Growth Potential of Clostridium perfringens from Spores in Acidified Beef, Pork, and Poultry Products during Chilling†

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ABSTRACT

The ability of Clostridium perfringens to germinate and grow in acidified ground beef as well as in 10 commercially prepared acidified beef, pork, and poultry products was assessed. The pH of ground beef was adjusted with organic vinegar to achieve various pH values between 5.0 and 5.6; the pH of the commercial products ranged from 4.74 to 6.35. Products were inoculated with a three-strain cocktail of C. perfringens spores to achieve ca. 2-log (low) or 4-log (high) inoculum levels, vacuum packaged, and cooled exponentially from 54.4 to 7.2°C for 6, 9, 12, 15, 18, or 21 h to simulate abusive cooling; the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) recommends a cooling time of 6.5 h. Total germinated C. perfringens populations were determined after plating on tryptose-sulfite-cycloserine agar and incubating the plates anaerobically at 37°C for 48 h. In addition, C. perfringens growth from spores was assessed at an isothermal temperature of 44°C. Growth from spores was inhibited in ground beef with a pH of 5.5 or below, even during extended cooling from 54.4 to 7.2°C in 21 h. In ground beef with a pH of 5.6, the growth was >1 log after 18 h of cooling from 54.4 to 7.2°C. However, 15 h of cooling controlled the growth to <1 log, regardless of the inoculum level. In addition, no growth was observed in any product with a pH ranging from 4.74 to 5.17, both during exponential abusive cooling periods of up to 21 h and during storage for 21 h at 44°C. While <1-log growth of C. perfringens from spores was observed in the pH 5.63 product cooled exponentially from 54.4 to 7.2°C in 15 h or less, the pH 6.35 product supported growth, even after 6 h of cooling from 54.4 to 7.2°C. These challenge tests demonstrate that adjustment of ground beef to pH of 5.5 or less and of barbeque products to pH of 5.63 or less inhibits C. perfringens spore germination and outgrowth during extended cooling periods from 54.4 to 7.2°C up to 15 h. Therefore, safe cooling periods for products with homogeneous, lower pHs can be substantially longer.

Enterotoxigenic Clostridium perfringens, a human pathogen, is a gram-positive, rod-shaped, spore-forming bacterium that is ubiquitous in the environment and has been found in soil, dust, water, food, and the intestinal tracts of animals and humans. Raw meat and poultry products are the most common food vehicles of transmission of the pathogen to humans and responsible for about 40% of foodborne illnesses (2). The Centers for Disease Control and Prevention estimates that approximately 965,958 cases of foodborne illness associated with C. perfringens infection occur annually in the United States (18). Illness occurs typically because of ingestion of potentially hazardous levels (ca. 10⁶ CFU/g) of viable vegetative cells in contaminated food that has been improperly cooled or held at room temperature for a substantially long time after cooking. The acidic conditions encountered in the stomach can, in fact, trigger the sporulation process of C. perfringens vegetative cells (7). The vegetative cells during sporulation in the intestine release an enterotoxin on sporangial autolysis, which leads to the gastrointestinal symptoms of food poisoning. While the illness is typically mild and exhibits self-limiting diarrhea with abdominal cramps and pain, fatalities can occur in infants, the elderly, or the immunocompromised, as was the case in the Oklahoma outbreak that occurred in late November 2001 (13).

C. perfringens vegetative cells do not survive the cooking processes (time and temperature) commonly used in the food service establishments. However, spores of C. perfringens that have a decimal reduction value at 100°C of 0.5 to 124 min (17) are likely to survive commercial cooking time and temperatures. Heat-activated spores can germinate, outgrow, and multiply if products are subsequently improperly cooled or temperature abused. Such scenarios can occur in food service establishments wherein foods are prepared in large quantities a day in advance or

