Evaluating the Impact of Cooling Techniques on Biosafety Level I *Escherichia coli* and *Bacillus cereus* Populations in Four Food Products

The Center of Excellence for
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Evaluating the Impact of Cooling Techniques on Biosafety Level I *Escherichia coli* and *Bacillus cereus* Populations in Four Food Products

Summary Report

The Center of Excellence for Food Safety Research in Child Nutrition Programs, Food Science Institute, and the Department of Animal Sciences and Industry

Kansas State University

Lindsay Beardall
M.S. Graduate Research Assistant
Animal Sciences and Industry

Dr. Paola Paez
Research Associate Professor
Center of Excellence for Food Safety Research in Child Nutrition Programs

Tracee Watkins
PhD Graduate Research Assistant
Center of Excellence for Food Safety Research in Child Nutrition Programs

Dr. Randall K. Phebus
Professor
Animal Sciences and Industry

Dr. Sara E. Gragg
Assistant Professor
Animal Sciences and Industry

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Executive Summary

School nutrition programs provide more than 31 million children with nutritious and safe meals in over 100,000 schools (U.S. Department of Agriculture Food and Nutrition Service, 2012). In school settings, certain foods may be cooked, cooled, and stored for later service. Therefore, proper food preparation practices are critical to preventing outbreaks among children, a high-risk population for severe illness and complications from foodborne illness. Time and temperature control have been identified as important foodborne pathogen control points in the United States Food and Drug Administration Food Code (U.S. Food and Drug Administration, 2013). Specifically, improper cooling has been identified as a contributing factor in some foodborne outbreaks associated with school nutrition programs (Pogostin et al., 2008; Venuto & Garcia, 2015), as improper cooling can lead to time and temperature parameters conducive for foodborne pathogen growth.

This study was designed to test the efficacy of cooling techniques used by school nutrition programs to control microbial growth. The study included the evaluation of cooling techniques for four food products, including pre-cooked taco meat, chili con carne with beans, low sodium marinara sauce, and brown rice. Food products were inoculated with a cocktail of four non-pathogenic American Type Culture Collection strains of *Escherichia coli*, surrogates to simulate behavior of Shiga Toxin-producing *Escherichia coli*, or a cocktail of two American Type Culture Collection strains of *Bacillus cereus*. Food products were then cooled in a walk-in refrigerator with an ice bath, or in a walk-in freezer, each with an assigned cover method. Food products were sampled over a 24-hour period (0-, 4-, 8-, 12-, and 24-hour time points) for enumeration of microbial populations.

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Data collected indicate that uncovered pans cooled more rapidly than those with a single layer of foil for air exposure or double layer of foil for no air exposure. However, cover method was not a statistically significant ($p>0.05$) variable for the microbial populations in any of the food products. For pre-cooked taco meat, chili con carne with beans, and brown rice, few cooling methods satisfied Food Code criteria by cooling from 57ºC (135ºF) to 21ºC (70°F) within two hours and to 5ºC (41°F) or below within a total of six hours (U.S. Food and Drug Administration, 2013). None of the methods tested on the low sodium marinara sauce product met both Food Code criteria for cooling.

The pre-cooked taco meat, chili con carne with beans, and low sodium marinara sauce products all exhibited a certain degree of microbial population decline over the 24-hour cooling period. The low sodium marinara sauce product was the exception, with a small recovery of bacteria observed over the cooling period; however, the difference in microbial populations was less than 0.50 $\log_{10}$ CFU/g. It is possible that these differences in observed populations were not the result of cooling failure or risk, but were due to non-uniform distribution of microorganisms and/or the variability of the nature of the food products. While some variable effects are statistically significant, they do not represent microbiological significance; this suggests all 12 cooling treatment combinations tested were low risk and effective at controlling microbial populations.

Our recommendations for rapid cooling based on these results include the following: when possible, leave pans uncovered or cover loosely with just one layer of aluminum foil or plastic food wrap, replacing or removing ice baths after several hours of cooling, and cooling foods at 2-inch product depths in the freezer. For microbiological control, the results indicate that variations observed in population for the cooling variables tested were less than 0.5 $\log_{10}$ CFU/g.

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Therefore, it is concluded that all 12 cooling combinations tested were effective at controlling microbial populations in the food products despite the inability of some cooling methods to meet Food Code (U.S. Food and Drug Administration, 2013) criteria with regard to temperature. Cooling food rapidly is a significant step toward preventing proliferation of microbial populations. However, these results suggest that despite the inability to achieve time and temperature limit criteria, none of the cooling methods tested outperformed the others with regards to control of microbial populations. Therefore, it is acceptable to allow a certain degree of flexibility with time and temperature limits.

