

## Research Note

# Effects of Chilling Rate on Outgrowth of *Clostridium perfringens* Spores in Vacuum-Packaged Cooked Beef and Pork

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### ABSTRACT

Cooked, chilled beef and cooked, chilled pork were inoculated with three strains of *Clostridium perfringens* (NCTC 8238 [Hobbs serotype 2], NCTC 8239 [Hobbs serotype 3], and NCTC 10240). Inoculated products were heated to 75°C, held for 10 min in a circulating water bath to heat activate the spores, and then chilled by circulating chilled brine through the water bath. Samples were chilled from 54.4 to 26.6°C in 2 h and from 26.6 to 4.4°C in 5 h. Differences in initial *C. perfringens* log counts and log counts after chilling were determined and compared with the U.S. Department of Agriculture (USDA) stabilization guidelines requiring that the chilling process allow no more than 1 log total growth of *C. perfringens* in the finished product. This chilling method resulted in average *C. perfringens* increases of 0.52 and 0.68 log units in cooked beef and cooked pork, respectively. These log increases were well within the maximum 1-log increase permitted by the USDA, thus meeting the USDA compliance guidelines for the cooling of heat-treated meat and poultry products.

In the United States, there is a trend toward minimally processed refrigerated foods. Food products that are packaged under vacuum and are then heat processed and chilled have been referred to as cook-in, cook-and-ship, pouch cooking, and sous vide products. The heat treatments these extended-shelf-life refrigerated foods undergo are less extensive than that required for commercial sterility (9, 14). Vegetative cells are destroyed, but spores survive this process. One spore-forming pathogenic anaerobic organism of concern in cook-in vacuum-packaged products is *Clostridium perfringens*. With a generation time of 7.1 min at 41°C, *C. perfringens* is one of the fastest growing bacteria; however, vegetative cells are inactivated at 60°C (3, 13). Heat-resistant spores can survive at 100°C for 1 h (4).

U.S. Department of Agriculture (USDA) stabilization guidelines require that the chilling process not allow more than 1 log total growth of *C. perfringens* in finished product (15). To insure that chilling guidelines are met, meat products should be chilled from 54.4 to 26.6°C in 90 min and from 26.6 to 4.4°C in 5 h or less. Customized stabilization processes are permitted, provided the procedure has been confirmed to meet USDA guidelines.

The objective of this study was to evaluate a customized chilling process involving cooling from 54.4 to 26.6°C in 2 h and from 26.6 to 4.4°C in 5 h or less for nonseasoned cooked beef and pork to determine if this process conforms to the USDA stabilization guidelines for the control of *C. perfringens*.

### MATERIALS AND METHODS

**Experimental design.** Three samples representing different lots of vacuum-packaged cooked beef and cooked pork were inoculated with a cocktail of *C. perfringens* spores. Inoculated samples were heat shocked and then chilled from 54.4 to 26.6°C in 2 h and from 26.6 to 4.4°C in 5 h. Log growth was measured and compared with USDA stabilization guidelines to validate the extended initial chilling period.

**Bacterial cultures and inoculum preparation.** Three strains of *C. perfringens* (NCTC 8238 [Hobbs serotype 2], NCTC 8239 [Hobbs serotype 3], and NCTC 10240 [Hobbs serotype 13]) were obtained from the Eastern Regional Research Center (USDA–Agricultural Research Service, Wyndmoor, Pa.). Individual strains were maintained at 4°C in cooked meat medium (Difco Laboratories, Sparks, Md.). Active cultures were prepared with freshly made fluid thioglycollate broth, and sporulation was carried out with Duncan and Strong sporulation medium (6). After sporulation, each strain was washed twice, resuspended in sterile distilled water, and stored at 4°C. A spore cocktail containing equivalent numbers of spores from each strain was prepared immediately prior to its use in experiments.

**Validation of chilling cycle.** Trimmed beef chuck, shoulder clods (North American Meat Processors Association [NAMP] no. 114C) (11), and pork shoulder, picnic, cushion boneless (NAMP no. 405B) (11) that had been denuded and chunked to an average size of 5.1 cm<sup>3</sup> were supplied by a commercial meat plant. Beef and pork chunk samples (454 g) were placed in separate cook-in bags (3 ml nylon-polyethylene with an O<sub>2</sub> transmission rate of 3.5 cm<sup>3</sup>/645.2 cm<sup>2</sup>/24 h at 21.1°C and a water vapor transmission rate of 0.6 g/645.2 cm<sup>2</sup>/24 h at 100% relative humidity; Koch Supplies Inc., Kansas City, Mo.) and vacuum packaged with a Multivac tabletop vacuum packager (Model 439532, Multivac,

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Kansas City, Mo.). Packaged beef and pork were steam cooked at 85°C in an Alkar oven (Model 450-VA, Alkar, Lodi, Wis.) to an internal temperature of 82.2°C and were then held at that internal temperature for 7 and 4 h, respectively, before chilling and subsequent storage at 4.4°C for 2 weeks. Three replications were performed, and separate packages were prepared for proximate analysis and for the determination of pH.

