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

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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The University of Wisconsin-Madison Center for Meat Process Validation provides science-based HACCP support to small meat processors in meeting state and federal mandates for safe food processing and handling.

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 Conditions	Log CFU per piece	
	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 Conditions	Log CFU per piece	
	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 Conditions	Log CFU per piece	
	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.

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