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stored for prolonged periods and then reheated prior to serving for consumption (6) or that were improperly cooled after a thermal process. The short generation time of the organism (6.3 min) in the rapid-growth temperature range between 43 and 47°C (11) indicates that rapid cooling of foods is absolutely critical after spore germination. Moreover, cooling process deviations can occur in commercial food processing operations, primarily because of power failures, refrigeration equipment malfunctions, or increased production volume, thereby enhancing the likelihood of *C. perfringens* germination and outgrowth during cooling of certain cooked meat and poultry products (23). These regulations specify that the cooling of certain cooked, cured, and uncured meat and poultry products must result in no more than 1-log multiplication of *C. perfringens* cells in the finished product. In addition, federal compliance guidelines have been developed to provide cooling options to federally inspected meat and poultry establishments in order to meet the stabilization performance standard. The first cooling option states that product’s maximum internal temperature during cooling should not remain between 54.4 to 26.7°C for more than 1.5 h, and cooling from 26.7 to 4.4°C should not take longer than 5 h (the preferred cooling option is total cooling time of 6.5 h). Alternatively, processors can follow the less conservative option of cooling cooked products from 48.9 to 12.8°C within 6 h, followed by additional cooling to 4.4°C prior to packaging (23). For meat and poultry products cured with a minimum of 100 ppm of in-going sodium nitrite, the guidelines allow cooling from 54.4 to 26.7°C in 5 h and from 26.7 to 7.2°C in 10 h (15 h of total cooling time). Products that comply with the stabilization performance standards for certain cooked, cured and uncured, ready-to-eat (RTE) meat and poultry have not been linked to *C. perfringens* illness.

The USDA-FSIS (22) nationwide microbiological data did not distinguish between vegetative cells and spores and reported total *C. perfringens* prevalence values of 10.4, 8.3, and 2.6% in market hogs, cows and bulls, and steers and heifers, respectively. In an industry survey, the incidence of *C. perfringens* spores in raw meat product mixtures ranged from 0.0% for cured and uncured whole-muscle product to 16.7% for uncured ground or emulsified product. The incidence of *C. perfringens* vegetative cells in raw meat product mixtures ranged from 1.6% for cured whole-muscle product to 48.7% for cured ground or emulsified product. For the cured ground or emulsified samples, the spore levels did not exceed 2.00 log CFU/g and averaged 1.56 log CFU/g. For the uncured ground or emulsified samples, the maximum spore level was 2.11 log CFU/g and averaged 1.75 log CFU/g. The total *C. perfringens* maximum and average populations ranged from 1.7 and 1.56 log CFU/g, respectively, for cured whole-muscle product, up to 2.92 and 2.22 log CFU/g, respectively, for uncured ground or emulsified product. The survey was based on a total of 445 whole-muscle and ground or emulsified raw pork, beef, and chicken product mixtures collected from four Midwestern meat processing facilities over a 10-month period (20).

*C. perfringens* spores were isolated from 80% of 54 spice and herb samples (3). Clearly, the commonly used addition of spices and herbs has an impact on the incidence and levels of *C. perfringens* spores found in raw, formulated RTE meat and poultry products before heat treatment. In products that did not meet USDA-FSIS cooling requirements, Kalinowski et al. (9) determined the levels of contamination in those cooked RTE meat and poultry products including cured meat and poultry, noncured poultry, roast beef, chili, barbeque products, beef brisket, and cooked ground taco beef, reporting that 95% of the samples had total *C. perfringens* populations of ≤100 CFU/g, and 5% had >100 CFU/g. More than 20% of barbeque products contained >100 CFU/g.

Our aim in the present study was to determine the potential germination and outgrowth of *C. perfringens* spores during abusive cooling of ground beef with a pH range 5.0 to 5.6 as well as in commercially cooked beef, pork, and chicken products containing barbeque sauce, chili sauce, or rubbed with seasoning. As we simulated exponential cooling of foods that can occur in the processing of foods in food service establishments, the cooling process validation data could be used by processors to prepare foods that meet the compliance guidelines for cooked meat and poultry products.

