 in (6.3 x 7.5 cm) site, each of twelve boneless skinless chicken breasts was inoculated with 0.017 oz (0.5 ml) spread over one half of the exposed surface, each of two chubs of ground beef round was inoculated over the entire upper surface with 0.03 oz (1.0 ml), the exterior of each of four fresh pork bratwursts was inoculated with 0.008 oz (0.25 ml) spread over the entire upper surface and four bratwursts were sliced open and for each bratwurst 0.008 oz (0.25 ml) was spread evenly inside the cut. After a 30-minute wait to allow bacterial attachment, the packaging material was placed back over the inoculated surface of beef, pork, and ground beef. Inoculated chicken breasts and bratwursts were individually placed into commercial zip-lock food storage bags. Inoculated meat products then remained in the 41°F (5°C) environmental chamber for 24 h until exposure to potential growth conditions.

Exposure of Inoculated Meat Products to Potential Growth Conditions After refrigeration, the inoculated mini-patties and small meat pieces were analyzed for initial inoculum levels or exposed to either room temperature (68 – 76°F / 20 - 24°C) for 1 h or 2 h, or 48 – 52°F (9-11°C, in a refrigerated incubator) for 2 , 4, 6, or 8 h. Because of the small amounts of meat/poultry studied, environment temperature was monitored, instead of product temperature, using a thermocouple probe (K-type) with an attached data-logger (Model SP-150, Dickson Instruments, Addison, IL). Inoculated commercial amounts of meat products were exposed to a temperature of either 50°F (10°C) for 8 hours or 72°F (22°C) for 2 hours. In trials with commercial amounts of products, the environmental chamber temperature was then returned to 41°F (5°C). Thermocouple probes (K-type) were inserted just under the surface of a beef round and bratwurst during the 50°F (10°C) and 72°F (22°C) trials. An attached data-logger recorded the time and temperature at 5-minute intervals.

Enumeration of Inoculum Organisms After the mini-patties or small meat pieces had been exposed to refrigeration and/or the various temperature/time combinations, surviving pathogens were enumerated. Enumeration was done for three samples following each storage treatment in each trial. Initially, 3.3 oz (99 ml) of BPD was added to a sample bag and the contents were stomached for 2 minutes using a Stomacher 400 lab blender (Fisher Scientific, Itasca, IL). Subsequent dilutions were made in BPD and spread-plates were prepared (one plate per dilution) on Sorbitol MacConkey agar

(SMAC; Oxoid, Inc., Ogdensburg, NY) and XLD agar (Oxoid) for enumeration of *E. coli* O157:H7 and *Salmonella* serovars, respectively. Plates were incubated at 95°F (35°C) for 24 h, typical colonies (white/colorless on SMAC, black on XLD) were counted and log CFU was calculated for each mini-patty or piece. To confirm that the colonies counted were the inoculum organisms, each of five typical colonies per plating medium (trials with ground beef, intact beef, pork, chicken) or one colony per trial (trials with bratwurst and corned beef) was transferred to BHIA and incubated for 24 h at 95°F (35°C). A presumptive *E. coli* O157:H7 colony from each BHIA plate was then tested for Gram reaction, cell morphology, oxidase reaction, presence of O157 antigen (DrySpot latex agglutination kit; Oxoid), and, for trials with ground beef, intact beef, pork, and chicken, appearance of growth on Levine's EMB agar (Difco). A presumptive *Salmonella* colony from each BHIA plate was tested for Gram reaction, cell morphology, oxidase reaction, and biochemical characteristics (API 20E kit; bioMerieux, Inc., Hazelwood, MO). Throughout the study, all presumptive colonies were confirmed as the appropriate inoculum species.

For trials with commercial amounts of product, samples were analyzed after 24 at 41°F (5°C) and then after 4 and 8 h exposure to 50°F (10°C) or after 1 and 2 h exposure to 72°F (22°C); samples were also analyzed 24 h after the 50°F (10°C) trials. Each sample was obtained by excising the inoculated area with a sanitized (70% ethanol) knife. The sample was transferred to a stomacher filter bag and analyzed as described above. Confirmation tests confirmed presumptive colonies as described above.

Statistical analysis For trials with small amounts (0.35 – 0.88 oz / 10 – 25 g; 0.98 – 3.22 in^2 / 6.3 – 20.8 cm^2 inoculated area) of meat, the log CFU/piece values for each sample after a given storage treatment in a trial were averaged. Then, the three resulting values (three trials) for a given storage treatment were averaged. The resulting value was compared to the value obtained after 24 h at 41°F (5°C) using the two-sample t-test (Minitab, release 12.22, Minitab, Inc., State College, PA) with a significance level of 0.05. No statistical analysis was done for trials with commercial amounts of meat.

RESULTS AND DISCUSSION

Initially, small amounts of ground beef, intact beef, pork, chicken dark meat, and chicken white meat; cured corned beef, and bratwurst were inoculated with multi-strain cocktails of *Salmonella* spp. and *E. coli* O157:H7. Small amounts of product were used because they would warm up much faster than the large amounts of meat used in meat processing plants, thus increasing the likelihood of pathogen growth. Samples were inoculated with approximately 5 log CFU of *E. coli* O157:H7 and *Salmonella* spp., refrigerated at 41°F (5°C) for 24 h (to be sure that the inoculum organisms were not reproducing, simulating their condition in previously refrigerated meat) and then exposed to either 48-52°F (9-11°C) for up to 8 h, or room temperature (68-76°F / 20-24°C) for up to 2 h. In these initial trials, changes in log CFU/piece were small after all storage treatments tested, ranging from an increase of 0.2 to a decrease of 0.2 (Table 1). No value

obtained after any storage treatment was significantly different ($P < 0.05$) from the initial value, with the exception of that for *Salmonella* on the ground beef mini-patties after 2 h at room temperature (panel A); however, the latter 0.2 increase in log CFU/piece is probably not of practical significance. It was clear from these results that short-term increases in raw meat temperature would have little effect on numbers of *E. coli* O157:H7 and *Salmonella* serovars present on meat and poultry products. Interestingly, the results suggested that short-term exposure of poultry pieces to temperatures above the 55°F (13°C) regulatory limit would not result in dangerous pathogen growth. The lack of growth observed in laboratory experiments was consistent with that predicted for *E. coli* O157:H7 and *Salmonella* by the United States Department of Agriculture, Agricultural Research Service PMP 6.1 computer-generated predictive model (USDA-ARS, Eastern Regional Research Center, Wyndmoor, PA). For a pH 6.5 broth system with 0.5% water-phase salt and no added sodium nitrite (least restrictive conditions in the model), the model predicted that *E. coli* O157:H7 would remain in lag phase for 54.0, 6.6, and 3.6 h at 50, 68, and 76°F (10, 20, and 24°C), respectively. Generation times for *E. coli* O157:H7 at these temperatures were predicted to be 5.3, 1.1 and 0.7 h, respectively. For *Salmonella* under the same broth culture conditions, the model predicted lag times of 63.4, 6.9, and 4.5 h and generation times of 9.4, 1.0 and 0.6 h, respectively.

