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Survival of *Clostridium perfringens, Staphylococcus aureus, Listeria monocytogenes* and *Salmonella enterica* in alternatively cured ham during cooking and process deviations

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ABSTRACT

Boneless hams were prepared with four different brines and inoculated on the surface and at a depth of 1 cm with multiple strains *of Clostridium perfringens, Staphylococcus aureus, Listeria monocytogenes and Salmonella enterica*. Hams were processed with a standard, low relative humidity or interrupted process cycle to an end point temperature of 70 °C. Microbiological populations were determined at the beginning, mid-point and end of the cycles. The change in population was calculated for each bacterium at each time point, by comparing the population to the initial inoculated population. There was no difference in the reductions in bacterial populations for all of the inoculated bacteria attributable to brine type. There were significant reductions in the populations of *Staphylococcus aureus, Listeria monocytogenes and Salmonella enterica* for both the surface and interior samples which were attributable to the end point temperature. Population reductions for *Clostridium perfringens* were approximately 1.8 $\log_{10}$ for both the surface and internal samples, and the population reductions for the surface samples were greatest with the interrupted cycle. The low Rh cycle resulted in the least reductions for the surface samples while the greatest population reductions for the surface samples were observed with the interrupted cycle.

1. Introduction

Consumers have demonstrated a preference for foods with minimal additives or with additives without chemical-sounding names, which have recently become known as "clean label" ingredients (Maruyama et al., 2021; Creswell, 2018). This has resulted in changes in formulation of cured meat products, with conventional curing ingredients being replaced with vegetable products which naturally contain curing ingredients (Sebranek & Bacus, 2007). Naturally cured or products labelled as uncured are not cured by the direct addition of sodium nitrite, but by the addition of products such as dehydrated celery juice and powder which contain a natural source of nitrate.

Product food safety becomes a major issue with alternatively cured meat products because they typically contain lower amount of nitrite levels when compared with traditionally cured meats (Jackson et al., 2011). Additionally, inclusion levels of celery juice powder and cherry powder are limited only by flavor and cost and yet currently less regulated than traditionally cured products. In the United States, alternatively-cured meat products are currently less regulated than their conventionally-cured counterparts, which typically use celery juice powder as a nitrite source and cherry powder as an ascorbic acid source. There are no regulatory limits on the use of these ingredients, although they tend to be self-limiting due to flavor and cost. On the other hand, their synthetic homologs are strictly regulated (Code of Federal Regualtions, 2019). Nitrites are known to be highly effective in controlling the growth of *Clostridium perfringens, Listeria monocytogenes* and many other foodborne pathogens (Myers et al., 2016; Sauter et al., 1977). However, because the antimicrobial efficacy of alternative cure

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[1] Shannon Cruzen was employed by Smithfield Foods when we initiated the project. She left Smithfield part way through the project and is now a private consultant. Perhaps it would be best to list her affiliation as "private consultant".

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ingredients is not fully determined and understood, these products are likely to be more susceptible to foodborne pathogens and may undergo additional scrutiny from a food safety perspective. Because of this, it was only recently that USDA had enough information to permit alternatively cured products to qualify for Stabilization Option 3 of USDA FSIS Compliance Guideline on Stabilization, Appendix B, which allows for slower cooling of products containing any source of nitrite and a cure accelerator (USDA–FSIS, 2017b).

Certain bacterial pathogens are associated with fresh and processed pork products. These include *Salmonella enterica*, *Clostridium perfringens* and *Staphylococcus aureus*. As a result, current USDA FSIS regulations are based on the control of these bacteria (USDA–FSIS, 2017a; USDA–FSIS, 2017b). The production of enterotoxin by *S. aureus* is also a concern with hams, as the microenvironment is conducive to both growth and toxin production (Smith et al., 1983). Although *Listeria monocytogenes* is commonly viewed as a post-processing contaminant in ready-to-eat meat and poultry products (USDA–FSIS, 2014), it is important to assure that the lethality process does not allow the survival of the bacterium in the fully cooked product.

Many studies have evaluated the survival of bacteria during ham processing with conventional and alternate cures (Jackson et al., 2011; King et al., 2015; Myers et al., 2016; Sullivan et al., 2012). However, many of these have been in model systems, and very few have examined the use of alternative cures in non-ideal situations, such as cooking with slow come-up times or during process deviations. Process deviations are unplanned events that unfortunately occur during normal operations, typically as a result of a loss of power or mechanical breakdown and may have repercussions on the survival and growth of bacterial pathogens. USDA–FSIS (2017a) provides guidance to meat and poultry processors on how to handle process deviations, with specific reference to deviations which exceed 6 h. The study presented here should provide additional information for a company to make an informed decision about a specific deviation.

As the development of uncured processes using natural cures grows, their impact on naturally occurring bacteria must be understood. The objective of this research was to evaluate the effects of different brines and process cycles on the populations of inoculated *C. perfringens*, *S. aureus, L. monocytogenes* and non-typhoidal *Salmonella enterica* on hams during processing.

