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6200 Aurora Ave., Suite 200W, Des Moines, IA 50322-2864

Lindsay Beardall,[1] Paola Paez,[2] Randall K. Phebus,[3] Tracee Watkins[2] and Sara E. Gragg[1*]

[1]Dept. of Animal Sciences and Industry, Kansas State University, 22201 W. Innovation Drive, Olathe, KS 66061, USA

[2]Center for Food Safety in Child Nutrition Programs, Kansas State University, 152 Justin Hall, 1324 Lovers Lane, Manhattan, KS 66506, USA

[3]Food Science Institute, Kansas State University, 1530 Mid-Campus Drive North, Manhattan, KS 66506, USA

Control of Surrogate *Escherichia coli* Populations in Three Food Products Using Common Food Service Cooling Methods

ABSTRACT

The United States Food and Drug Administration (U.S. FDA) has identified improper ("slow") cooling as an important contributing factor in foodborne illness outbreaks. The efficacy of post-thermal treatment cooling techniques on controlling surrogate *Escherichia coli* growth in taco meat, chili con carne with beans, and low-sodium marinara sauce was evaluated. Each product was cooked to 73.9°C, portioned to 2- and 3-inch depths in stainless steel steam table pans, and cooled to 60°C ± 5°C before inoculation with *E. coli*. Pans were prepared with different cover methods to allow or restrict air exposure and then placed inside a -20°C commercial walk-in freezer or situated in ice water baths inside a 4°C commercial walk-in refrigerator. Product temperatures were recorded for 24 hours. Microbial populations were enumerated at 0, 4, 8, 12, and 24 hours. Temperature data for taco meat and chili con carne with beans revealed that few cooling methods met the 2017 FDA Food Code cooling criteria, while data for low-sodium marinara sauce showed that no cooling method met the Food Code chilling criteria. Population changes were < 0.50 $\log_{10}$ CFU/g over 24 hours in all products, indicating that all cooling methods were low risk for microbial proliferation and were therefore effective at controlling *E. coli* within these products.

INTRODUCTION

In the United States, the School Breakfast Program and the National School Lunch Program combined serve over 7.3 trillion meals annually to school children *(23)*. Schools are associated with foodborne illness more often than other institutional foodservice settings. Compared with prisons, camps, daycare facilities, or cafeterias, schools account for nearly half of outbreaks arising in institutional foodservice settings, with a median outbreak size second only to those striking correctional facilities *(9)*. The greater size of outbreaks may be attributed to the number of meals served in schools. Considering that children are the primary users of school nutrition programs and that the severity of foodborne illness and the frequency of complications in young children have been well documented, outbreaks in this population are

*Author for correspondence: Phone: +1 913.307.7371; Fax: +1 913.541.1488; Email: saragragg@k-state.edu

of particular concern *(3, 21, 30)*. Underdeveloped immune systems and low body weights contribute to the increased susceptibility of young children *(3)*. The large number of children served at schools, combined with the classification of children as an at-risk population, makes proper food preparation practices important in a school meal setting.

School nutrition programs are required to have a food safety program based on the principles of Hazard Analysis Critical Control Point (HACCP); proper cooling practices are an integral part of the food safety program *(5)*. Improper cooling is considered a proliferation risk factor by the Centers for Disease Control and Prevention (CDC), meaning that improper cooling can lead to microbial growth, including growth of pathogens, in food products *(4)*. The FDA identifies time/temperature control as a critical control point for preventing foodborne illness *(24–27)*. Improper cooling has been recognized as one of the contributing factors to foodborne outbreaks in schools *(18, 29)*. Because slow cooling is a public health risk, the United States Food and Drug Administration (U.S. FDA) Food Code was updated in 2009 to require food products to be cooled to 21.1°C within 2 hours of cooking and to 5°C within a total of 6 hours *(26)*, cooling parameters that are consistent with what is published in the more recent 2017 FDA Food Code *(28)*, which is the version referenced herein. Researchers evaluating cooling techniques commonly used in school nutrition programs for various food products have concluded that very few techniques meet the FDA Food Code requirement *(12, 16, 17, 19)*.

Exposure to *E. coli* O157:H7 often results in symptoms that are severe, with 46% of cases resulting in hospitalization and a small number of cases resulting in death *(20)*. Between 1998 and 2016, *E. coli* O157:H7 was associated with 11 outbreaks in school and university foodservice settings *(4)*. Exposure to *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* (STEC) is a critical concern for young children (age 1 to 9 years), as they experience a higher infection rate than adults and have an increased likelihood of developing chronic sequelae, such as hemolytic uremic syndrome (HUS) *(3)*, which can lead to kidney failure. STEC infections such as *E. coli* O157:H7 infections are most commonly contracted via the fecal-oral route, often by consuming contaminated food or water *(7, 13)*. Improper hygiene and cross-contamination are two ways STEC may contaminate food products after they have been cooked; infectious food handlers are often implicated in outbreaks of gastrointestinal foodborne illness in school settings *(8, 29)*.

Krishnamurthy and Sneed *(12)* reported that taco meat and chili are two foods that are commonly prepared and cooled in over half of schools. Similarly, taco meat, chili with beans, and marinara sauce are food products commonly served in other foodservice establishments. For example, chili with beans and products with marinara sauce as an ingredient are served in jails *(6)*. Therefore, understanding the risk for microbial proliferation during cooling of these products is important.

The objective of this study was to evaluate cooling methods commonly used in school nutrition programs to quantify their effect on *E. coli* populations over a 24-hour period in three food products commonly served in school nutrition programs: taco meat, chili con carne with beans, and low-sodium marinara sauce. The research protocol for this project used, as a foundation, previous research on cooling methods conducted by the Center for Food Safety in Child Nutrition Programs. While the research protocol was designed to mimic school nutrition program practices, other food foodservice facilities also serve some or all of the food products investigated in this study. As a result, the data presented herein will be useful for other foodservice facilities that serve chili con carne with beans, taco meat, and marinara sauce.