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Background

With the use of biological safety level I (BSL I) organisms, two foodborne pathogens were simulated in this research study, including *Bacillus cereus* (*B. cereus*) and Shiga toxin-producing *Escherichia coli* (STEC) (i.e. *Escherichia coli (E. coli)* O157:H7). *B. cereus* causes more than 63,000 illnesses annually, and 100% of these illnesses are foodborne in origin (Scallan et al., 2011). Spores of *B. cereus* are ubiquitous in the environment and may reside at low populations in certain food products such as uncooked rice. Spores lie dormant in food products and can survive the cooking process. Once the food product is cooled, spores may germinate and outgrow in the temperature "danger zone" of 5-57.2°C (41-135°F). When compared to other pathogens, *B. cereus* generally causes mild and self-limiting symptoms of diarrhea or vomiting, contributing to the fact that it is a markedly underreported foodborne illness (Granum & Lindbak, 2013; Granum, 2007; Bottone, 2010; Arnesen, Fagerlund, & Granum, 2008). Rice is a dish served in schools and daycares that has been implicated in several emetic-type *B. cereus* foodborne outbreaks in the United States (Center for Disease Control and Prevention (CDC), 1994; Gilbert, Stringer, & Peace, 1974; Mortimer & McCann., 1974).

Exposure to *E. coli* O157:H7 results in a similar annual number of illnesses as *B. cereus*; however, the symptoms are often more severe, with 46% of cases resulting in hospitalization and a small number of cases resulting in death (Scallan et al., 2011). Exposure to *E. coli* O157:H7 is a critical concern for young children (age 1-9 years), as they experience a higher infection rate than adults and have an increased likelihood of developing chronic sequelae like hemolytic uremic syndrome (HUS) (Buzby, 2001), which can lead to kidney failure. In fact, the World Health Organization (WHO) has identified HUS as the most common cause of acute renal failure

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in children (Tserenpunstag, Chan, Smith, & Morse, 2005; WHO, 2016). STEC infections, like *E. coli* O157:H7, are most commonly contracted via the fecal-oral route, often by consuming contaminated food or water (Croxen et al., 2013; Lim, Yoon, & Hovde, 2010). Beef, leafy vegetables, and other produce products have been associated with outbreaks of *E. coli* O157:H7 (CDC, 1993; Heiman, Mody, Johnson, Griffin, & Gould, 2015; Rangel, Sparlin, Crowe, Griffin, & Swerdlow, 2005). Improper hygiene and cross contamination are two ways *E. coli* O157:H7 may contaminate food products after they have been cooked; infectious food handlers are often implicated in outbreaks of gastrointestinal foodborne illness in school settings (Venuto & Garcia, 2015; Daniels et al., 2002).

Clostridium perfringens (*C. perfringens*) is another foodborne pathogen associated with improper cooling and is responsible for over 965,000 foodborne illnesses annually (Scallan et al., 2011). One study provided evidence that prolonged cooling (taking longer than 15 hours to reach 7.2°C or 44.96°F) could result in 4-5 $\log_{10}$ CFU/g growth of *C. perfringens* within a cooked ground beef product (Juneja, Snyder, & Cygnarowicz-Provost, 1994). Another study demonstrated that *C. perfringens* in a chili product was also able to grow if cooling of the product to 4.4°C (39.92°F) took longer than 2 hours (Blankenship, Craven, Leffler, & Custer, 1988). However, review of the literature indicates that evidence of a proper surrogate to model *C. perfringens* under cooling conditions is lacking. In the Food and Drug Administration (FDA) Safe Practices for Food Processes, Chapter 6: Microbiological Challenge Testing, *Clostridium sporogenes* is mentioned as a surrogate for *Clostridium botulinum*, but does not contain information regarding a surrogate for *C. perfringens* (U.S. FDA, 2003). 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

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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 research study.

When food is cooked in mass quantities, as it is in school settings, large outbreaks of foodborne illness can occur (Gould et al., 2013; Levine, Smart, Archer, Bean, & Tauxe, 1991; Matsui et al., 2004; Michino et al., 1999). Foodborne outbreak data collected during the years 1998-2008 revealed that schools were the institutional setting associated with the largest number of outbreaks (286) and illnesses (17,266) when compared to other institutions such as daycares, workplace cafeterias, and prisons or jails (Gould et al., 2013). Large outbreaks may be due, in part, to the fact that The National School Lunch Program (NLSP) serves over 31 million children each day in the United States (United States Department of Agriculture (USDA) Food and Nutrition Service, 2013). However, outbreaks in this population are of particular concern, as the severity of foodborne illness and frequency of complications in young children have been well documented (Buzby, 2001; Tserenpuntsag et al., 2005; WHO, 2016). An underdeveloped immune system and a low body weight contribute to the increased susceptibility of young children (Buzby, 2001). The large population of children served at school, combined with the classification of children as an at-risk population, makes proper food preparation practices especially critical in a school lunch setting.