The surface temperature for small pieces of meat would rapidly increase to at or near the storage temperature, with the interior temperature also increasing rapidly. In intact-whole muscle product (beef, pork, and chicken

pieces), pathogenic bacteria are only expected to be on the surface, since the interior of muscle tissue is virtually free of microbes. The surface is the first part of the samples to warm or cool when exposed to changing temperatures. Within practical limits, temperature changes within intact muscle have little effect on surface microbial growth. Ground meat (mini-patties and bratwurst) represents a different case since microbes are distributed throughout, and the slowest-cooling part of the ground meat mass is the key area when evaluating microbial growth. Compared to the samples used in our study, larger masses of meat would take considerably longer to increase in temperature, and thus there would be a smaller likelihood of pathogen growth. In addition, though, large masses of ground meat would take a longer time to cool once they did increase in temperature. Ideally, meat and poultry processors should determine actual processing plant and product temperatures at various times during the processing day, over an extended time, to fully understand their product temperature history. Additionally, processors should obtain microbiological testing data for indigenous microorganisms, e.g. coliform count, Aerobic Plate Count, for use in validating critical limits. If no increase in numbers of these indigenous microbes occurs when the proposed critical limits are followed, then the critical limits could be considered fully validated. Such an approach was taken by Brashears et al. (2002) to validate a beef fabrication critical limit of processing room temperature at 57°F (14°C) or lower for no more than 4 hours.

Results of subsequent trials with commercial amounts of meat (Table 2) confirmed the findings of trials with small meat pieces. There was no evidence of

growth on any of the products during exposure to either 50°F (10°C) for 8 hours or 72°F (22°C) for 2 hours. In 50°F (10°C) trials the temperatures just below the surface of the beef round (largest piece of meat) and the bratwurst (smallest piece) did not exceed 47°F (8.3°C) during 8 hours and were back to 41°F (5°C) within 5.5 and 0.5 hours, respectively, after the room was re-cooled. In 72°F (22°C) trials, the beef round temperature did not exceed 55°F (13°C) during the 2 h exposure time, and had cooled to 41°F (5°C) in 1.5 h after the room was re-cooled. The bratwurst surface temperature rose to 66°F (19°C) during 2 h at 72°F (22°C), but had cooled to 41°F (5°C) within 1.25 h after the room was re-cooled. Samples analyzed 24 h after the end of the 50°F (10°C) exposure showed no evidence of pathogen growth. These findings suggest that pathogen growth is unlikely in re-cooling raw meat and poultry products after short-term exposure to 50 – 72°F (10 - 22°C) temperatures.

Given that 41°F (5°C) is widely regarded as a safe temperature for preventing growth of non-psychrotrophic pathogenic bacteria in potentially hazardous foods (United States Food & Drug Administration, 2001), critical limits for a specific HACCP plan could address the time that the relevant temperature (product or processing room) is above this temperature. With temperature and microbiological data in hand, processors could establish scientifically valid critical limits involving processing plant or product temperatures and times, and design their monitoring programs accordingly. Our results suggest that the following times and temperatures should be considered potential critical limits for preventing the growth of *Salmonella* spp. and *E. coli* O157:H7 on raw meat and

poultry: products can be exposed to temperatures between 41 and 50°F (5 and 10 °C) for not more than 8 hours or to temperatures between 41 and 72°F (5°C and 22°C) for not more than 2 hours. The existing regulatory limit for raw poultry temperature during processing is supported by our results. Furthermore, our poultry results could be useful in evaluating poultry processing deviations involving short-term temperature elevation.

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TABLE 1.

LOG CFU/G OF SALMONELLA SEROVARS AND E. COLI O157:H7 ON SMALL AMOUNTS (0.35 – 0.88 oz / 10 – 25 g; 0.98 – 3.22 in^2 / 6.3 – 20.8 cm^2 INOCULATED AREA) OF MEAT AND POULTRY PRODUCTS STORED AT 41°F (5°C) FOR 24 H AND THEN AT EITHER 50°F (10°C) OR ROOM TEMPERATURE (RT; 68-76°F / 20-24°C). PRODUCTS ARE GROUND BEEF MINI-PATTY (PANEL A), INTACT BEEF (B), INTACT PORK (C), CHICKEN WHITE MEAT (D), CHICKEN DARK MEAT (E), BRATWURST EXTERIOR (F), BRATWURST INTERIOR (G), AND CURED CORNED BEEF (H).

A = ground beef mini-patty

Storage Treatment	Log CFU per piece [A]	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	4.7 (0.1)	4.5 (0.1)
1 h at RT	4.7 (0.1)	4.5 (0)
2 h at RT	4.8 (0.1)	4.7 (0.1)[B]
2 h at 50°F	4.7 (0)	4.5 (0)
4 h at 50°F	4.7 (0.1)	4.5 (0)

[A] VALUES ARE MEANS (N = 3) WITH STANDARD DEVIATIONS IN PARENTHESES. ABSENCE OF A SUPERSCRIPT INDICATES NO SIGNIFICANT DIFFERENCE ($P \geq 0.05$) FROM INITIAL VALUE).

[B] VALUE IS SIGNIFICANTLY DIFFERENT ($P < 0.05$) FROM INITIAL VALUE.

B = beef piece

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	4.6 (0.1)	4.6 (0.1)
1 h at RT	4.7 (0.1)	4.6 (0.1)
2 h at RT	4.8 (0.1)	4.6 (0.1)
2 h at 50°F	4.7 (0)	4.5 (0.1)
4 h at 50°F	4.8 (0.1)	4.6 (0)

C = pork piece

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	4.6 (0)	4.5 (0.1)
1 h at RT	4.5 (0)	4.5 (0.1)
2 h at RT	4.5 (0.1)	4.5 (0.1)
2 h at 50°F	4.6 (0.1)	4.5 (0.1)
4 h at 50°F	4.5 (0.1)	4.5 (0.1)

D = chicken white meat piece

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	4.7 (0.2)	4.3 (0.2)
1 h at RT	4.6 (0.1)	4.2 (0.2)
2 h at RT	4.7 (0.2)	4.3 (0.1)
2 h at 50°F	4.8 (0.1)	4.3 (0.1)
4 h at 50°F	4.7 (0.1)	4.2 (0.1)

E = chicken dark meat piece

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	4.7 (0)	4.3 (0.1)
1 h at RT	4.7 (0.2)	4.4 (0.1)
2 h at RT	4.8 (0.1)	4.3 (0.1)
2 h at 50°F	4.6 (0.1)	4.3 (0.1)
4 h at 50°F	4.7 (0.1)	4.4 (0.1)

F = exterior of bratwurst

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	5.3 (0.1)	5.2 (0.1)
4 h at 50°F	5.1 (0.2)	5.0 (0.2)
6 h at 50°F	5.2 (0.1)	5.1 (0.1)
8 h at 50°F	5.2 (0.1)	5.1 (0.2)

G = interior of bratwurst

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	5.5 (0.1)	5.4 (0.1)
4 h at 50°F	5.5 (0)	5.4 (0.1)
6 h at 50°F	5.5 (0.1)	5.3 (0.2)
8 h at 50°F	5.5 (0.1)	5.3 (0)

H = corned beef piece

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	5.9 (0.1)	5.8 (0.1)
4 h at 50°F	5.9 (0.1)	5.8 (0)
6 h at 50°F	5.9 (0.2)	5.8 (0.1)
8 h at 50°F	5.8 (0.2)	5.7 (0.2))

TABLE 2.

LOG CFU/G OF SALMONELLA SEROVARS AND E. COLI O157:H7 ON COMMERCIAL AMOUNTS OF MEAT AND POULTRY PRODUCTS STORED AT 41°F (5°C) FOR 24 H AND THEN AT EITHER 50°F (10°C) OR ROOM TEMPERATURE (68-76°F / 22°C). PRODUCTS ARE BEEF ROUND (PANEL A), BEEF BRISKET (B), PORK LOIN (C), CHICKEN BREAST (D), GROUND BEEF (E), BRATWURST EXTERIOR (F), AND BRATWURST INTERIOR (G).