MATERIALS AND METHODS

Escherichia coli strains

Four *E. coli* strains were chosen from the ATCC® Non-pathogenic *Escherichia coli* Surrogate Indicators Panel (ATCC® MP-26™) to serve as surrogates for *E. coli* O157:H7 *(1, 11, 15)*. The four strains that were utilized in a cocktail consisted of ATCC® BAA-1427, BAA-1429, BAA-1430, and BAA-1431, which have been recommended by the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) for use in research evaluating changes in microbial populations in the food processing environment during validation studies *(1)*. The ATCC isolates were rehydrated in tryptic soy broth (TSB; BD Difco™, Franklin Lakes, NJ) and incubated separately at 37°C for 24 hours. One-ml portions of each culture were frozen in microcentrifuge tubes with 10% glycerol (Fisher Scientific, Waltham, MA) and stored at -80°C until later use.

Escherichia coli inoculum preparation

One day prior to inoculation of pre-cooked taco meat and chili con carne, a micro-centrifuge tube of each frozen *E. coli* strain was thawed to room temperature (20°C) and transferred separately into four 50-ml centrifuge tubes containing 25 ml of buffered peptone water (BPW; BD Difco™, Franklin Lakes, NJ). These cultures were incubated at 37°C for 18 to 24 hours. For the marinara sauce (pH 4.18; Education pH meter; Fisher Scientific, Waltham, MA), each thawed *E. coli* strain was grown separately at 37°C for 18 to 24 hours in 25 ml of TSB with 1% glucose in order to produce acid-adapted cultures, per Buchanan and Edelson *(2)*. This acid-adaptation protocol is supported by the National Advisory Committee for the Microbiological Criteria of Foods (NACMCF) for challenge studies with low-pH foods *(14)*. Although the chili con carne with beans recipe used in this study used a tomato base, this product was not highly acidic (pH 4.68), and culture die off was

not observed in the inoculated product. As a result, a study of acid adaptation of *E. coli* was not pursued for chili con carne with beans. After incubation, the 25-ml culture tubes were centrifuged at 5,000 × g for 15 minutes at 4°C. The supernatant was discarded, and the pellets were re-suspended in 25 ml of 0.1% BD Bacto™ Peptone Water (PW; Fisher Scientific, Waltham, MA), after which all four strains were combined in a sterile 100-ml container, resulting in a master inoculum cocktail containing approximately 10^9 CFU/ml. To achieve a target concentration of 10^4 CFU/g of product, the inoculum for each pan was prepared in 0.1% PW based on the weight of food product within each pan, with the liquid of the inoculum comprising no more than 1% of the food product *(14)*.

Food product preparation

All food products met the nutritional standards for Child Nutrition Programs *(22)*. Food products and ingredients were ordered from a foodservice distributor. Pre-cooked, frozen taco meat was thawed in a commercial refrigerator at 4°C for several days prior to each experimental replication. On day 0 of each experimental replication, five-pound bags were placed in 2-inch stainless steel steam table pans and heated in commercial steamers (Electrolux Air-o-Steam Touchline Combi Oven and Cleveland SteamChef Electric Countertop Steamer) to 73.9°C, which was determined by use of a Taylor 9842FDA waterproof digital thermometer (Taylor Precision Products, Oak Brook, IL) at several locations throughout the product. Canned, low-sodium marinara sauce was cooked to 73.9°C in a commercial tilt skillet (Cleveland Tilt Skillet). Chili was prepared according to a recipe used by a local school nutrition program following USDA guidelines and was cooked to 73.9°C in the same commercial tilt skillet as used for the marinara sauce. After the food products were reheated or cooked, they were portioned in 2- and 3-inch depths in 2½- and 4-inch deep stainless steel steam table pans. Products portioned to 2-inch depths were placed into pans with dimensions of (W × L × D) 30.5 cm × 50.8 cm × 6.4 cm (12-inch × 20-inch × 2.5-inch), while products portioned to 3-inch depths were in pans with dimensions of (W × L × D) 30.5 cm × 50.8 cm × 10.2 cm (12-inch × 20-inch × 4-inch).

Food product inoculation

The temperature of all products was monitored with a Taylor 9842FDA waterproof digital thermometer (Taylor Precision Products, Oak Brook, IL), and all food products were stirred and allowed to cool to 60°C ± 5°C prior to inoculation. After the calculated volume of inoculum was added to each pan, food was manually stirred for approximately 2 minutes to distribute the bacterial cells. Inoculation times were recorded for each pan upon completion of stirring, and time points were set accordingly.

Sampling

After inoculation, samples were collected from each pan after 0, 4, 8, 12, and 24 hours of cooling. At each time point, a composite sample was obtained by using a plastic disposable tablespoon to gather food from four or five randomly chosen sub-surface areas within each pan, where the food was likely warmer than at the product surface and therefore more at risk for microbial growth. Collecting food from five locations in every pan at each sampling point was the goal. If pans stored in the freezer began to solidify, making it difficult to collect food during sampling, at least four random locations were sampled. Samples were collected at random to account for the possibility that, following stirring of the product, pockets of elevated temperature may have remained.

Composite samples were homogenized by hand mixing, after which a 25-g sample from each composite was diluted with 225 ml of BPW and stomached for one minute at 230 rpm (Stomacher® 400 Circulator; Seward, Bohemia, NY). Serial dilutions of the samples were then prepared in 9-ml tubes of BPW, and dilutions were spread plated onto MacConkey (MAC; Remel, Lenexa, KS) agar. MAC plates were incubated at 37°C for 18 to 24 hours, and representative colonies were counted.

