Survival of *Clostridium perfringens*, *Staphylococcus aureus* and *Salmonella enterica* in Alternatively Cured Bacon During Cooking and Process Deviations

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Abstract

Pork bellies were injected with four different alternative curing brines. The bellies were inoculated on the surface and at a depth of 1 cm with multiple strains *of Clostridium perfringens, Staphylococcus aureus and Salmonella enterica*. The bellies were processed using either a standard process cycle or an interrupted process cycle to simulate a process deviation. Additionally, laboratory simulation of the same cycles was conducted where surface inoculated pork belly samples (22 ± 1 g) were processed in a circulating water bath. Microbiological populations were determined at the beginning, mid-point and end of the cycles, and the change in population was calculated for each bacterium at each time point, by comparing the population to the initial inoculated population. Irrespective of the brine or process cycle, the populations of all of the inoculated bacteria on both the surface and interior samples had decreased by the end of the process. There was no difference in the reductions in bacterial populations for all of the inoculated bacteria by brine type or by sample location ($P>0.30$). There were differences in the microbial population reductions for *C. perfringens* attributable to the processing cycle ($P<0.001$), with less population reductions associated with the standard cycle when compared to the interrupted cycle. However, no differences ($P>0.10$) were observed in the population reductions between the two processing cycles for either *S. aureus* or *S. enterica*.

Keywords
bacon, process deviation, Clostridium, Staphylococcus, Salmonella

1. INTRODUCTION

Cured meat products have traditionally been formulated with sodium nitrite. In addition to the development of cured color and antioxidant effects, sodium nitrite has a significant role in microbial inhibition. Specifically, nitrite is known to inhibit the outgrowth of the spore-formers *Clostridium botulinum* and *Clostridium perfringens* (Osterbauer et al., 2017). Nitrite is also known to affect growth of the pathogens *Salmonella* and *Staphylococcus aureus*, (Collins-Thomson et al., 1984; King et al., 2016). In the past decade, the use of celery juice or powder as a natural source of nitrate/nitrite has risen in popularity, especially with the advent of pre-converted celery juice or powders which don't require the user to convert nitrate to nitrite. While studies have documented the antimicrobial effectiveness of the nitrite contained in these powders (King et al., 2015), many of these have been in model systems, and none have examined its use in non-ideal situations such as cooking with slow come-up times or during process deviations (i.e., loss of steam, power outage, or mechanical breakdown). These non-ideal disruptions in the process may present an opportunity for growth and enterotoxin production by *S. aureus* and *C. perfringens* (Portocarrero et al., 2013). Both organisms will grow in the general range of 10–46 °C (50–115 °F), with *C. perfringens* able to tolerate slightly higher temperatures (Doyle, 2002 & King et al., 2015). The staphylococcal enterotoxin is heat stable, so subsequent heat treatments at normal temperatures will not destroy it and it will remain a hazard. *C. perfringens*, if given

enough time to grow and sporulate, will form heat-resistant spores that are unaffected by most cooking temperatures below retort. *Salmonella* and *L. monocytogenes* are also pathogens of concern in pork products, especially those that may not reach 69^{o}C (156°F) internal temperature (Lavieri et al., 2015) during processing.

Alternatively cured meat products are currently less regulated than conventionally cured products. Inclusion levels of celery juice powder and cherry powder are limited only by flavor and cost, whereas their synthetic homologs are strictly regulated. In contrast, because the antimicrobial efficacy of alternative cure ingredients is less well understood, these products may undergo additional scrutiny from a food safety perspective. For example, it was only in 2017 that USDA had the information to allow alternatively cured products to qualify for Stabilization Option 3 of Appendix B, which allows slower cooling of products containing any source of nitrite and a cure accelerator (USDA-FSIS, 2017)

As the use of curing ingredients from natural sources increases, it is imperative to understand if microorganisms in processed meats with alternate curing ingredients may respond differently from those in processed meats with conventional synthetic ingredients when exposed to process deviations or atypical heating processes. This information is important first and foremost for food safety, but also to provide the industry with standards and limits based on relevant science. This study was initiated to fill the gaps in the knowledge regarding the use of pre-converted celery juice powder and natural cure accelerators. 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* and non-typhoidal *Salmonella enterica* on pork bellies during processing.