2. Materials and methods

2.1. Ham preparation and processing

Boneless ham muscles were obtained from a commercial federally inspected slaughter establishment. The meat was injected with the different brines (Table 1) to 110% of the initial meat weight, tumbled separately for 2 h, and allowed to marinate overnight. The brine formulations were based on formulations currently in commercial production. The hams (approximately 5 pounds each) were made by stuffing the injected meat into ham netting. The hams were vacuum packaged and held at 33 °F (0.5 °C) until inoculation and processing.

Ham processes were developed in consultation with industry professionals and University extension faculty (Table 2). The three cycles were a standard cycle, an interrupted cycle and a low Rh cycle. Although designated as a smoke cycle, the smoke unit was turned off for this process, so that this was a thermal cycle and only the effect of temperature and brine would be measured. Temperature measurements were taken in two hams from each brine for each replication, and the process was stopped when average temperature of the hams reached a minimum of 70 °C (158 °F).

2.2. Bacterial cultures

The bacteria used in these experiments are described in Table 3. *Clostridium perfringens* strains were cultured in fluid thioglycolate medium and in Duncan-Strong sporulation medium (Duncan & Strong, 1968) as described by Juneja et al. (2021). Briefly, the cultures grown in fluid thioglycolate medium were used to prepare primarily vegetative cells in late logarithmic growth stage, while those grown in Duncan-Strong medium were grown primarily to produce spores. The

Table 1
Brine formulations.

Brine Description	Brine 1	Brine 2	Brine 3	Brine 4
	Alternative Cure	Conventional Cure	Conventional Cure	No cure
Nitrite concentration (ppm)	100	100	200	0
Erythorbate concentration (ppm)	250	250 (ascorbate)	547	0
NaCl concentration	0.8%	0.8%	0.8%	0.8%
Brine Ingredient	**Brine 1**	**Brine 2**	**Brine 3**	**Brine 4**
Water	45.4[a]	45.4	45.4	45.4
Sea Salt	3.63	2.95	2.81	3.63
Cane Sugar	2.27	2.27	2.27	2.27
Kerry XP30	1.49			
Acerola Cherry Powder	0.33			
Erythorbate		0.11 (ascorbate)	0.25	
Prague Powder		0.73	0.87	

[a] Weight in kg.

Table 2
Ham thermal processing cycles.

Time (Hours)	Dry Bulb °C	Wet Bulb °C	Relative Humidity %
Standard & interrupted			
2[a]	65.6	37.8	17.8
2	68.3	46.1	29.3
2	73.9	57.2	43.0
2	85.0	65.6	41.5
To internal of 70 °C	87.8	76.7	62.5
Low Rh			
2	65.6	37.8	17.8
2	68.3	43.3	25.0
2	73.9	47.8	25.0
2	85.0	57.2	26.2
To internal of 70 °C	87.8	60.0	27.1

[a] For process deviation, the smokehouse was turned off at 30 min into the cook schedule for 5 h, then restarted at the 30 min timepoint where it left off.

Table 3
Strains of bacteria used to inoculate hams.

Bacterium	Strains
C. perfringens	ATCC 10258 ATCC 3124 ATCC 12917
S. aureus	Swine Isolate (Iowa State University Veterinary Diagnostic Laboratory) Pork Skin isolate (Iowa State University Veterinary Diagnostic Laboratory) ATCC 29737
S. enterica	Typhimurium ATCC 700720 Montevideo (clinical isolate) Newport ATCC 6962
L. monocytogenes	H7769 4b (food isolate associated with outbreak) H7764 1/2a (food isolate associated with outbreak) Scott a

cells and spores were harvested separately by centrifugation (9,500 g, 10 min, 4 °C) and were then resuspended in 1/10 volume of physiological saline (0.85% sodium chloride, wt/vol). Microscopic evaluation of the spore preparation showed very few intact vegetative cells. The populations of the vegetative cell and spore preparations were enumerated separately, and prior to inoculation of the hams the harvested cells and spores were mixed in equal quantities, to prepare an inoculated population that was composed of both vegetative cells and spores in an approximate 1:1 ratio.

Staphylococcus aureus, Listeria monocytogenes and *Salmonella enterica* were grown to late logarithmic growth stage in trypticase soy broth at 37 °C for 18–24 h. The cultures were harvested by centrifugation as described above, and then re-suspended in 1/10 volume of buffered peptone water. The *S. aureus* strains obtained from the Iowa State University Veterinary Diagnostic Laboratory were isolated from swine and confirmed to produce toxin, although they were not specifically tested for enterotoxin. There was no information regarding toxin production for the ATCC strain.

2.3. Inoculation

An inoculum culture was prepared by combining equal volumes of *C. perfringens* (spores and vegetative cells), *S. aureus, L. monocytogenes,* and *S. enterica.* The combined inoculum had initial populations of approximately 10^7–10^8 colony forming units (cfu) per ml for *S. aureus, L. monocytogenes,* and *S. enterica* and approximately 10^6 to 10^7 cfu per ml for *C. perfringens* (spores and vegetative cells). The hams were surface inoculated with the mixed culture of the with a foam paint brush, with initial populations in the range of 10^6 to 10^7 cfu per cm^2. The hams were also inoculated sub-surface by injecting approximately 0.2 ml of the same mixed culture to a depth of 1 cm below the surface, with initial populations in the range of 10^5 to 10^6 cfu per gramfor *C. perfringens* and approximately 10^6 for *S. aureus, L. monocytogenes,* and *S. enterica*. The inoculated hams were covered with plastic to prevent surface drying and stored at 5 °C for 24 h prior to processing. This resulted in inoculated bacteria being in a physiological state which would be typical of those seen in commercial meat processing.