Treatments and cooling

After the food products were inoculated and time point 0 samples obtained, each pan was fitted with a Lascar EL-USB-2-LCD USB temperature data logger (Lascar Electronics, Erie, PA) in the center of the pan, as previously described by Roberts et al. *(19)*, to record the product temperature every 60 seconds for 24 hours. Pans were then prepared with three cover types: uncovered, covered with a single layer of aluminum foil over the top of the pan, or double covered to restrict air exposure. Double-covered pans had one layer of plastic wrap (for marinara sauce product, because of its acidity) or aluminum foil (pre-cooked taco meat and chili con carne with beans) directly contacting the top surface of the food product and another layer of aluminum foil over the top of the pan (air space present between layers). Each cover type was applied to pans with both a 2- and 3-inch food product depth and prepared in duplicate so that one pan could be stored at 4°C (Standard Deviation: 1.34°C) in a walk-in refrigerator and the duplicate pan could be stored at -20°C (Standard Deviation: 3.83°C) in a walk-in freezer. Pans in the refrigerator were situated into ice baths as suggested in the FDA Food Code *(28)*. The ice baths were prepared by filling 4- and 6-inch stainless steel steam table pans three-quarters full with crescent-shaped ice (for use with the 2½- and 4-inch depth pans, respectively). Thus, six pans of each inoculated food product were stored for cooling and sampling in each of the storage locations (refrigerator or freezer). To prevent food products from becoming completely frozen and thus unable to be sampled, pans in the freezer were transferred to a shelf in the refrigerator immediately after the 8-hour sampling.

Statistical analyses

All experimental procedures were replicated three times. *E. coli* population data and temperature data were analyzed using liner mixed modeling with a compound symmetry covariance structure, a compound symmetry with heterogeneous time variances structure, or an unstructured covariance matrix combined with a PROC MIXED procedure in the Statistical Analysis Software 9.4 (SAS; Cary, NC). These covariance structures were chosen on the basis of Akaike's information criterion (AIC) to obtain the best covariance structure for microbial population data from each product. This was considered a four-factor, repeated-measures experiment and was analyzed accordingly. A Type III test for fixed effects was also conducted.

Least squares means (LSMEANS) of microbial populations were calculated by use of the LSMEANS statement in SAS and were used to compare the significance of effects on variables and variable interactions at a significance threshold of $P \leq 0.05$. To reduce variability in temperature data, five values near each time point were averaged. Effects of temperature data variables and variable interactions were also compared at a significance threshold of $P \leq 0.05$.

RESULTS

Temperature data analysis

The effects of cover type, storage location, and depth variables (main effects), as well as variable interactions, were analyzed for statistical significance. To perform the cooling study, temperature was decreased dramatically over time during the cooling process, and time was therefore not included as a main effect; rather, all main effects and interactions were analyzed at six individual time points (0, 2, 4, 8, 12, and 24 hours). The significance of effects of specific temperature variables will be discussed below.

For taco meat, no variable had a significant effect at time point 0 hours. At the 2-hour time point, effects of cover type and storage location by product depth were significant. Uncovered pans were significantly cooler than single-covered or double-covered pans. The 2-inch depth in the freezer was cooler than both the 3-inch depth in the freezer and the 2-inch depth with an ice bath in the refrigerator. However, the 3-inch product depth stored in the refrigerator with an ice bath was significantly the coolest at the 2-hour time point.

At 4- and 8-hour time points, the effects of storage location, storage location by product depth, and cover type were significant for taco meat. The freezer cooled 2-inch product depths more rapidly than 2-inch product depths in the refrigerator at 4 and 8 hours of cooling. The 3-inch product depths cooled more rapidly in the refrigerator for the first 4 hours, but by time point 8, products at 3-inch depths were at lower temperatures in the freezer. At 8 hours of cooling, the 2-inch product depth in the refrigerator cooled less rapidly than the 3-inch product depth in the refrigerator.

Uncovered pans of taco meat cooled more rapidly than single-covered or double-covered pans at 4 and 8 hours. Storage in the refrigerator or freezer was the only significant factor for cooling at the 12- and 24-hour time points. Pans removed from the freezer and placed in the refrigerator after the 8-hour time point remained at a lower temperature than those stored in the refrigerator with an ice bath.

No variable had a significant effect at the 0-hour time point for chili con carne with beans. At 2 hours of cooling, storage location, product depth, storage location by product depth, and cover type all had a significant effect. The 2-inch product depths in the freezer were cooler than 3-inch depths in the freezer. The 2- and 3-inch product depths stored in the refrigerator with an ice bath were cooler than 3-inch depths in the freezer. Uncovered product depths were also significantly cooler than single- or double-covered product depths at the 2-hour time point.

At the 4-hour time point, the effects of depth, storage location by depth, and cover type were significant for the cooling of chili con carne with beans. The pans stored in the freezer at 2-inch product depths cooled more quickly 3-inch depths stored in the freezer. The 3-inch product depths cooled more quickly in the refrigerator than in the freezer after 4 hours of cooling. The uncovered pans cooled more rapidly during the first 4 hours than single- or double-covered pans.

At the 8-hour time point, the effects of location, storage location by depth, and cover were significant, or nearly significant ($P = 0.0518$) at 12-hours, for chili con carne with beans. At these time points, the 2-inch product depth in the freezer cooled most rapidly. The uncovered pans were cooler than the double covered pans after 8 and 12 hours of cooling.

At the 24-hour time point, the effects of storage location and depth by cover type were significant for chili con carne with beans. Pans in the refrigerator at the 24-hour time point were cooler by a small (1.3°F), but statistically significant, amount. The 3-inch product depths stored uncovered were the lowest in temperature at the 24-hour time point, with the double-covered 2-inch pans the second coolest in comparison, though the difference between the 3-inch uncovered pans and 2-inch double-covered pans was not significant ($P = 0.0535$).