A = beef round

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars.
Initial (24 h, 41°F)	4.8	4.7
1 h at 72°F	5.3	5.3
2 h at 72°F	4.7	4.5
Initial (24 h, 41°F)	5.3	5.1
4 h at 50°F	5.5	5.4
8 h at 50°F	5.3	5.1

B = beef brisket

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	5.1	5.1
1 h at 72°F	4.7	4.5
2 h at 72°F	4.6	4.4
Initial (24 h, 41°F)	5.3	4.8
4 h at 50°F	5.5	5.1
8 h at 50°F	4.6	4.4

C= Pork Loin

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	4.8	4.7
1 h at 72°F	4.5	4.1
2 h at 72°F	4.2	3.9
Initial (24 h, 41°F)	4.7	4.7
4 h at 50°F	4.8	4.6
8 h at 50°F	4.5	4.4

D = Chicken breast

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	4.9	4.8
1 h at 72°F	4.8	4.7
2 h at 72°F	4.8	4.6
Initial (24 h, 41°F)	4.6	4.1
4 h at 50°F	4.6	4.3
8 h at 50°F	4.9	4.7

E = Ground beef

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	4.9	4.8
1 h at 72°F	4.6	4.5
2 h at 72°F	4.7	4.6
Initial (24 h, 41°F)	4.6	5.3
4 h at 50°F	5.2	4.9
8 h at 50°F	5.0	4.8

F = Bratwurst exterior

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	4.6	4.3
1 h at 72°F	4.8	4.4
2 h at 72°F	4.7	4.4
Initial (24 h, 41°F)	4.7	4.3
4 h at 50°F	4.7	4.5
8 h at 50°F	4.7	4.4

G = Bratwurst Interior

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* serovars
Initial (24 h, 41°F)	5.4	5.1
1 h at 72°F	4.9	4.8
2 h at 72°F	5.3	5.0
Initial (24 h, 41°F)	5.5	5.2
4 h at 50°F	5.1	5.1
8 h at 50°F	5.0	5.2

Report #3 Short-term temperature abuse of cooked but not shelf-stable meat and poultry products

Laboratory-Based Evidence Supporting Simple Critical Limits for Use with Cured Meat and Poultry Products in the "Heat Treated But Not Fully Cooked, Not Shelf-Stable" HACCP Category

Steven C. Ingham[1*], Jill A. Losinski[1], and Dennis R. Buege[2]

University of Wisconsin-Madison

[1]Department of Food Science

[2] Department of Animal Sciences

* Corresponding author:

1605 Linden Drive, Madison, WI 53706-1565

Phone 608-265-4801, fax 608-262-6872, e-mail scingham@wisc.edu

ABSTRACT Some processors receive or produce refrigerated fully cooked cured meat products, then subdivide and repackage the product into single portions for refrigerated or frozen distribution. During portioning and packaging, the product is not re-heated and, because the consumer is directed to fully cook the product, it is assigned to the United States Department of Agriculture (USDA) Hazard Analysis Critical Control Point (HACCP) plan category of “heat treated but not fully cooked, not shelf-stable”. In a HACCP plan for this category, a primary Critical Control Point is usually the step at which the product is warmest. This study was done to support the designation of simple Critical Limits that could be incorporated in HACCP plans for heat treated but not fully cooked, not shelf-stable products to prevent growth of *Salmonella* spp. and *E. coli* O157:H7. Single-portion cured pork chops, turkey slices, and ham slices, intended for re-cooking by the consumer, were inoculated with multi-strain cocktails of *Salmonella* spp. and *E. coli* O157:H7, refrigerated 24 h at 41°F (5°C), and then exposed to either 50°F (10°C) for 2, 4, or 6 h, or room temperature (70°F / 21°C) for 1, 3, or 5 h. The greatest increase in pathogen numbers during the short-term exposure of pork chops to 50 – or 70°F (10 or 21°C) was 0.5 log CFU/piece with a statistically significant increase ($P < 0.05$) only observed after 5 h at 70°F (21°C) for *Salmonella* spp. (0.3 log CFU/piece increase). On ham and turkey slices, there was a 0.3 – 0.4 log CFU/piece increase in pathogen numbers when the products were first removed from refrigeration, which was probably attributable to recovery of injured cells. However, there was no significant increase in pathogen numbers thereafter, indicating that neither species was

growing. For full validation of Critical Limits, the individual processor should obtain actual plant and product temperature data, as well as microbiological data for their products. However the Critical Limits suggested by our results are that cured products in the heat treated but not fully cooked, not shelf-stable product category should not be between 41 and 50°F (5 and 10 °C) for more than 6 hours, or between 41 and 70°F (5°C and 21°C) for more than 5 hours.

INTRODUCTION Some processors receive or produce refrigerated fully cooked cured meat products, subdivide them into single portions, and repackage the products for refrigerated or frozen distribution. During this process, the products are not re-heated, and, because the consumer is directed to fully cook the products before consuming them, the products are assigned to the United States Department of Agriculture (USDA) Hazard Analysis Critical Control Point (HACCP) plan category of “heat treated but not fully cooked, not shelf-stable”. The major Critical Control Point (CCP) in HACCP plans for meat products in this category is usually the step in the process at which the product is warmest and conditions are most conducive for pathogen growth. For not fully cooked products, regulatory officials expect processors to use Critical Limits (time and temperature) for a CCP(s) that have been scientifically validated for preventing growth of *Salmonell*a spp. and *Escherichia coli* O157:H7. Although validation of Critical Limits should ideally involve in-plant microbiological testing (1), wide-scale microbiological testing may not be feasible for all processors. In particular, challenge studies involving pathogenic bacteria should not be conducted in a

commercial meat processing facility. The objective of this study was to provide initial evidence, based on laboratory studies of pathogen growth, supporting simple Critical Limits that could be incorporated in the HACCP plan for cured meat and poultry products in the heat treated but not fully cooked, not shelf-stable HACCP category.

MATERIALS AND METHODS

Overview Pork chops cut from cooked, cured pork loin and cured turkey and ham slices were inoculated with multi-strain cocktails of *Salmonella* spp. and *E. coli* O157:H7, refrigerated at 41°F (5°C) for 24 h (to be sure that the inoculum organisms were not actively growing and to simulate the situation in previously refrigerated meat) and then exposed to 50°F (10°C) for 2, 4, or 6 h or to room temperature (70°F / 21°C) for 1, 3, or 5 h. Growth of the inoculum organisms during the short-term temperature increases was determined by plating on selective media. The experiment was performed in triplicate to obtain three independent trials.

Pork Chops Pork chops cut from fully cooked and smoked cured pork loins were received via refrigerated truck from a processor. The pork loins had been cured with a mixture of water, salt, dextrose, sodium phosphates, sodium erythorbate, and sodium nitrite. The pork chops were approximately 0.7 in (1.8 cm) thick and were vacuum-packaged prior to shipping. Upon receipt, the pork chops were stored at 41°F (5°C) until used.

Ham and Turkey Slices Individually vacuum-packaged cured ham and turkey slices (about 0.8 in / 2 cm thick) were received from a processor in insulated coolers containing ice packs. Other than ham and turkey, the ingredient statements for the two products were identical, reading “water, sodium lactate, salt, sugar, sodium phosphate, sodium ascorbate, sodium nitrite”. The ham and turkey slices were stored at 41°F (5°C) until used.