**MATERIALS AND METHODS**

**Test organisms and preparation of spore cocktail.** Three different enterotoxin-producing *C. perfringens* strains NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3), and ATCC 10288 (Hobbs serotype 13) were obtained from our culture collection. An aliquot (0.1 ml) from the stock culture was inoculated into freshly prepared fluid-thioglycollate medium to prepare an active culture. Spores of each strain were prepared separately in modified Duncan and Strong sporulation medium by using our previously described procedure (5). The spore crops were harvested by centrifugation, washed twice with sterile distilled water, resuspended in 10 ml of water, and then stored in a refrigerator at 4°C. Sppore populations were determined by spiral plating (model D, Spiral Systems Plating Instruments, Cincinnati, OH) appropriate dilutions, in duplicate, made in 0.1% peptone water on tryptose-sulfite-cycloserine agar. A mixture of spores containing all three *C. perfringens* strains was prepared immediately prior to conducting the experiments by mixing equivalent proportions of spores from each suspension.

**Preparation of meat samples and inoculation.** Ground beef (93% lean) was obtained from a local grocery store and stored frozen (−5°C) during the course of the study (a maximum of 40 days). Organic, unpasteurized, filtered Spectrum Neutrals apple cider vinegar (Spectrum, Boulder, CO) of 5% acidity was used to adjust pH of meat in the range of 5.0 to 5.6. Commercial beef, pork, and chicken products were received from Dr. David Baker, David Baker and Associates, Atlanta, GA. Duplicate 5-g portions of each sample were weighed into low-oxygen-transmission Whirl Pak bags (18-oz [532-ml] capacity, 0.125 ml of oxygen transmission per 100 in² [645.16 cm²] in 24 h; product no. B01300WA, Nasco, Ft. Atkinson, WI). While ground beef samples were inoculated with 1 ml of the non–heat-shocked *C. perfringens* spores, the cured and uncured whole-muscle product to % *C. perfringens* had strains was prepared immediately after spore germination and outgrowth during cooling of certain cooked meat and poultry products (5). The spore crops were thawed and concentrated 100-fold, and 0.1 ml of the concentrated spore suspension was added to 99.9 ml of sterile 10% sodium chloride solution. The spore populations were determined by spiral plating (model D, Spiral Systems Plating Instruments, Cincinnati, OH) appropriate dilutions, in duplicate, made in 0.1% peptone water on tryptose-sulfite-cycloserine agar. A mixture of spores containing all three *C. perfringens* strains was prepared immediately prior to conducting the experiments by mixing equivalent proportions of spores from each suspension.

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perfringens spore cocktail, commercial products were inoculated with 1 ml of the heat-shocked (75°C/20 min) spore cocktail to obtain a final concentration of spores of ca. 2.0-log (low) or 4.0-log (high) spores per g. Since low-temperature cooking of ground beef served to heat shock C. perfringens spores, spores were not heat shocked prior to inoculation. On the other hand, commercial products were inoculated with heat-shocked spores, because these products were not heated or cooked prior to cooling. The bags were massaged manually for even distribution of the spores in the meat sample and then pressed on a solid surface to flatten the samples to uniform thicknesses of about 2 mm. Negative controls consisted of bags containing noninoculated meat samples. The bags were then evacuated to a negative pressure of 1,000 mbar (1,000 MPa) and vacuum sealed with a Multivac gas-packaging machine (model A300/16, Multivac, Inc., Kansas City, MO).

**Growth studies, cooking, and cooling procedures.** Growth of C. perfringens from spores was assessed by incubating the bags containing commercial products at an isothermal temperature of 44°C for 21 h and removed periodically for microbial enumeration. For heating and cooling studies, the bags containing ground beef or commercial products were sandwiched between stainless steel wire racks, as described previously (21). Ground beef samples (pH 5.0 to 5.6) were submersed completely in a circulating water bath (NESLAB RTE-221, NESLAB Instruments, Inc., Newington, NH). Thereafter, meat was cooked by increasing the temperature of the water bath in a linear fashion to 71°C in 1 h. For exponential chilling, cooked ground beef bags as well as beef, pork, or chicken products bags containing heat-shocked spores were submersed completely in a circulating water bath set at 54.4°C. The bath was allowed to equilibrate at this temperature for 10 min and then chilled exponentially from 54.4 to 7.2°C within 6-, 9-, 12-, 15-, 18-, and 21-h target chilling times.