2.4. Microbiological analysis

Samples were taken from the hams prior to the beginning of the process, at a mid-point during the process and at the end of the process. Surface samples consisted of a pre-determined surface area (2 × 2 cm) aseptically removed with a sterile scalpel and forceps. Internal samples were obtained by excising the tissue around the injection site to a depth of approximately 2 cm, with the weight of the sample recorded.

C. perfringens populations were enumerated by surface plating on Perfringens agar with tryptose sulfite cycloserine and egg yolk emulsion (Oxoid, Basingstoke, UK) and incubated at 35 °C in anaerobic jars for 48 h (USDA–FSIS, 1998). *S. aureus* populations were enumerated by surface plating on Baird-Parker agar with egg yolk tellurite emulsion, and incubated at 37 °C for 48 h. *S. enterica* were enumerated using the thin agar layer method of Kang and Fung (2000) to recover thermally injured cells, with Xylose Lysine Deoxycholate (XLD) agar as the selective layer and trypticase soy agar as the non-selective layer. The plates were incubated at 37 °C for 48 h. *L. monocytogenes* were enumerated on Modified Oxford Medium (MOX) and incubated at 35 °C for 48 h. The detection limit of all of the assays was <1 $\log_{10}$ colony forming units (cfu) per g or cm^2.

2.5. Chemical analysis

The fully cooked hams were chilled for 18–24 h at <5 °C, after which a core of the ham was removed and analyzed for residual nitrite, salt and water activity. Briefly, the samples were ground and three representative sub-samples from each ground sample were analyzed for residual nitrite for cured meat using a colorimetric method (AOAC 973.31). Salt was analyzed by a silver nitrate titration procedure and a Chloride test kit CD-51 (HACH Company, Loveland CO, method 8207). Water activity was determined using an AquaLab series 4 water activity meter (Meter Group, Inc., Pullman WA), following the instructions for the instrument.

2.6. Experimental design

Each trial was independently replicated three times, with duplicate samples for each sampling point within replication. Microbial populations were transformed to $\log_{10}$ cfu/cm^2 (surface samples) or g (internal samples).

The change in population was calculated as:

Log_{10} Population change = $\log_{10}$ (population at sampling time) - $\log_{10}$ (initial population).

The population changes were analyzed by bacterial type for the main effects of brine type, thermal processing cycle type (standard v. interrupted v. low Rh), time and sample location (surface v. internal) and their interactions. All statistical analyses were conducted using SigmaStat 4.0 (Systat Software, Inc; San Jose CA). Following the approach of Wasserstein et al. (2019), we decided not to declare a level of significance (i.e., $P < 0.05$) and simply include the actual p values for the reader's consideration.

3. Results

The results of the chemical analyses are presented in Table 4. There were observed differences in the residual nitrite levels by cycle ($P = 0.034$) and by brine ($P = 0.073$). Brines 1 and 2 had consistently higher residual nitrite than either Brines 3 or 4. There was no observed interaction between brine and cycle ($P = 0.23$). The results of the residual NaCl concentrations were similar to the residual nitrite in regard to the cycle ($P = 0.032$). However, there were no differences between brine ($P = 0.22$) or interaction between brine and cycle ($P = 0.51$). The residual NaCl concentration was higher in the low Rh cycle than with the interrupted cycle, as a result of increased product dehydration. The water activity was affected by Brine ($P = 0.09$) but not by cycle or the interaction between brine or cycle. Brine 4 had a statistically lower a_w than the other brines, but this was a very minor difference (approximately 0.004) and within experimental error.

The wet bulb, dry bulb and internal temperatures of the hams during processing are shown in Fig. 1. The internal temperatures of both the standard and low Rh cycles closely paralleled each other, although the low Rh cycle took slightly longer to reach the end point temperature of 70.0 °C. As expected, the temperature profile of the interrupted cycle

Table 4
Chemical analyses of the fully cooked boneless hams.

Cycle	Analysis	Brine 1	Brine 2	Brine 3	Brine 4
Standard	Nitrite (ppm)	12.7 (13.9)[A]	9.91 (6.4)	1.0 (0.1)	1.17 (0.2)
	NaCl (%)	2.11 (0.035)	1.96 (0.094)	2.35 (0.290)	2.27 (0.320)
	A_w	0.98 (0.01)	0.97 (0.01)	0.97 (0.0)	0.97 (0.0)
Interrupted	Nitrite (ppm)	2.29 (1.16)	2.28 (1.43)	1.9 (0.3)	1.16 (0.1)
	NaCl (%)	1.88 (0.23)	2.01 (0.26)	2.21 (0.27)	2.25 (0.52)
	A_w	0.97 (0.01)	0.98 (0.01)	0.97 (0.0)	0.97 (0.01)
Low Rh	Nitrite (ppm)	2.71 (1.04)	1.62 (0.19)	1.24 (0.2)	0.74 (0.59)
	NaCl (%)	2.60 (0.15)	2.2 (0.06)	2.32 (0.18)	2.52 (0.36)
	A_w	0.97 (0.0)	0.97 (0.0)	0.97 (0.01)	0.97 (0.0)

[A] Mean (standard deviation).

