

Influence of Four Retail Food Service Cooling Methods on the Behavior of *Clostridium perfringens* ATCC 10388 in Turkey Roasts following Heating to an Internal Temperature of 74°C

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ABSTRACT

The influence of four food service cooling methods (CM) on growth of *Clostridium perfringens* ATCC 10388 in cooked turkey roasts was evaluated. Raw whole turkey roasts were inoculated with *C. perfringens* spores (~4.23 log CFU per roast), vacuum packaged, and heated to an internal temperature of 74°C. The cooked roasts were cooled as follows: whole roast cut into four quarters and held at 4°C (CM1); whole roast held in a blast chiller (CM2); whole roast loosely wrapped and held at 4°C (CM3); and whole roasts (three per bag) held at 4°C (CM4). The roasts were analyzed for *C. perfringens* using Shahidi-Ferguson *perfringens* agar and anaerobic incubation (37°C, 24 h). None of the cooling methods met the amended 2001 U.S. Food and Drug Administration Food Code guidelines for safe cooling of potentially hazardous foods. Times taken for roasts to cool from 57 to 21°C using CM1, CM2, CM3, and CM4 were 2.27, 3.11, 6.22, and 8.71 h, respectively. Times taken for roasts (21°C) to reach 5°C ranged from 6.33 (CM1) to 19.45 h (CM4). Based on initial numbers of *C. perfringens*, no growth occurred in roasts cooled by CM1 or CM2, whereas numbers increased by 1.5 and 4.0 log in whole roasts cooled via CM3 and CM4, respectively. These findings indicate that certain food service cooling methods for whole cooked turkey roasts may result in proliferation of *C. perfringens* and increase the risk of foodborne illness by this pathogen.

Clostridium perfringens is a gram-positive, anaerobic spore-forming pathogenic bacterium whose spores are common contaminants of a variety of foods (12). This pathogen is an ongoing concern in retail food service where large volumes of food are prepared in advance and cooled before reheating for service (27). Improper cooling of food was identified as the number one factor that contributed to the occurrence of 1,918 outbreaks of *C. perfringens* food poisoning in the United States from 1961 to 1982 (2). From 1983 to 1992 there were an estimated 654 reported outbreaks of *C. perfringens* food poisoning resulting in 248,520 cases (14). Based on surveillance information in the United States from 1993 to 1997, *C. perfringens* is responsible for 8.7% of all reported outbreaks (3). *C. perfringens* has been linked to foodborne illness involving roast beef, turkey, meat-containing Mexican foods, and other meat dishes (2).

Cooking meat products in retail food service is normally sufficient to destroy *C. perfringens* vegetative cells but not the spores of this pathogen. For example, cooking beef roasts (1.45 kg each) to an internal temperature of 60°C for 2.3 h or longer can result in a 12-log reduction of *C. perfringens* vegetative cells (18). However, spores of this pathogen typically survive the cooking process and are heat shocked, triggering them to germinate during improper cooling. While heating at 70 to 80°C for about 20 min is required to heat shock *C. perfringens* spores to induce ger-

mination (8, 12), the use of lower temperatures (e.g., 55 to 60°C) in low-temperature, long-time cooking of meats can also serve to activate spores (1). Improper cooling allows outgrowth of *C. perfringens* vegetative cells, which can multiply rapidly to reach high numbers within slow-cooling bulk products such as turkey roasts. As cooked meat dishes cool they will inevitably traverse the growth temperature range (50 to 15°C) for *C. perfringens* (12). Therefore, rapid cooling of cooked foods is crucial to prevent proliferation of this pathogen, whose population can double every 7.1 min between 41 and 46°C (13).

According to the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service guidelines for cooling of meat products, the internal temperature of cooked meats should not remain between 54.4 and 26.7°C for over 1.5 h nor between 26.7 and 4.4°C for more than 5 h during cooling (24). If meat processors are unable or not willing to meet these cooling guidelines, they must prove that an alternative cooling regimen will give less than 1-log increase in growth of *C. perfringens* and no growth of *Clostridium botulinum* (24). According to the performance standards for the cooling of ready-to-eat meat products that were published in the *Federal Register* 66(39) in February 2001, there should not be more than 1.0-log multiplication of *C. perfringens* within these products during cooling (26). In 2003, the U.S. Food and Drug Administration (FDA) amended their 2001 Food Code (28, 29) and recommended that all potentially hazardous foods be cooled from 57 to 21°C in 2 h and then from 21 to 5°C in an additional 4 h (29). Based on observational studies, retail

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food service procedures might not be meeting these FDA cooling recommendations. Henroid and Sneed (5) found that in most school food service operations employees were not measuring food temperatures. In operations where food temperatures were measured, often the data were not documented, employees did not calibrate thermometers, and documentation of calibration was nonexistent in all schools studied. Also, six of ten school food service operations were not in compliance with FDA recommendations for cooling potentially hazardous foods (5). Snyder and Labalestra (20) found that health departments were not conducting cooling studies to verify the FDA-recommended 6-h cooling time in retail food service and that this standard was a very difficult one to meet using typical food service refrigeration facilities.

