PE5.04 Complying with Stabilization Microbiological Performance Standards Using Alternative Cooling Procedures for Large, Intact Meat Products 331.00

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***Abstract*—Achieving the United States Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) stabilization microbiological performance standards for cooling procedures proves to be challenging for processors of large, whole-muscle meat products. This study was conducted to determine if slower cooling times than those defined USDA- FSIS performance standards impact log growth of *Clostridium perfringens*. Large (10.43-12.25 kg), cured bone-in hams (n = 110) and large (≥ 9.07 kg), uncured beef inside rounds (n = 100) were utilized. Ham stabilization treatments investigated extending the times taken to reduce internal product temperature from 54.5°C to 26.7°C and from 26.7°C to 7.2ºC, independently. Further, a "worst case scenario" and a control defined by current USDA-FSIS Appendix B guidelines also were assessed. The "worst case" treatment evaluated the effects of cooling product at room temperature (approximately 22.8°C) in place of normal cooling procedures in a temperature controlled environment. Roast beef stabilization treatments investigated extending the times taken to reduce internal product temperature from 54.5°C to 26.7°C and from 26.7°C to 4.5ºC, independently. A "worst case scenario" also was assessed. Stabilization showed less than 1-log growth of *C. perfringens* for all treatments, with the exception of the "worst case" scenario for roast beef. As expected, > 1 log growth of *C. perfringens* was reported for uncured roast beef maintained at room temperature for cooling. This study supports product safety with the use of cooling times much slower than those specified by USDA-FSIS Appendix B. The results demonstrate that industry may have increased flexibility associated with cooling large, whole-muscle cuts while still complying with the required performance standards.**

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***Index Terms*—*Clostridium perfringens*, performance standards, stabilization**

I. INTRODUCTION

During the production of ready-to-eat and partially cooked meat and poultry products, establishments must meet microbiological performance standards set in place by the United States Department of Agriculture, Food Safety Inspection Service (USDA-FSIS). These standards, found in Chapter 9 of the Code of Federal Regulations (CFR), "set forth levels of pathogen reduction and limits on pathogen growth that official meat and poultry establishments must achieve in order to produce unadulterated products" [3]. More specifically, no more than 1-log growth of *Clostridium perfringens* may occur during product stabilization [1, 2, 4].

In January of 1999, USDA-FSIS published compliance guidelines for meeting lethality and stabilization performance standards for some ready-to-eat and partially cooked meat and poultry products [1, 2]. Then, on February 27, 2001, USDA-FSIS published a proposed rule in the Federal Register that suggested these standards be extended to all ready-to-eat and partially heat-treated meat and poultry products [4]. These compliance guidelines contain time and temperature recommendations for cooling procedures that produce products which meet the performance standards. However, achieving USDA-FSIS stabilization microbiological performance standards for cooling procedures proves to be challenging for processors when manufacturing large, whole-muscle meat products. Failing to satisfy the USDA-FSIS "safe harbor" compliance guideline processing parameters for cooling processes may

result in lack of compliance with the performance standards, and as a result, a deviation from a critical limit will occur and corrective actions must be performed on all products associated with the deviation. By examining effects of longer cooling times, alternative times that meet the stabilization performance standards may be achieved. This change in acceptable cooling parameters will reduce the incidence of deviations and the false assumption of unsafe product.

"Appendix B Compliance Guidelines for Cooling Heat-Treated Meat and Poultry Products (Stabilization)" [2] states that the entire cooling process should allow no more than 1-$\log_{10}$ total growth of *Clostridium perfringens*. These guidelines state that *C. perfringens* can be used alone in an inoculation study to test the performance standards of a cooling process because controlling the outgrowth of *C. perfringens* spores to one log or less also would prevent outgrowth of *C. botulinum* spores. Spores and vegetative cells of *C. perfringens* are present on raw meat. The cooking process of ready-to-eat products will kill the vegetative cells, but may activate the spores to germinate. During the cooling process, germinated spores will grow until the product reaches a cool enough temperature to prevent such outgrowth. The chilling process is a critical step in controlling *C. perfringens*. According to the compliance guidelines for cooling, the most rapid growth for clostridia is between 54.4°C and 26.7°C [2]. Excessive dwell time in this range is hazardous, and thus product should be cooled as rapidly as possible.

II. MATERIALS AND METHODS

A. Raw materials

One-hundred-and-ten bone-in hams (IMPS # 401A) [3], weighing between 10.43 and 12.25 kg, and one-hundred boneless beef inside rounds (IMPS # 168) [3], weighing greater than 9.07 kg, were purchased from a commercial processing facility and shipped frozen to the Rosenthal Meat Science and Technology Center at Texas A&M University.

