Research Note

# Evaluation of *Listeria monocytogenes* and *Staphylococcus aureus* Survival and Growth during Cooling of Hams Cured with Natural-Source Nitrite

JIAN WU, JENNIFER ACUFF, KIM WATERMAN, AND MONICA PONDER*

*Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA*
*(ORCID: https://orcid.org/0000-0001-7047-3127 [M.P.])*

## ABSTRACT

Growing consumer demand for clean-label "natural" products has encouraged more meat processors to cure meat products with natural sources of nitrate or nitrite such as celery juice powder. One challenge for these producers is to identify safe cooling rates in products cured with celery juice powder where extended cooling could allow growth of pathogens. The Food Safety and Inspection Service of the U.S. Department of Agriculture recently added guidelines for stabilization of meat products cured using naturally occurring nitrites based on control of *Clostridium* spp. However, a knowledge gap exists for safe cooling rates that prevent the growth of *Listeria monocytogenes* and *Staphylococcus aureus,* potential postlethality contaminants, in naturally cured ham. The study was conducted to investigate the temperature profiles of naturally cured hams of typical sizes during refrigerator cooling and to determine the behavior of *S. aureus* and *L. monocytogenes* on ham during these cooling periods. Whole hams (14 lb [6,300 g]), half hams (6 lb [2,700 g]), and quarter hams (3 lb [1,400 g]) were slowly cooked in a smokehouse until internal temperatures reached a minimum of 140°F (60°C) and then were immediately transferred into a walk-in cooler (38°F [3.3°C]). Cooling times for hams of all sizes were within the requirements for cured products but not for uncured products. Worst-case scenarios of postprocessing surface contamination were simulated by inoculating small naturally cured ham samples with *S. aureus* or *L. monocytogenes*. These inoculated hams were then cooled under controlled conditions of 130 to 45°F (54.4 to 7.2°C) for 720 to 900 min. By the end of cooling, small decreases (0.5 to 0.6 log CFU/g) were found for each inoculum. These findings may help small ham processors evaluating production and quality control methods to determine whether recommended concentrations of natural curing agents used to prevent growth of clostridial pathogens may also prevent growth of other pathogens during meat cooling.

## HIGHLIGHTS

- Refrigerator cooling of naturally cured hams of various sizes was evaluated.
- All hams cooled met U.S. requirements from Appendix B option 3 but not options 1 or 2.
- *L. monocytogenes* and *S. aureus* populations did not increase during extended cooling.

Ready-to-eat (RTE) meats, including hams, have been implicated in a variety of human illnesses including botulism, staphylococcal endotoxin poisoning, and listeriosis *(1, 10, 15)*. The Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) *(13, 14)* has published compliance guidelines to help operators of small meat processing plants establish safe cooking and cooling procedures that ensure lethality of vegetative bacteria and prevent growth of spore-forming bacteria. Postcooking contamination of RTE meats, including whole pieces before slicing, has been documented after contact with contaminated equipment or water and with airborne microorganisms in the processing environment *(7, 8, 16)*. Because of concerns about the growth of spore-forming bacteria such as *Clostridium botulinum* and *Clostridium perfringens* and postcooking contamination by pathogens such as *Staphylococcus aureus* and *Listeria monocytogenes,* the use of antimicrobials is recommended for RTE meats sold in the United States.

Nitrites are key ingredients of RTE meats, contribute to color and flavor, and have antimicrobial properties. Nitrites prevent growth of *Clostridium* spp., including *C. perfringens* and *C. botulinum,* and must appear on the ingredient label when added in the purified form of sodium nitrite. Consumers have shown increased interest in clean-label

* Author for correspondence. Tel: 540-231-5031; Fax: 540-231-9293;
  E-mail: mponder@vt.edu.

meat products due to concerns about the adverse health effects of meat products traditionally cured with sodium nitrite *(11, 12)*. In response, processors, including both large corporations and small-scale artisans, are changing their recipes to replace synthetic nitrites with vegetable juices containing high nitrate concentrations. King et al. *(4)* found that natural sources of nitrites (e.g., celery juice powder [CJP]) can be used to control growth of *C. perfringens* in RTE meats, provided a source of ascorbate (e.g., cherry powder) is used. The FSIS guideline for stabilization of meat and poultry products (Appendix B) *(14)* recommends use of prereduced vegetable powders such as CJP to achieve the target concentrations of 100 ppm of nitrite and 250 ppm of ascorbate in RTE meats to limit growth of *C. perfringens* to <1 log CFU when fully cooked products are cooled from 130 to 80°F (54.4 to 26.7°C) for ≤5 h and then from 80 to 45°F (7.2°C) for ≤10 h (15 h total cooling time).