**Time and temperature for cooling.** The mathematics for determining the cooling times and temperatures come from Pflug and Blaisdell (16) and Dickerson and Read (4):

\[ k \Delta t = \log(T_{\text{actual}} - T_{\text{cold source}}) - \log(T_{\text{start}} - T_{\text{cold source}}) \]

where \( k \) is the slope of the cooling line.

To calculate the slope \( k \) of the cooling line, the equation is written as:

\[ k = \frac{\{\log(T_{\text{actual}} - T_{\text{cold source}})\} - \{\log(T_{\text{start}} - T_{\text{cold source}})\}}{\Delta t} \]

To find the actual product temperature after time, the equation is:

\[ T_{\text{actual}} = T_{\text{cold source}} + (T_{\text{start}} - T_{\text{cold source}}) \times 10^{k\Delta t} \]

To find the actual time to reach a temperature:

\[ \Delta t = \frac{\{\log(T_{\text{actual}} - T_{\text{cold source}})\} - \{\log(T_{\text{start}} - T_{\text{cold source}})\}}{k} \]

This means if the difference between the center temperature of the sample and the cold source (e.g., cold bath) is plotted on semilog paper, the result is a straight line when the bottom point on the y axis plotted as 1° above the cold source temperature. It is 1°, because the sample center temperature can only approach the temperature of the cold source, but will never reach the temperature of the cold source.

**Enumeration of C. perfringens.** Isothermal growth experiment samples as well as samples after cooking and cooling were enumerated for total C. perfringens population by spiral plating. The plates were incubated at 37°C in an anaerobic chamber (Bactron IV, Sheldon Laboratories, Cornelius, OR) for 48 h. Thereafter, typical colonies of C. perfringens population were counted and recorded as log CFU per gram of meat.

**Statistical analyses.** Two independent replications, in duplicate, were performed for isothermal temperature growth studies as well as for each of the exponential chilling times (6, 9, 12, 15, 18, and 21 h). Data (in log CFU per gram) were analyzed with the analysis of variance of the General Linear Model procedure of the Statistical Analysis System (release 8.01, SAS Institute Inc., Cary, NC). Means of the C. perfringens populations (in log CFU per gram) were separated with the Bonferroni least-squares method (14).

**RESULTS AND DISCUSSION**

Commercial beef, pork, and chicken products were assessed in challenge-validation studies to evaluate the potential germination and outgrowth of C. perfringens after abusive chilling times (Table 1). While eight products contained barbeque sauce, rotisserie-cooked pork shoulder was rubbed with seasoning, and boiled beef contained chili sauce. Among the barbeque sauce ingredients were vinegar, water, catsup, molasses, sugar, salt, Worcestershire sauce, and seasoning. The pH of the products ranged from pH 4.74 for pit-cooked pork shoulder to pH 6.35 for rotisserie-cooked pork shoulder. The pH of the barbeque products ranged from pH 4.77 to 5.17.

The pH growth range for C. perfringens is between 5.0 and 9.0, with an optimum pH between 6.0 and 7.0 (7). While an acidic food with pH of 5.0 or less would restrict growth of C. perfringens, other ingredients in meat product formulations, such as type of antimicrobial agents, organic acids, or ingredients in the sauce, could act synergistically with acidic pH to affect the growth of C. perfringens from spores in cooked products (10). Nevertheless, growth from spores of C. perfringens in cooked products can occur if the rate and extent of cooling is not adequate. In the present study, C. perfringens growth from spores was assessed in ground beef adjusted to various pH values between 5.0 and 5.6, after 6, 9, 12, 15, 18, and 21 h of exponential cooling. Growth was inhibited in ground beef with a pH of 5.5 or less, even during extended cooling from 54.4 to 7.2°C in 21 h (Table 2). In comparison with the controls (beef pH of 6.5), growth was lower (\( P \leq 0.05 \)) in ground beef, pH 5.6, after the abusive chilling regimes of 21 or 18 h, regardless of the initial inocula of C. perfringens spores. In ground beef, pH 5.6, exponential chilling from 54.4 to 7.2°C in 21 h resulted in germination and outgrowth of C. perfringens spores from an initial population of ca. 3.0 to ca. 6.0 log CFU/g, and from ca. 2.0 to ca. 4.0 log CFU/g. After 18 h of chilling from 54.4 to 7.2°C, the growth of C. perfringens from spores was greater than 1 log, regardless of the initial inoculum level, in ground beef with a pH of 5.6. Thus, 18 h of chilling of ground beef, pH 5.6, from 54.4 to 7.2°C will not meet the USDA-FSIS stabilization performance standards of ≤1-log growth of C. perfringens during cooling deviations. However, a 15-h cooling controlled the growth, regardless of the inoculum level (data not shown).

