

## Validation of Bacon Processing Conditions To Verify Control of *Clostridium perfringens* and *Staphylococcus aureus*

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### ABSTRACT

It is unclear how rapidly meat products, such as bacon, that have been heat treated but not fully cooked should be cooled to prevent the outgrowth of spore-forming bacterial pathogens and limit the growth of vegetative cells. *Clostridium perfringens* spores and vegetative cells and *Staphylococcus aureus* cells were inoculated into ground cured pork bellies with and without 1.25% liquid smoke. Bellies were subjected to the thermal profiles of industrial smoking to 48.9°C (120°F) and normal cooling of bacon (3 h) as well as a cooling phase of 15 h until the meat reached 7.2°C (45°F). A laboratory-scale bacon smoking and cooling operation was also performed. Under normal smoking and cooling thermal conditions, growth of *C. perfringens* in ground pork bellies was <1 log regardless of smoke. Increase of *S. aureus* was 2.38 log CFU/g but only 0.68 log CFU/g with smoke. When cooling spanned 15 h, both *C. perfringens* and *S. aureus* grew by a total of about 4 log. The addition of liquid smoke inhibited *C. perfringens*, but *S. aureus* still achieved a 3.97-log increase. Staphylococcal enterotoxins were detected in five of six samples cooled for 15 h without smoke but in none of the six samples of smoked bellies. In laboratory-scale smoking of whole belly pieces, initial *C. perfringens* populations of  $2.23 \pm 0.25$  log CFU/g were reduced during smoking to  $0.99 \pm 0.50$  log CFU/g and were  $0.65 \pm 0.21$  log CFU/g after 15 h of cooling. Populations of *S. aureus* were reduced from  $2.00 \pm 0.74$  to a final concentration of  $0.74 \pm 0.53$  log CFU/g after cooling. Contrary to findings in the ground pork belly system, the 15-h cooling of whole belly pieces did not permit growth of either pathogen. This study demonstrates that if smoked bacon is cooled from 48.9 to 7.2°C (120 to 45°F) within 15 h, a food safety hazard from either *C. perfringens* or *S. aureus* is not likely to occur.

Traditional bacon is a heat-treated but not fully cooked product made from cured pork bellies that are smoked, cooled, pressed, sliced, and packaged. Sliced bacon is a refrigerated product that is not shelf stable and must be cooked, usually by frying or heating in a microwave or conventional oven, before consumption. Retail packages are usually vacuum sealed, whereas food-service bacon is boxed in bulk. A portion of the bacon market is represented by fully cooked product that is ready to heat and serve.

Bacon has a long history (many years) of safe consumption without being linked to reported outbreaks of bacterial illness (8). Joint efforts by industry and government helped establish appropriate concentrations of added sodium nitrite (NaNO<sub>2</sub>) in pork bellies necessary to ensure inhibition of *Clostridium botulinum* (types A and B) in vacuum-packaged bacon for the entire shelf life (3). The universal historical safety of bacon can be attributed to the use of preservatives such as NaCl and NaNO<sub>2</sub> and to the cooking step prior to consumption.

In the United States, regulatory checks on industrial hazard analysis critical control point systems often require the designation of at least one critical control point (CCP) in every process. Although CCPs may be chemical, physical, or biological, many bacon processors have identified spore-forming toxigenic bacteria as biological hazards rea-

sonably likely to be found during bacon processing and have selected the cooling stage after smoking as a CCP. Such a CCP is selected despite the fact that bacon is cooked prior to consumption, thereby greatly reducing vegetative cell populations. The tendency to identify cooling as a CCP in bacon processing probably stems from the reliance on “safe harbor” cooling guidelines that were outlined by the U.S. Department of Agriculture Food Safety and Inspection Service (FSIS) and guaranteed to control certain spore-forming bacteria (22). These cooling guidelines, which were intended for fully cooked meats, have been cited by processors and regulators as critical limits for bacon cooling despite the fact that bacon is not fully cooked during processing. The cooling guidelines for cured meats dictate that cooling times between 54.4 and 7.2°C (130 and 45°F) not exceed 15 h and specifically that the product temperature dwell no longer than 5 h between 54.4 and 26.7°C (130 and 80°F) and no more than 10 h between 26.7 and 7.2°C (80 and 45°F). However, during smoking, bacon usually achieves maximum temperatures of only 47 to 52°C (116.6 to 125.6°F), with ranges depending on processor preferences, equipment, and other variables. Because the FSIS cooling guidelines are not designed for meats that are heat treated but not fully cooked, questions exist about the safety of a product cooled as long as 15 h between the peak smoking temperatures and 7.2°C (45°F). In previous studies, fully cooked cured pork products did not support *Clostridium perfringens* growth when cooling intervals from 54.4 to

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7.2°C (130 to 45°F) spanned 15 h (20, 24). However, these studies involved *C. perfringens* spore inocula that were subjected to cooking temperatures prior to cooling. The behavior of this organism during bacon production, not including cooking temperatures, has not been reported.

A variety of microorganisms can routinely be found in bacon, including gram-positive bacteria (e.g., *Lactobacillus* and *Bacillus*) and gram-negative bacteria (e.g., *Moraxella* and *Escherichia coli*) (23). A survey of 263 samples of vacuum-packaged bacon revealed an 89% positive rate for presumptive *Clostridium* (14). *C. perfringens* was found in 24% of the samples, and *C. botulinum* was found in 4.2%. No growth of either pathogen was observed at storage temperatures of 15, 20, and 25°C, but other species increased in numbers at these temperatures.

Staphylococci are also typically part of the microflora of bacon (4, 8). In a survey, nine *Staphylococcus aureus* isolates were found in 10 bacon samples, along with eight isolates of *Streptococcus faecalis*, six of *C. perfringens*, six of *Bacillus cereus*, four of *E. coli*, and one of *Pseudomonas aeruginosa* (15). These organisms can be introduced to all areas of pork bellies during injection of brine solutions. The behavior of *S. aureus* is of interest in slowly cooled bacon because this pathogen is salt tolerant and capable of producing heat-stable enterotoxin(s). There is potential for some growth in a product that is heat treated but not fully cooked because temperatures necessary for vegetative cell death will not be attained in the meat during production. When temperatures increase and decline during production, lengthy periods occur within the growth range of the organism, and there may be further potential for growth and even enterotoxin production if a cooling stage is inordinately long. It is not known how *S. aureus* would respond in bacon cooled for 15 h.