At time point 0, the effect of product depth was significant, as 3-inch product depths were observed to have a significantly higher temperature than 2-inch product depths for the low-sodium marinara sauce. At 2 or 4 hours of cooling, there were no significant effects on cooling. However, the effect of depth was approaching significance at 2 hours ($P = 0.0681$) and at 4 hours ($P = 0.0533$), with the 2-inch product depths cooler than the 3-inch depths. The effects of storage location and depth were significant at the 8-hour time point. Freezer-cooled pans had cooled to lower temperatures at this time point, and 3-inch product depths continued to be significantly higher in temperature than 2-inch product depths. Storage

TABLE 1. Cooling technique combinations that achieved FDA Food Code criteria for pre-cooked taco meat, chili con carne with beans, and marinara sauce

	Pre-Cooked Taco Meat		Chili con Carne with Beans		Marinara Sauce	
Cooling Technique Combination	**2 hours**	**6 hours**	**2 hours**	**6 hours**	**2 hours**	**6 hours**
2-inch Refrigerated ice bath Single cover			✓			
2-inch Refrigerated ice bath Double cover						
2-inch Refrigerated ice bath Uncovered			✓	✓	✓	
3-inch Refrigerated ice bath Single cover						
3-inch Refrigerated ice bath Double cover						
3-inch Refrigerated ice bath Uncovered	✓	✓	✓	✓		
2-inch, freezer Single cover		✓		✓		✓
2-inch, freezer Double cover		✓		✓		✓
2-inch, freezer Uncovered	✓	✓	✓	✓		✓
3-inch, freezer Single cover						
3-inch, freezer Double cover						
3-inch, freezer Uncovered		✓		✓		✓

location had a significant effect for the 12- and 24-hour time points, with temperatures of freezer-cooled pans lower than those of refrigerated pans.

Temperature data and FDA Food Code criteria

The temperature data for each cooling technique combination were compared to the 2017 FDA Food Code criteria *(28)*. *Table 1* summarizes data on whether or not the FDA Food Code criteria were satisfied for each food product. *Figures 1–3* illustrate the cooling curves for each cooling technique combination in each product.

Temperature data collected from taco meat indicate that two cooling technique combinations (freezer-cooled, uncovered, 2-inch depth product as well as refrigerator and ice bath-cooled uncovered, 3-inch depth product) met the 2017 FDA Food Code criteria *(28)* *(Fig. 1)*. As *Table 1* highlights, three freezer-stored cooling techniques satisfied the 6-hour 2017 FDA Food Code *(28)* criterion, but not the 2-hour criterion.

Temperature data on chili con carne with beans indicate that three cooling technique combinations (refrigerator and ice bath-cooled, uncovered, 2-inch and 3-inch product depths as well as freezer-cooled, uncovered, 2-inch product depth) achieved the 2017 FDA Food Code *(28)* criteria *(Fig. 2)*. Four additional cooling technique combinations satisfied either the 2-hour or 6-hour 2017 FDA Food Code criterion *(28)*, but not both criteria *(Table 1)*.

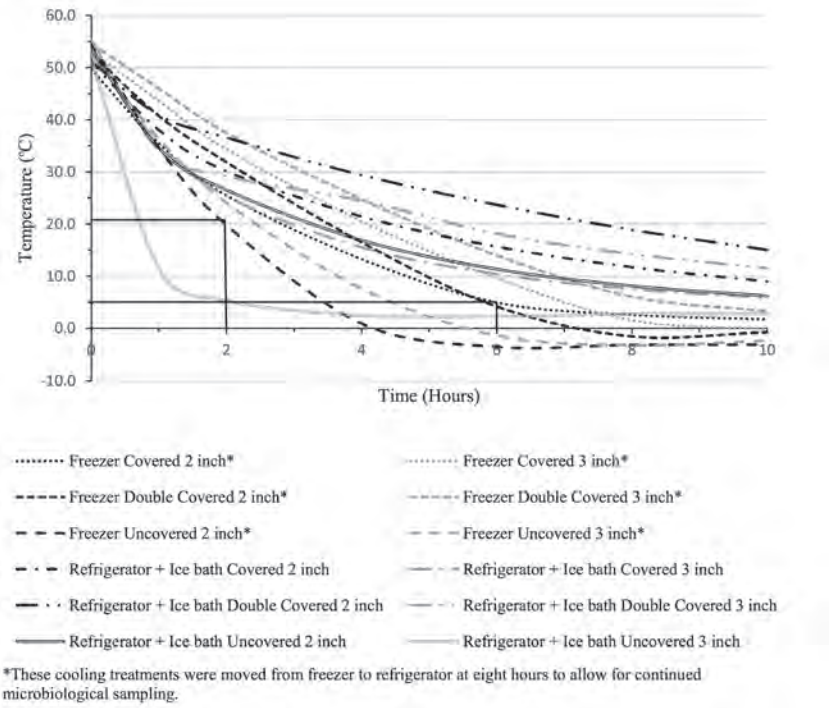

FIGURE 1. Cooling curves for all cooling technique combinations tested for pre-cooked taco meat. Black lines represent the two FDA Food Code time and temperature criteria.