While C. perfringens growth can occur between 15 and 50°C, the optimum temperature range for growth is between 43 and 45°C (12). Beef, pork, or chicken products were inoculated with approximately 2.5 or 3.5 log CFU/g of C. perfringens spores and then spore germination and out-
growth was assessed at an isothermal temperature of 44°C. Interestingly, C. perfringens growth from spores was restricted in products with pH of 5.17 or less, even after 21 h of storage at 44°C. However, C. perfringens spore germination and outgrowth (P ≤ 0.05) at 44°C was observed in rotisserie-cooked pork shoulder (pH 6.35) or boiled beef (pH 5.63). The levels of C. perfringens in the pH 6.35 product at 44°C in 6 h increased significantly (P ≤ 0.05) from 3.59 and 2.72 log CFU/g to 8.29 and 7.79 log CFU/g, respectively (Fig. 1). In the pH 5.63 product stored at 44°C for 6 h, C. perfringens growth from spores significantly increased (P ≤ 0.05) by about 4 log CFU/g, regardless of the initial inoculum level.

Figure 2A and 2B and Table 3 show that growth of C. perfringens from spores can occur in meat, pork, and poultry products, depending on the pH of the product and the rate and extent of cooling. Regardless of the initial inoculum levels, no growth was observed in products with pH ranging from 4.74 to 5.17 during exponential abusive cooling periods of up to 21 h (Fig. 2). However, exponential cooling of rotisserie-cooked pork shoulder, pH 6.35, from 54.5 to 7.2°C in 21 h resulted in germination and outgrowth of C. perfringens spores and a significant increase (P ≤ 0.05) from an initial population of 4.18 to 8.75 log CFU/g and from 2.70 to 7.03 log CFU/g was observed. Growth from spores of C. perfringens was also observed in boiled beef, pH 5.63, after cooling from 54.5 to 7.2°C in 21 h; the levels significantly increased (P ≤ 0.05) by 2.85 and 1.82 log CFU/g, with the initial population levels of 4.07 and 2.63 log CFU/g, respectively. Thus, low initial levels of C. perfringens substantially restricted C. perfringens extent of growth in pH 5.63 product. These results are in agreement with the findings of Kalinowski et al. (9). In their study, cooked, uncured RTE turkey inoculated with 1 log CFU/g cooled from 48.9 to 12.8°C resulted in 0.83 log of C. perfringens in 6 h. However, a 2.25-log CFU/g increase was observed when the inoculum level was 3.23 log CFU/g. As observed in ground beef with pH 5.6, ≤1-log growth of C. perfringens from spores was observed in boiled beef, pH 5.63, cooled exponentially from 54.4 to 7.2°C in 15 h or

TABLE 1. pHs and formulations of commercial beef, pork, and chicken products used in the challenge–validation studies