Given the salt tolerance of *S. aureus* and the potential to produce heat-stable enterotoxin, the behavior of this pathogen and of *C. perfringens* during smoking and cooling of bacon should be explored. Because *S. aureus* also is able to grow and produce enterotoxins in temperature-abused precooked bacon (13, 17), limiting growth of this pathogen during smoking and cooling is also of interest for these products. This study was designed to evaluate the microbiological safety of bacon with respect to *C. perfringens* and *S. aureus* under commonly used processing times and temperatures and smoke application. The ultimate goal was to establish safe cooling time and temperature guidelines for heat-treated but not fully cooked bacon to control both pathogens.

## MATERIALS AND METHODS

**Strains and inocula.** *C. perfringens* ATCC 3624 (which does not produce enterotoxin, *ent*<sup>-</sup>) and FD1041 (which does, *ent*<sup>+</sup>) were provided by Dr. Ronald Labbe (University of Massachusetts, Amherst). Another *ent*<sup>+</sup> strain, ATCC 12916, and one biochemically confirmed isolate from a raw meat blend (designated JM2) with unknown enterotoxin-producing ability were also included as inocula. Stock cultures of strains were prepared by inoculating broth cultures into freshly prepared cooked meat medium (CMM, pH 7.1; BBL, Difco, Becton Dickinson, Sparks,

Md.) supplemented with 10% (vol/vol) glycerol (Sigma, St. Louis, Mo.) and incubating for 24 h at 37°C. Aliquots of active CMM cultures were then stored in cryogenic vials (NalgeNunc International, Naperville, Ill.) at -80°C. Periodically, frozen stocks were thawed and inoculated into separate tubes of fluid thioglycollate medium (FTG, pH 7.5; Becton Dickinson) and incubated for 24 h at 37°C. One milliliter of each strain of actively growing FTG culture was inoculated into 100 ml of freshly prepared modified Duncan and Strong sporulation medium with caffeine (1 g/liter) and raffinose (4 g/liter) and incubated for 20 to 24 h at 37°C. Sporulation of each strain was confirmed microscopically using the Schaeffer-Fulton endospore stain technique (5). Spores were harvested and concentrated using a modification of the technique described by Garcia-Alvarado et al. (7) by repeated low-speed centrifugation and washing with cold deionized water. Fifty milliliters of sporulated cultures was dispensed into 50-ml conical centrifuge tubes (Becton Dickinson) and centrifuged at 4.4°C once for 10 min at 3,000 × *g* using a refrigerated bench-top centrifuge (Rotina 35R, Helmer, Noblesville, Ind.). After decanting the supernatant, 25 ml of cold (4.4°C) deionized water was added to the tube, which was vortexed and then centrifuged again at 500 × *g*. The slow centrifugation step was repeated at least five times to separate spores from vegetative cells. Spore populations in suspensions of each strain were confirmed by removing portions, heat shocking at 75°C for 15 min, and plating on Shahidi-Ferguson *perfringens* agar without egg yolk (SFP, pH 7.5; BBL, Difco, Becton Dickinson) supplemented with 370 mg/liter D-cycloserine (Sigma). Concentrations were 10<sup>4</sup> spores/ml, and total *C. perfringens* recovered without heat shocking was also 10<sup>4</sup> cells/ml. These spore-vegetative cell suspensions were stored at 4.4°C and used as inocula within 21 days.

Three strains of *S. aureus* were used: ATCC 27664 (enterotoxin E), ATCC 13565 (enterotoxin A), and ATCC12600. They were grown separately in tryptic soy broth (TSB, pH 6.8; Becton Dickinson) at 35°C for 24 h prior to inoculation.

**Product temperature probing.** Smoking and blast chilling time and temperature profiles from two commercial processing facilities were acquired by use of portable wireless data-logging temperature probes. For each product, at least three Tracksense II temperature probes (Ellab, San Jose, Calif.) and at least three Datatrace temperature probes (FRB Micropack, Mesalabs, Lakewood, Colo.) were inserted into the approximate geometric center and on the surface of separate raw pork bellies in various rack locations in the smokehouses. Probing occurred on at least two separate dates. Probes were retrieved when smoked bellies had been cooled to ≤7.2°C (45°F). Data were downloaded using probe-accompanying data-logging software and transferred to Excel (Microsoft Corp., Redmond, Wash.). Temperature profiles exhibiting the longest chilling period from 48.9 to 7.2°C (120 to 45°F) were selected for simulation in laboratory experiments to study the observed worst cases of those processes during normal operation. Chilling regimes observed in plants were also extended to 15 total hours between 48.9 to 7.2°C (120 and 45°F) in laboratory experiments.

**Positive growth controls.** To monitor growth in optimal menstra, *C. perfringens* spore-vegetative cell suspensions of each strain were diluted and inoculated separately at 1 to 3 log CFU/ml into screw-cap test tubes (16 mm inside diameter by 150 mm long) containing freshly steamed FTG medium. *S. aureus* strains were diluted in the same way and inoculated into TSB at 1 to 3 log CFU/ml. These tubes were immersed into the water bath and weighed down.

**Ground pork belly system.** Raw pork bellies retrieved from three separate commercial bacon-processing facilities just prior to smoking were vacuum packaged and shipped to the lab overnight in insulated containers at  $\leq 4.4^{\circ}\text{C}$ . Raw bellies contained 12% (wt/wt) curing solution consisting of water, brine, sugar, sodium phosphate, sodium erythorbate, and sodium nitrite. Proximate analysis revealed that on average bellies contained 49.5% moisture, 11.5% protein, 41.4% fat, 1.6% salt, and 2.9% brine concentration. Upon receipt, raw bellies were cut into thin strips that were ground through a  $\frac{3}{8}$ -in. (0.32-cm) plate using a sanitized meat grinder (model 4822, Hobart, Troy, Ohio). Ground pork bellies were placed into separate WhirlPak bags (B01239, Nasco, Fort Atkinson, Wis.), frozen at  $-7^{\circ}\text{C}$ , and then thawed overnight prior to use.