2. MATERIALS AND METHODS

2.1 Pork bellies: Fresh pork bellies were obtained directly from a federally inspected establishment. Two bellies for each treatment were used in each replication, and the experiment was indeopendently replicated three times. The bellies were prepared using the brine solutions described in Table 1. The brines were formulated to achieve the specific levels in the pumped bellies. Brine 1 was an alternative cure (100 ppm nitrite, 250 ppm erythorbate), Brine 2 was a conventional cure (100 ppm nitrite, 250 ppm erythorbate), Brine 3 was also a conventional cure (120 ppm nitrite, 547 ppm erythorbate) and Brine 4 did not contain curing agents (NaCl and sucrose only). All of the brines were formulated to a target level of 0.8% NaCl, which would be considered a lower sodium brine formulation when compared to conventional processes.

2.2 Meat preparation: The pork bellies for both the smokehouse studies and the laboratory-based studieswere injected to 12% of the green weight of the bellies. The injected bellies were vacuum tumbled for 30 minutes on a continuous cycle at slow speed in a processing room at or below 10^{o}C. The bellies were then vacuum packaged and held at 1^{o}C until inoculating and smoking.

2.3 Bacterial Cultures: The bacteria used in these experiments are described in Table 2. *Clostridium perfringens* strains were cultured in fluid thioglycolate medium and in Duncan-Strong sporulation medium (Duncan & Strong, 1968) as described by Juneja et al. (1993). 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 cells and spores were harvested separately by centrifugation (9,500 g, 10 min, 4^{o}C) and were then resuspended in 1/10 volume of physiological saline (0.85% sodium chloride, wt/vol). Prior to inoculation of the pork bellies, 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 and *Salmonella enterica* were grown to late logarithmic growth stage in trypticase soy broth at 37^{o}C for 18-24 hours. 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 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.

2.4 Inoculation and processing: The vacuum packaged pork bellies were removed from the packages and then surface inoculated on the lean side of the belly with a mixed culture of the *C. perfringens* (spores and vegetative cells), *S. aureus,* and *S. enterica* with a foam paint brush, with initial populations in the range of 10^6 to 10^7 colony forming units (cfu) per cm^2 or g. The bellies 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 on the lean side. For the laboratory-based simulation experiments using a water bath, 22 ± 1 g were removed from the previously prepared pork bellies and the samples were surface inoculated with the same mixed culture and massaged manually for 30 sec. All of the inoculated belly samples were covered with a plastic overlay (Saran) to

prevent surface drying and stored at 5^{o}C for 24 hours prior to processing. This resulted in inoculated bacteria being in a physiological state which would be typical of those seen in commercial meat processing. Water bath samples were then vacuum-packaged in plastic bags (Clear-Tite 31 5.5x11 CLR Mast, Oshkosh, WI) using a Multivac packaging machine (Model A300/16, SEPP Haggenmüller, Kg, Germany).

A bacon process was developed in consultation with industry professionals and University extension faculty (Table 3). Although designated as a smoke cycle, the smoke unit was turned off for this process so that smoke would not be a variable in the experiment, and only the effect of temperature and brine would be measured. Temperature measurements were taken in two bellies from each brine for each replication, and the process was stopped when average temperature of the bellies reached a minimum of 51.1^{o}C (124^{o}F).

In addition to the industrial bacon processing, a laboratory experiment to simulate the same processing parameters was conducted. Inoculated vacuum packaged belly samples were immersed in a microprocessor-controlled programmable circulating water bath (RTE-211, Neslab, Portsmouth, NH) capable of maintaining cooking and chilling temperatures equivalent to the product processes being simulated or the deviation modifications thereof. Preliminary experiments determined the programming schedules necessary to achieve time and temperature profiles inside belly samples identical to those of represented industrial processes. Samples were removed from the bath at preselected time intervals between given temperatures. Calibrated submersible data loggers (HiTemp 140, 20°C - 140°C (accuracy ±0.1°C), MadgeTech, Inc., Warner, NH) were inserted into two of the uninoculated samples to cover the entire length of the tip

portion. Temperature was monitored throughout the laboratory simulation of the cooking and chilling.

2.5 Microbiological analysis: Samples were taken from the bellies 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 (2x2 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. Laboratory simulation samples weighed 22 ± 1 g. Samples were homogenized in buffered peptone water and serially diluted as appropriate.