<table>
<thead>
<tr>
<th>pH</th>
<th>Meat product</th>
<th>Barbeque sauce</th>
<th>Grind size</th>
<th>Sauce ingredients&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Textured vegetable protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.82</td>
<td>Pit-cooked beef brisket</td>
<td>Yes</td>
<td>0.75-in. (1.9-cm) plate grind</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>4.89</td>
<td>Pit-cooked pork shoulder</td>
<td>Yes</td>
<td>1.25-in. (3.2-cm) plate grind</td>
<td>B</td>
<td>No</td>
</tr>
<tr>
<td>4.8</td>
<td>Pit-cooked pork shoulder</td>
<td>Yes</td>
<td>1.25-in. (3.2-cm) plate grind</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>5.17</td>
<td>Pit-cooked pork shoulder</td>
<td>Yes</td>
<td>3-in. (7.6-cm) sliced pulled</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>4.97</td>
<td>Pit-cooked pork shoulder</td>
<td>Yes</td>
<td>1.25-in. (3.2-cm) plate grind</td>
<td>C</td>
<td>No</td>
</tr>
<tr>
<td>6.35</td>
<td>Rotisserie-cooked pork shoulder</td>
<td>No</td>
<td>3-in. (7.6-cm) sliced pulled</td>
<td>Rubbed with seasoning</td>
<td>No</td>
</tr>
<tr>
<td>4.74</td>
<td>Pit-cooked pork shoulder</td>
<td>Yes</td>
<td>0.75-in. (1.9-cm) plate grind</td>
<td>B</td>
<td>Yes</td>
</tr>
<tr>
<td>4.84</td>
<td>Pit-cooked pork shoulder</td>
<td>Yes</td>
<td>0.75-in. (1.9-cm) plate grind</td>
<td>D</td>
<td>Yes</td>
</tr>
<tr>
<td>5.63</td>
<td>Boiled beef</td>
<td>No</td>
<td>0.625-in. (0.16-cm) plate ground</td>
<td>Chili sauce</td>
<td>Yes</td>
</tr>
<tr>
<td>4.77</td>
<td>Rotisserie-cooked chicken</td>
<td>Yes</td>
<td>0.625-in. (0.16-cm) chop</td>
<td>A</td>
<td>—&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> A, vinegar, water, catsup, molasses, sugar, salt, Worcestershire sauce, seasoning; B, vinegar, water, sugar, salt, Worcestershire sauce, seasoning; C, catsup, water, sugar, molasses, salt, Worcestershire sauce, seasoning; D, vinegar, water, catsup, sugar, Worcestershire sauce, seasoning.

<sup>b</sup> —, no data.

TABLE 2. Growth of Clostridium perfringens from spores in ground beef, pH 5.0 to 5.6, cooked to 60°C in 1 h and then chilled from 54.4 to 7.2°C in 21 or 18 h<sup>a</sup>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chill treatment:</th>
<th>21 h</th>
<th>18 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High inoculum</td>
<td>Low inoculum</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>7.72 ± 0.08</td>
<td>7.67 ± 0.02</td>
</tr>
<tr>
<td>pH 5.0</td>
<td></td>
<td>3.11 ± 0.15</td>
<td>2.27 ± 0.17</td>
</tr>
<tr>
<td>pH 5.1</td>
<td></td>
<td>3.10 ± 0.11</td>
<td>1.60 ± 0.35</td>
</tr>
<tr>
<td>pH 5.2</td>
<td></td>
<td>3.15 ± 0.06</td>
<td>1.82 ± 0.28</td>
</tr>
<tr>
<td>pH 5.3</td>
<td></td>
<td>3.19 ± 0.03</td>
<td>1.41 ± 0.36</td>
</tr>
<tr>
<td>pH 5.4</td>
<td></td>
<td>3.20 ± 0.10</td>
<td>1.73 ± 0.49</td>
</tr>
<tr>
<td>pH 5.5</td>
<td></td>
<td>3.82 ± 0.33</td>
<td>1.99 ± 0.36</td>
</tr>
<tr>
<td>pH 5.6</td>
<td></td>
<td>5.91 ± 0.15</td>
<td>4.27 ± 0.39</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data represent means of two replicate experiments, each performed in duplicate and expressed as mean ± standard deviation. Initial C. perfringens population after cooking was ca. 3 log CFU/g (high inoculum) and ca. 2 log CFU/g (low inoculum).