Serial dilutions of cell suspensions were made in phosphate-buffered saline (PBS, pH 7.2; BBL, Becton Dickinson). Dilutions containing ca.  $10^4$  CFU/ml for each strain were then combined into a test tube and vortexed. From that combined strain suspension, 0.5 ml was added to each 50-g portion of ground pork belly in small WhirlPak bags (B01065; Nasco). The contents of each bag was mixed thoroughly by hand massaging for 2 min. To determine the effect of smoking on bacterial growth, 0.63 ml of liquid smoke (Charsol Supreme, Red Arrow, Manitowoc, Wis.) was added to some bags prior to mixing. The liquid smoke had a pH of 2.2 to 2.8, 14.0 to 16.0% total acidity (as acetic acid), 15.0 to 23.0 mg/ml smoke flavor compounds, and 24.0 to 30.0% carbonyls.

Meat in bags was spread evenly to 5-mm thickness encompassing the width of the bag (10 cm) and was 10 cm in length. The inoculated meat inside the bags was completely immersed in a water bath. The meat within one bag per experiment was probed with a calibrated thermocouple (20-ga; K-type, Omega, Stamford, Conn.), which was connected to a temperature data logger (HH 611PL4F, Omega). Time and temperature data were verified after each experiment. Worst-case plant cooling of bacon bellies involved a decline from 50 to  $7.2^{\circ}\text{C}$  in less than 4 h.

For extended cooling experiments, the goal was to examine 15 h of exponential cooling from a peak smoking temperature of  $48.9^{\circ}\text{C}$  ( $120^{\circ}\text{F}$ ) to  $7.2^{\circ}\text{C}$  ( $45^{\circ}\text{F}$ ). In practice, cooling of meat within these temperature ranges typically involves two cooling rates. To achieve biphasic cooling, two cooling rate constants,  $K_{\text{cool}1}$  and  $K_{\text{cool}2}$ , were calculated using a formula modified from Juneja et al. (10), from which the water/air temperature component was removed:

$$K_{\text{cool}} = \ln(T2/T1)/tc$$

where  $T1$  is the initial product temperature,  $T2$  is the temperature at the end of the cooling phase, and  $tc$  is the cooling time in hours. For  $K_{\text{cool}1}$ , the value for  $tc$  was 5 h and the values for  $T1$  and  $T2$  were  $48.9$  and  $26.7^{\circ}\text{C}$ , respectively. For  $K_{\text{cool}2}$ ,  $tc$  was 10 h and the values for  $T1$  and  $T2$  were  $26.7$  and  $7.2^{\circ}\text{C}$ , respectively. These cooling constants were substituted in the following formula:

$$Tn = (Ti)e^{K_{\text{cool}1}t} \text{ or } K_{\text{cool}2}t$$

where  $Tn$  is the next temperature at each constant time interval and  $Ti$  is the previous temperature. These simple calculations generated numbers for accurate programming of a microprocessor-controlled programmable circulating water bath (RTE-211, Neslab, Portsmouth, N.H.) for dual cooling rates. Typical smoking temperatures were programmed to precede both 15 h of cooling and worst-case cooling observed in plants.

**Smoking and cooling whole belly pieces.** In addition to experiments with ground pork bellies, a laboratory incubator and liquid smoke were used to smoke inoculated whole pork belly

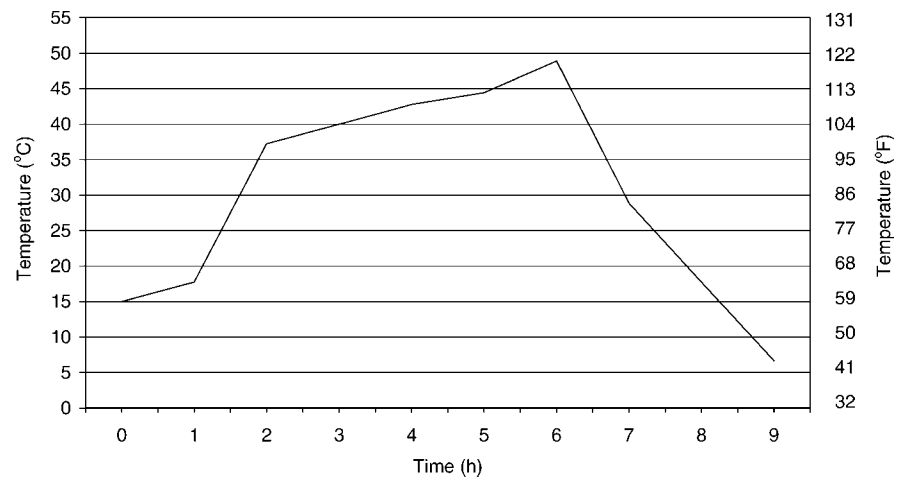
pieces followed by 15 h of cooling in the water bath. Vacuum-packaged raw cured pork bellies were received from the same three commercial plants and stored at  $-1.1^{\circ}\text{C}$  before use. Proximate analyses of these bellies were similar to those for ground pork bellies. On the day of the experiment, bellies were sliced into strips, and each strip was divided into several pieces weighing  $50 \pm 2$  g (6 by 3 by 4 cm). Suspensions of *C. perfringens* and *S. aureus* strains were diluted (target  $10^4$  CFU/ml) separately before combining each diluted strain into a single inoculum. Each belly piece was then injected at three sites with 0.5 ml of the inoculum using a sterile 3-ml syringe (309586; Becton Dickinson) and an 8-cm hypodermic needle (7020-1, Popper & Sons, Inc., New Hyde Park, N.Y.). Three aliquots of  $<0.2$  ml each were injected into each piece to ensure adequate distribution of the 0.5 ml of inoculum within the piece. The pieces were then pierced on bacon combs (six pieces per comb), which were hung on wire racks inside a programmable incubator (model 818, Precision, Winchester, Va.). The incubator temperature was reprogrammed to increase hourly to approximate smoking times and temperatures observed in plants.