Preparation of Inoculum Although contamination of raw meat or poultry by a single pathogen strain is possible in a plant setting, the present study used multi-strain “cocktails” of *Escherichia coli* O157:H7 and *Salmonella* spp. to account for potential strain-to-strain differences. The following *Escherichia coli* O157:H7 strains were used: ATCC 43894, 51657, 51658, and 43895 (obtained from American Type Culture Collection, Manassas, VA; the first three strains were originally from infected patients and the fourth was from ground beef implicated in an outbreak), and USDA-FSIS-380-94 (obtained from Dr. John Luchansky, Food Research Institute, University of Wisconsin-Madison; originally from salami implicated in an outbreak). *Salmonella* spp. strains used were *S. hadar* S21, *S. typhimurium* S9, *S. infantis* S20, *S. enteritidis* E40, *S. anatum* S14, and *S. heidelberg* S13. All of the salmonellae were obtained from Dr. Eric Johnson, Food Research Institute, University of Wisconsin-Madison. The original sources were unknown for strains S21 and S20, while strains S9, S13, and S14 were originally isolated at the Wisconsin State Laboratory of Hygiene. Strain E40 was a chicken ovary isolate. Frozen stock cultures were maintained

in Brain Heart Infusion (BHIB; Difco, Becton Dickinson, Sparks, MD) with 10% (v/v) added glycerol (Fisher Scientific, Itasca, IL). Working cultures were prepared by growing each strain for two passages in BHIB and then streaking on Brain Heart Infusion Agar (BHIA; Difco). Following growth on BHIA for 24 h at 95°F (35°C), the working cultures were stored at 41°F (5°C). A colony of each strain was streaked on BHIA and grown for 24 h at 35°C, following which a colony of each culture was grown separately in 0.3 oz (9 ml) of BHIB for 24 h at 95°F (35°C). All cultures for each species were combined, and then centrifuged at 5,000 x g for 10 minutes. Each resulting pellet was then re-suspended to original volume in Butterfield's Phosphate Diluent (BPD, Nelson-Jameson, Marshfield, WI) and cocktails for the two species were combined.

Inoculation of Meat Pieces Pork chops, ham slices, and turkey slices were laid on aluminum foil that had previously been treated with 70% (v/v) ethanol in a laminar flow bio-safety hood. Each pork chop was inoculated with 0.3 ml of a 1:1,000 dilution (BPD) of the two-species cocktail which was then spread evenly using a sterile bent plastic rod (Daigger, Inc., Vernon Hills, IL). After inoculation, the pork chops, ham slices, and turkey slices were allowed to dry for 15 min., and then flipped over, and the inoculation procedure was repeated. Each inoculated piece was aseptically transferred to a vacuum-packaging bag (FoodSaver, Tilia, Inc., San Francisco, CA), vacuum-packaged, and refrigerated for 24 h at 41°F (5°C) to ensure that inoculum cells were not actively growing. After this 24 h refrigeration period, enumeration of inoculum

organisms was done for three pieces per storage treatment for each trial of each product type, as described below.

Exposure of Inoculated Meat to Potential Growth Conditions After refrigeration, the inoculated pork chops, ham slices, and turkey slices were analyzed for initial inoculum levels or exposed to either 50°F / 10°C (in a refrigerated incubator) for 2, 4, or 6 h or room temperature (70°F / 21°C in an incubator) for 1, 3, or 5h.

Enumeration of Inoculum Organisms After the pork chops, ham slices, and turkey slices had been exposed to refrigeration and/or the various temperature/time combinations, surviving pathogens were enumerated. Enumeration was done for three samples following each storage treatment in each trial. Each pork chop, ham slice, or turkey slice was aseptically removed from its package and a 1 in x 1 in 0.25 in thick (2.5 cm x 2.5 cm x 0.6 cm thick) piece of meat was excised from one side of the chop or slice and transferred to a sterile sample bag. Then, 3.3 oz (99 ml) of BPD was added to the sample bag and the contents were stomached at medium speed for 2 minutes using a Stomacher 400 lab blender (Fisher Scientific, Itasca, IL). Subsequent dilutions were made in BPD and spread-plated (one plate per dilution) on Sorbitol MacConkey agar (SMAC; Oxoid, Inc., Ogdensburg, NY) and XLD agar (Oxoid) for enumeration of *E. coli* O157:H7 and *Salmonella* spp., respectively. Plates were incubated at 95°F (35°C) for 24 h, typical colonies (white/colorless on

SMAC, black on XLD) were counted, and log CFU was calculated for each piece of pork chop. To confirm that colonies counted were the inoculum organisms, each of four typical colonies per plating medium was transferred to BHIA and incubated for 24 h at 95°F (35°C). Presumptive *E. coli* O157:H7 colonies were then tested for Gram reaction, cell morphology, oxidase reaction, and presence of O157 antigen (latex agglutination kit; Oxoid). Presumptive *Salmonella* spp. colonies from each BHIA plate were tested for Gram reaction, cell morphology, oxidase reaction, and biochemical characteristics (API 20E kit; bioMerieux, Inc., Hazelwood, MO). Throughout the study, the identity of all presumptive colonies was confirmed.

Statistical analysis The log CFU/piece values for each sample after a given storage treatment in a trial were averaged (n = 3). Then, the three resulting values (three trials) for a given storage treatment were averaged. The resulting value was compared to the value obtained after 24 h at 41°F (5°C) using the two-sample t-test (Minitab, release 12.22, Minitab, Inc., State College, PA) with a significance level of 0.05.

RESULTS AND DISCUSSION For pork chops, the changes in pathogen numbers, expressed in log CFU/piece, were small after all storage treatments tested, ranging from an increase of 0.3 to no change (Table 1). Except for *Salmonella* after 5 h at 70°F (21°C) and *E. coli* after 3 h at 70°F / 21°C (Table 1), no value obtained after any storage treatment was significantly different (P <

0.05) from the initial value. In the latter case, the 0.2 increase in log CFU/piece was probably not of practical importance because no significant increase in *E. coli* O157:H7 numbers was observed after a longer period of 70°F (21°C) storage (5 h). It is clear from these results that short-term increases in product temperature will have little effect on numbers of *E. coli* O157:H7 and *Salmonella* spp. present on the pork chops.

For the ham and turkey slices, there was a statistically significant decrease in pathogen numbers during the 24 h of storage at 41°F (5°C) (Tables 2 and 3). This decrease was 0.7 – 0.8 log CFU/piece. During the first 2 h at 50°F (10°C) and the first 1 h at 70°F (21°C), pathogen numbers increased to levels 0.4 log CFU/piece lower than those before the 24 h 41°F (5°C) storage. Because pathogen numbers did not significantly increase during subsequent exposure to 50 or 70°F (10 or 21°C), we conclude that this initial increase was caused by recovery of cold-injured cells, rather than by actual growth in numbers.

In the present study, we monitored the temperature of the meat storage environment, as well as the actual meat temperature. The meat temperature increased to near that of the environment. Thus monitoring room temperature for processing of relatively small products like refrigerated pork loin, ham slices, or turkey slices may be an appropriate approach for Critical Limit monitoring. Ideally, meat and poultry processors should determine actual processing plant and product temperatures at various times during the processing day, over an extended time, to fully understand their product temperature history. Additionally, processors should obtain microbiological testing data for indigenous

microorganisms, e.g. coliform count, Aerobic Plate Count, for use in validating Critical Limits. No increase in numbers of these indigenous microbes would support the validity of the proposed Critical Limits. Such an approach was taken by Brashears et al. (1) to validate a beef fabrication Critical Limit of processing room temperature at 57°F (14°C) or lower with meat exposure for no more than 4 hours.