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^{o}C in anaerobic jars for 48 h. *S. aureus* populations were enumerated by surface plating on Baird-Parker agar with egg yolk tellurite emulsion, and incubated at 37^{o}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. A spiral plater was used for plating (WASP 2, Microbiology International, Frederick, MD). The plates were incubated at 37^{o}C for 48 h and counted with an automated colony counter (aCOLyte 3 HD, Synbiosis, Frederick, MD).

2.6 Chemical Analysis: The bellies were chilled for 18 – 24 hours at <5^{o}C, and were then sampled at a point approximately 15 cm from the ham end of the belly. A horizontal strip approximately 2.5 cm wide was taken from each belly across the entire

width of the belly and analyzed for residual nitrite, salt and water activity. The entire samples were ground (lean and fat), thoroughly mixed and three representative sub-samples from each ground sample were analyzed for residual nitrite for cured meat using a colorimetric method (AOAC 973.31). The nitrite levels are expressed as the weight of $NaNO_2$. Salt was analyzed for the whole belly experiment using the silver nitrate titration procedure and a Chloride test kit CD-51 (HACH Company, Loveland CO, method 8207). For the water bath experiment, salt content was analyzed using a colorimetric method (AOAC 935.47). Water activity was determined using an AquaLab series 4 water activity meter (Meter Group, Inc., Pullman WA), following the instructions for the instrument.

2.7 Experimental Design: Each trial was independently replicated three times, with duplicate samples for each sampling point within each replication. Microbial populations were transformed to $\log_{10}$ colony forming units/ 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 formulation (1 to 4) , cycle type (standard v. interrupted), time (midpoint and final) sample location (surface v. internal), replication 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 readers consideration.

3. RESULTS

At the end of the processing the pork bellies reached an average temperature across all brines and replications of of 51.15^{o}C (Figure 1; standard error of the mean, 0.64; data not shown). During the interrupted cycle (Figure 1B), the pork belly samples in the water bath remained at a higher temperature than the bellies in the smokehouse for the 3 hour interruption, and the temperature difference ranged from 4^{o}C to 7^{o}C. The temperature of the bellies in the smokehouse fell from 35.5^{o}C at the start of the interruption to 27.7 ^{o}C when the process was re-started, while the temperature of tes in the waterbath fell from 37.1^{o}C at the start of the interruption to 35.2^{o}C when the process was re-started.

The different temperature profiles for the interrupted cycle were evaluated with the USDA- ARS Pathogen Modeling Program (PMP; USDA-ARS, 2021). When the temperature profiles were entered for the dynamic model for *C. perfringens*, the model predicted an overall population increase of 0.43 $\log_{10}$ for the smokehouse temperature profile and a 0.35 $\log_{10}$ for the waterbath model. Under practical applications, it would be very difficult to resolve a 0.08 $\log_{10}$ difference between the two system. The PMP did not allow for dynamic modeling of the growth of either *S. aureus* or *S. enterica*. The genereation time for *S. aureus* at 28^{o}C,the lowest temperature in the smokehouse, was 0.6 hours, while the generation time at 35^{o}C, the lowest temperature that the samples reached in the water bath, was 0.4 hours. The maximum temperature that the PMP would predict for *S. enterica* was 30^{o}C, and the generation time was 0.4 hours.

When analyzed as a main effect, the different brine formulations had no effect (*P* >0.50) on the change in population of any of the bacteria on either the surface or internal samples, irrespective of cycle type. Because of this, the data from all four brines was pooled in the analyses. (Table 4). There was no difference in the change in populations attributable to the cycle (standard or interrupted) for either *S. aureus* or *S. enterica* (for both bacteria, P >0.14). The final populations were reduced by, at least 1.6 $\log_{10}$ (surface) and at least 1.2 $\log_{10}$ (internal) for *S. aureus*, while the final populations were reduced by approximately 1.0 $\log_{10}$ for both surface and internal samples for *S. enterica*, with the exception of the surface samples with the standard cycle. In that case, the average population was reduced by approximately 1.5 $\log_{10}$.

There were observed differences in the population reductions for *C. perfringens* by cycle type for both surface (P=0.022) and internal (P=0.006) samples. The $\log_{10}$ population changes were greater for the interrupted cycle for both surface and internal samples. The final population change for the surface and internal samples for the standard cycle ranged from -0.3 to -0.6 $\log_{10}$, while the population changes for the interrupted cycle ranged from -1.5 to -1.9 $\log_{10}$.