Concentrated liquid smoke was diluted to 75% of original strength with sterile deionized water and applied to the belly pieces with a spray bottle. This smoke application represented liquid smoke atomization schedules that occur in some plants at roughly 4 and 5 h into the heating-drying process for bellies. Because the phenolic fraction of smoked bacon derived from liquid smoke is similar to that of traditionally smoked bacon (12), the simulation also represented natural smoking. The incubator door was opened, and each piece was sprayed with ca. 5 ml of the diluted liquid smoke at 4 h and again at 5 h. An upper and lower rack in the incubator was used to hang bacon combs, and each rack had four combs: three holding belly pieces from each of the three plants and one holding belly pieces inserted with thermocouples. Two thermocouples were inserted into two pieces on both racks. Metal pans were placed beneath the pieces to collect the runoff of liquid smoke. Upon completion of the smoking process, combs were removed from the incubator and pieces were transferred to small WhirlPak bags. The bags were closed and immersed in a water bath set at  $48.9^{\circ}\text{C}$  and programmed to cool to  $7.2^{\circ}\text{C}$  in 15 h according to the calculated biphasic cooling cycle. Pieces with thermocouples were also transferred to the bath.

**Enumeration and analysis.** Inoculated bags were analyzed for populations of *C. perfringens* and *S. aureus*, and samples were taken again at the peak smoking temperature ( $48.9^{\circ}\text{C}$  [ $120^{\circ}\text{F}$ ]) and after cooling to  $7.2^{\circ}\text{C}$  ( $45^{\circ}\text{F}$ ).

For ground bacon bellies, 25-g portions were transferred to filtering stomacher bags and combined with 25 ml of PBS, and the contents were stomached for 1 min at medium speed. Whole belly pieces (50 g) were combined with 50 ml of PBS before stomaching. One milliliter of the diluent was spread plated (0.25 ml each) onto four SFP or Baird-Parker (BP, pH 6.9; BBL, Becton Dickinson) agar plates. Diluted samples were also spiral plated (model D, Spiral Systems, Inc., Cincinnati, Ohio) on SFP and BP. Concurrently, samples were withdrawn from inoculated FTG and TSB tubes at each temperature to establish behavior of *C. perfringens* and *S. aureus* in optimal growth media under dynamic temperature conditions. SFP plates were overlaid with egg yolk-free SFP agar containing cycloserine, allowed to absorb for ca. 30 min, placed upright into rectangular anaerobic jars, and incubated for 48 h at  $37^{\circ}\text{C}$  before counting *C. perfringens* colonies. BP plates were inverted and incubated similarly under aerobic conditions. All bacon substrates subjected to extended cooling were also analyzed for staphylococcal enterotoxins using an en-

FIGURE 1. Thermal profile of ground pork bellies during water-bath simulation of plant temperatures for smoking and normal cooling of bacon.



zyme-linked fluorescent antibody assay (VIDAS, SET2, bio-Mérieux/Vitek, Inc., Hazelwood, Mo.).

Raw and smoked samples were analyzed for water activity ( $a_w$ ) using a water activity meter (Aqualab series 3, Decagon Devices, Inc., Pullman, Wash.) and for pH using a pH meter (model 320, ThermOrion, Beverly, Mass.). Samples were also analyzed for nitrite as described by the Association of Official Analytical Chemists method 973.31 (2). Phenol content of laboratory-smoked bacon was measured and compared with that of commercially produced bacon to verify the degree of penetration and quantity of liquid smoke in laboratory samples. Following the method of Tucker (21), 50-g meat samples were blended with 200 ml of an alcohol-water mixture (1:1), and the solution was then filtered and chilled. Sample extracts (5 ml) were combined with 5 ml of a 0.5% sodium borate solution and 1 ml of 2,6-dichloroquinonechloroimide solution (0.05% in 7% ethanol). After standing at 25°C for 1 h, the blue indophenol was extracted from the samples with 15 ml of *n*-butyl alcohol. The extract was filtered through Whatman no. 2 filter paper, and the color intensity (percent transmittance) was read on a spectrophotometer (Genesys 20, ThermoSpectronic, Rochester, N.Y.). A linear relationship of five diluted concentrations of phenol standard versus the logarithm of transmission percentages was graphed. Using the graph, log percent transmission values of actual bacon samples were converted to mg of phenol per 100 g of bacon.

**Statistical analysis.** Cured pork bellies from three plants were used to perform three replicates of each experiment. Populations of *C. perfringens* and *S. aureus* were determined before heating, at the endpoint of smoking, and after cooling by taking the log values of duplicate samples from three replicates (six total values) and calculating the means and standard deviations at each sample point. Average log increases for *C. perfringens* and *S. aureus* in ground belly experiments were analyzed with a one-way repeated measures analysis of variance (ANOVA) and compared between experiments using Fisher's least significant difference (LSD) test at  $\alpha = 0.05$  (SigmaStat, version 2.03, SPSS, Inc., Chicago, Ill.). The same analysis was applied to phenol concentrations in bacon systems. Phenol concentrations in bacon, ground bacon, and bacon pieces were also analyzed by one-way ANOVA and compared using the LSD test.

## RESULTS AND DISCUSSION

Surveys of time and temperature parameters in two large bacon-processing establishments served as guidelines for these laboratory experiments. Variations do exist in in-

dustry, but the data collected from two large commercial plants are representative of general industrial bacon processing. Bacon processing generally spans an 8-h period for smoking and cooling to 7.2°C. During that period, marinated pork bellies rapidly increased from refrigerated temperatures during the first hour to about 25°C (77°F) and then slowly increased to a temperature of no less than 46.7°C (116°F). Generally, peak temperatures for proper drying and smoke application range from 50 to 52°C (122 to 125.6°F), but our probe data revealed that certain areas of bellies can reach as high as 55.2°C (131.3°F) in some smokehouses. Laboratory experiments targeted a peak temperature of 48.9°C (120°F) to account for the low temperatures to which some processed bacon is exposed. These conditions also allowed us to study *C. perfringens* without exceeding its growth temperature range. During the heating process, natural smoke (generated from hardwood sawdust) is applied to the bellies or liquid smoke is atomized inside the smokehouse. Industrial observations of cooling of smoked bellies indicate that cold brine showering followed by blast chilling cools the product most rapidly. Some processors utilize only blast chilling, but maximum times observed to cool those bellies to 7.2°C were  $\leq 3$  h. Observed cooling times simulated here represent those for blast-only operations. Only gross human error or equipment malfunction could lead to unintentional extension of the cooling period to 15 h.