Given that 41°F (5°C) is widely regarded as a safe temperature for preventing growth of non-psychrotrophic pathogenic bacteria in potentially hazardous foods (2), Critical Limits for a specific HACCP plan could address the time that the relevant temperature (product or processing room) is above 41°F (5°C). With temperature and microbiological data in hand, processors can establish scientifically valid Critical Limits involving processing plant or product temperature and times, and design their monitoring programs accordingly. As a guideline for developing these Critical Limits, our results suggest the following general Critical Limits for preventing the growth of *Salmonella* spp. and *E. coli* O157:H7 on pork chops, ham slices, or turkey slices in the heat treated but not fully cooked, not shelf-stable product category: products should not be between 41 and 50°F (5 and 10 °C) for more than 6 hours, or between 41 and 70°F (5°C and 21°C) for more than 5 hours.

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Table 1. Mean (3 trials, each trial containing triplicate samples) log of Colony Forming Units (CFU) of *Salmonella* spp. and *E. coli* O157:H7 per pork chop sample after refrigeration (24 h at 41°F / 5°C) and subsequent storage at 50 or 70°F (10 or 21°C). Values are means with standard deviations in parentheses. Absence of a superscript indicates no significant difference ($P \geq 0.05$) from initial value.

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* spp.
Initial (24 h, 41°F)	4.6 (0.1)	4.4 (0.1)
2 h at 50°F	4.8 (0.3)	4.6 (0.3)
4 h at 50°F	4.8 (0.1)	4.6 (0.3)
6 h at 50°F	4.7 (0.1)	4.6 (0.1)
1 h at 70°F	4.6 (0.1)	4.5 (0.2)
3 h at 70°F	4.8 (0.2) [A]	4.6 (0.3)
5 h at 70°F	4.7 (0.1)	4.7 (0.1) [A]

[A]Value is significantly different ($P < 0.05$) from initial value.

Table 2. Mean (3 trials, each trial containing triplicate samples) log of Colony Forming Units (CFU) of *Salmonella* spp. and *E. coli* O157:H7 per ham slice sample at inoculation, after refrigeration (24 h at 41°F / 5°C), and after subsequent storage at 50 or 70°F (10 or 21°C). Values are means with standard deviations in parentheses. Absence of a superscript indicates no significant difference ($P \geq 0.05$) from initial value.

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* spp.
At inoculation	5.2 (0.1) [A]	5.0 (0) [A]
Initial (24 h, 41°F)	4.4 (0.1)	4.3 (0.2)
2 h at 50°F	4.8 (0.1) [A]	4.6 (0.3)
4 h at 50°F	4.8 (0.2)	4.7 (0.1) [A]
6 h at 50°F	4.8 (0.3)	4.7 (0.1) [A]
1 h at 70°F	4.8 (0.2)	4.7 (0.2)
3 h at 70°F	4.9 (0.1) [A]	4.8 (0.1) [A]
5 h at 70°F	4.8 (0.2)	4.7 (0.2) [A]

[A]Value is significantly different ($P < 0.05$) from initial value.

Table 3. Mean (3 trials, each trial containing triplicate samples) log of Colony Forming Units (CFU) of *Salmonella* spp. and *E. coli* O157:H7 per turkey slice sample at inoculation, after refrigeration (24 h at 41°F / 5°C), and after subsequent storage at 50 or 70°F (10 or 21°C). Values are means with standard deviations in parentheses. Absence of a superscript indicates no significant difference ($P \geq 0.05$) from initial value.

Storage Treatment	Log CFU per piece	
Conditions	*E. coli* O157:H7	*Salmonella* spp.
At inoculation	5.2 (0.1) [A]	5.0 (0.2) [A]
Initial (24 h, 41°F)	4.4 (0.1)	4.3 (0.1)
2 h at 50°F	4.8 (0.1)	4.6 (0.2)
4 h at 50°F	4.8 (0.1) [A]	4.5 (0.2)
6 h at 50°F	4.7 (0.1)	4.5 (0.1)
1 h at 70°F	4.8 (0.1)	4.6 (0.1)
3 h at 70°F	4.9 (0.2) [A]	4.7 (0.2) [A]
5 h at 70°F	4.7 (0.1)	4.5 (0.1)

[A]Value is significantly different ($P < 0.05$) from initial value.

Report #4 Survival of *Listeria monocytogenes* on head cheese

Validation of Head Cheese Formulation as an Antimicrobial Agent Against *Listeria monocytogenes*

Steven C. Ingham[1*], Jill A. Losinski[1], and Dennis R. Buege[2]

University of Wisconsin-Madison

[1]Department of Food Science

[2] Department of Animal Sciences

* Corresponding author:

1605 Linden Drive, Madison, WI 53706-1565

Phone 608-265-4801, fax 608-262-6872, e-mail scingham@wisc.edu

INTRODUCTION

The formulation of head cheese involves addition of large amounts of vinegar to pieces of meat. The finished product typically has a pH below 4.6 and, in some cases, the pH may be less than the 4.4 level which is cited as the minimum pH allowing growth of *Listeria monocytogenes* (2). At the request of a head cheese processor, the present study was conducted to validate a traditional head cheese manufacturing formulation as an effective antimicrobial agent against *L. monocytogenes*. Such a validation would allow the processor to operate under Alternative 2 of the USDA interim final rule addressing the control of *Listeria monocytogenes* on ready-to-eat meat and poultry products (1).

MATERIALS AND METHODS

Overview Head cheese from three different lots was inoculated with a multi-strain cocktail of *Listeria monocytogenes*. After samples were inoculated they were vacuum packed, and stored at 41°F (5°C). Three inoculated samples and one uninoculated sample were analyzed at day 0, day 8, and day 31 to determine the number of *L. monocytogenes* cells present.

Head Cheese Three loaves of head cheese representing three separate lots were shipped overnight to the laboratory in cooler boxes with ice packs and stored at 41°F 5°C until used. The pH values of the three lots were 4.4, 4.2, and 4.4. For this study, each loaf of head cheese was divided into 10 slices with

dimensions of 4.25 inches x 2.25 inches x 0.75 inches (10.8 cm x 5.7 cm x 1.9 cm).

Preparation of Inoculum Table 1 shows the strains used in this study. Frozen stock cultures were maintained in Brain Heart Infusion (BHIB; Difco, Becton Dickinson, Sparks, MD) with 10% (v/v) added glycerol (Fisher Scientific, Itasca, IL). Working cultures were prepared by growing each strain for two passages in BHIB and then streaking on Brain Heart Infusion Agar (BHIA; Difco). Following growth on BHIA for 24 h at 95°F (35°C), the working cultures were stored at 41°F (5°C). A colony of each strain was streaked on BHIA and grown for 24 h at 95°F (35°C), following which a colony of each culture was grown separately in 9 ml of BHIB for 24 h at 95°F (35°C). All cultures were combined, and then centrifuged at 8,000 x g for 10 minutes. Each resulting pellet was then re-suspended to original volume in Butterfield's Phosphate Diluent (BPD, Nelson-Jameson, Marshfield, WI).

Table 1. Organisms used in this study.

Microorganism	Strain Designation	Original Source
Listeria monocytogenes	Scott A[a]	Human isolate- MA epidemic
Listeria monocytogenes	LM 101[a]	Hard salami
Listeria monocytogenes	LM 108[a]	Hard salami
Listeria monocytogenes	LM 310[a]	Goat cheese
Listeria monocytogenes	V7[a]	Raw milk

[a] Obtained from the laboratory of Eric Johnson, Food Research Institute, University of Wisconsin-Madison

Inoculation of Head Cheese For inoculation, slices of head cheese were placed on aluminum foil that had previously been treated with 70% (v/v) ethanol in a laminar flow bio-safety hood. Each slice was inoculated with 0.01 oz (0.3 ml) of a 1:10 dilution (BPD) of the cocktail. The inoculum was spread evenly over the surface of the piece of head cheese using a sterile bent plastic rod (Daigger, Inc., Vernon Hills, IL). After inoculation, the pieces were allowed to dry for 15 min., flipped, and the procedure was repeated on the other side. Then, slices were aseptically transferred to a vacuum-packaging bag (FoodSaver, Tilia, Inc., San Francisco, CA), vacuum-packaged, and stored at 41°F (5°C). Uninoculated control slices of head cheese were packaged and stored in the same way.