The change in populations between the surface and internal samples was compared at the end of the processing cycles, approximately 7 (standard) and 10 (interrupted) hours after the start of the process (Figure 2). Only the results from the end of the cycles were used in the analysis. Although the results for *S. aureus* were numerically greater for the interrupted cycle when compared to the standard cycle, neither cycle (P=0.14) nor sample location (P=0.46) had important main effects.

The population changes seen with the laboratory procedure were very different from those determined in the smokehouse ($P<0.001$). In almost every case, the laboratory experiments resulted in increases or slight decreases in the populations of *C. perfringens* and *S. enterica*, while the smokehouse data showed decreases in the populations (Table 6). In the laboratory experiments, *C. perfringens* populations were >1-$\log_{10}$ and >2-$\log_{10}$ higher than the data from the smokehouse for both the standard and interrupted cycles, respectively. Similarly, *S. enterica* populations for both standard and interrupted cycles were >1-$\log_{10}$ higher than smokehouse data except for Brine 3 for the interrupted cycle. However, the trend was different for *S. aureus*, as the laboratory samples showed greater (>1 $\log_{10}$) population decreases than the smokehouse data ($P<0.001$) for the standard cycle. For the interrupted cycle, the population changes were not different ($P=0.376$) for *S. aureus*.

The results of the chemical analyses are presented in Table 5. There were differences noted between the two preparation methods ($P<0.001$) and the cycles ($P=0.003$), for both theresidual nitrite and NaCl.. There were no differences noted between the different brines ($P=0.89$) and there were no significant interactions between preparation method, cycle or brine ($P>0.38$). There were differences in the NaCl content based on the preparation method ($P=0.004$), but not between brines or cycles ($P>0.22$). As with the water activity, there no significant interactions between the different variables. There were differences observed with residual nitrite between both the preparation method ($P<0.001$) and brine formulation ($P=0.07$). There were no observed differences between cycle ($P=0.97$).

4. DISCUSSION

This study used random bellies supplied from a federally inspected meat processing establishment. The main objectives were to examine the effects of brine formulation and interrupted processing cycle on the survival of the three bacterial pathogens. Because of this, there was inherent variability between individual bellies as well as variability between replications. However, this is the same variability which would be expected during actual processing conditions.

The results demonstrated that the alternative cures in both standard and process deviation cycles resulted in a reduction in the inoculated bacteria in the smokehouse. This is in contrast to the results of Taormina & Bartholomew (2005), who reported that bacon samples produced in a model system showed an approximate 0.8 $\log_{10}$ increase in the populations of both *C. perfringens* and *S. aureus* in belly samples at the "peak smoking temperature", 48.9 °C at 6 h. The results presented here did indicate a similar increase in population of the same two bacteria at the mid-point in the process (Table 4), but showed decreases in population at the end of the cycle (Fig. 1). The Taormina & Bartholomew (2005) study used conventionally cured bellies obtained from commercial sources in a model system. Differences in bacterial strains, cures, process and processing system (model system vs. smokehouse) may well account for these observed differences. Of these, the cures and process may be the largest contributors to the observed differences. The process used in the model system was completed in 6 hours to an end point temperature of 48.9^{o}C, while the one presented in this study took approximately 7 to 7.5 hours (10 to 10.5 for the interrupted cycle) to reach an end point temperature of 51.1^{o}C.

Sindelar et al. (2019) reported that the populations of *C. perfringens* (spores and vegetative cells) at internal locations of pork bellies increased by less than 1 $\log_{10}$ and declined significantly (approximately 3 $\log_{10}/cm^2$) on the surface of the bellies during an extended bacon process. In that study, conventionally cured bellies were processed for approximately 15.5 hours to an end point temperature of 51.7^{o}C. That study incorporated several 3-hour "holds" into the process to simulate extended smoke times, although no smoke was added to the product. The present study found minor decreases in the population of *C. perfringens* in internally inoculated samples after processing (Table 4 and Fig. 1). The increase in population in the previous study may well have been attributable to the extended process cycle.