The gap between FDA cooling recommendations and verification of cooling standards, coupled with observations of noncompliance of retail food service cooling procedures, served as impetus for this study. To our knowledge there is no published research on cooling time, product temperature, and growth of *C. perfringens* as affected by four different food service cooling methods employed for USDA commodity turkey roasts (5.1 kg each). Accordingly, the objectives of this research were to (i) develop time and temperature cooling curves for USDA commodity turkey roasts using selected food service cooling methods, and (ii) evaluate the influence of these cooling methods on the behavior of *C. perfringens* ATCC 10388 in turkey roasts following heating to an internal temperature of 74°C.

MATERIALS AND METHODS

Bacterial strain and spore suspension. *C. perfringens* strain ATCC 10388, isolated from lamb that developed enterotoxemia, was obtained from the American Type Culture Collection. *C. perfringens* spores were maintained in cooked meat medium (Difco, Becton Dickinson, Sparks, Md.) at 4°C and used as stock culture throughout the present study. The organism was cultured in fluid thioglycollate medium and sporulation was induced in modified Duncan-Strong sporulation medium (4) as described by Juneja et al. (9). The spore crop was harvested by centrifugation (10,000 × *g*, 10 min, 4°C), resuspended in physiological saline (0.85% wt/vol sodium chloride), then diluted in fresh saline to give a final spore concentration of approximately 1.7×10^4 CFU/ml. The diluted spore suspension was combined with filter-sterilized green food coloring (four drops of coloring per 10 ml of spore suspension) immediately before injection into turkey roasts.

Sample preparation and inoculation. Raw boneless turkey roasts (5.1 kg each) measuring 39.8 cm (length) and 13.9 cm (diameter), and packaged in a heat-sealed plastic film, were obtained from the USDA's Food and Nutrition Service. The roasts were kept frozen (−17.7°C) for no more than 2 months before use. Prior to each experiment, roasts were placed in a walk-in refrigerator at 4°C for 48 h until they were completely thawed. All roasts were inoculated using the same procedure, with a slight modification for roasts that were to be cut into quarters after heating. Thawed turkey roasts were inoculated with *C. perfringens* spores to give a final spore concentration of approximately 4.23 log CFU per roast. Inoculation was performed via use of a 15.2-cm 20-gauge needle (Fisher Scientific Co., Pittsburgh, Pa.) to inject 1.0 ml of the green-colored spore suspension in the geometric center of each roast. The geometric center (6.98 cm) of each roast

TABLE 1. Treatment code for and description of each retail food service cooling method (CM) used for turkey roasts

Treatment code	Description of cooling method
CM1	Roast quartered, uncovered, in walk-in refrigerator
CM2	Whole roast loosely wrapped, in blast chiller
CM3	Whole roast loosely wrapped, in walk-in refrigerator
CM4	Whole roasts (three per bag) on sheet pans, in walk-in refrigerator