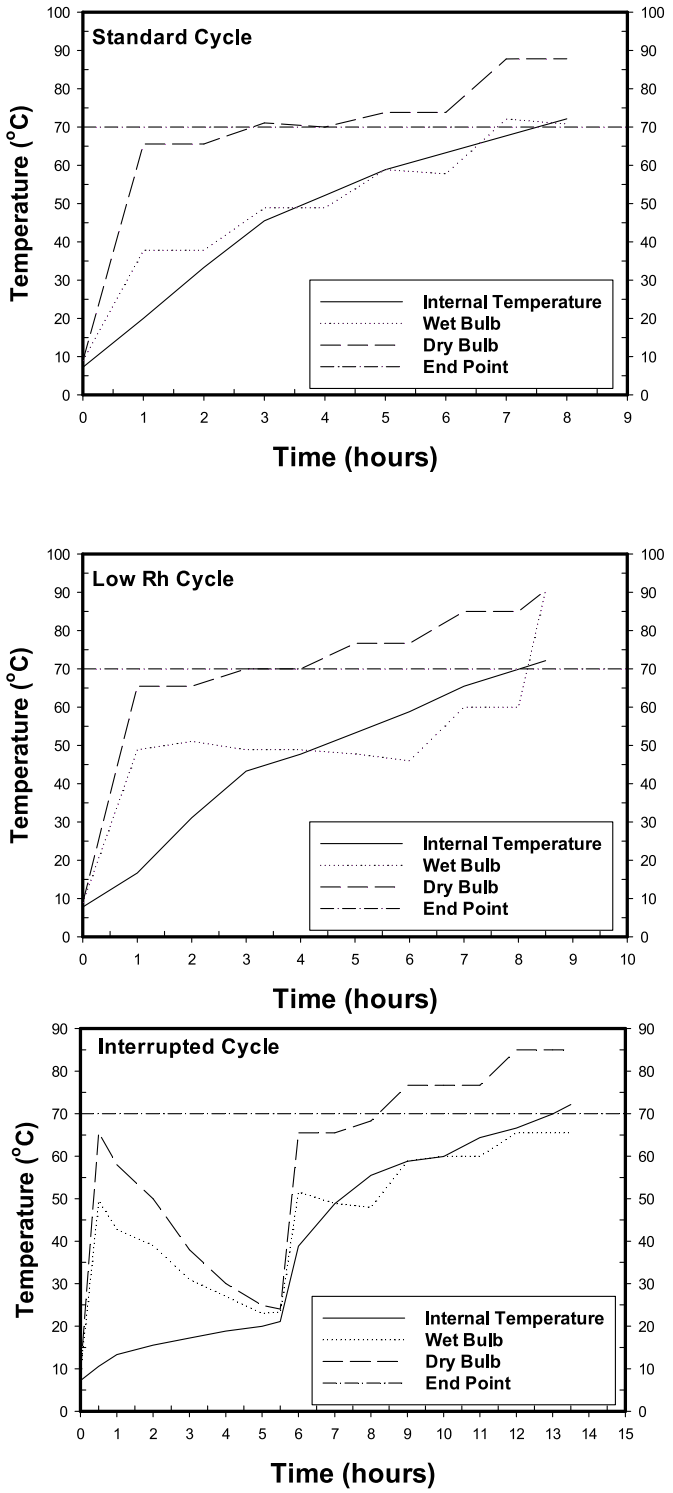

Fig. 1. Average internal temperatures of hams processed with the standard, low Rh and interrupted cook cycle.

resulted in the internal temperatures gradually rising from 10 °C to 21 °C during the 5 h process interruption and taking an additional 5 h to reach the endpoint temperature.

The analysis of the changes in microbial populations for all of the bacteria studied indicated that there were no differences between brine types (types 1 to 4; $P > 0.60$). Therefore, the data from all four of the brines was pooled for each bacterium for further analysis. The results of the population reductions for *Clostridium perfringens* after cooking are shown in Fig. 2. The samples were collected at the midpoint of the cycle and at the end of the cook cycle, when the hams reached 70 °C. The midpoint of the standard and low Rh cycles was at 4 h, while the midpoint of the interrupted cycle was defined as the point at the end of the 5 h process interruption, 5.5 h into the cycle. Population reductions were observed at the end of the cycle than at the midpoint of the cycle ($P < 0.001$) for the surface and internal samples. The standard and low Rh cycles had slight population reductions on the surface samples (ca. 0.5 $\log_{10}$) at the midpoint of the cycle when compared to the initial populations. However, the interrupted cycle had essentially no change in population between the beginning of the cycle and immediately after the cycle interruption.