Enumeration of Inoculum Organisms After inoculation, enumeration for three inoculated samples was done. For enumeration, each vacuum sealed bag was first treated with 70% ethanol, then opened using scissors that had been flame sterilized. Next, a 1 inch x 1 inch x 0.25 inch section was aseptically excised from one randomly chosen side of the slice and placed into a whirl pack bag. Then, 3.3 oz (99 ml) of BPD was added to the sample bag and the contents were stomached for 2 minutes on medium speed using a Stomacher 400 lab blender (Fisher Scientific, Itasca, IL). Subsequent dilutions were made in BPD and spread-plated on LSA agar (Oxoid, Inc., Ogdensburg, NY) with added Listeria Selective Supplements (Oxford formulation; Oxoid) for enumeration of *L. monocytogenes*. LSA plates were incubated at 95°F (35°C) for 48 h, typical

colonies (brown to black colonies with a zone of black precipitate) were counted, and the logarithm of Colony-Forming Units (log CFU) was calculated for each sample. In addition to the enumeration described above, day 8 samples were also diluted and plated on BHIA, incubated 48 h at 95°F (35°C). This analysis provided an estimate of all aerobic bacteria on the product surface, including injured (but viable) and uninjured *L. monocytogenes*. To confirm that colonies counted on LSA were *L. monocytogenes* , one typical colony per plating per lot of head cheese was transferred to BHIA and incubated for 24 h at 95°F (35°C). Presumptive *L. monocytogenes* colonies from each BHIA plate were tested for Gram reaction, cell morphology, oxidase reaction, and biochemical characteristics (API Listeria; bioMerieux, Inc., Hazelwood, MO). Throughout the study, all presumptive colonies were confirmed as the *L. monocytogenes*.

RESULTS AND DISCUSSION

The results (Table 2) clearly show that *L. monocytogenes* rapidly died off on the surface of the head cheese. Population decreases after 8 days averaged about 2.8 logs as determined using LSA and 2.5 logs as determined with BHIA plating. All colonies observed on BHIA after the day 8 plating had typical *L. monocytogenes* morphology. Counts on BHIA after 8 days of refrigeration were 0– 0.4 logs higher than on LSA, indicating the presence of some injured, but viable, *L. monocytogenes* cells on the head cheese. However, by 31 days no survivors were detected on LSA. In order to claim that an antimicrobial agent is effective in preventing growth of *L. monocytogenes* on a ready-to-eat meat or

poultry product, the agent must not allow more than a 1 log increase in *L. monocytogenes* populations (2). In the head cheese, the *L. monocytogenes* population decreased, so the processor may consider the product formulation to be an effective antimicrobial agent. Other head cheese processors should determine the typical pH of their product. If the pH is within the 4.2 – 4.4 range described in the present study, the processor may consider the product formulation to be an effective antimicrobial agent, allowing operation under Alternative 2 of the USDA regulations (1).

Table 2. Log CFU/g (standard deviation in parentheses) of *Listeria monocytogenes*.

	A	B	C
Day 0	5.9 (0.1)	5.7 (0.1)	5.8 (0.1)
Day 8	3.2 (0.3)	2.0[a] (0)	3.1 (0.4)
Day 8 (BHIA)	3.6 (0.3)	2.2 (0.4)	3.1 (0.1)
Day 31	0.9[b] (0.0)	0.9[b] (0.0)	0.9[b] (0.0)

a- no LM detected on 1:100 dilution, assigned value of 1.9 log CFU/g

b- no LM detected on 1:10 dilution, assigned value of 0.9 log CFU/g

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Report #5. Survival of Listeria monocytogenes on Ready-to-Eat Meat Products

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Survival of *Listeria monocytogenes* During Storage of Ready-to-Eat Meat Products Processed by Drying, Fermentation, and/or Smoking

Steven C. Ingham*[1], Dennis R. Buege[2], Brenda K. Dropp[1], and Jill A. Losinski[1]

University of Wisconsin-Madison

[1] Department of Food Science

[2] Department of Animal Sciences

*Corresponding author

University of Wisconsin-Madison, Department of Food Science

1605 Linden Drive, Madison, WI 53706-1565

Phone 608-265-4801

Fax 608-262-6872

e-mail scingham@wisc.edu

ABSTRACT

The survival of *Listeria monocytogenes* was evaluated on 15 ready-to-eat meat products made using drying, fermentation, and/or smoking. The products were obtained from 6 processors and included summer sausage, smoked cured beef, beef jerky, snack stick, and pork rind/cracklin products. The water activity (a_w) of the products ranged from 0.27 (pork rinds/cracklins) to 0.98 (smoked cured beef slices). Products were inoculated with a 5-strain cocktail of *L. monocytogenes*, re-packaged under either vacuum or air, and then stored at either room temperature (70°F / 21°C) or under refrigeration (41°F / 5°C) for 4- 11 weeks. Numbers of *L. monocytogenes* fell for all products during storage, ranging from a decrease of 0.8 log CFU on smoked cured beef slices during 11 weeks under vacuum at 41°F (5°C) to a decrease of 3.3 log CFU on a pork rind product stored 5 weeks under air at 70°F (21°C). All of the products tested could be produced under Alternative 2 of the United States Department of Agriculture (USDA) regulations mandating control of *L. monocytogenes* on ready-to-eat meat and poultry products. For many of the products, 1 week of post-processing storage prior to shipment would act as an effective post-lethality treatment and would allow processors to operate under Alternative 1 of these regulations.

INTRODUCTION 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 (*2*). This rule went into effect October 6, 2003 and has already had a major effect on processors of these products. The rule is intended to encourage processors of RTE products to take one or more specific steps to ensure the absence of *L. monocytogenes* from their products. These steps range from focused sanitation procedures to adding formulation or processing steps designed to kill *L. monocytogenes* or inhibit its growth. 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*.

Most small-scale meat processors in Wisconsin have chosen Alternative 3 for controlling *L. monocytogenes*. This alternative provides the least assurance of safety and requires the most frequent testing of food contact surfaces among the three alternatives. For many RTE meat products though, the reduction of water activity, accomplished through the addition of salt and cooking or drying, could serve as an antimicrobial process by making the finished product unsuitable for *L. monocytogenes* growth. Similarly, the reduction of pH via fermentation or addition of an acidulant, or surface-deposition of inhibitory compounds via smoking could also be effective antimicrobial processes. Items produced in these ways could thus fall under Alternative 2. Compliance guidance from USDA (*3*) has stated that an effective antimicrobial process will allow no more than a 1.0 log increase in *L. monocytogenes* on an RTE product throughout its shelf-life. This guidance also summarized scientific studies indicating that *L. monocytogenes* will not multiply at a water activity of < 0.92 or a pH of <4.39.

It is also possible that some RTE products could fall under Alternative 1, when the above-mentioned antimicrobial processing techniques are combined with short-term storage prior to distribution that effectively serves as a post-lethality treatment. The compliance guidance from USDA states that an effective post-lethality treatment must reduce numbers of *L. monocytogenes* by at least 1 log.

The regulation requires that post-lethality treatments must be scientifically validated and that evidence must be provided to substantiate the effectiveness of

antimicrobial processes. Unfortunately, most small-scale processors are unable to provide this evidence.