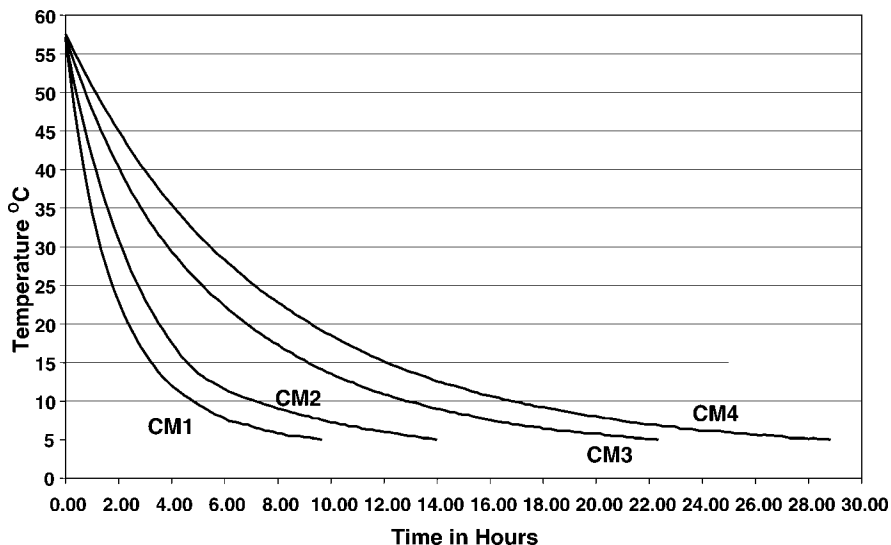
was determined by measuring the circumference (*C*) of the respective roasts and applying the mathematical formula $C = \pi D$. This formula was used to obtain the radius (0.5*D*), which represented the center of the roast in which the spore suspension was injected. Roasts that were to be cut into quarters after heating were marked (using a permanent marker) into four 9.9-cm-thick sections. The roasts were inoculated with 1.0 ml of the spore suspension in the center of one of the two inner sections.

A thermocouple type-K probe (Fisher Scientific Co.) was inserted next to the inoculum at the same depth (6.98 cm), in the center of each turkey roast. For roasts that were to be cut into quarters, a type-K probe was inserted into the center of a delineated inner section that was not inoculated. All puncture holes in the plastic packaging film were sealed using a glue gun immediately after inoculation and insertion of the probes. Each roast was placed in a separate Rival VSB2 Seal-a-Meal heat-resistant plastic bag (the Holmes Group Inc., Milford, Mass.) with the thermocouple probe extending out of the opening of the outer bag. A glue gun was used to seal the probe at the opening of the bag while allowing space at either side of the sealed probe for evacuating air from the roasts during vacuum packaging. All roasts were packaged under vacuum with a Multivac model A 300/51 vacuum packaging machine (Multivac Inc., Kansas City, Mo.). Each type-K probe was attached to an Atkins model 37313 data recording digital thermometer (Atkins Technical Inc., Gainesville, Fla.) set to record temperature data at 10-min intervals.

Heating and cooling. The turkey roasts were heated in a steamer at full steam to an internal temperature of 74°C, according to 2001 FDA Food Code (28, 29). After reaching 74°C, roasts were removed from the steamer and assigned to one of four cooling methods (CM1, CM2, CM3, and CM4) described in Table 1. In all treatments, each of four type-K probes (one for each cooling method) remained attached to a separate Atkins data recording thermometer to record cooling temperatures, until turkey roasts reached 5°C. For treatment 1, the heated roast was cut into four 9.9-cm sections using a sterile knife. Each section was spread apart, leaving a gap of approximately 6.85 cm between sections for cooling, and cooled uncovered in a walk-in refrigerator at 4°C. For treatment 2, the roast was loosely covered and cooled in a Servolift Eastern blast chiller, shock freezer model HCM51-20 (Servolift Eastern, Boston, Mass.). For treatment 3, the roast was loosely covered and refrigerated (4°C). For treatment 4, three heated roasts were placed next to one another on a sheet pan. The entire sheet pan with roasts was tightly covered with plastic then refrigerated. The middle roast was inoculated and fitted with a type-K probe.

Microbiological analysis. Viable populations of *C. perfringens* survivors in cooked turkey roasts were determined within 2 h after heating to 74°C and when roasts were cooled to 5°C. Roasts were cut open using a sterile knife and the highly visible green cores of the roasts were aseptically excised. The excised

FIGURE 1. Temperature profiles for cooling of cooked turkey roasts from 57 to 5°C via application of four retail food service cooling methods (CM). Roast cut into quarters and held in walk-in refrigerator (CM1); whole roast loosely wrapped and held in blast chiller (CM2); whole roast loosely wrapped and held in walk-in refrigerator (CM3); whole roasts (three per bag) on sheet pans in walk-in refrigerator (CM4).