After 2 weeks, cooked meat and broth were aseptically transferred into stomacher bags (17.8 by 29.9 cm; Biosciences International, Rockville, Md.). Bags were manually massaged, then stomached (Lab Blender 400, Tekmar Co., Cincinnati, Ohio) for 2 min, and 25 g of homogenate was placed in another cook-in bag, inoculated with the *C. perfringens* spore cocktail, and vacuum packaged. A noninoculated control was prepared for each species to monitor the chilling rate by inserting a thermocouple (T-type, 32 gauge; Omega, Stamford, Conn.) into the center of the product prior to vacuum packaging.

Heat shocking and chilling were carried out with a circulating water bath (Model N2, Haake, Germany) by using a programmable temperature controller (Model CN3200, Omega). Inoculated beef and pork were heated in 75°C water until the internal temperature of the meat reached 75°C, and the temperature was then held at 75°C for 10 min to heat activate spores. Next, the meat was chilled from 54.4 to 26.6°C in 2 h and from 26.6 to 4.4°C in 5 h with -1°C brine in the water bath. Samples were then held in a 1.1°C cooler for enumeration the following day.

**Spore inoculation level.** After inoculation, the initial load of *C. perfringens* spores was determined. An additional 25-g sample from each replication of vacuum-packaged inoculated meat was prepared and heat shocked as described previously and was then immediately chilled to <10°C in an ice water bath. Samples were placed in a 1.1°C cooler until enumeration the following day.

***C. perfringens* enumeration.** A 25-g sample was placed in a filtered stomacher bag, homogenized with 25 ml of sterile 0.1% peptone water diluent, and stomached for 2 min. Serial dilutions were plated on tryptose sulfite cycloserine agar under anaerobic conditions by Fung's double-tube method (1) and incubated for 10 to 12 h at 37°C.

**Proximate analysis and pH.** The fat, moisture, and protein contents and the pH values of cooked beef and pork were determined. Each product was homogenized by blending cooked meat and broth in a food processor (Model 702R, Silex Inc., Hamilton Beach, Washington, N.C.) for approximately 2 min. Homogenate was placed into a polyethylene bag (15.2 by 30.5 cm; Consolidated Plastics, Twinsburg, Ohio), heat sealed with an Impulse heat sealer (Model mp12, Midwest Pacific, Taiwan), and stored at 4°C until it was analyzed within 24 h. Immediately prior to sample analysis, homogenized meat was tempered at 25°C for 30 min and was then manually massaged for 30 s prior to the removal of a test aliquot. Homogenate (10 g) was combined with 90 ml of deionized water and stirred for 5 min before pH was determined with a Corning pH meter 320 (with a temperature-compensating Corning 300 electrode). Fat analysis was performed by ether extraction according to AOAC International (2) methods 991.36, 960.39, and 920.39. Moisture content was determined by placing a sample into an aluminum tin, weighing the sample, and then placing the sample in a 125°C oven (Model OV-490-2, Blue M Electric, Blue Island, Ill.) for 4 h. After cooling in a desiccator, the samples were reweighed and the moisture percentage was calculated and recorded. The percentage of protein was determined by AOAC International (2) method 981.10.

**Statistical analysis.** Data were analyzed with proc GLM, and mean separation was carried out with Fisher's least significant difference test (12). The significance level was set at  $P < 0.05$ .

## RESULTS AND DISCUSSION

The moisture, fat, and protein contents of cooked beef were similar to those of cooked pork ( $P > 0.05$ ). The average fat contents for beef and pork were 5.91 and 5.96%, respectively. Average moisture contents were 72.51 and 72.69% for beef and pork, respectively, and beef and pork contained 21.68 and 20.56% protein, respectively. Since the proximate analysis revealed no differences between beef and pork, it was assumed that these factors would not affect differences in log counts as a result of procedures involving heating and subsequent chilling. The growth level of *C. perfringens* during the chilling of beef and pork from 54.4 to 26.6°C in 2 h and from 26.6 to 4.4°C in 5 h was <1 log CFU/g. There was a growth increase of 0.52 log CFU/g (from 3.02 to 3.54 log CFU/g;  $P < 0.05$ ) for beef as a result of the chilling procedure used in this study, while the growth increase for pork was 0.68 log CFU/g (from 3.40 to 4.08 log CFU/g;  $P < 0.05$ ). The procedure used to chill beef and pork met USDA stabilization guidelines requiring that the chilling process allow no more than 1 log total growth of *C. perfringens* in the finished product (15).

The *C. perfringens* log growth level for beef was lower than that for pork ( $P < 0.05$ ). The differences in the pH values of cooked beef and cooked pork may be partly responsible for this observation. Cooked beef had a pH of 5.9, which was more acidic ( $P < 0.05$ ) than that of cooked pork, which had a pH of 6.3. The pH values for cooked beef in this study are similar to those found in other studies (5, 10). Cooked pork pH values were within the 5.8-to-6.3 range reported for cooked cubed pork (5). *C. perfringens* grows best at pHs of 6.0 to 7.0 (8). Generation time increased from 80.1 to 122.1 min at 28°C in ground beef when the pH was reduced from 7.0 to 5.5 (7).

In summary, the chilling time for cooked beef and cooked pork could be extended from 90 min to 2 h during the initial cooling period when meat is cooled from 54.4 to 26.6°C and still meet USDA guidelines allowing no more than 1 log total growth of *C. perfringens* in finished product. The outgrowth of *C. perfringens* was more extensive in the cooked pork than in the cooked beef during the chilling process; however, both products met safety criteria established by USDA.

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