There was a difference in the *C. perfringens* population reductions between the standard and interrupted cycles, with greater reductions seen with the interrupted cycle (Fig. 1). The differences were surprising as the opposite effect was anticipated based on temperatures and the published growth rates of *C. perfringens*. However, Sindelar et al. (2019) did report a reduction of approximately 2.8 $\log_{10}$ in the populations of *C. perfringens* on the surface of bacon processed with an extended cycle. That same study reported a small (< 1 $\log_{10}$) increase in population in the internal samples from the same process. An important part of the experimental design was the incorporation of the 24 hour refrigerated storage period prior to processing, to simulate commercial operations. Novak et al. (2001) found that the decimal reduction values of *C. perfringens* vegetative cells at 60^{o}C were significantly lower for cells which been held at 4^{o}C for 24 hours after the initial incubation period. The interrupted cycle would have exposed the cells to additional thermal processes. In addition, it is likely that the *C. perfringens* spores

began to germinate during the extended process cycle. Once germinated, the vegetative cells of *C. perfringens* could have been more susceptible to the thermal lethality of the process than when they were in the spore state. The interrupted cycle would have allowed more time for spore germination and exposed the vegetative cells to additional thermal processing.

Current cured meat processing conditions appear to be adequate to control *C. perfringens*. Taormina et al. (2003) surveyed commercially processed products and found that only 1.6% of the whole muscle samples were positive, and spores were not detected in any of the positive samples. When present, the bacteria populations did not exceed 1.70 $\log_{10}$ CFU/g and averaged 1.56 $\log_{10}$ CFU/g. The Food and Drug Administration's Bad Bug Book (2012) states that foodborne illness from *C. perfringens* occurs "after consumption of foods containing large numbers (>10^6 live vegetative cells or >10^6 spores) of *C. perfringens* capable of producing the enterotoxin". The low reported incidence of *C. perfringens* in cured meats may be in part attributable to the observed reductions associated with bacon processing.

Both processes resulted in a decrease in the populations of *S. aureus* (Fig. 1). This is in contrast to the study of Taormina & Bartholomew (2005), who reported approximately a 1.5 $\log_{10}$ increase in *S. aureus* using a model bacon process with ground pork bellies. However, Sindelar et al. (2019) reported approximately a 2.8 $\log_{10}$ reduction in the population of *S. aureus* on the surface of pork bellies processed with an extended processing cycle, which is similar to the results presented here. The same authors reported a slight decrease in the populations on the internal samples. Both of

the previous studies used different brines and processing cycles, and the Taormina & Bartholomew (2005) study used ground pork bellies in a model system.

Stiles & Witter (1965) reported decimal reduction values of 25.2 and 17.6 minutes at 48.9^{o}C and 51.6^{o}C, respectively, for *S. aureus* in laboratory media containing 7.5% NaCl. Shebuski et al. (2000) showed that growth at a water activity of 0.94 (~9%NaCl) increased heat resistance in *S. aureus*. In the present study, the brine formulation was adjusted to approximately 0.8%NaCl, and the final NaCl concentrations in the bellies after processing ranged from 0.5% to 0.99% (Table 5). This would suggest that the decimal reduction values in these bellies were less than the values published by Stiles & Witter (1965) and would explain the population reductions seen with *S. aureus*.

The populations observed in the present study for *S. enterica* (Fig. 1) were similar to those reported by Burnham et al. (2006), who found that the populations were reduced by approximately 1 $\log_{10}$ during a 6 hour bacon processing cycle in a model system under “high humidity” conditions. Presumably this would be similar to the processing conditions in the study presented here. Sindelar et al. (2019) reported population reductions of >2 $\log_{10}$ for *S. enterica* on pork bellies processed with an extended processing cycle. It is likely that the longer thermal cycle contributed to the greater reductions reported by Sindelaret al. (2019). Bacon is generally considered to be a low risk product for foodborne *Salmonella*, and there has only been one reported outbreak with even a tentative link to bacon between 1997 and 2017 (CDC, 2020). Comparing the laboratory preparation method to the smokehouse method of preparation was surprising. The laboratory procedure has frequently been used to simulate many procedures (Taormina et al., 2003; Taormina & Bartholomew, 2005) and

has been considered to be adequate for process validation. However, the results presented in this study differ substantially from previously published results (Taormina et al., 2003), which may be attributable to different brines and different processing conditions. It is interesting to note the difference in responses between bacteria, in that the laboratory procedure overpredicted the survival of the inoculated populations of *C. perfringens* and S. *enterica*, and yet predicted less survival for *S. aureus* during the standard cycle. Further research may be needed to determine the specific parameters which resulted in these differences, but clearly the method of the procedure impacted both the results and the interpretation of the results. While there were observed differences in the temperature profiles, especially during the interrupted cycles, these would not be sufficient to account for the differences in the population trends between the two different systems. The predicted generation times or predicted growth from the USDA-ARS PMP (2021) would not account for the observed differences in the changes in populations in the two systems.