The first experiments were designed to determine the behavior of *C. perfringens* and *S. aureus* in a cured ground pork belly system subjected to temperature conditions observed during smoking and blast chilling in plants. Figure 1 depicts the thermal profile of ground bacon in a water bath during simulation of plant smoking and normal cooling temperatures. The smoking step spanned 6 h, and cooling from 48.9°C (120°F) to 7.2°C (45°F) was completed in 3 h. Growth of the individual strains of *C. perfringens* and *S. aureus* in broth during the 9-h period is shown in Figure 2. All *C. perfringens* strains demonstrated marginal to good growth in broth, whereas the enterotoxin-negative *S. aureus* ATCC 12600 grew better than did other strains of that species. These findings clearly demonstrate that under ideal conditions both pathogens can achieve substantial growth

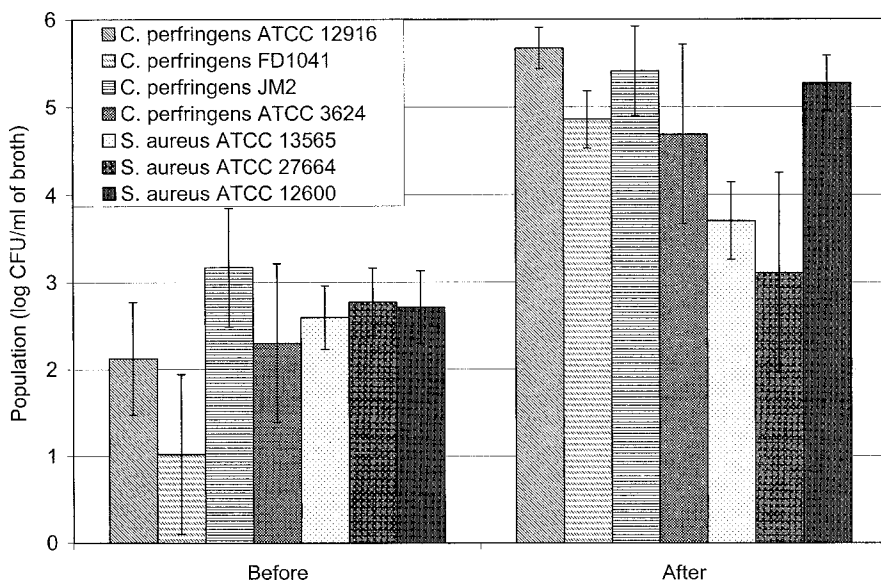


FIGURE 2. Growth of individual strains of *C. perfringens* and *S. aureus* in FTG and TSB, respectively, during laboratory simulation of smoking and normal cooling of bacon. Average counts of strains are noted by bars before smoking and after cooling. Error bars represent standard deviations.

at the temperatures to which the ground pork bellies were subjected. However, Table 1 reveals that slight increases in total *C. perfringens* (average of <1 log) occurred in the ground belly substrate during normal cooling. This growth inhibition may have been due to salts and phosphates in the marinade along with competitive inhibition from the normal microflora. When liquid smoke was mixed with the cured ground pork belly substrate at a concentration meant to mimic that applied to bellies in certain large smokehouses, growth of *C. perfringens* was limited further. *S. aureus* increased by 2.38 log in nonsmoked ground pork bellies subjected to normal smoking and cooling times and temperatures (Table 2). Log increases in *S. aureus* in ground belly with liquid smoke averaged only 0.68 log CFU/g, which was significantly less ( $P < 0.05$ ) than log growth in the nonsmoked substrate. The presence of liquid smoke at 1.25% in ground pork bellies inhibited both pathogens; average growth of both pathogens was <1 log CFU/g. These data demonstrate the safety of the current production practices for bacon with respect to these pathogens.

To further examine the safety of bacon processing, the cooling phase after smoking was extended to 15 h until 7.2°C was reached (Fig. 3). Both *C. perfringens* and *S. aureus* grew in the slowly cooled cured ground pork bellies by roughly 1 log CFU/g during smoking and by a total of ca. 4 log CFU/g after cooling for 15 h (Tables 1 and 2). Under the same time and temperature conditions, all strains

of *C. perfringens* grew by ca. 5 log CFU/ml in broth (Fig. 4). Growth of *S. aureus* strain ATCC 12600 in broth was also very good, whereas growth of ATCC 27664 was inconsistent and that of ATCC 13565 was limited. Incorporation of liquid smoke into the bacon substrate resulted in the inhibition of *C. perfringens*, but *S. aureus* growth was not affected, and a ca. 4-log increase was observed.

Comparisons of *C. perfringens* and *S. aureus* growth among experiments are also shown in Tables 1 and 2, respectively. Growth of *C. perfringens* in the substrate without liquid smoke that was subjected to 15 h cooling was significantly greater ( $P < 0.05$ ) than that in the other experiments. However, growth of *C. perfringens* was marginal in the presence of liquid smoke for both normal and 15-h cooling periods and was not significantly different from that for normal cooling of ground bellies without smoke. Under normal smoking and cooling temperatures, growth of *S. aureus* was significantly reduced by the presence of liquid smoke. However, growth of *S. aureus* was not significantly reduced by liquid smoke in substrates subjected to the 15 h of cooling.