For meat products, added natural nitrites are just as effective as chemical nitrites for controlling growth of *Clostridium* spp. during extended cooling processes *(4)*. Thus, natural nitrites incorporated into ham have been assumed to be equally effective for controlling growth of other major pathogens, such as *S. aureus* and *L. monocytogenes*. However, limited information is available on the effects of natural nitrates on these other postlethality contaminants. Sodium nitrite from either chemical or natural sources inhibits the growth of *L. monocytogenes* during extended refrigerated storage *(2, 5)*. However, very few studies have addressed the inhibitory effect of naturally sourced nitrites on *L. monocytogenes* or *S. aureus* during an extended cooling process when meat products are exposed for prolonged periods to the temperature zone that is conducive to growth of those pathogens. Because of variability in product formulations, processing facilities, and resource accessibility, validation of individual cooling processes may be difficult for some processors, especially very small establishments. In this study, pilot-scale extended cooling processes with ham products were conducted to investigate the cooling pattern of whole hams of different sizes, and a universal laboratory-scale process was developed to evaluate survival of *S. aureus* and *L. monocytogenes* during extended cooling of hams produced with natural nitrites. A recent outbreak of listeriosis was attributed to fully cooked, whole sugar-cured hams *(1)*; thus, postlethality contamination of RTE meats still occurs. Findings from the present study will be a useful reference for food safety professionals and meat processors on how naturally occurring nitrites affect the growth of potential postlethality contaminants during the cooling process.

## MATERIALS AND METHODS

**Bacterial cultures.** *L. monocytogenes* strains 1/2a FSL R2-499 (sliced turkey isolate, Centers for Disease Control and Prevention, Atlanta, GA [CDC]), 1/2b FSL R2-502 (chocolate milk isolate, CDC), and 4b ScottA (1985 outbreak isolate from milk, University of Georgia, Athens) and *S. aureus* strains ATCC 12600 and ATCC 13565 (food-poisoning isolate from ham) were used in this study. Each strain was taken from frozen culture stock, streaked onto modified Oxford agar or mannitol salt agar, and incubated at 37°C for 24 h. A single colony from each plate was

TABLE 1. *Smokehouse program settings for ham heating*

| Program step | Heating time (h:min) | Relative humidity (%) | Temp, °F (°C) |
|---|---|---|---|
| Preheat | 1:00 | 25 | 160 (71.1) |
| 1 | 1:00 | 25 | 160 (71.1) |
| 2 | 0:45 | 30 | 160 (71.1) |
| 3 | 1:00 | 40 | 170 (76.7) |
| 4 | 1:30 | 50 | 180 (82.2) |
| 5 | Until core temp reaches 140°F (60°C) | 60 | 190 (87.8) |

then transferred into tryptic soy broth (TSB) and incubated for 24 h at 37°C for two generations. The second-generation TSB cultures of *L. monocytogenes* and *S. aureus* strains were mixed into single-species cocktails and serially diluted to approximately 6 to 7 log CFU/mL for ham inoculation.

**Ham temperature during extended cooling.** Bone-in whole hams (ca. 14 lb [6,300 g] each) were purchased from a grocery store; boned half hams (ca. 6 lb [2,700 g] each) and quarter hams (ca. 3 lb [1,400 g] each) were purchased from the Virginia Tech Meat Center. Hams were placed on a stainless steel cart and heated in a programmable smokehouse (Alkar 1000, Alkar-RapidPak, Lodi, WI) until the internal temperature reached 140°F (60°C). Temperature programs are presented in Table 1. After heating, the cart with shelved hams was transported into a walk-in cooler (7T95, Hillphoenix, Chino, CA) and held overnight at 40°F (4.4°C). Surface temperatures and internal temperatures at different distances from the surface (shallow, ~3 mm; mid, 36 to 56 mm; core, 56 to 95 mm) were measured with T-type needle thermocouples (CNL, Ecklund-Harrison, Fort Myers, FL) and recorded with a temperature data logger (RDXL12SD, Omega, Stamford, CT). The cooling temperature profile of the whole ham was used as the reference to simulate extended cooling processes.