The population reductions at the end of the standard cycle were approximately 1.8 $\log_{10}$ for both the surface and internal samples. The low Rh cycle resulted in the lowest population reductions for the surface samples at the end of the cook cycle (ca. 1 $\log_{10}$), although the population reductions for the internal samples were similar to those for the standard cycle. The greatest population reductions for the surface samples were observed with the interrupted cycle, while the population reductions on the internal samples were comparable to the standard and low Rh cycles.

The population reductions with *Salmonella enterica* are shown in Fig. 3. All of the cycles resulted in substantial population reductions at the end of the cook cycle for both surface and internal samples and the population reductions ranged from approximately 4.3 to 5.3 $\log_{10}$.

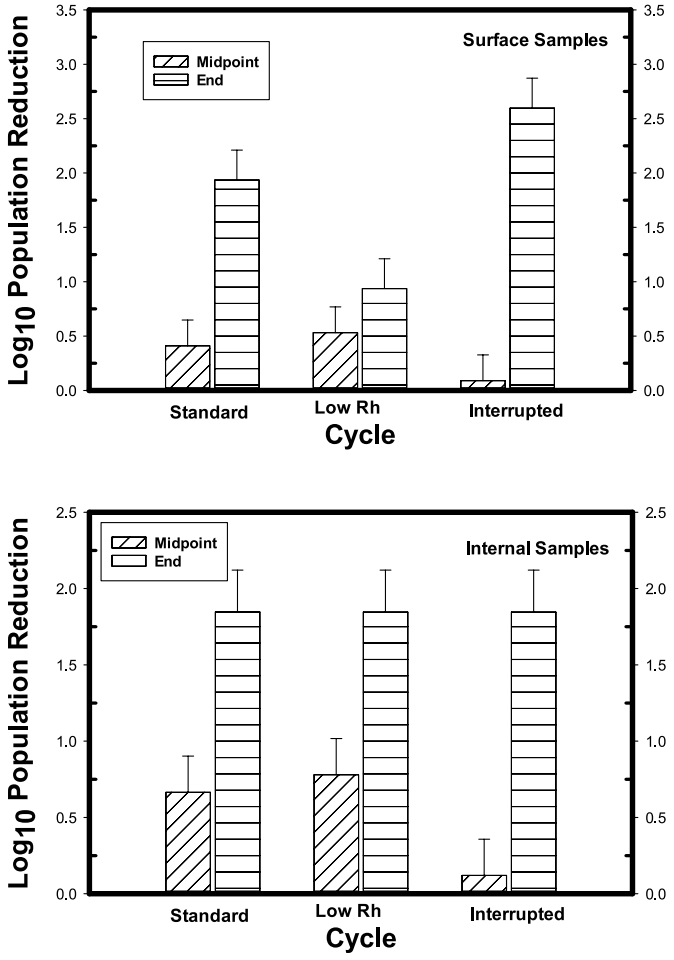

Fig. 2. Population reductions of *Clostridium perfringens* in boneless hams at the midpoint (4 or 5.5 h) and end (8 or 13 h) of the cycle. There was no difference ($P > 0.60$) in the reductions between the individual brines, so the data from all four brines was pooled for further analysis. The initial populations were approximately $\log_{10}$ 5.9/cm^2 (surface) and $\log_{10}$ 5.4/g (internal).