Similar laboratory methods have been used in the development of predictive models, and while the predictive models are very useful, it is worth considering that the models may not always be an accurate representation of what happens under actual processing conditions (Fakruddin et al., 2011).

The results of the chemical analysis of the processed and chilled bellies generally were as expected for water activity and residual NaCl (Table 5). The water activity of the samples processed in the water bath are slightly higher than those observed with the smokehouse process because the water bath samples were in sealed bags, while the smokehouse process exposed the bellies to the environment. The

observed water activity was similar to the values reported by Fernandez-Salguero et al. (1993) for bacon, approximately 0.97. The brines were formulated to 0.8% NaCl, and the residual NaCl content in the bellies ranged from 0.57 to 1.03%. The relatively minor observed differences could be due to the preparation of the initial samples or the method of analysis. The observed differences in residual nitrite levels is puzzling, as the brine formulations for both preparation methods were prepared from the same ingredients using the same formulations, although the analyses were conducted in two different laboratories. There should have been no residual nitrite with the samples from Brine 4 prepared in the smokehouse, as Brine 4 formulation did not contain nitrite. However, all of the bellies processed in the smokehouse had residual nitrite levels, and the samples from the interrupted cycles had numerically lower concentrations ($P=0.009$). It may be possible that the sea salt used in the formulations contained some small amounts of nitrite (Sebranek & Bacus, 2007), but the water bath processed Brine 4 samples did not have residual nitrite. Since bellies with different brines were processed in the smokehouse at the same time, it is conceivable that there was cross contamination between the bellies, although the bellies were physically separated and gloves were changed between collecting the samples.

Fresh meat also has also been reported to have some low levels of nitrite (Iacumin et al., 2019). Okayama et al. (1991) reported that nitrite decomposition during cooking was influenced more by time at a given temperature than by the temperature itself. The interrupted cycle extended the processing time by three hours, which may explain the lower observed residual nitrite concentrations in the bellies processed with the interrupted cycle when compared to the standard cycle.

5. CONCLUSIONS

The alternative cure brines evaluated in this study resulted in similar microbial population reductions to previous studies using conventional cure brines. This suggests that the alternative cure brines provide the same level of safety as conventional cures. The simulated process deviation resulted in either similar (*S. aureus* and *S. enterica*) or greater (*C. perfringens*) population reductions than the standard process. The results of simulated processes with water baths should be viewed with some caution, as that method tended to overpredict the survival of both *C. perfringens* and *S. enterica*. This does not negate the value of laboratory simulations or predictive models derived from those simulations, but simply suggests that in some cases the actual processes may well produce different results than the simulations. While every process deviation is different and should be evaluated independently, the significance of the process deviation reported in this study during bacon processing may not be as great as has been previously thought.

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Authorship Contribution Statement

Shannon M. Cruzen: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing - review & editing.

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Conflict of Interest Statement

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, *other than those declared in the author affiliations*.

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Table 1. Formulations of the brines used to inject the pork bellies. The brines were formulated to achieve the specific concentrations (in ppm or %) in the pumped bellies.

	Brine 1	**Brine 2**	**Brine 3**	**Brine 4**
Brine Description	Alternative Cure	Conventional Cure	Conventional Cure	No cure
Nitrite concentration (ppm)	100	100	120	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. Strains of bacteria used to inoculate pork bellies and bone-in 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

Table 3. Belly processing schedule for standard and interrupted (process deviation) cycles

Step	Time (hours)	Elapsed Time (h)	Step Type	Dry Bulb °C	Wet Bulb °C	Relative Humidity (%)
1	0	0	-	-	-	-
2	2*	2	Cook	48.9	40.5	59.5
3	5	7	Cook	54.4	36.1	61.4
4	-	-	Cook Hold to 51.1°C internal	60.0	51.7	63.0

* For the interrupted (process deviation) cycle, the process was stopped after 1 hour of elapsed time, held for 3 h without temperature control and then restarted from the point where the process stopped.