**Ham samples cured with natural-source nitrite.** Ground pork (homogenized raw pork leg meat), salt (2.1%), preconverted CJP (100 ppm of verified ingoing nitrite equivalent; Veg Stable 506 2.25% nitrite, Florida Food Products, Eustis, FL), cherry powder (250 ppm of ingoing ascorbic acid; Veg Stable Cherry 525 17% ascorbic acid, Florida Food Product), tapioca starch (2%), dextrose (2%), and water were mixed to formulate a homogeneous ham sample with 40% pickup weight (pH 6.5 ± 0.1, water activity = 0.96). Ham samples (25 g) were aseptically weighed into filtered blender bags (Whirl-Pak, Nasco, Fort Atkinson, WI), flattened to a thickness of 2 to 3 mm, preheated to 165°F (74°C) for 1 min in a hot water bath, then cooled to room temperature. Five hundred microliters of the *L. monocytogenes* cocktail inoculum or the *S. aureus* cocktail inoculum containing 6 to 7 log CFU/mL was added to each sample for a final inoculation level of 4 to 5 log CFU/g on the ham. The inoculated ham samples were hand massaged for 15 s before being used in the simulated whole ham cooling process.

**Extended cooling process for inoculated ham samples.** Inoculated ham samples were placed into a water bath (2870, Thermo Scientific, Marietta, OH) and then a low-temperature incubator (Innova 4230, New Brunswick Scientific, Edison, NJ), resulting in two cooling processes with extremely low cooling rates to simulate worst-case scenarios. The first cooling process simulated refrigerator cooling of a whole ham, where the

FIGURE 1. *Temperature changes of ham samples during cooling. Temperatures of whole ham, half ham, and quarter ham are the average core temperature from triplicate cooling processes. Simulated and extended simulated cooling temperatures are the average of samples of inoculated small hams during lab-scale cooling processes. Shaded zone around each curve represents the standard deviation.*
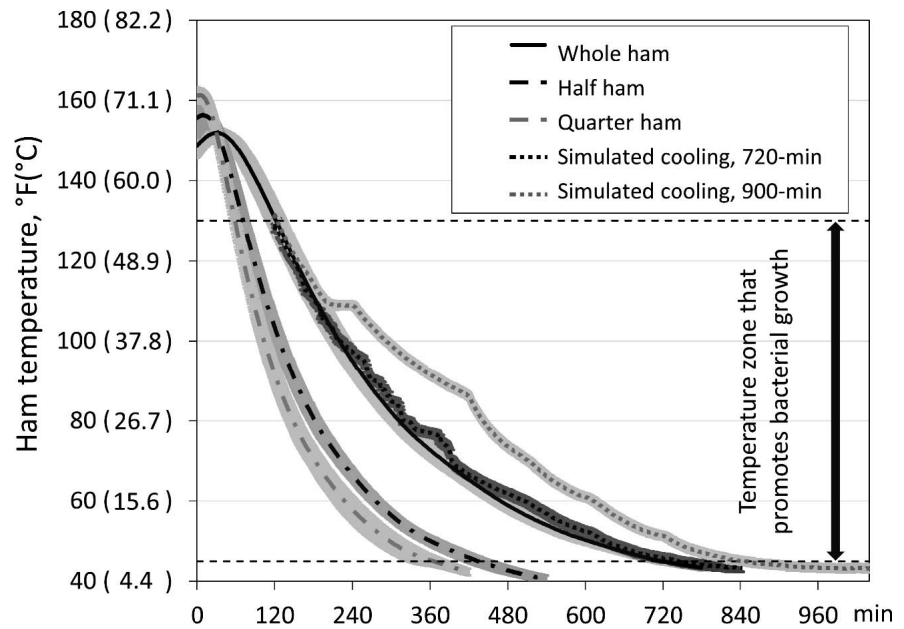
inoculated samples were first held in a water bath microadjusted from 130 to 80°F within ca. 3.5 h and then moved into the low-temperature incubator until the temperature was reduced to 45°F after an additional 7 h. The second cooling process simulated the longest cooling allowed by FSIS Appendix B option 3, in which inoculated samples were cooled from 130 to 80°F in ca. 5 h in a water bath and from 80 to 45°F in the low-temperature incubator in an additional 10 h. Temperatures of the water bath, incubator, and ham samples were monitored and recorded with the thermocouples and the data logger.

**Enumeration of bacterial populations on ham samples.** As ham samples were removed from the cooling process, 75 mL of sterile 1% peptone water was added, and samples were mixed for 1 min with a laboratory blender (BagMixer 400 CC, Interscience, Woburn, MA). The blended mixture was serially diluted and spiral plated (WASP Touch, Don Whitley Scientific Co., Bingley, UK). on modified Oxford agar for samples inoculated with *L. monocytogenes* or mannitol salt agar for samples inoculated with *S. aureus*. Colonies on agar plates were enumerated with a plate counter (ProtoCOL SR/HR, version 1.47.0, Synbiosis, Cambridge, UK) after 24 h of incubation at 37°C.