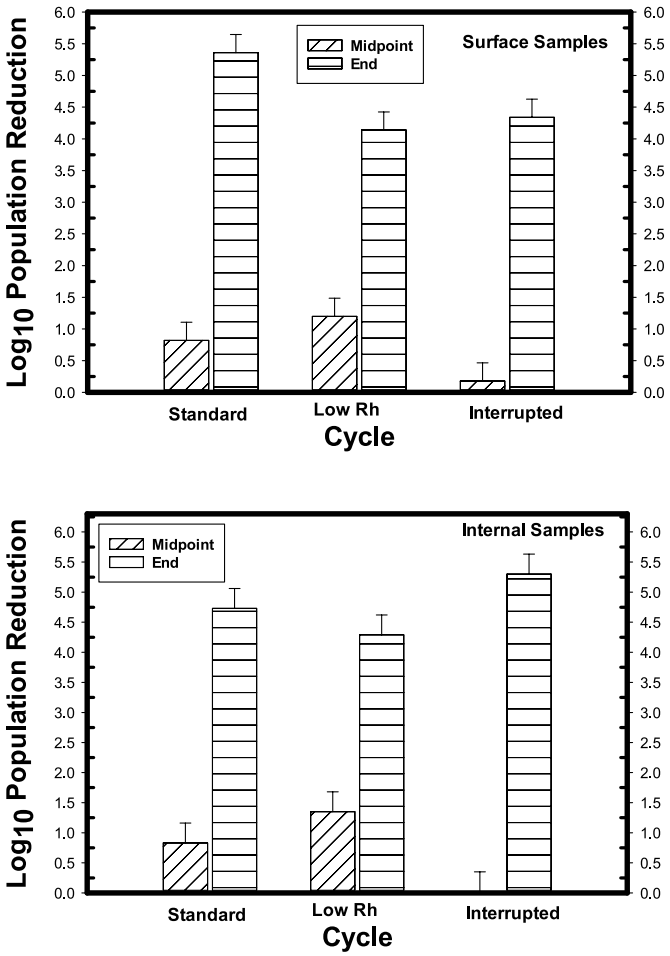

Fig. 3. Population reductions with *Salmonella enterica* in boneless hams at the midpoint (4 or 5.5 h) and end (8 or 13 h) of the cycle. There was no difference ($P > 0.60$) in the reductions between the individual brines, so the data from all four brines was pooled for further analysis. The initial populations were approximately $\log_{10}$ 6.5/cm^2 (surface) and $\log_{10}$ 6.1/g (internal).

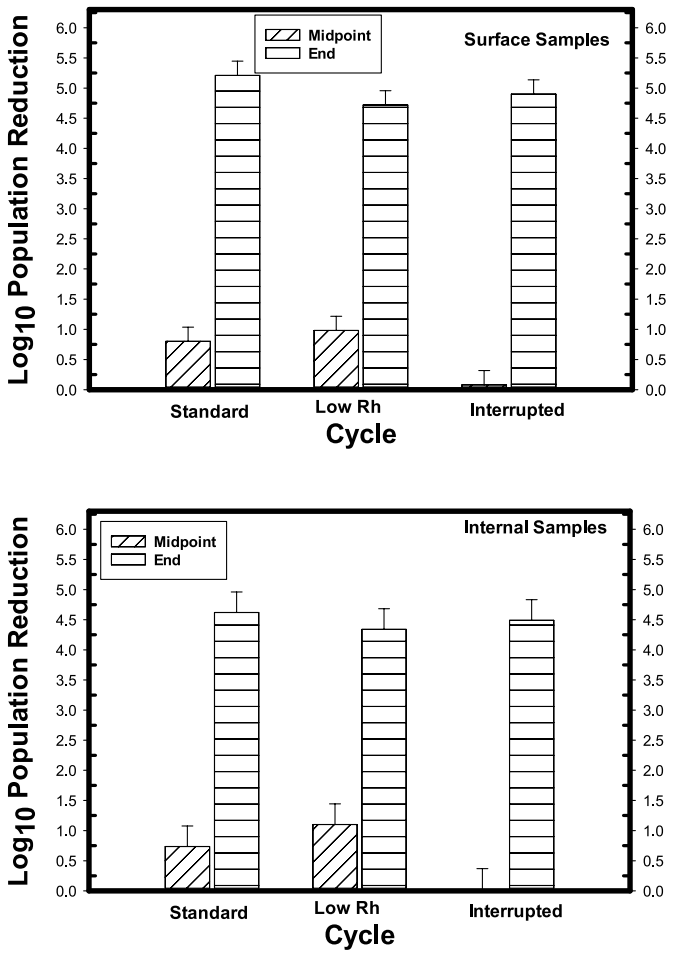

Fig. 4. Population reductions of *Staphylococcus aureus* in boneless hams at the midpoint (4 or 5.5 h) and end (8 or 13 h) of the cycle. There was no difference ($P > 0.60$) in the reductions between the individual brines, so the data from all four brines was pooled for further analysis. The initial populations were approximately $\log_{10}$ 6.7/cm^2 (surface) and $\log_{10}$ 6.0/g (internal).

However, the populations on both the surface and internal samples were below the detectable limits of the assay (<1 $\log_{10}$ cfu/g or cm^2) at the end of the cook cycle. The numerically largest reductions were seen on the surface samples of the standard cycle and the internal samples of the interrupted cycle. As observed with the *C. perfringens* results, the reductions were greater at the end of the cook cycle than at the midpoint of the cycle ($P < 0.001$) for the standard and low Rh cycles. The standard and low Rh cycles had population reductions of approximately 1 $\log_{10}$ at the midpoint of the cycle when compared to the initial populations, while the interrupted cycle had essentially no change in population between the beginning of the cycle and immediately after the cycle interruption.

The population reductions for *S. aureus* are shown in Fig. 4. The population reductions at the end of the cycles ranged from approximately 4.9 to 5.2 $\log_{10}$ for the surface samples, while the observed reductions in the internal samples were approximately 4.5 $\log_{10}$. The pattern of reduction between the midpoint and final points in the cycle were similar to those observed with *S. enterica*, with small reductions observed at the midpoint and large reductions observed at the end of the cook cycle. As with the *S. enterica* results, any surviving populations at the end of the cook cycle were below the detectable limits of the assay.

The population reductions with *L. monocytogenes* are shown in Fig. 5. There were observed differences in the population reductions between cycles for the surface samples ($P < 0.01$), with the standard cycle resulting in the greatest population reduction (5.5 $\log_{10}$) but were not different between interrupted and low Rh cycles (3.4 $\log_{10}$). The population reductions for the internal samples ranged from approximately 4.3 to 5.2 $\log_{10}$, with the low Rh cycle having the lowest population reduction, while the standard and interrupted samples had equivalent reductions. As with the other vegetative bacteria, viable bacteria were not recovered in either surface or internal samples at the end of the cook cycles.