core samples were mixed with 100 ml of sterile 0.1% peptone water in sterile stomacher bags (Fisher Scientific Co.) and pummeled at normal speed for 60 s using a Seward Stomacher 400 lab blender (Seward Ltd., Norfolk, UK). Tenfold serial dilutions of the meat homogenate were prepared in sterile 0.1% peptone water. Samples of meat homogenate or appropriate dilutions were pour-plated in duplicate using Shahidi-Ferguson perfringens agar base (Difco) with added filter-sterilized D-cycloserine (Sigma Chemical Company, St. Louis, Mo.) solution. All inoculated Shahidi-Ferguson perfringens D-cycloserine agar plates were incubated in anaerobic jars with Oxoid AnaeroGen AN35 sachet bags (Oxoid Ltd., Basingstoke, Hampshire, UK) at 37°C for 24 h before counting bacterial colonies. Black colonies were considered presumptive positive for *C. perfringens*. Randomly selected presumptive *C. perfringens* isolates from each sample were confirmed as gram-positive, nonmotile rods that reduced nitrate, fermented lactose, and liquefied gelatin in motility-nitrate and lactose gelatin media as described by Labbe (13).

Data analysis. Three independent replicate experiments were conducted, and results are reported as means. Cooling curves were developed from the downloaded Atkins 37313 recording thermometer data. Reported populations of viable *C. perfringens* in turkey roasts were determined by calculating the log value of bacterial counts on duplicate plates and the mean and standard deviation of three replicates using Microsoft Excel 2003 Software (Microsoft Inc., Redmond, Wash.) (15). Data from changes in viable counts resulting from the cooling methods were analyzed using the general linear models procedure of the Statistical Analysis System software program (SAS Institute Inc., Cary, N.C.)

TABLE 2. Mean time (hours) required for cooling turkey roasts from 57 to 21°C (I), 21 to 5°C (II), and 57 to 5°C (III) using four retail food service cooling methods

Cooling method	Cooling time (h)		
	I	II	III
CM1	2.27 (±0.10) ^a	6.33 (±0.86)	8.60 (±0.92)
CM2	3.11 (±0.20)	9.33 (±1.42)	12.44 (±1.58)
CM3	6.22 (±0.35)	13.80 (±2.40)	20.02 (±3.08)
CM4	8.71 (±0.10)	19.45 (±0.51)	28.16 (±0.60)

^aMean (± standard deviation) of three replicate experiments.

(16). Statistical analyses included analysis of variance and comparison of means by Tukey ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Figure 1 shows the cooling curves (from 57 to 5°C) for turkey roasts subjected to four retail food service cooling methods. The method of cooling that resulted in the fastest decrease in temperature of turkey roasts involved cutting the cooked roasts into quarters and refrigerating them uncovered at 4°C (CM1). Generally, the thickness or distance from the surface to geometric center of a food product is a major factor that affects the rate of heat loss. The smaller the distance the more rapid the heat loss. The cut sections of the quartered roasts had the smallest distance from the cut surface to the center (4.95 cm) compared with the thickness of whole roasts (6.98 cm). This effectiveness of reduced product thickness in facilitating heat loss is evident when the cooling time for refrigerated quartered roasts is compared with that for a whole roast in a blast chiller. An internal temperature of 21°C was achieved in 2.27 and 3.11 h for refrigerated quartered roast (CM1) and blast-chilled whole roast (CM2), respectively (Table 2).

The problem of slow cooling is further exacerbated when whole roasts are held under refrigerator conditions. For example, refrigerated single whole roast (CM3) and refrigerated roasts (three per bag; CM4) cooled the slowest compared with all other methods evaluated. Times required for the internal temperature of roasts to cool from 57 to 21°C were 6.22 and 8.71 h, respectively, for CM3 and CM4 (Table 2). Two factors that most likely contributed to the very slow heat loss from the three-bagged roasts subjected to CM4 are (i) the insulating effect of the two roasts that flanked the center roast (with probe), and (ii) the plastic bag that trapped heat around all three roasts. Roasts subjected to CM3 and CM4 also took the longest to cool from 21 to 5°C. These results dramatically illustrate the heat-insulating effect of food cooled in bulk quantities.

Initial *C. perfringens* populations (4.23 log CFU per roast) decreased to 2.70 log CFU per roast in roasts analyzed within 2 h after heating to an internal temperature of

TABLE 3. Changes in viable counts of *C. perfringens* in turkey roasts based on differences between viable counts after cooling to 5°C and initial numbers in the raw product (4.23 log CFU per roast) or numbers in the cooked product (2.70 log CFU per roast) at 2 h after heating to 74°C

Cooling method	Viable count after cooling to 5°C	Log change in viable count ^a	Log change in viable count ^b
CM1	2.78 (±0.26) ^c	-1.45 (±0.10) A	0.08 (±0.26) A
CM2	3.37 (±0.15)	-0.86 (±0.17) A	0.67 (±0.15) A
CM3	5.73 (±0.12)	1.50 (±0.70) B	3.03 (±0.12) B
CM4	8.23 (±0.36)	4.00 (±0.61) C	5.53 (±0.36) C

^a Viable counts in roasts (5°C) minus initial viable numbers in raw product.