**Statistical analysis.** Extended heating and cooling processes for each ham size were repeated three times. Simulated cooling processes were conducted three times at each of two settings. Duplicate samples with each inoculum were enumerated from each cooling process at each sampling point. Standard plate counts for each bacterial type were log transformed. Within each cooling process and each inoculum type, levels for each bacterial species were compared at each sampling time point with a one-way analysis of variance (ANOVA) and Dunnett's multiple comparison in JMP Pro 15 (SAS Institute, Cary, NC). Results were considered significant at $P < 0.05$.

## RESULTS AND DISCUSSION

**Cooling behavior of ham.** The temperature of the core of the ham, which is the coldest area, was plotted against cooling time (Fig. 1). The core temperature of the ham continued to rise during the first 10 to 30 min after heating

stopped, depending on the size of the ham. On average, quarter hams required 303 ± 23 min to cool from 130 to 45°F; cooling times for half hams and whole hams were 369 ± 18 and 598 ± 16 min, respectively. For each size of ham, the portion of the cooling temperature curve between 130 and 45°F can be best expressed ($R^2 > 0.99$; Excel, Microsoft, Redmond, WA) by a second-order polynomial equation (data not shown). The time requirements and the actual cooling times of ham are summarized in Table 2.

FSIS guidelines for small producers of ready-to-eat meats (Appendix B) provide three options for cooling cooked meat products that focus on time and temperature profiles. These options are intended to assure that growth of *C. perfringens* does not exceed <1 log CFU; however, they do not consider potential growth of introduced *L. monocytogenes* or *S. aureus*, both of which may be postcooking contaminants. For uncured products such as fully cooked uncured hams, the cooling time requirements for options 1 and 2 are fairly short *(14)*: option 1 requires ≤1.5 h between 130 and 80°F and ≤5 h between 80 and 40°F; option 2 requires ≤1 h between 120°F (48.9°C) and 80°F and ≤5 h between 80 and 55°F (12.8°C) followed by continuous cooling to 45°F. For fully cooked products cured with at least 100 ppm of sodium nitrite from purified or natural sources and 250 ppm of sodium ascorbate, the cooling time is extended because the antimicrobial agents prevent growth of the intended pathogen for control (option 3). For option 3 *(14)*, the cured products must chill from 130 to 80°F in <5 h but cannot exceed 10 h between 80 and 40°F. Our results indicate that each of the typical sizes of ham produced by our surveyed small processors in Virginia were cooled as per the requirements of option 3 by placement in a walk-in refrigerator without other assistive cooling methods such as brine chilling or cold shower. However, additional cooling methods may be needed if the refrigerator were overly packed or the power were insufficient, making it necessary for processors to monitor meat temperatures. Even the smallest size of ham tested (quarter ham) did not meet the

TABLE 2. *Stabilization options of three FSIS Appendix B options and cooling times for ham*

| Parameter | Option 1: uncured and cured products | | Option 2: uncured and cured products | | Option 3: cured products with ≥100 ppm of nitrite and ≥250 ppm of ascorbic acid | |
|---|---|---|---|---|---|---|
| Temp zone | 130–80°F (54.4–26.7°C) | 80–40°F (26.7–4.4°C) | 120–80°F (48.9–26.7°C) | 80–55°F (54.4–12.8°C) | 130–80°F (54.4–26.7°C) | 80–45°F (26.7–7.2°C) |
| Maximum time allowed (min) | 90 | 300 | 60 | 300 | 300 | 600 |
| Cooling time (min) | | | | | | |
|   Quarter ham | 90 | 332[a] | 78[a] | 109 | 90 | 217 |
|   Half ham | 111[a] | 336[a] | 94[a] | 129 | 111 | 253 |
|   Whole ham | | | | | 193 | 407 |
|   Simulation cooling (720-min process) | | | | | 202 | 423 |
|   Simulation cooling (900-min process) | | | | | 318[a] | 404 |

[a] Time that the product was exposed to the required temperature zone exceeded the requirements of Appendix B.

cooling requirements of either option 1 or option 2, indicating that chilling or cooling methods in addition to refrigerator cooling are needed for uncured hams >3 lb to ensure *C. perfringens* growth is <1.0 log CFU/g. Individual processing facilities should take product size and weight into account and test the capabilities of their own cooling equipment. For small processors that are unable to invest in additional types of cooling equipment, use of preconverted natural sources of nitrite and ascorbate will prevent production of an adulterated product.