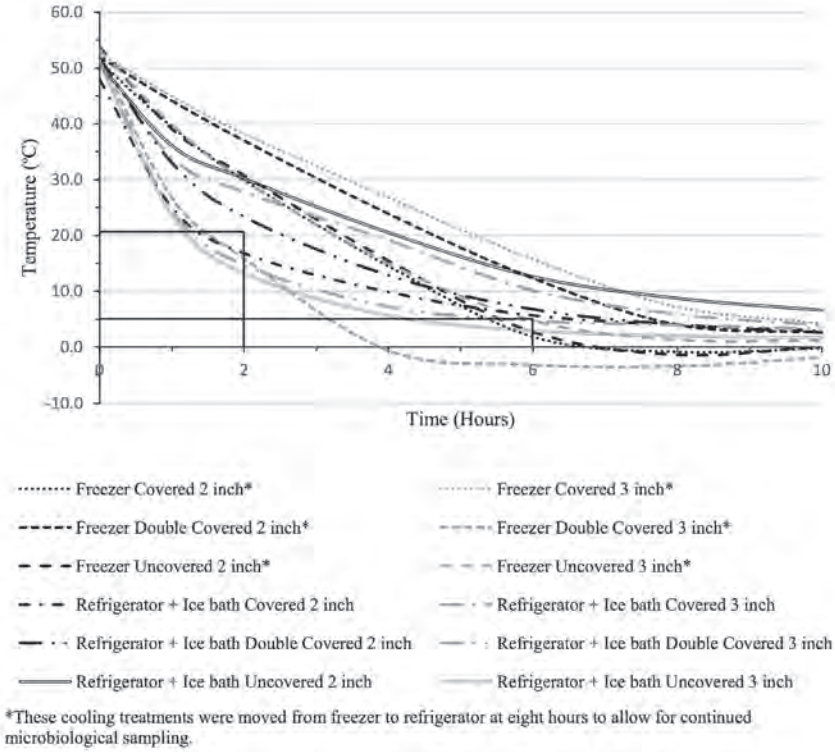

FIGURE 2. Cooling curves for all cooling technique combinations tested for chili con carne with beans. Black lines represent the two FDA Food Code time and temperature criteria.

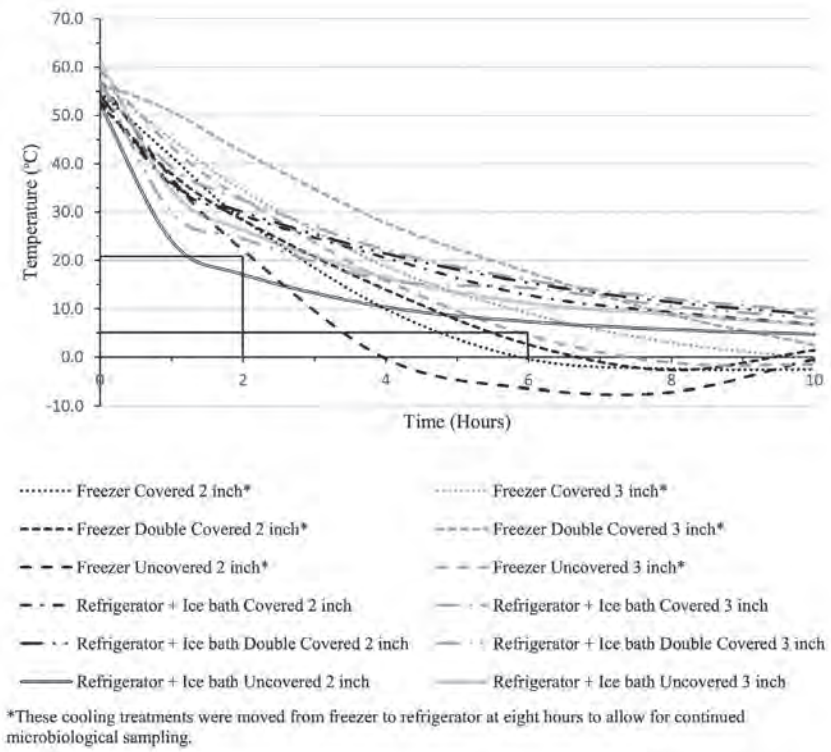

FIGURE 3. Cooling curves for all cooling technique combinations tested for low-sodium marinara sauce. Black lines represent the two FDA Food Code time and temperature criteria.

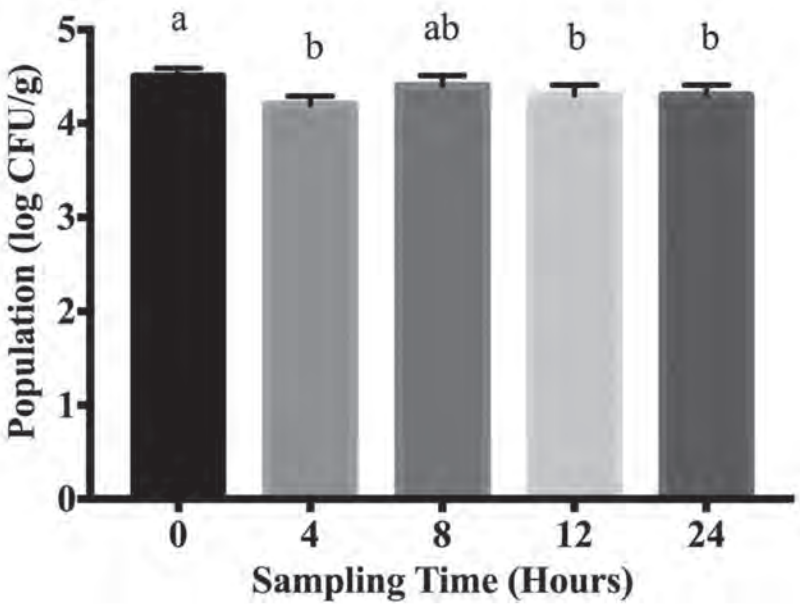

FIGURE 4. Surrogate *Escherichia coli* populations (log_{10} CFU/g) in pre-cooked taco meat analyzed by time. Time was the only significant variable ($P = 0.0022$). Therefore, data associated with all cover types, depth, and storage location are displayed as time alone. [a,b,c]Different superscripts indicate statistically significant differences. Error bars represent the standard error of the mean.

No cooling technique combinations achieved the 2017 FDA Food Code criteria *(28)* for cooling marinara sauce *(Fig. 3)*. Five cooling technique combinations satisfied either the 2 hour or 6 hour criteria, but not both 2017 FDA Food Code criterion *(28)* *(Table 1)*.