^b Viable counts in roasts (5°C) minus numbers of survivors at 2 h after heating to 74°C.

^c Mean (± standard deviation) of three replicate experiments. Mean values with different letters in the same column are significantly different ($P < 0.05$).

74°C. Taormina and Dorsa (22) suggested that heating can alter the levels of *C. perfringens* spores and vegetative cells in heated foods entering the cooling process. Shigehisa et al. (17) demonstrated that heating *C. perfringens* H-3 spores from 20 to 60°C in fluid thioglycollate medium at rates of 25, 20, and 13°C/h (1.6-, 2-, and 3-h heating time, respectively) resulted in reduction of initial spore populations. Those same authors reported an increase in total counts (vegetative cells and spores) of *C. perfringens* before death of vegetative cells occurred above 50°C. Also, there was a 2-log decrease in initial numbers of spores following heating from 20 to 60°C for 5.7 h (heating rate of 7°C/h). In the present study the turkey roasts took 3 h for their internal temperature to increase from 4 to 74°C (heating rate 23°C/h) (data not shown). The observed change in viable population of *C. perfringens* in cooked roasts entering the cooling process represented a 1.45-log reduction in the initial viable spore count. It is likely that germination and outgrowth of some spores occurred during relatively slow heating of the turkey roasts. Spores that germinated and produced vegetative cells would have become more susceptible to heat as the temperature became lethal, leaving only those spores that had not yet germinated to enter the cooling process.

Table 3 shows changes in viable counts of *C. perfringens* in turkey roasts based on differences between viable counts after cooling to 5°C and initial numbers in the raw product (4.23 log CFU per roast) or numbers in the cooked product (2.70 log CFU per roast) at 2 h after heating to 74°C, in reference to the time that each method took to reduce the internal temperature of the roasts from 57 to 5°C. No increase in initial viable counts was observed in roasts cooled using CM1 or CM2. In fact, roasts cooled by both of these methods exhibited varying decreases in initial viable counts of the pathogen (Table 3). The refrigerated whole roast (CM3) and whole roasts (three per bag) (CM4) exhibited a 1.50- and 4.00-log increase in viable counts, respectively. Both of these cooling methods took the lon-

gest to reduce the internal temperature of the roasts from 57 to 21°C (Table 2), thus allowing enough time (6.22 and 8.71 h) for proliferation of *C. perfringens* at temperatures that are conducive to rapid growth of the pathogen. *C. perfringens* is mesophilic and grows rapidly between 37 and 45°C (6), with 20 and 50°C representing lower and upper growth temperature limits, respectively (7). Under optimal conditions *C. perfringens* exhibits a generation time as short as 7 min (7). Therefore, it was not surprising that both of these cooling methods resulted in relatively large increases in initial populations of *C. perfringens*.

When the number of *C. perfringens* survivors in roasts (at 2 h after heating to 74°C) was used to determine changes in viable counts during cooling, larger increases in populations of the pathogen were realized (Table 3). All cooling methods resulted in increases in numbers of the pathogen; however, these increases from CM1 and CM2 (0.08 and 0.67 log CFU per roast, respectively) were not significantly different ($P > 0.05$). In contrast, CM3 and CM4 resulted in log increases of 3.03 and 5.53, respectively ($P < 0.05$). These increases in viable counts of *C. perfringens* resulting from use of CM3 and CM4 far exceed the USDA Food Safety and Inspection Service safe cooling standard criteria (23). The criteria allow no more than a 1.0-log increase between 48.9 and 12.8°C within 6 h and represent compliance guidelines to control growth of *C. perfringens* to dangerous levels in cooked meat and poultry products. While the refrigeration of three roasts together in a bag (CM4) is not a commonly used cooling method, it has been observed in practice and serves as a worst-case scenario for comparison purposes.