Table 4. Log_{10} change in bacterial populations on surface and internal samples as affected by process cycle. A positive result indicates an increase in population, while a negative result indicates a decrease in population. Since brine formulationwas not a factor (the minimum calculated P value for brine was >0.5) for all bacteria, these results are pooled from all four brine formulations. Initial populations: *C. perfringens* 5.1 ± 0.7 cfu/g or cm^2; *S. aureus* 6.2 ± 0.6 cfu/g or cm^2; *S. enterica* 6.7 ± 0.8 cfu/g or cm^2.

Cycle[a]	Sample[b]	Sample Point[c]	*C. perfringens*	*S. aureus*	*S. enterica*
Standard	Surface	Mid-point	-0.94 (0.16)[d]	0.50 (0.27)	-0.00 (0.25)
Interrupted	Surface	Mid-point	-0.55 (0.16)	0.61 (0.27)	0.17(0.25)
Standard	Surface	Final	-0.63 (0.16)	-1.43 (0.27)	-1.55(0.25)
Interrupted	Surface	Final	-1.97 (0.16)	-2.10 (0.27)	-0.99(0.25)
Standard	Internal	Mid-point	-0.19 (0.22)	0.12 (0.24)	-0.38 (0.21)
Interrupted	Internal	Mid-point	-0.51 (0.22)	-0.16 (0.24)	-0.12 (0.21)
Standard	Internal	Final	-0.31 (0.22)	-1.28 (0.24)	-0.95 (0.21)
Interrupted	Internal	Final	-1.56 (0.22)	-1.88 (0.24)	-0.99 (0.21)

a Interrupted cycle: process was stopped after 1 h elapsed time, held for 3 h without temperature control, and then restarted

b Surface sample (2 x 2 cm); Internal sample (1cm below the surface)

c Sampling point: approximately 3 hours and 7 hours (standard) or 4 hours and 10 hours (interrupted)

d Log_{10} population change (standard error of the mean)

e Means with different superscripts within columns

Table 5. Comparison of the chemical properties of the products produced with different brines and cycles between the smokehouse and laboratory method.

Brine Formulation	Cycle	Process	Nitrite(ppm)[A]	NaCl (%)	A_w
1	Standard	Smokehouse	4.78 (19.67)[B]	0.996 (0.129)	0.981 (0.002)
		Laboratory	49.83 (13.91)	1.017 (0.091)	0.991 (0.002)
	Interrupted	Smokehouse	0.98 (19.67)	0.800 (0.129)	0.977 (0.002)
		Laboratory	22.5 (13.91)	0.950 (0.091)	0.988
2	Standard	Smokehouse	2.82 (19.67)	0.572 (0.129)	0.982 (0.002)
		Laboratory	47.67 (13.91)	0.883 (0.091)	0.991
	Interrupted	Smokehouse	1.21 (19.67)	0.717 (0.129)	0.976 (0.002)
		Laboratory	42.33 (13.91)	1.033 (0.091)	0.986
3	Standard	Smokehouse	2.74 (19.67)	0.790 (0.129)	0.981 (0.002)
		Laboratory	43.83 (13.91)	0.933 (0.091)	0.992
	Interrupted	Smokehouse	0.62 (19.67)	0.710 (0.129)	0.981 (0.002)
		Laboratory	86.33 (13.91)	0.733 (0.091)	0.989
4	Standard	Smokehouse	4.31 (19.67)	0.651 (0.129)	0.979 (0.002)
		Laboratory	0.833 (13.91)	0.867 (0.091)	0.992
	Interrupted	Smokehouse	0.347 (19.67)	0.864 (0.129)	0.981 (0.002)
		Laboratory	0.00 (13.91)	1.033 (0.091)	0.985

A – nitrite levels are expressed as the weight of $NaNO_2$

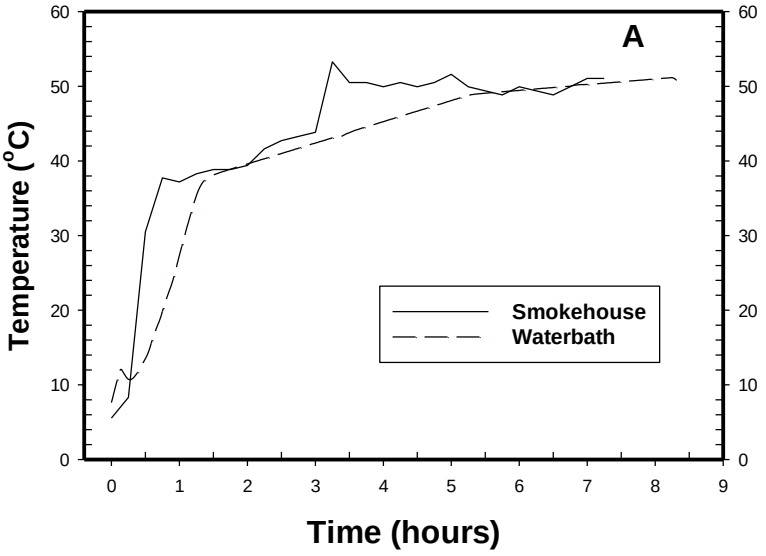
B - Least Squares mean (standard error)