The objective of the present study was to evaluate the survival, and perhaps death, of inoculated *L. monocytogenes* during storage (after re-packaging) on a variety of RTE meat products made using drying, fermentation and/or smoking techniques. Information obtained in the study could thereby provide evidence needed by processors to implement Alternatives 1 or 2 in their RTE meat product operations.

MATERIALS AND METHODS Packages of RTE meat products were submitted by six processors. Product ingredients are summarized in Table 1. Depending on the product, 1 – 3 lots of product were tested. A representative sample from each lot of product was vacuum packaged and sent to a commercial testing laboratory to be analyzed for water activity, pH, and % water-phase salt (forced air oven determination of moisture – AOAC method 950.46Bb and potentiometric method for salt – AOAC method 980.25; *1*). For each product, the lowest % water-phase salt value, and the highest water activity and pH values, *i.e.* conditions least restrictive to microbial growth, are reported in Table 2. Because post-lethality contamination of RTE products by *L. monocytogenes* will occur only on the product surface, 0.6 cm thick sections of product, with surface dimensions of 1.5 in x 1.5 in (3.7 x 3.7 cm), were cut from each product using a knife [previously sanitized using 70% (v/v) ethanol] and placed in a biosafety hood on aluminum foil that had previously been sanitized with ethanol and

ultraviolet light. For beef jerky, the 1.5 in x 1.5 in (3.7 x 3.7 cm) pieces were cut from individual jerky slices. For the pork rind/cracklin products, existing individual pieces, each about 1 in x 1 in (2.5 x 2.5 cm), were individually inoculated.

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, and are listed in Table 3 . Stock cultures were maintained at -4°F (-20°C) in Brain Heart Infusion broth (BHIB; Difco, Becton-Dickinson, Sparks, MD) with 10% (w/v) added glycerol (Fisher Scientific, Itasca, IL). Working cultures, maintained at 40°F (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 95°F (35°C) for 18-24 h in BHIB, streaked to a BHIA plate, incubated at 95°F (35°C) for 18-24 h and examined for purity, and then stored at 40°F (4°C). Inoculation cultures were prepared for each strain by transferring a loopful of growth from the working culture plate to 0.3 oz (9 ml) of BHIB and incubating at 95°F (35°C) for 20-24 h. To prepare the 5-strain inoculum cocktail, the BHIB cultures were combined and distributed evenly into two 1.7-oz (50-ml) sterile plastic centrifuge tubes, and centrifuged for 10 minutes at 5,000 x g. The supernatant in both tubes was decanted and the pellets were resuspended to the original volume in Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, WI). The resulting cocktail was serially diluted in BPD and plated to determine cell concentration. To inoculate the meat or pork rind/cracklin pieces, a 0.0008 oz (0.025 ml) volume of the undiluted cocktail was pipetted onto the product surface and distributed as

evenly as possible using a sterile plastic spreader. The product pieces were then allowed to dry for at least 15 minutes and then vacuum packaged (0.8 atm.; Food Saver bags and packaging machine; Tilia, Inc.; San Francisco, CA) and stored at either 41 or 70°F (5 or 21°C). Pork rind/cracklin pieces were allowed to dry and then stored aerobically in zip-lock plastic bags at 70°F (21°C).

At the start of the study and after 1and 4, 5, or 11 weeks of storage, samples were analyzed for the number of *L. monocytogenes* cells per sample. The sample bag was aseptically opened, BPD (1.7 oz / 99 ml) was added, and the sample was stomached for 2 minutes at medium speed (Stomacher 400 lab blender; Fisher). Serial dilutions were made in BPD as needed. For the initial dilution, 0.03 oz (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, 0.003 oz (0.1 ml) was spread on one LSA plate per dilution. Plates were incubated at 95°F (35°C) for 48 h and then examined for typical *L. monocytogenes* colonies (small-medium, grey-brown-to-black; raised, flat, or sunken colonies surrounded by a black precipitate zone). Replica plating from Nutrient agar (Difco) to LSA was performed for some summer sausage samples but there was little evidence of cell injury. Furthermore, injured cells would be unlikely to survive the low-pH stress of human gastric juice if products were ingested. Therefore, direct plating on LSA was used throughout the study. For each product lot analyzed, one presumptive *L. monocytogenes* colony was selected at each sampling time for confirmation

testing. The colony was transferred to Nutrient Agar and after incubation was 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*.

With the exception of the pork rind/cracklin products, where only a small amount of sample was available, three pieces were analyzed at each sampling time for each lot of a particular product. For the pork rind/cracklin products, only one sample was analyzed per sampling time for each lot. The log CFU was calculated for each piece. Then the mean log CFU per piece was calculated for each lot and the mean of all lots tested was determined for the product at that sampling time. A value of 0.9 log CFU/g was assigned when no colonies were present for the least dilute plating.

RESULTS AND DISCUSSION The RTE meat products varied widely in water activity, pH and % water-phase salt (Table 2). Given that higher water activity and pH and lower % water-phase salt would increase the likelihood of *L. monocytogenes* growth, the product judged most likely to allow growth of this pathogen was the smoked cured beef slices and the products judged least likely to support growth were the pork rind/cracklin products and beef jerky. Numbers of *L. monocytogenes* recovered from stored products were consistent with these compositional factors. Levels of *L. monocytogenes* fell rapidly on the pork rind/cracklin products during room temperature storage (Table 4), with decreases

of 1.2 – 2.1 log CFU in the first week of storage, and subsequent decreases to levels at or near the detection limit for samples stored 5 weeks. Similar results were obtained for the beef jerky (Table 4). Processing RTE products to yield water activity of $\leq$ 0.75, combined with 1 week of 21°C storage, appears to effectively allow the processor to operate under Alternative 1, with the processing technique as the antimicrobial process and the one-week storage as the post-lethality treatment. It is not known whether the decrease in *L. monocytogenes* numbers during storage occurred at a uniform rate or was early in the one-week period. The latter situation would result in a shorter pre-shipment storage period serving as the post-lethality treatment.

The sausage products (summer, elk, buffalo) had maximum water activity of 0.94 – 0.96, considerably higher than for beef jerky. However, fermentation of the summer sausage products during processing resulted in a lower finished product pH than for the pork rind/cracklin products and jerky. (Table 2). Neither the water activity nor the pH of these products was low enough to predict, based on USDA compliance guidance, that *L. monocytogenes* would not grow. The combination of somewhat reduced water activity and pH seemed to effectively inhibit growth and cause death of *L. monocytogenes*. *L. monocytogenes* decreased in numbers by $\geq$1.0 log at room temperature (Table 4) or during refrigeration (Table 5). These results strongly suggest that sausage products, with a mandatory one-week pre-distribution storage period, could also be produced under Alternative 1. To do so, processors would be required to either ensure that their summer sausage had water activity and pH at least as low as

those products used in the present study, or have a challenge study conducted to validate the post-lethality treatment for their products.

Somewhat less death of *L. monocytogenes* occurred during storage of snack stick products (Table 5). It is clear that the snack stick products could be produced under Alternative 2; case-by-case studies would be necessary to determine if Alternative 1 could be chosen for these products. Room-temperature storage of snack stick products was not evaluated, although many products of this type are stored at ambient temperatures.