B. Treatment structure

Ten hams were assigned randomly to each of the eleven ham cooling treatments and ten inside rounds were assigned randomly to each of the ten roast beef cooling treatments. For both ham and roast beef, each stabilization treatment (n = 10) for either ham or roast beef, was conducted twice, with each run (n = 5) taking place on separate days. Treatments are outlined in Table 1.

Table 1. Time parameters (h) by treatment for ham stabilization

	Ham	Roast Beef
Treatment Number	Treatment Length (h) 54.5°C to 26.7°C / 26.7°C to 7.2°C	Treatment Length (h) 54.5°C to 26.7°C / 26.7°C to 4.5°C
1	5.0 / 10.0	"worst case"
2	6.0 / 10.0	2.0 / 5.0
3	7.0 / 10.0	2.5 / 5.0
4	8.0 / 10.0	3.0 / 5.0
5	9.0 / 10.0	3.5 / 5.0
6	5.0 / 11.0	2.0 / 5.5
7	5.0 / 12.0	2.0 / 6.0
8	5.0 / 13.0	2.0 / 6.5
9	5.0 / 14.0	2.0 / 7.0
10	9.0 / 14.0	3.5 / 10.5
11	"worst case"	*

* Roast beef were subjected to ten treatments, not eleven.

Ham cooling treatments included a control as defined by Appendix B, which recommends that the maximum internal temperature be reduced from 54.4 to 26.7ºC in 5 h and from 26.7 to 7.2ºC in 10 h (15 h total cooling time) [2].

Both ham and roast beef cooling treatments included a "worst case" scenario as defined by removing the products from the smokehouse upon completion of thermal processing and reducing the temperature from 54.4 to 26.7ºC by allowing the products to equilibrate at room temperature (approximately 22.8ºC). For all stabilization treatments, samples were taken from each ham or roast, and plate counts were used to determine log growth of *C. perfringens*, once desired time and temperature parameters were reached.

C. Raw material preparation

Preparation of each ham and roast took place before treatment application. For each treatment group, frozen hams and roasts were removed from the freezer (-40ºC) and were allowed to thaw at approximately 1.1ºC. Each thawed ham or roast was weighed and trimmed free of intermuscular fat and connective tissue required to expose the *M. gracilis* and *M. semimembranosus* muscles. Trimming of the product allowed increased uniformity between products and a fresh lean surface for microorganism attachment during inoculation. During the weighing and trimming process, each ham and roast were assigned an individual identification number and an associated treatment group (run). Following trimming, each ham and roast were re-weighed to assess

compliance with the weight parameters set forth in the proposal for this experiment; this weight is referred to as the "trimmed weight." After initial product preparation, hams were cured. Using a curing pump with a four-needle hand-valve injector, hams were stitch pumped to 20% of their raw, trimmed weights with a brine solution consisting of 2% sodium chloride, 2% sucrose, 200 ppm sodium nitrite, 540 ppm sodium erythorbate, and 5000 ppm of sodium tripolyphosphate. Pumped hams were weighed to verify initial brine retention (≥ 20% of initial raw trimmed ham weight), placed in gondolas (by run), covered with plastic, and allowed to equilibrate at approximately 1.1°C for 12 to 15 h prior to thermal processing. Post-equilibration, each ham was re-weighed to determine final brine retention.

D. Inoculation procedures

For inoculation of ham and roast beef, a core and cheesecloth method was used. Cheesecloth was prepared by cutting cheesecloth sheets into 7 x 40 cm strips and overlaying two strips to form a cross. Ten cheesecloth pairs were each separated with white paper, and each set of ten pairs was wrapped in a white paper envelope for autoclaving. Cheesecloth packages were autoclaved at 121°C for 15 min.

Following aseptic procedures, four cores were removed from each ham or roast using a 3.3 cm autoclaved corer (5 cores were taken from the ham or roast used as the control). Each core was removed and a 2.5 cm long portion was cut from the internal end of each core. One uninoculated 2.5 cm portion from each inoculation day was placed in a sterile stomacher bag as the negative control. All other 2.5 cm long core portions were inoculated by injecting 0.1 ml of 107 *C. perfringens* spore suspension into the center of each core. Each inoculated 2.5 cm core was wrapped in the center of a cheesecloth pair, introduced back into the original ham or roast, and covered with the remaining core portion. One extra core portion per run (day) was inoculated and immediately placed in a sterile stomacher bag as a positive control. The stomacher bags containing the positive and negative controls were placed in an insulated plastic cooler with refrigerant packs and transported to the Food Microbiology Lab for further analysis.