An important food safety implication of *S. aureus* growth is whether or not enterotoxin is produced. Enterotoxin was detected in five of six samples of bacon without smoke that were cooled for 15 h, whereas presence of smoke inhibited enterotoxin production by growing populations of *S. aureus* in the bacon. Although growth of en-

TABLE 1. Comparison of *C. perfringens* growth during bacon processing simulations using ground pork belly substrate

Bacon treatment and cooling cycle	<i>C. perfringens</i> population (log CFU/g)			
	Initial	48.9°C (120°F)	Final	Log increase <sup>a</sup>
Ground bellies, normal cooling	1.60 ± 0.55	2.34 ± 0.37	2.44 ± 0.43	0.84 B
Smoked ground bellies, normal cooling	1.78 ± 0.13	1.95 ± 0.08	2.02 ± 0.56	0.24 BC
Ground bellies, 15 h cooling	2.17 ± 0.42	3.18 ± 0.98	6.10 ± 0.89	3.93 A
Smoked ground bellies, 15 h cooling	2.01 ± 0.40	1.89 ± 0.21	2.34 ± 0.07	0.33 BC

<sup>a</sup> Average log increases were calculated from differences in final and initial populations for each replicate. Increases not followed by the same letter are significantly different ( $P < 0.05$ ).

TABLE 2. Comparison of *S. aureus* growth in bacon processing simulations using ground pork belly substrate

Bacon treatment and cooling cycle	<i>S. aureus</i> population (log CFU/g)				Log increase <sup>a</sup>	Enterotoxin <sup>b</sup>
	Initial	48.9°C (120°F)	Final			
Ground bellies, normal cooling	2.87 ± 0.56	4.14 ± 0.27	5.25 ± 0.49		2.38 B	NT
Smoked ground bellies, normal cooling	2.48 ± 0.46	1.36 ± 0.66	3.16 ± 0.63		0.68 C	NT
Ground bellies, 15 h cooling	2.83 ± 0.08	3.53 ± 0.11	6.88 ± 0.13		4.05 A	5/6
Smoked ground bellies, 15 h cooling	1.29 ± 0.21	0.77 ± 0.07	5.26 ± 0.23		3.97 A	0/6

<sup>a</sup> Average log increases were calculated from differences in final and initial populations for each replicate. Increases not followed by the same letter are significantly different ( $P < 0.05$ ).

<sup>b</sup> No. of positive samples/no. of samples tested. NT, not tested.

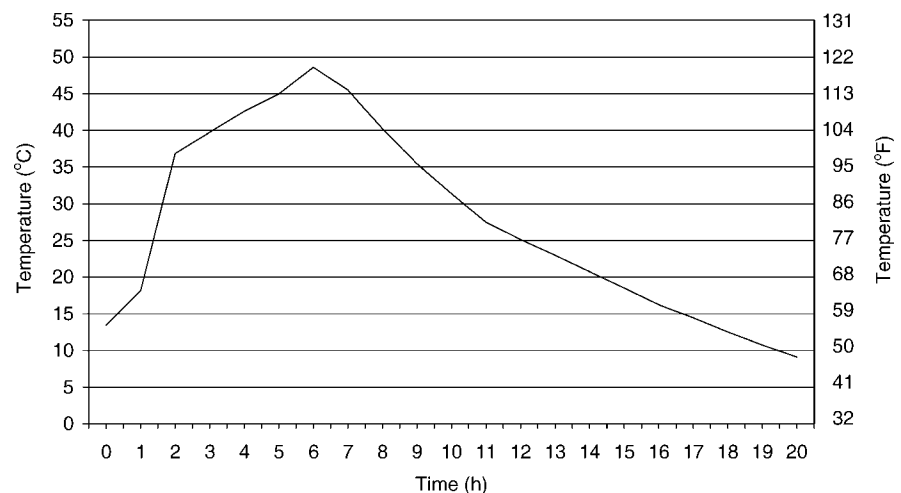
terotoxin-positive strains of *S. aureus* in broth was less than that of strain ATCC 12600, these strains apparently grew in bacon without smoke, as indicated by the presence of enterotoxins. The increase in *S. aureus* counts by almost 4 log is not desirable in any food product, but the absence of enterotoxins from any sample of smoked pork bellies indicates that the product would remain safe if bacon were properly cooked prior to consumption.

Experiments performed in ground pork belly systems did not replicate exactly the conditions of actual bacon bellies during smoking and cooling. There are obvious surface area differences between ground bellies and intact bellies, so experiments were designed to verify the smoke effects observed in ground pork bellies using whole belly pieces. This design was necessary because some researchers have reported that liquid smoke application to bacon allows little diffusion of phenols, with major flavoring occurring mostly on the surface layers (12). Wood smoke and its associated phenols possess broad spectrum antimicrobial activity (1), and our work in pork belly systems has demonstrated the inhibitory power of liquid smoke against *C. perfringens* and *S. aureus* enterotoxin production. The smoking process reduces the surface moisture of bacon with a corresponding increase in NaCl concentrations and a decrease in  $a_w$ . These changes occur in addition to the deposition of bacteriocidal smoke components (8). Liquid smokes applied to meats strongly inhibit some staphylococci, motile lactobacilli, micrococci, and *S. faecalis* (8). There also appears to be a

residual bacteriostatic effect of smoke components in meat, which is attributable mostly to phenols.

The final experiment was designed to smoke inoculated belly pieces in a laboratory incubator to clarify findings in the ground pork belly system. Figure 5 shows the thermal profile of the laboratory-scale smoking and cooling process and the associated counts of the pathogens before and after smoking and after cooling for 15 h to 7.2°C. The temperature control during smoking was affected when the incubator door was opened for smoke application. However, because the temperature remained near or above optimal growth temperatures for both pathogens during those times, it is not likely that the temperature fluctuations reduced opportunity for growth. Spray applications of liquid smoke clearly reduced counts of *C. perfringens* and *S. aureus* recovered after the smoking phase. The initial *C. perfringens* population of  $2.23 \pm 0.25$  log CFU/g was reduced during the smoking process to  $0.99 \pm 0.50$  log CFU/g. After extended cooling spanning 15 h, the population further declined to a final concentration of  $0.65 \pm 0.21$  log CFU/g. The average inoculation concentration of *S. aureus* was  $2.00 \pm 0.74$  log CFU/g. The smoking process reduced *S. aureus* populations to  $0.72 \pm 0.37$  log CFU/g, with little change occurring during the 15-h cooling period before a final concentration of  $0.74 \pm 0.53$  log CFU/g was detected. Six of the six bacon pieces tested were negative for staphylococcal enterotoxins. Contrary to findings in the ground pork belly system, the slow cooling of bacon pieces did not

FIGURE 3. Thermal profile of ground pork bellies during water-bath simulation of temperatures for smoking and 15 h of cooling (48.9 to 26.7°C in 5 h and 26.7 to 7.2°C in 10 h) of bacon.