4. Discussion

There were no observed differences in the population reductions of the individual bacteria between the four different brines. This is consistent with the work of Cruzen et al. (2022), who used the identical brines for bacon processing. Although it is possible that the brines would have had some impact on the populations, the effect of the lethality cycles was far greater than any difference between the individual brines.

All of the processing cycles resulted in substantial reductions in the populations of the inoculated bacteria on both the surface and interior of the hams. As expected, the populations of *C. perfringens* were reduced to a lesser extent than those of the vegetative bacteria, but even these were reduced by at least 1 $\log_{10}$ in the surface samples with the low Rh cycle, and greater than 2 $\log_{10}$ in the surface samples of the interrupted cycle. The greater reductions observed in the interrupted cycle appear at odds with the temperature profile, where the hams were held in the lower growth temperature range for *C. perfringens*. However, the USDA

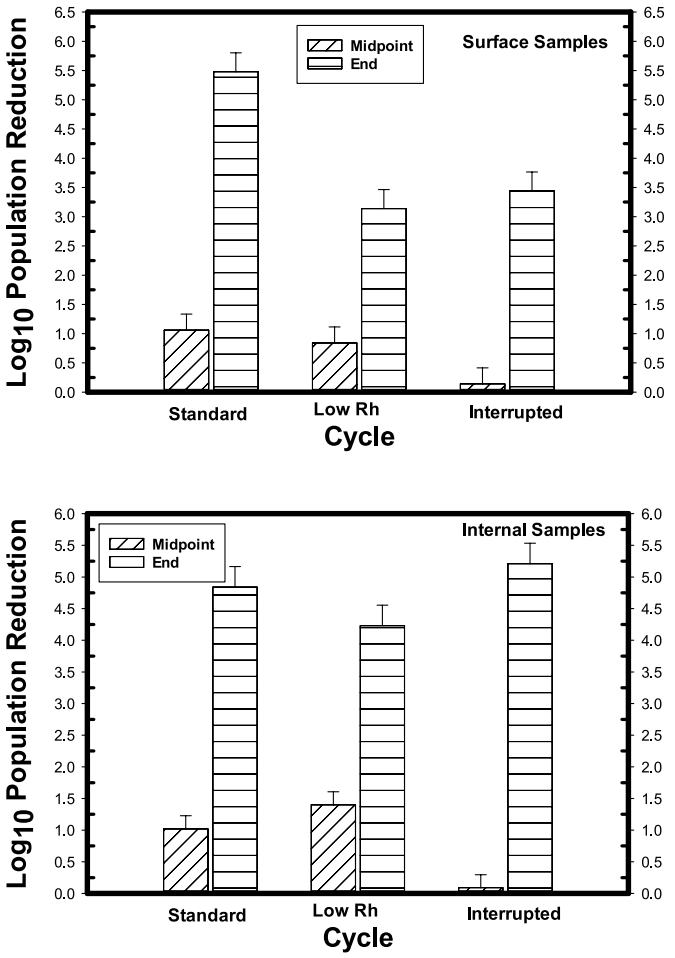

Fig. 5. Population reduction of *Listeria monocytogenes* in boneless hams at the midpoint (4 or 5.5 h) and end (8 or 13 h) of the cycle. There was no difference ($P > 0.60$) in the reductions between the individual brines, so the data from all four brines was pooled for further analysis. The initial populations were approximately $\log_{10}$ 6.3/cm^2 (surface) and $\log_{10}$ 5.8/g (internal).

Pathogen Modeling Program (USDA-ARS, 2021; Juneja et al., 2001) predicted only minimal growth with the interrupted cycle temperature profile. The sample analysis showed that there was essentially no change in the population during the cycle interruption, which is in agreement with the model predictions.

The population of *C. perfringens* represents the total population of both spores and vegetative cells. Although the population of spores and vegetative cells were not enumerated separately, the population reductions reflect an impact of the lethality process on both spores and vegetative cells. The populations of spores and vegetative cells were approximately equal in the initial inoculum. If both populations were initially 10^6 ($\log_{10}$ 6), then the combined inoculum of 10^6 spores and 10^6 cells would have been $\log_{10}$ 6.3. If the lethality process destroyed all of the vegetative cells without impacting the spore population, the surviving population would have been 10^6 ($\log_{10}$ 6) spores. The maximum population reduction achievable by destroying only vegetative cells would have been 0.3 $\log_{10}$. The population reductions at the end of the cycles were greater than 1 $\log_{10}$.