**Survival of bacteria in ham with natural-source nitrite.** Survival of *L. monocytogenes* and *S. aureus* is shown in Figure 2. For the 720-min cooling process derived from whole ham cooling, inoculation levels were 5.9 ± 0.1 and 5.5 ± 0.1 log CFU/g, respectively. For the 900-min cooling process, which was designed to represent surface contamination of the ham followed by a worst-case scenario of slow cooling, inoculation levels were 4.6 ± 0.1 and 4.2 ± 0.1 log CFU/g, respectively. Neither bacterium had significant growth over the cooling process; according to the ANOVA and Dunnett's multiple comparison, populations of

*L. monocytogenes* decreased significantly at the end of the cooling processes ($P < 0.05$). A similar small but not significant decrease in *S. aureus* occurred at the end of 720 min of cooling ($P = 0.076$). The production of staphylococcal enterotoxins was not tested because the population was below the threshold for toxin generation *(6)*. In previous studies with a ground beef model system designed to investigate the potential outgrowth of vegetative *S. aureus* cells, no growth was observed during 18 h of cooling from 130 to 45°F *(3)*, a longer cooling period than that in our study. In our extended ham cooling system, which was based on refrigerated cooling rates of actual ham products with natural-source nitrite, a similar trend was found; the population of vegetative cells did not increase during the 15-h cooling period. Although the antimicrobial effects were not assessed after 15 h, no growth of *C. perfringens* or *L. monocytogenes* was previously reported in fully cooked deli-style turkey breast formulated with celery juice nitrates during extended storage at 4°C *(2, 4)*. In the present study, growth results for *S. aureus* and *L. monocytogenes* fill a knowledge gap for controlling vegetative cells of post-lethality contaminants during the cooling of naturally cured
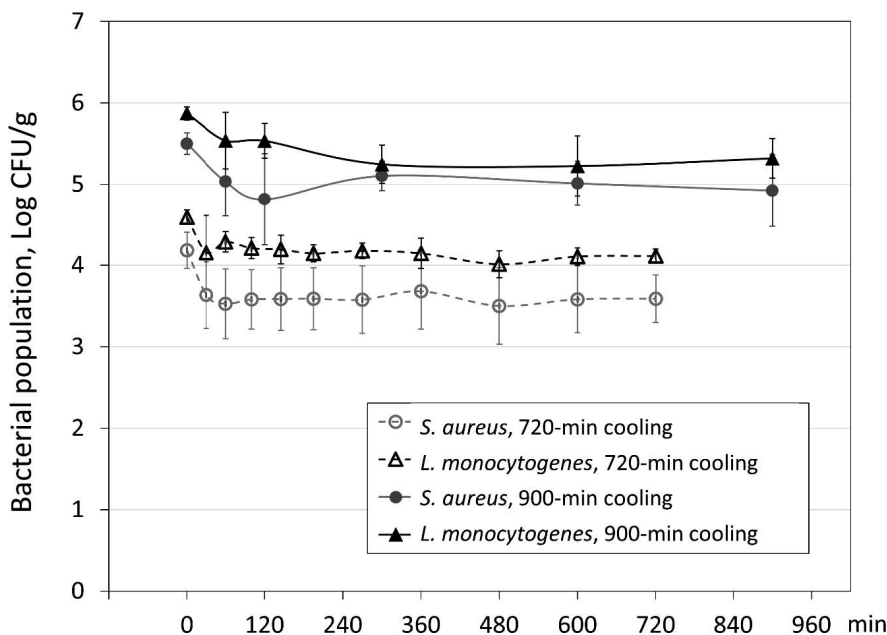


FIGURE 2. *Populations of* L. monocytogenes *and* S. aureus *on samples of ham cured with natural-source nitrite during simulated cooling processes. Values are average plate counts from duplicate samples and three trials. Error bars represent the standard deviation.*

meat pork products, providing a valuable reference and resource for meat processors.

Small meat processing establishments can have their products inspected by state health departments or other state agencies when sales remain within the state. However, meat product sales across state borders require USDA inspection, which is typically paid for by the processor. For large products such as whole hams, cooking and cooling processes may take longer than the average workday shifts, which requires processors to pay inspectors for prolonged working hours. Although cooling processes can be expedited by upgrading the cooling facility, upgrades may require a large initial capital investment. In the present study, naturally cured ham that was cooled within the requirements of FSIS Appendix B to control the growth of *C. perfringens,* growth of *L. monocytogenes* and *S. aureus* was also controlled. This information can be useful when processors are designing their production systems.

Effective lethal treatments and minimization of post-processing contamination remain the most important strategies for improving food safety *(9)*. However, extended cooling processes of hams formulated with natural-source nitrite (CJP) did not allow growth of the postprocessing contaminants *L. monocytogenes* and *S. aureus*.

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