The intact and sliced smoked cured beef had the highest water activity and lowest % water-phase salt of the products studied (Table 2). As expected, *L. monocytogenes* survival was best on the sliced smoked cured beef product (Table 5) with decreases of only 0.8 log CFU during 11 weeks of refrigerated storage. Surprisingly, *L. monocytogenes* numbers decreased 3.0 log CFU on the surface of intact smoked beef during this same storage period. This difference in survival could reflect the somewhat lower water activity of the intact product, localized areas on the product surface possibly having even lower water activity, and perhaps the presence of anti-microbial smoke-derived compounds on the surface of the smoked beef product. The smoked cured beef results suggest that processing of these products should be done under Alternative 2 of the regulations.

In conclusion, the smoking, cooking, and drying processes used to make the tested products can be considered effective antimicrobial processes under the USDA regulations mandating control of *L. monocytogenes* on ready-to-eat meat

and poultry products. To verify that their own processing techniques allow operation under Alternative 2, processors should either verify that their products have water activity, pH, % water-phase salt levels at least as restrictive as those in the present study, or have a challenge study conducted to validate the lack of *L. monocytogenes* growth on their products. For many of the products studied, a one- week period of post-packaging room temperature storage prior to shipment could act as an effective post-lethality treatment and would allow processors to operate under Alternative 1 of the USDA *L. monocytogenes* regulations. Again, processors should verify similar product composition or conduct a challenge study to validate the post-lethality treatment lethality.

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Table 1. Ingredients of ready-to-eat meat products evaluated for survival of inoculated *Listeria monocytogenes* during storage (after re-packaging).

Product Description	Processor	Ingredient Summary
Smoked cured beef piece/slices	A	Beef, cured with water, salt, sugar, sodium erythorbate, sodium nitrite
Summer sausage	A	Beef and pork, salt, dextrose, natural spices, lactic acid starter culture, sodium erythorbate, sodium nitrite
Summer sausage	B	Beef, beef hearts[1], salt, corn syrup solids, dextrose, spices, mustard seed, sodium erythorbate, lactic acid starter culture, sodium nitrite
Summer sausage	C	Beef, pork, water, salt, corn syrup solids, dextrose, spices, ground mustard, lactic acid starter culture, sodium erythorbate, sodium nitrite
Buffalo summer sausage	C	Buffalo, pork, water, salt, sugar, pepper, mustard seed, garlic, monosodium glutamate, sodium erythorbate, sodium nitrite
Elk summer sausage	C	Elk, pork, water, salt, sugar, black pepper, mustard seed, garlic, monosodium glutamate, lactic acid starter culture, sodium erythorbate, sodium nitrite

Venison snack sticks	F	Venison, pork, water, salt, sugar, corn syrup solids, spices, dextrose, dehydrated garlic, red pepper, lactic acid starter culture, sodium nitrite
Beef snack sticks	A	Beef, salt, dextrose, natural spices, natural flavoring, lactic acid starter culture, sodium erythorbate, sodium nitrite
Pork rinds	D	Pork rinds, seasoning[2]
Pork cracklins	D	Pork cracklins (fried out pork fat with attached skin), salt
Beef jerky	E	Beef, water, salt, flavoring, sodium nitrate and sodium erythorbate

[1] One other lot also contained pork; another tested lot also contained pork and garlic.

[2] Seasonings differed for regular, smoke flavor, and BBQ flavor.

Table 2. Chemical and physical characteristics of ready-to-eat meat products evaluated for survival of inoculated *Listeria monocytogenes* during storage (after re-packaging).

Product Description	Processor	a_w	pH	water-phase salt (%)[a]
Smoked cured beef piece	A	0.96	5.5	2.9
Smoked cured beef slices	A	0.98	5.6	2.5
Summer sausage	A	0.96	4.7	3.9
Summer sausage	B	0.95	4.9	5.2
Summer sausage	C	0.96	4.8	5.0
Buffalo sausage	C	0.95	5.2	6.5
Elk sausage	C	0.96	5.3	4.5
Venison snack sticks	F	0.91	4.8	7.6
Beef snack sticks, small	A	0.95	5.0	5.6
Beef snack sticks, large	A	0.93	5.0	5.9
Pork rinds	D	0.29	6.0	56.9
Pork rinds, smoke flavor	D	0.27	6.1	60.7
Pork rinds, BBQ flavor	D	0.27	6.1	69.3
Pork cracklins	D	0.28	6.7	69.2
Beef jerky	E	0.75	5.6	14.4

[a] Also referred to as “brine content”.

Table 3. Strains of *Listeria monocytogenes* used to inoculate ready-to-eat meat products prior to re-packaging and storage.

Genus and species	**Strain Designation**	**Original Source**
Listeria monocytogenes	Scott A	Human outbreak isolate
Listeria monocytogenes	LM 101	Hard salami
Listeria monocytogenes	LM 108	Hard salami
Listeria monocytogenes	LM 310	Goat cheese
Listeria monocytogenes	V7	Raw milk

Table 4. Survival of inoculated *L. monocytogenes* on ready-to-eat meat products stored after re-packaging under air or vacuum at 70°F (21°C).

Product Description (processor)	Log CFU/sample (mean) with n and range in parentheses		
	Start	1 week	5 weeks
Summer sausage (B)	4.2 (1,0)	1.0 (1,0)	0.9 (1,0)[ab]
Summer sausage (C)	3.4 (1,0)	1.5 (1,0)	0.9 (1, 0)
Elk sausage (C)	4.0 (1,0)	2.4 (1,0)	0.9 (1,0)
Buffalo sausage (C)	3.1 (1,0)	0.9 (1,0)	0.9 (1,0)
Pork rinds (D)	4.3 (2, 0.1)	2.2 (2, 0.6)	1.1 (2,0.4)
Pork rinds, smoke flavor (D)	4.4 (1, 0)	2.7 (1, 0)	1.7 (1,0)
Pork rinds, BBQ flavor (D)	4.2 (1,0)	3.0 (1,0)	0.9 (1,0)
Pork cracklins (D)	4.2 (2,0.4)	2.6 (2,1.6)	1.1 (2, 0.4)
Beef jerky (E)	3.6 (3, 1.2)	1.2 (3, 0.2)	0.9 (3,0)

[a] 0.9 is value assigned when no cells were detected.

[b]Value obtained after 4 weeks of 70°F (21°C) storage.

Table 5. Survival of inoculated *L. monocytogenes* on ready-to-eat meat products stored after re-packaging under vacuum at 41°F (5°C).

Product Description (processor)	Log CFU/sample (mean) with number of lots and range in parentheses				
	Start	1 week	4 weeks	5 weeks	11 weeks
Smoked beef piece (A)	4.5 (2, 0.2)	3.7 (2, 0.9)	NT[a]	NT	1.5 (2,1.1)
Smoked beef slices (A)	4.3 (2, 0.1)	4.2 (2, 0.2)	NT	NT	3.5 (2,0.5)
Summer sausage (A)	3.6 (3, 0.6)	2.3 (3, 0.4)	0.9 (3, 0)	NT	NT
Summer sausage (B)	3.9 (3, 0.3)	1.5 (3, 1.8)	NT	NT	0.9 (3, 0)
Summer sausage (C)	3.4 (1,0)	1.5 (1,0)	NT	NT	0.9 (1,0)
Buffalo sausage (C)	3.7 (1,0)	2.6 (1,0)	NT	NT	1.5 (1,0)
Elk sausage (C)	3.8 (1,0)	3.2 (1,0)	NT	NT	2.4 (1,0)
Venison snack sticks (F)	4.0 (1, 0)	3.6 (1, 0)	NT	1.1 (1, 0)	NT
Beef snack sticks, small (A)	3.5 (2, 0.2)	2.5 (2, 0.3)	NT	2.1 (2, 1.0)	NT
Beef snack sticks, large (A)	3.7 (1, 0)	3.0 (1,0)	NT	2.0 (1, 0)	NT

[a]Not tested.