<sup>b</sup> ND, not determined.
Growth of (A) high inoculum levels and (B) low C. perfringens spores germinated in raw beef without prior heat shock. Low-temperature, lengthy growth of inoculated spores. If cooking temperatures are designed to inactivate C. perfringens spores, the products' organoleptic attributes are likely to be negatively impacted. Therefore, traditional pasteurization–cooking temperatures used to cook foods will result in foods containing surviving, heat-activated spores. In the present study, we cooked ground beef inoculated with C. perfringens spores by linearly increasing the temperature to 71°C in 1 h to heat shock the spores; commercial beef, pork, and chicken products were inoculated with heat-shocked spores before being subjected to abusive chilling regimes. Our intent was to increase the rate of germination of spores of C. perfringens. Barnes et al. (1) reported that almost all spores germinated after beef received a heat treatment as compared with only 3% of inoculated C. perfringens spores germinated in raw beef without prior heat shock. Cooking time and temperature also reduce population densities of competing spoilage microflora. Increased anaerobic environment in cooked foods and reduced populations of indigenous microflora create an environment conducive for C. perfringens growth from surviving heat-activated spores during cooling, provided the rate and extent of chilling is not sufficient.

Research on control of C. perfringens spore germination and outgrowth in meat and poultry products of various pH values has been published in scientific literature. Buffered vinegar and a blend of lemon juice concentrate and vinegar in ground turkey supplemented with 1.5% sea salt and turbinado sugar (0.5%) restricted C. perfringens growth from spores to <1 log CFU/g after abusive chilling for 21 h, regardless of the pH (5.57 to 5.62) of the product (24). In a study by Velugoti et al. (25), when the pH of injected pork containing minimal levels of salt (0.85% NaCl), potato starch (0.25%), and potassium tetra pyrophosphate (0.2%) at a 12% pump rate was adjusted to pH 5.75 by using 2% calcium lactate, extended chilling of up to 21 h restricted C. perfringens growth to <1 log. In another study (19), pale, soft, and exudative pork meat (pH 5.29) containing sodium pyrophosphate dibasic and sodium acid pyrophosphate blend (0.3%) controlled C. perfringens germination and outgrowth to <1 log CFU/g after abusive chilling of 21 h, thereby meeting the USDA-FSIS stabilization performance standards. Thippareddi et al. (21) also reported C. perfringens population increases of 1.51 and 3.70 log in cooked beef and pork, respectively, subsequent to exponential cooling from 54.4 to 4.4°C in 18 h. The authors stated that these differences in the extent of growth could be attributed to the higher pH (6.11) of the injected pork compared with injected beef (pH 5.62). When turkey roast (pH 5.94) was cooled from 54.4 to 4.4°C for the same period of time, i.e., 18 h, a 4.66-log CFU/g population increase of C. perfringens was observed (8). These reports suggest that in addition to pH, meat species and muscle type can affect the germination and outgrowth of C. perfringens spores cooled when subject to similar time–temperature cooling profiles.

Our findings will assist in preparation of safe cooked meat, pork, and poultry products in retail food service industries (15). Low-temperature, lengthy cooking of contaminated meat will not eliminate C. perfringens spores. If cooking temperatures are designed to inactivate C. perfringens spores, the products’ organoleptic attributes are likely to be negatively impacted. Therefore, traditional pasteurization–cooking temperatures used to cook foods will result in foods containing surviving, heat-activated spores. In the present study, we cooked ground beef inoculated with C. perfringens spores by linearly increasing the temperature to 71°C in 1 h to heat shock the spores; commercial beef, pork, and chicken products were inoculated with heat-shocked spores before being subjected to abusive chilling regimes. Our intent was to increase the rate of germination of spores of C. perfringens. Barnes et al. (1) reported that almost all spores germinated after beef received a heat treatment as compared with only 3% of inoculated C. perfringens spores germinated in raw beef without prior heat shock. Cooking time and temperature also reduce population densities of competing spoilage microflora. Increased anaerobic environment in cooked foods and reduced populations of indigenous microflora create an environment conducive for C. perfringens growth from surviving heat-activated spores during cooling, provided the rate and extent of chilling is not sufficient.