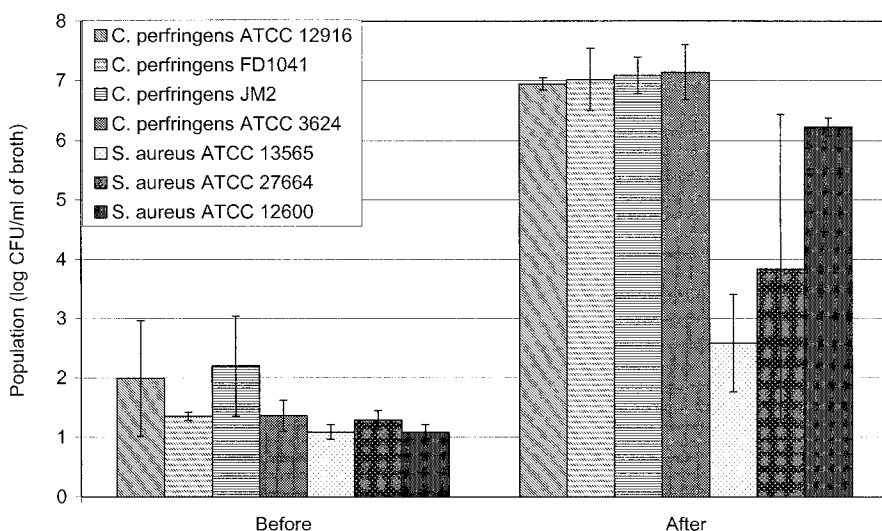


FIGURE 4. Growth of individual strains of *C. perfringens* and *S. aureus* in FTG and TSB, respectively, during laboratory simulation of smoking and 15 h of cooling (48.9 to 26.7°C in 5 h and 26.7 to 7.2°C in 10 h) of bacon. Error bars represent standard deviations.

permit growth of either pathogen. The inhibition of *C. perfringens* reported for ground pork bellies was confirmed with the whole belly pieces using the laboratory-scale smoking and cooling operation. However, *S. aureus* growth observed in the ground belly system was not evident during laboratory-scale smoking and 15 h of cooling of whole belly pieces.

Other researchers have observed no growth of *S. aureus* on sliced smoked bacon at 5°C but some growth (1 log CFU/g) when bacon was held at 25°C (8). No differences were seen in that study between lean meat and fat as a growth medium for *S. aureus*, a finding that has important implications in the present study for comparison of the ground belly system and whole belly pieces. If there are no differences in growth on lean meat versus fat, then differences in behavior of *S. aureus* in the two substrates could be narrowed to surface area differences, nutrient availability, and smoke effects. In a related cooling challenge study, *S. aureus* was inoculated into autoclaved ground beef at ca. 1.8 log CFU/g, and no growth was observed after meat was heated to 60°C and cooled from 54.4 to 7.2°C in 21 h (11).

Smith et al. (18) found that *C. perfringens* growth in

uncured ground beef was greater in vacuum-sealed Spiral Biotech bags than in WhirlPak bags. These differences were attributed to greater oxygen transmission through the WhirlPak bags compared with the Spiral Biotech bags. In our study, ground pork bellies without smoke permitted a 3.93-log increase of *C. perfringens* during cooling for 15 h (Table 1), indicating that adequate growth can occur in unsealed WhirlPak bags. There was a ca. 5-log increase in growth in broth subjected to the same temperatures (Fig. 4). The experiments of Smith et al. differed from those in our study in that they used an uncured ground beef substrate that was vacuum sealed in bags and evaluated at longer cooling times (21 h). Our study incorporated variables in a laboratory that most closely followed actual bacon-processing conditions. Smoking and cooling of bacon in plants does not occur in vacuum-sealed pouches of any kind, and therefore *C. perfringens* occurring in bellies would not be subjected to severe oxygen deprivation. WhirlPak bags were used to obtain precise temperatures in uniform meat samples via a water bath. The use of an oxygen-impermeable sealed pouch would have created an un-

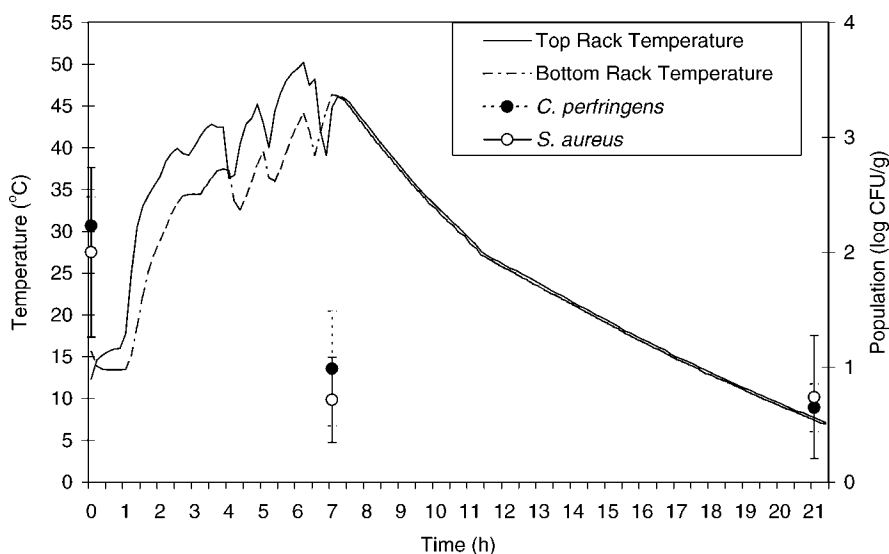


FIGURE 5. Inhibition of *C. perfringens* and *S. aureus* and associated thermal profiles during laboratory simulation of smoking of whole belly pieces and 15 h of cooling (48.9 to 26.7°C in 5 h and 26.7 to 7.2°C in 10 h). Error bars represent standard deviations.