Our findings are consistent with those of previous studies on the growth of *C. perfringens* in cooked turkey meat during cooling. Kalinowsky et al. (11) demonstrated that cooling uncured cooked turkey from 48.9 to 12.8°C for 6 h resulted in less than 1.0-log (0.83-log) increase in growth of *C. perfringens*. For that same critical temperature range, Steele and Wright (21) reported that 8.9 h of cooling was the maximum time necessary to prevent a 1.0-log increase in *C. perfringens* in uncured cooked turkey roasts. Data from cooling profiles generated in the present study indicated that CM1, CM2, CM3, and CM4 took 3.7, 5.6, 10.4, and 14.0 h, respectively, to reduce the internal temperature of turkey roasts from 48.9 to 12.8°C. CM1 and CM2 were the only two cooling methods that reduced the internal temperature of the roasts from 48.9 to 12.8°C in less than 6 or 8.9 h and restricted growth of the pathogen to less than 1.0 log (Table 3).

The USDA Pathogen Modeling Program (PMP) version 7.0 for estimating *C. perfringens* growth at changing temperatures during cooling of cooked meats was used to predict the growth of the pathogen in cooked turkey roasts cooled via the four different cooling methods used in the present study. The model predicted that cooling cooked meats from 48.9 to 12.8°C in 3.7 (CM1), 5.6 (CM2), 10.4 (CM3), and 14.0 h (CM4) would result in relative log growth of 0.10, 0.23, 1.68, and 3.39, respectively. Based on data from the present study, cooling turkey roasts using CM1, CM2, CM3, and CM4 resulted in 0.08-, 0.67-, 3.03-,

and 5.53-log growth of the pathogen, respectively. Predictions from the PMP and the results of the present study both suggest that CM1 and CM2 would restrict growth of the pathogen to less than 1.0 log, whereas CM3 and CM4 would allow growth of the pathogen by more than 1 log. For two of the cooling methods (CM3 and CM4), the PMP markedly underpredicted the extent of growth of *C. perfringens* in the turkey roasts. These results may be attributed to faster than predicted growth of *C. perfringens* (strain ATCC 10388) in cooked turkey roast during cooling. The actual model (10) that formed the basis of the PMP to predict growth of *C. perfringens* at changing temperatures during cooling of cooked meat products was used in a validation study recently conducted by Smith and Schaffner (19). Those researchers demonstrated that the model consistently underpredicted increases in growth of the pathogen when exponential cooling occurred at two different rates in the cooling process. On comparing their experimental data with the PMP predicted values from single cooling rate studies, the researchers suggested that the model could be improved for describing the growth of *C. perfringens* during exponential cooling.

The four cooling methods for turkey roasts evaluated in the present study represent practices observed in school food service operations (5). None of the cooling methods achieved the amended 2001 Food Code recommendations for cooling potentially hazardous foods from 57 to 21°C in 2 h and from 21 to 5°C in an additional 4 h (29). However, CM1 and CM2 met the USDA stabilization performance standard (25) in that less than 1.0-log increase in growth of *C. perfringens* was achieved using these two cooling methods. In contrast, CM3 and CM4 failed to meet this standard. The walk-in refrigerator used in the present study was not the forced-air type of cooling system and therefore simulated the refrigeration conditions commonly observed in school food service establishments. Turkey roasts were cooled uncovered or loosely covered (except for CM4) with the doors of the cooling equipment closed during the entire cooling process. These practices represent a conservative scenario for the cooling of foods and do not account for variables in actual food service establishments that could potentially increase cooling time. Examples of such variables include frequent opening and closing of a refrigerator door and wrapping hot food tightly with aluminum foil or film before cooling.

This study simulated actual food service cooling methods observed in school food service operations. Measurements of time, temperature, and growth of *C. perfringens* during cooling of turkey roasts provided data that were compared with cooling recommendation of the amended 2001 FDA Food Code standards and USDA Food Safety and Inspection Service safe cooling standard criteria for cooked meat and poultry products. Significant findings of the study indicated that time standards were not met, and typical cooling methods, based on measurements of cooling time and temperature, are unacceptable. The most significant finding of the study was that two cooling methods (CM3 and CM4) caused an increase in *C. perfringens* populations, enough to cause foodborne illness. Results of the

present study indicate that normal refrigeration methods typically used in school food service operations are unacceptable for cooling whole turkey roasts to ensure the microbiological safety of these meat products. If food service operations are unable to invest in a blast chiller then it is imperative that whole cooked turkey roasts be cut into quarters prior to refrigeration (4°C) to facilitate rapid cooling. The data generated in the present study are useful for educating food service professionals about the microbial safety of cooling methods for potentially hazardous foods. This approach may prove effective in curtailing the practice of potentially dangerous cooling methods in food service operations to avoid outbreaks of foodborne disease caused by *C. perfringens*.

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