The greater reduction at the end of the cook cycle suggests that the spore portion of the inoculum may have begun to germinate during the cycle interruption, with the resulting vegetative cells being more sensitive to heat than the original spores. Grecz and Arvay (1982) reported that approximately 40% of non-heat activated *C. botulinum* spores had initiated germination after 4 h at 14 °C. De Jong et al. (2004) determined that the growth rate of *C. perfringens* was not affected by adaptation to low temperatures, and that germination was observed over a temperature range of 3 °C to 37 °C. However, regarding germination, that study studied time periods significantly greater than those of the interrupted process of this study. Spore germination can begin at temperatures as low as -1 °C (Knaysi, 1964) and heat resistance decreases rapidly during germination (Moir & Cooper, 2016). Sindelar et al. (2019) may have observed a similar phenomenon with an extended ham process, which took approximately 21–24 h to reach an end point temperature of 65.6 °C. In that study, the authors observed population reductions of 2.5–3 $\log_{10}$ in both surface and internal bacon samples and 1 to 2.5 $\log_{10}$ in ham samples. The extended process (slow come up times) in that study would have created a similar environment to that of the interrupted cycle in the current study, allowing more of the spores to germinate and become less heat resistant.

The population reductions for the vegetative bacteria were what was expected, based on published D_{10} values for the specific bacteria. The USDA FSIS (2017) compliance guideline indicates that reaching 70 °C results in an instant compliance with the regulation, requiring a 6.5 $\log_{10}$ reduction in *Salmonella* spp. While the results of these experiments did not achieve a 6.5 $\log_{10}$ reduction in *S. enterica* per se, viable *S. enterica* were not detected at the end of the cook cycle. The observed reductions indicate that the processes and brines have a substantial protective effect for public health. Grant & Patterson (1995) reported that the D_{70} value of *S. enterica* was 0.09 min in roast beef. Doyle and Mazzotta (2000) and Jarvis et al. (2016) have published comprehensive reviews of the thermal tolerance of *S. enterica*, and while few studies have examined the heat resistance at 70 °C, it is apparent from the published results that even the most heat resistant strains of *S. enterica* would be substantially reduced at an end point temperature of 70 °C. An internal end point temperature of 70 °C is sufficient to assure the safety of the fully cooked hams.

The substantial reductions in the populations of *S. aureus* in both the surface and internal samples supports previously published research. Silliker et al. (1962) reported that *S. aureus* did not survive after processing fully cooked hams. Palumbo et al. (1977) reported that viable *S. aureus* could not be detected in hot dog batter inoculated to 8 $\log_{10}$/g and heated to 71.1 °C. Similarly, Firstenberg-Eden et al. (1977) reported that the decimal reduction time of *S. aureus* in milk at 70 °C was 0.1 min and Li et al. (2005) reported that the decimal reduction values at 70 °C in liquid egg for *S. aureus* ranged from 0.33 to 0.43 min. This is consistent with the results reported here, where viable *S. aureus* were not recovered from the fully cooked hams.

As with the other vegetative bacteria, the surviving populations of *L. monocytogenes* were below the detectable limits of the assay. Carlier et al. (1996) reported that the decimal reduction value at 60 °C *for L. monocytogenes* in ham was 1.82 min. All of the ham process cycles maintained internal temperatures at or above 60 °C for at least 2 h. McMinn et al. (2018) reported a decimal reduction value of 0.27 min for *L. monocytogenes* in ham at 71.1 °C, which is approximately equal to the end point temperature of the ham processes in this study. In other products, Huang (2013) reported a decimal reduction value of less than 10 s (0.17 min) at 66 °C in chicken meat and Li et al. (2005) reported that the decimal reduction values at 70 °C in liquid egg for *L. monocytogenes* ranged from 0.07 to 0.13 min. The typical end point temperatures of ham processes are more than sufficient to eliminate any naturally occurring *L. monocytogenes* in the raw hams.

An important aspect of this research was determining the potential increase in bacterial populations because of the process interruption. The process interruption allowed the ham internal temperature to rise from approximately 10 °C–20 °C over a period of 5 h. In every case, there was essentially no change in the populations of the inoculated bacteria, suggesting that a process interruption at an early stage of the process may not necessarily result in an increase in the populations of the bacteria of concern. Modeling the potential growth of the bacteria with the Pathogen Modeling Program (USDA-ARS, 2021) confirmed the laboratory findings, with relatively long lag phases and long generation times

predicted for all of the bacteria at these temperatures and growth conditions.

5. Conclusions

As previously observed with bacon processing (Cruzen et al., 2022), there was no observed effect of the brines on the populations of the inoculated bacteria. All of the processes reduced the populations of *S. enterica*, *S. aureus* and *L. monocytogenes* to less than the detection limit of the quantitative assay at the end of the cycle. The populations of *C. perfringens* on the surface of the hams were reduced to the greatest extent in the interrupted process cycle, which we speculate is attributable to the germination of spores within the inoculum, resulting in more vegetative cells with a lower heat resistance than the spores.

CRediT authorship contribution statement

Shannon M. Cruzen: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Hayriye Cetin-Karaca:** Data curation, Formal analysis, Methodology, Resources, Validation, Writing – review & editing. **Rodrigo Tarté:** Conceptualization, Formal analysis, Resources, Visualization, Writing – review & editing. **Joseph G. Sebranek:** Conceptualization, Formal analysis, Resources, Visualization, Writing – review & editing. **James S. Dickson:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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