TABLE 3. *Properties of plant-produced bacon and experimental bacon substrates*

Bacon type	pH	$a_w$	NaNO <sub>2</sub> (ppm)	Phenol (mg/100 g) <sup>a</sup>
Plant-produced bacon	6.11 ± 0.08	0.967 ± 0.008	49.0 ± 21.4	2.75 ± 0.13 c
Cured ground bellies	6.29 ± 0.03	0.965 ± 0.009	59.2 ± 24.8	
Cured ground bellies + 1.25% liquid smoke	5.85 ± 0.29	0.963 ± 0.003	59.2 ± 24.8	5.05 ± 0.81 B
Laboratory-smoked whole belly pieces	5.89 ± 0.18	0.965 ± 0.007	58.3 ± 36.2	10.66 ± 0.57 A

<sup>a</sup> Phenol concentrations not followed by the same letter are significantly different ( $P < 0.05$ ).

realistic growth environment that would not closely approximate bacon processing conditions.

Properties of commercially produced bacon were similar to those of the bacon substrates used in this study except for phenol content (Table 3). The average pH of the belly pieces after cooling was 5.89 (range, 5.68 to 6.11), which was slightly lower than the average pH of commercially produced bacon (6.11). Differences were not seen between the internal and external pH of the belly pieces, suggesting that smoke penetration was thorough. When belly pieces were stomached in PBS diluent, the pH of the diluent decreased to 5.23, probably because of release of smoke components from meat into the homogenate. However, the homogenates were plated within a few minutes of stomaching, and the surface pH of the agar media was not altered when plated. Water activity and NaNO<sub>2</sub> values were similar between plant-produced bacon and experimental bacon systems. The average phenol concentration in commercially produced bacon (2.75 mg/100 g) was significantly lower ( $P < 0.05$ ) than that of both experimental bacon substrates. Ground pork bellies supplemented with 1.25% liquid smoke had an average phenol concentration of 5.05 mg/100 g, whereas the laboratory-smoked whole belly pieces, which received more smoke, had phenol concentration averaging 10.66 mg/100 g. To determine whether less smoke could have been used, ground bellies were prepared with 0.8% liquid smoke, but detectable phenol concentrations were not different from those in the bellies with 1.25%. This finding and the pH values similar to those of plant produced bacon suggest that the amount of smoke applied to ground pork bellies was appropriate and that the information generated should be applicable to industrially produced bacon. However, the phenol concentrations in whole belly pieces indicate that too much smoke was applied. Therefore, observed death of both pathogens in whole belly pieces may not occur during plant production of bacon in smokehouses if subjected to these cooling conditions.

Eklund et al. (6) evaluated liquid smoke on heat-processed whitefish steaks for efficacy against *C. botulinum*. They found that surface-inoculated *C. botulinum* spores were inhibited in the fish when the outer 4-mm layer of fish contained 3.7 mg of phenol per 100 g of fish. However, spores that were injected into the muscle were less inhibited by phenol at 4.0 mg/100 g in inner layers of muscle. This effect was attributed to reaction of smoke components with fish proteins, differences in phenol mi-

gration into muscle, and less heating of the inner layers compared with the outer layers. In our analyses, phenol concentrations did not differ markedly between surface and inner layers of bacon pieces. Smoke phenols, carbonyls, and organic acids present in liquid smoke clearly penetrated the whole belly pieces during smoking, resulting in effective inhibition of both pathogens.

The specific components of liquid smoke flavoring have been identified (9, 12), and the phenolic fraction includes several compounds that have strong and broad antimicrobial properties against spoilage and pathogenic microorganisms (19). The antibacterial properties of commercial smoke preparations (liquid and solid) against gram-negative and gram-positive foodborne pathogens have been associated with the concentration of phenols (19). The phenols and guaiacols themselves contain numerous known antimicrobials. Eight phenols, such as cresol and ional, have been detected, and 11 to 12 guaiacols have been detected (including eugenol and vanillin). These components appear to be retained in bacon even after cooking (16). Several organic acids are also present in liquid smoke, including acetic and propionic acids.

Other workers have stated that with respect to *S. aureus*, *Salmonella*, *C. perfringens*, and *C. botulinum*, vacuum-packaged bacon is a safe product as evidenced by its lack of association with foodborne illness (8). Our assessment is that bacon is a safe wholesome meat product that is unlikely to be a vehicle for *C. perfringens* or *S. aureus* foodborne illness. *C. perfringens* did not proliferate in the presence of liquid smoke in any of our experiments or in substrates without smoke when subjected to plant-derived heating times and temperatures. Although *S. aureus* grew in ground bellies with smoke during extended cooling, no enterotoxins were produced.

Data generated in ground pork bellies can be used to assess the safety of bacon processing in controlling *C. perfringens* and *S. aureus*. The results of this study indicate that if smoked bacon is cooled to 7.2°C (45°F) within 15 h, the product will remain safe from both pathogens. Double-smoking of bacon, in which liquid smoke is applied to the pork bellies via brine injection and then reapplied during heating and drying, would probably provide an even greater degree of safety than the single-smoking process studied here. It is not known how the pathogens would respond in reduced-salt pork bacon or beef bacon.

The use of a 15-h cooling schedule from 48.9 to 7.2°C (120 to 45°F) does not constitute a food safety hazard in



bacon from either *C. perfringens* or *S. aureus*. Therefore, processors are justified in selecting a 15-h cooling schedule as a critical limit if physical properties of their bacon match those of our ground pork belly substrates. However, in light of the impact of smoke on *C. perfringens* and *S. aureus*, the better option may be to state that growth of these pathogens is not reasonably likely to occur during cooling and that cooling is a simple control point rather than a CCP. Another perhaps more valid alternative is to make smoke addition a CCP, given the clear inhibitory effect of liquid smoke against *C. perfringens* and *S. aureus* demonstrated in the present study. Processors should consider the total time bacon is held within the growth temperature range of these pathogens rather than considering only the cooling phase.

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