E. Thermal processing

Following preparation and inoculation, the hams and roasts were thermally processed, in a smokehouse, to an internal temperature of 64.4ºC for a minimum of 107 s to achieve lethality as suggested by Appendix A [1]. After thermal processing, the products underwent one of the assigned cooling treatments previously outlined in Table 1.

F. Microbiological analysis

For all stabilization treatments, core samples were taken when the internal temperature reached 54.4°C and 7.2°C for ham, and 54.4°C and 4.5°C for roast beef and plate counts were used to determine log growth of *Clostridium perfringens*, once desired time and temperature were reached. Microbiological analyses taken after each cooling treatment demonstrated which treatments met the FSIS stabilization microbiological performance standards.

G. Statistical analysis

Data were analyzed using PROC GLM of SAS (SAS Institute, Inc., Cary, NC). Least squares means were generated for main effects and separated using PDIFF option when appropriate with an alpha-level of $P < 0.05$.

III. RESULTS AND DISCUSSION

Microbiologically significant spore outgrowth is reported as any *C. perfringens* growth greater than 1 $\log_{10}$ (CFU/g). All ham stabilization treatments resulted in post-stabilization samples with < 1 log growth of *C. perfringens.* Therefore, as reported in Table 2, no significant growth of *C. perfringens* was seen across the ham stabilization treatments.

Table 2. Least squares means for treatment effect on $\log_{10}$ (CFU/g) growth of *C. perfringens* spores after stabilization for all hams

Treatment Number	$\log_{10}$ (CFU/g)
1	-0.3^{a}
2	-0.5^{ab}
3	-0.3^{ab}
4	-0.2^{a}
5	-0.2^{a}
6	-0.2^{a}
7	-0.3^{ab}
8	-0.6^{ab}
9	-0.3^{a}
10	-0.1^{ab}
11	-0.9^{b}
[1]SEM	0.12

Least squares means within a column with different letters (*a-d*) differ ($P < 0.05$)

[1]SEM = Standard error of the least squares means

As expected, the roast beef stabilization phase of this experiment resulted in post-stabilization samples with < 1 log growth *C. perfringens* on all treatments except treatment 1 (Table 3). Treatment 1, defined as the "worst case" scenario for roast beef, differed ($P < 0.05$) from all other roast beef stabilization treatments.

Table 3. Least squares means for treatment effect on $\log_{10}$ (CFU/g) growth of *C. perfringens* spores after stabilization for all roast beef

Treatment Number	$\log_{10}$ (CFU/g)
1	1.9^{a}
2	-0.1^{d}
3	0.1^{cd}
4	0.4^{bcd}
5	0.9^{b}
6	0.1^{d}
7	0.2^{bcd}
8	0.3^{bcd}
9	0.3^{bcd}
10	0.9^{bc}
11	*
[1]SEM	0.18

Least squares means within a column with different letters (*a-d*) differ ($P < 0.05$)
[1]SEM = Standard error of the least squares means

As displayed in Table 3, treatments 5 and 10 are nearing significant levels of spore outgrowth, with 0.9-$\log_{10}$ (CFU/g) growth achieved by both treatments. Upon review of the stabilization treatment structure for roast beef, treatments 5 and 10 vary greatly in overall treatment length; however, both required 3.5 h from 54.4ºC to 26.7ºC. Due to the excessive dwell time that occurred from 54.4ºC to 26.7ºC for both treatments, inference can be made that a 3.5 h cool down from 54.4ºC to 26.7ºC and any length of time thereafter may be in danger of violating microbiological performance standards for stabilization. Therefore, these data support the use of all roast beef stabilization treatments except 1, 5, and 10 to ensure definite product safety. However, treatments 5 and 10 may be utilized while still meeting microbiological performance standards, but some caution should be exercised because these treatments have a significantly lower margin of safety.

IV. CONCLUSION

Data from this study support product safety with slower cooling times than those defined in Appendix B for both cured bone-in ham and uncured roast beef. The identification of slower cooling times that meet the FSIS stabilization microbiological performance standards will permit the processing industry to explore more accommodating processing procedures. This will allow extended processing times to be utilized without the concern of producing an unsafe product. In turn, processing deviations, associated corrective actions, and resulting product disposal may be greatly minimized. The results demonstrated that industry may have increased flexibility associated with cooling large, whole-muscle cuts while still complying with the required performance standards.

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