Microbiological data analysis

Time was the only factor that had a significant effect on the cooling of pre-cooked taco meat *(Fig. 4)*. No statistically significant difference in *E. coli* populations was observed for the cover type (two layers, one layer, uncovered), storage location (refrigerator vs. freezer), or product depth (2-inch vs. 3-inch) variables (data not shown), and there were no significant variable interactions. With regard to time, 0.15 $\log_{10}$ CFU/g was the largest increase in populations, occurring between the 4- and 8-hour time points *(Fig. 4)*.

The effect of time and the product depth by time interaction were significant for chili con carne with beans. Populations increased in the 2-inch product depths between 0 and 24 hours (0.11 $\log_{10}$ CFU/g), whereas they decreased in the 3-inch product depths between 0 and 24 hours (0.15 $\log_{10}$ CFU/g) *(Fig. 5)*. A statistically significant difference in *E. coli* populations was not detected for cover type (two layers, one layer, uncovered), storage location (refrigerator vs. freezer), or product depth (2-inch vs 3-inch) for this product (data not shown).

The main effects of product depth and time were statistically significant for low-sodium marinara sauce. The difference in *E. coli* populations between 2-inch (4.20 $\log_{10}$ CFU/g) and 3-inch (3.79 $\log_{10}$ CFU/g) product depths was 0.41 $\log_{10}$ CFU/g *(Fig. 6)*. With regard to time, 0.21 $\log_{10}$ CFU/g was the largest population increase, occurring between the 0- and 8-hour time points *(Fig. 7)*. No statistically significant differences in populations were observed for the cover (covered two layers, covered one layer, uncovered) or storage location (refrigerator vs. freezer) variables (data not shown), and no interaction combinations tested were significant.

DISCUSSION

All food products were inoculated with a 10^4–10^5 CFU/g concentration of *E. coli*. This concentration was chosen based on parameters described in a publication delineating parameters for microbial challenge studies *(14)*. As a study to monitor potential growth, a 10^4 to 10^5 CFU/g concentration was chosen to reflect a pre-stationary phase population. The inoculum concentration was higher than the suggested concentration of 10^2 to 10^3 CFU/g to ensure that the bacterial populations would remain detectable with use of the enumeration methods described herein. More specifically, this safeguards against (1) possible population declines at

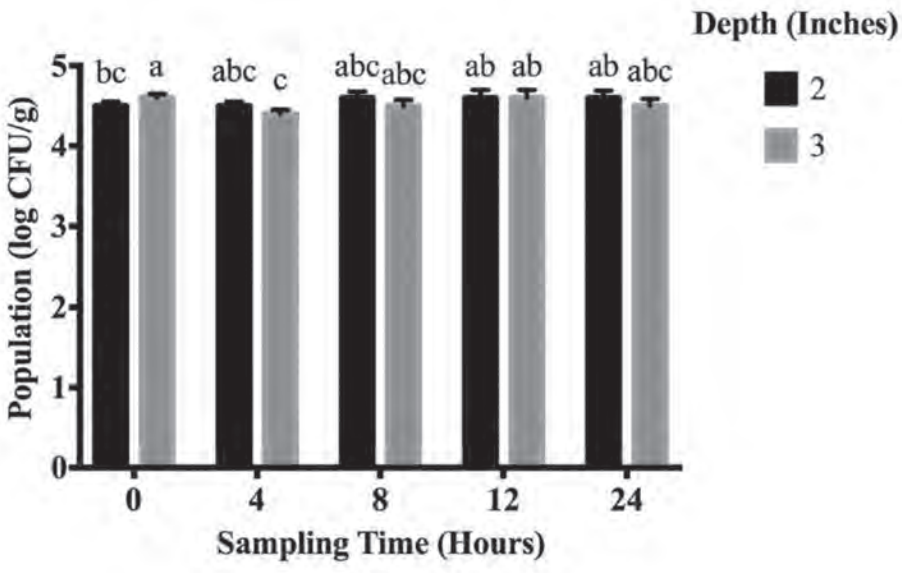

FIGURE 5. Surrogate *Escherichia coli* populations ($\log_{10}$ CFU/g) in chili con carne with beans analyzed by product depth and time. The depth by time interaction was significant ($P = 0.0197$). Therefore, data associated with all cover types and storage locations are displayed as product depth and time. Time was a significant variable ($P = 0.0015$), but data are not presented as time alone because of the depth by time interaction.
[a,b,c]Different superscripts indicate statistically significant differences.
Error bars represent the standard error of the mean.

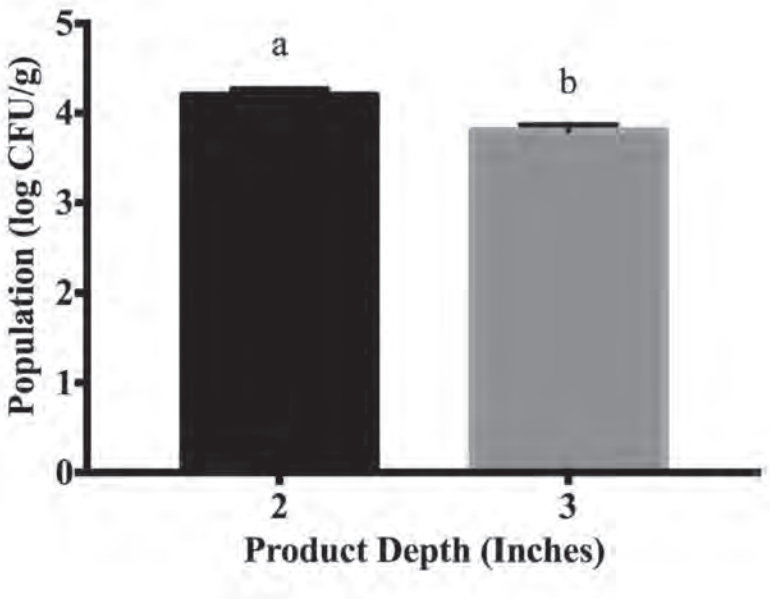

FIGURE 6. Surrogate *Escherichia coli* populations (log_{10} CFU/g) in low-sodium marinara sauce analyzed by product depth. Product depth was significant ($P < 0.0001$). Therefore, data from all time points associated with all cover types and storage location are displayed as depth alone.
[a,b,c]Different superscripts indicate statistically significant differences.
Error bars represent the standard error of the mean.