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Our findings will assist in preparation of safe cooked meat, pork, and poultry products in retail food service industries.
operation, without posing a food safety hazard associated with \( C. \) \( \text{perfringens} \) spore outgrowth. The results of the present study indicate that ground beef with \( pH \) 5.6, as well as the beef, pork, and poultry barbecue products assessed in this study with \( pH \) of 5.63 or less, cooled exponentially from 54.4 to 7.2 °C in 15 h or less would restrict the growth of \( C. \) \( \text{perfringens} \) to \( \leq 1 \) log CFU/g. However, rotisserie-cooked pork shoulder (\( pH \) 6.35) supported growth even during 6 h of cooling from 54.4 to 7.2 °C. This challenge study also demonstrated that barbecue products (\( pH \) \( \leq 5.17 \)) would not allow \( C. \) \( \text{perfringens} \) growth from spores when incubated at 44 °C for 21 h because of addition of acidic food ingredients. Thus, achieving the equilibrium \( pH \) of 5.17 or less prior to the onset of \( C. \) \( \text{perfringens} \) growth can allow the cooling process schedule to be extended beyond the USDA-FSIS stabilization guidelines. These results emphasize the importance of \( pH \) of products in controlling the germination and outgrowth of \( C. \) \( \text{perfringens} \) during cooling of the cooked products. However, it is worth pointing out that factors other than \( pH \), such as temperature (chill rate), oxygen, water activity, curing salts, organic acids, also affect the growth of \( C. \) \( \text{perfringens} \).

Consumers demand moist, less acidic, minimally processed foods that have homemade appeal. As such, there has been an increased interest in applying mild, multiple barriers as a more ideal approach to ensure microbiological safety as well as organoleptic acceptability of thermally processed foods. Therefore, investigation of multiple food formulation factors by using the hurdle approach to control germination and outgrowth of \( C. \) \( \text{perfringens} \) spores during cooling of commercially prepared foods is warranted. Incorporation of these hurdles in commercial products could play an additive or synergistic role in order for products to meet the USDA-FSIS stabilization performance standards or policy–guidance for cooked, heat-treated, not-shelf-stable meat and poultry products.

**ACKNOWLEDGMENTS**

We thank Angie Martinez for her technical assistance with performing laboratory studies and John Phillips for statistical analyses of the data.

**REFERENCES**


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**TABLE 3. Population counts of \( \text{Clostridium perfringens} \) in beef and pork products cooled from 54.4 to 7.2 °C in 6 to 18 h**

<table>
<thead>
<tr>
<th>Meat product</th>
<th>Cooling time (h)</th>
<th>Inoculum level:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before chilling</td>
<td>After chilling</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>Before chilling</td>
<td>After chilling</td>
</tr>
<tr>
<td>Rotisserie-cooked pork shoulder</td>
<td>18</td>
<td>3.57 ± 0.07</td>
</tr>
<tr>
<td>Boiled beef</td>
<td>18</td>
<td>3.56 ± 0.13</td>
</tr>
<tr>
<td>Rotisserie-cooked pork shoulder</td>
<td>15</td>
<td>3.58 ± 0.07</td>
</tr>
<tr>
<td>Boiled beef</td>
<td>15</td>
<td>3.51 ± 0.07</td>
</tr>
<tr>
<td>Rotisserie-cooked pork shoulder</td>
<td>12</td>
<td>3.52 ± 0.04</td>
</tr>
<tr>
<td>Boiled beef</td>
<td>12</td>
<td>3.36 ± 0.07</td>
</tr>
<tr>
<td>Rotisserie-cooked pork shoulder</td>
<td>9</td>
<td>3.56 ± 0.06</td>
</tr>
<tr>
<td>Rotisserie-cooked pork shoulder</td>
<td>6</td>
<td>3.68 ± 0.02</td>
</tr>
</tbody>
</table>

\(^a\) Values are expressed as means ± standard deviations. The beef products’ \( pH \) was 5.63; the pork products’ \( pH \) was 6.35.
pathogens—microbiology and molecular biology. Caister Academic Press, Norfolk, UK.