Table 6. Observed population changes in bacteria: smokehouse results in comparison to laboratory results. The population difference compares the population at the completion of the cycle to the population at time 0. Negative values indicate a population decrease, positive values indicate a population increase.

Bacterium	Cycle	Preparation Method	Brine 1	Brine 2	Brine 3	Brine 4
C. perfringens	Standard	Smokehouse	0.02 (0.42)[A]	-0.11 (0.42)	0.09 (0.42)	-0.18 (0.42)
		Laboratory	1.74 (0.21)	1.48 (0.21)	1.30 (0.21)	2.16 (0.21)
	Interrupted	Smokehouse	-1.18 (0.30)[A]	-0.90 (0.30)	-0.96 (0.30)	-0.57 (0.30)
		Laboratory	1.80 (0.18)	2.20(0.18)	1.53(0.18)	2.04 (0.18)
S. enterica	Standard	Smokehouse	-0.90 (0.48)	-1.37 (0.48)	-1.03 (0.48)	-1.80 (0.48)
		Laboratory	0.20 (0.24)	-0.18 (0.24)	-0.09 (0.24)	0.29 (0.24)
	Interrupted	Smokehouse	-0.67 (0.32)	-0.94 (0.32)	-0.79 (0.32)	-0.78 (0.32)
		Laboratory	0.64 (0.19)	0.43 (0.19)	0.02 (0.19)	0.85 (0.19)
S. aureus	Standard	Smokehouse	-0.99 (0.38)	-1.33 (0.38)	-1.36 (0.38)	-1.17 (0.38)
		Laboratory	-2.72 (0.19)	-3.05 (0.19)	-3.05 (0.19)	-2.81 (0.19)
	Interrupted	Smokehouse	-1.76 (0.66)	-1.97 (0.66)	-1.95 (0.66)	-1.41 (0.66)
		Laboratory	-1.42 (0.38)	-1.36 (0.38)	-1.39 (0.38)	-1.53 (0.38)

A Least squares mean (standard error)

Figure 1. Average internal temperatures of pork bellies during smokehouse and waterbath process for the standard (A) and interrupted (B) processes.



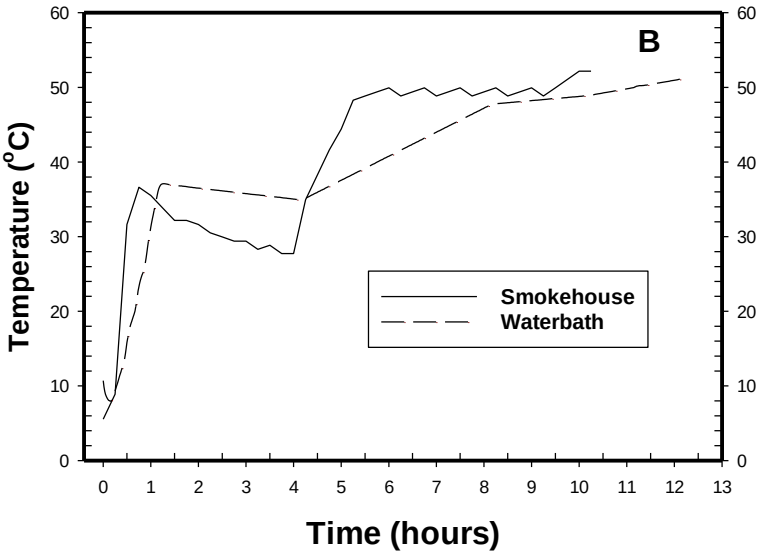


Figure 2. Comparison of log population changes in surface and internal samples at the end of the processing cycle for the bellies processed in the smokehouse.