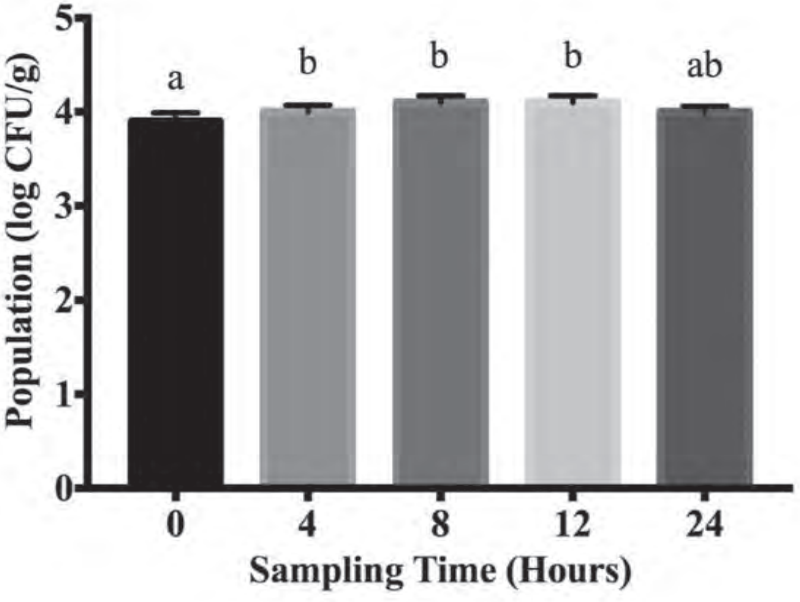

FIGURE 7. Surrogate *Escherichia coli* populations (log_{10} CFU/g) in low-sodium marinara sauce analyzed by time. Time was significant ($P = 0.0312$). Therefore, data associated with all cover types, depth, and storage location are displayed as time alone.
[a,b,c]Different superscripts indicate statistically significant differences.
Error bars represent the standard error of the mean.

inoculation (i.e., shock from temperature, pH, etc. of food products), and (2) population declines that may occur during the 24-hour cooling process.

Temperature data

Statistical analysis of the temperature data collected indicate that storage location, product depth, storage location by product depth, and cover type often had significant effects on the cooling of these food products. In general, the freezer cooled more consistently to lower temperatures, 2-inch product depths cooled more quickly than 3-inch product depths, and uncovered pans cooled most rapidly. Although differences were not statistically significant, it is noteworthy that in all three products, the 3-inch product depths stored in the freezer cooled less effectively in the first four hours than did the 3-inch product depths in the refrigerator with an ice bath. However, at 4 to 5 hours, the ice bath did not facilitate further cooling of the food product but held it at a steady temperature, which could have been caused by the ice of the ice-bath melting and reducing the removal of heat from the product. Conversely, the freezer continued to cool to a lower temperature at a stable rate. The refrigerator with ice bath cooling method was most effective for the first 4 hours of the cooling process, but the freezer cooled in a more controlled, predictable manner—and to lower temperatures—for the remainder of the cooling process.

In general, the temperature data results reflect conclusions similar to those of previously published research *(16, 17, 19)*. The present study identified several refrigerator and ice bath cooling combinations that achieved FDA Food Code criteria, which previous studies did not identify for pre-cooked taco meat and chili con carne with beans *(16, 17, 19)*. Previous researchers hypothesized that chili and taco meat products may be too dense for refrigerator and ice bath methods to effectively cool them to FDA Food Code criteria *(16, 17, 19)*. This may be due to the composition of the ice water baths, as this study utilized pans filled three-quarters full of ice with no water added. In contrast, other research *(19)* concluded that 2-inch product depths of tomato sauce cooled in the freezer met both cooling requirements of the FDA Food Code *(28)*, which was not consistent with the findings presented herein. In the present study, the freezer-cooled, uncovered, 2-inch product depth missed achieving the 2-hour criteria of the 2017 FDA Food Code; however, this technique satisfied the 6-hour time and temperature criteria.

The three main studies just compared evaluated the cooling of chili, meatless tomato sauce, beef taco meat, and steamed rice; in these studies, the freezer and refrigerator were not opened once the cooling process had begun *(16, 17, 19)*. In order to access the food products for microbiological sampling at the five time points for this study, the -20°C walk-in freezer and 4°C walk-in refrigerator were opened after the cooling process had begun. It must also be taken into consideration that food products went directly from heating to cooling in the two previous studies. In the present study, food products were cooled to 60°C ± 5°C, to facilitate inoculation at a temperature not lethal to the *E. coli* cells, before placement in the -20°C walk-in freezer and 4°C walk-in refrigerator *(16, 19)*. The four food products were also left uncovered in the previous cooling studies *(16, 19)*, which, based upon the data presented herein, likely influenced cooling and the differences between cooling results.

Microbiological data

The most significant decrease in *E. coli* populations present in taco meat occurred between time point 0 and 4 hours (0.31 $\log_{10}$ CFU/g), and overall, *E. coli* populations decreased 0.20 $\log_{10}$ CFU/g between time point 0 and 24 hours *(Fig. 4)*. Although statistically significant, this population decrease is not notable from a biological viewpoint. Thus, it should be considered that *E. coli* population variability within the taco meat, or variability introduced by the plating method itself, were responsible for this population decrease. This, combined with the lack of other main effects or interactions, demonstrates effective control of *E. coli* populations by the cooling methods evaluated.

Temperature data collected from the chili con carne with beans product indicate that product depth was significant in the first 4 hours of the cooling process, as 3-inch product depths cooled less rapidly and recorded a higher temperature than 2-inch product depths at the 4-hour time point. The retention of heat in 3-inch product depths of chili con carne with beans may have resulted in pockets of lethal (73.9°C) temperature, which led to a small, but significant, population decline (0.28 $\log_{10}$ CFU/g) during the first 4 hours of cooling *(Fig. 5)*. This statistically significant difference in population, as well as the others reported herein, were well under 0.5 $\log_{10}$ CFU/g; thus, a difference in population of this magnitude may have been the result of natural variation in populations throughout the food product or the result of variability introduced as a limitation of the plating method. These results, along with the lack of statistical differences of effects among cover type and storage location variables, indicate that the cooling methods evaluated were effective at controlling *E. coli* populations in chili con carne with beans.

With regard to the low-sodium marinara sauce, the *E. coli* population at 3-inch product depths was considered significantly lower than at 2-inch product depths *(Fig. 6)*, and temperature data also suggest that the effect of product depth was significant within the first four hours of cooling, with 3-inch product depths being significantly higher in temperature than 2-inch product depths. It must be considered that the 3-inch product depths may have facilitated the retention of pockets of lethal (73.9°C) temperature, which could have reduced the bacterial population at inoculation. Even though stirring took place to cool the product prior to inoculation, and again for approximately 2 minutes to

distribute inoculum, it is possible that the product did not cool evenly.

Though time had a statistically significant effect for marinara sauce, 0.21 $\log_{10}$ CFU/g was the largest increase in populations, occurring between the 0- and 8-hour time points. Natural variation of the *E. coli* population within the product, or variability introduced by plating methods, may have been more responsible than the cooling procedure for these population changes *(Fig. 7)*. It could be hypothesized that heat combined with acidity (pH 4.18; Education pH meter; Fisher Scientific, Lenexa, KS) injured the cells, causing them to lag initially and then recover over time. However, this is simply a hypothesis, as evaluating sublethal injury was beyond the scope of this study. Overall, these results indicate that all cooling method variables suppressed growth to the same degree, suggesting that all were effective at controlling *E. coli* populations in marinara sauce.

According to performance standards outlined in Annex 3, Section 3-501.19 of the 2017 FDA Food Code, hot foods held in the absence of temperature control should not exceed 1 $\log_{10}$ growth of *Clostridium perfringens* (*C. perfringens*) and *Bacillus cereus (28)*. A limitation of this study was the inability to model *Clostridium perfringens*, another foodborne pathogen associated with improper cooling, which is responsible for over 965,000 foodborne illnesses annually as well as 24 outbreaks in schools, colleges and universities between 1998 and 2016 *(4, 20)*. A review of the literature indicated that evidence of a proper surrogate to model *C. perfringens* under cooling conditions is lacking. The *FDA Safe Practices for Food Processes*, Chapter 6: Microbiological Challenge Testing, mentions *Clostridium sporogenes* as a surrogate for *Clostridium botulinum* but does not contain information regarding a surrogate for *C. perfringens (10)*. In order to effectively simulate food preparation and product cooling in a school setting, it was necessary to utilize commercial scale food preparation equipment, coolers, and freezers. These resources were not available for use with pathogenic microorganisms within the control of a biosafety level II (BSL II) laboratory. Therefore, it was not possible to model *C. perfringens*, a BSL II microorganism, in this study.

The *E. coli* strains chosen from the ATCC® MP-26™ panel have been investigated and found to be appropriate surrogates under cooling conditions not only for Shiga-toxin producing *E. coli* but also for another important enteric pathogen, *Salmonella enterica (11, 15)*. Therefore, the results of this research can provide insight into the behavior of several enteric pathogens under cooling conditions, which is advantageous, considering that *Salmonella* has been identified as one of the top pathogens implicated in foodborne illness outbreaks in school settings *(8, 29)*.

The 2017 FDA Food Code also states that the performance standard is no more than 1 $\log_{10}$ CFU/g growth of the non-sporeformer, *Listeria monocytogenes*, in cold foods held without temperature control *(28)*. Although the data presented in the current study represent the cooling of hot foods, it is relevant to mention that *E. coli*, also a non-sporeformer, exhibited growth (0.21 $\log_{10}$ CFU/g) in only one food product (low-sodium marinara sauce). Not only is this growth marginal, but it is also well below the 1 $\log_{10}$ CFU/g of *Listeria monocytogenes* growth allowable in cold food products.

SUMMARY

The methods used to cool food in this study provided equivalent microbiological control. Data suggest that all 12 cooling combinations tested can be utilized as an effective strategy for controlling *E. coli* populations in these food products, despite the inability of some cooling methods to meet 2017 FDA Food Code criteria *(28)* with regard to temperature. While microbiological data suggests that all methods effectively controlled populations of surrogate *E. coli*, it is recommended that the cooling techniques that satisfied 2017 FDA Food Code *(28)* requirements for cooling be preferentially used by school nutrition programs and other foodservice operations. As noted before, considering that the food products used in this study could be prepared, cooled, and served in a variety of foodservice operations, results are not limited to the school nutrition environment.

It must be emphasized that these data are limited in scope with regard to food products and microorganisms investigated. Furthermore, because generic *E. coli* cultures were used, these data merely provide an indication as to how *E. coli* O157:H7 might behave under simulated cooling conditions. More research is necessary, as this study is not exhaustive, and more combinations should be explored in future experimentation.

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