Improper cooling is considered a proliferation risk factor by the CDC, meaning improper cooling can lead to microbial growth, including pathogens, in food products (CDC, 2016). Similarly, the FDA has consistently identified time/temperature control as a critical control point for preventing foodborne illness (U.S. FDA, 2000; U.S. FDA, 2004; U.S. FDA, 2009). Cooling is an integral part of the food preparation process for school nutrition programs, and 78% of

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school foodservice managers report cooling leftovers to reheat for service at another meal (Krishnamurthy & Sneed, 2011). Improper cooling has been identified as one of the contributing factors to foodborne outbreaks in schools (Pogostin, et al., 2008; Venuto et al., 2015). One way to identify and prevent high-risk food preparation practices is to utilize the principles of Hazard Analysis and Critical Control Point (HACCP). Accordingly, an important aspect of The Child Nutrition and WIC Reauthorization Act of 2004 was the requirement that schools utilize food safety programs based on HACCP principles (USDA Food and Nutrition Service, 2005). FDA also instituted an update to the Food Code in 2005 to address the risks of cooling, that requires cooked food products to be cooled from 57ºC (135ºF) to 21ºC (70°F) within two hours and 5ºC (41°F) or below within a total of six hours (U.S. FDA, 2015).

To meet these cooling criteria, the FDA has suggested certain methods to effectively cool food products to the target temperatures in the required amount of time, such as storing food in shallow pans, in smaller or thinner portions, and using rapid cooling equipment (U.S. FDA, 2015). Appendix A provides references to sections 3-501.14 and 3-501.15 of Chapter 3 of the FDA Food Code that delineate time and temperature criteria and suggested cooling methods.

Previous studies, including research conducted by the Center of Excellence, provided the information necessary to design this experiment to accurately reflect school nutrition programs practices and relevant food products (Krishnamurthy et al., 2011; Olds & Sneed, 2005; Olds, Roberts, Sauer, Sneed, & Shanklin, 2013; Roberts, Olds, Shanklin, Sauer, & Sneed, 2013). These studies were conducted to provide evidence of effective cooling methods that could be utilized in foodservice operations and included a focus on food products served by school nutrition programs. The cooling of food products with several methods including refrigerators, ice baths, freezers, and blast chillers was evaluated (Krishnamurthy et al., 2011; Olds & Sneed,

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2005; Olds, Roberts, Sauer, Sneed, & Shanklin, 2013; Roberts, Olds, Shanklin, Sauer, & Sneed, 2013).

Food products were chosen based on those most commonly served in school nutrition programs, which were identified via survey of school foodservice managers, and included taco meat filling, chili, spaghetti sauce, and rice (Krishnamurthy et al., 2011). These food products were also considered to be among those that could be incorporated as ingredients in multiple dishes and would, therefore, have an increased likelihood of leftovers being cooled and served at a later time. Olds et al. (2005) concluded that the blast chiller, a form of rapid cooling equipment, was one of the few cooling methods that produces results that meet FDA Food Code criteria. However, blast chillers represent a significant financial investment and only 8% of schools nationwide own and use them (Olds et al., 2005; Krishnamurthy et al., 2011). Another common barrier is a lack of adequate freezer space, with schools reporting 20% free or open space for cold storage (Roberts et al., 2013). As reported by Roberts et al. (2013), few cooling techniques meet the criteria of the 2013 FDA Food Code. Thus, the focus of this research study was to scientifically characterize cooling methods that are both feasible and effective at preventing microbial growth in meals prepared in school nutrition program settings, as it is critical to public health.

Objectives

1) Evaluate common cooling methods and their effect on microbial populations over a 24-hour period in four food products commonly served by school nutrition programs.

2) Develop recommendations for school nutrition personnel regarding best cooling practices for preventing foodborne illness.

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Materials and Methods

Bacterial Strains

E. Coli

Four *E. coli* strains were chosen from the American Type Culture Collection® (ATCC) Non-pathogenic *Escherichia coli* Surrogate Indicators Panel (ATCC® MP-26™) to serve as surrogates for Shiga Toxin-producing *E. coli* (STEC). The four strains were utilized in a cocktail that included ATCC® BAA-1427, BAA-1429, BAA-1430, and BAA-1431. All four strains were originally isolated from cattle hides, and each are recommended by the United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) for use in research when evaluating changes in microbial populations in the food processing environments during validation studies (ATCC®, 2015). *E. coli* was evaluated in pre-cooked taco meat, low sodium marinara sauce, and chili con carne with beans to serve several research purposes. First, the *E. coli* strains chosen from the ATCC® MP-26™ panel have been investigated and found to be appropriate surrogates under cooling conditions for Shiga-toxin producing *E. coli*, and are also an appropriate surrogate for another significant enteric pathogen, *Salmonella eneterica* (Keeling et al., 2009; Niehbuhr et al., 2008). Therefore, the results of this research can provide insight into the behavior of several enteric pathogens under cooling conditions, which is advantageous considering *Salmonella* has been identified as one of the top pathogens implicated in foodborne illness outbreaks in school settings (Daniels et al., 2002; Venuto et al., 2015). Second, proper hand hygiene and cross contamination in food preparation environments, including school settings, continue to be contributing factors in outbreaks of foodborne illness (Daniels et al., 2002; Jackson et al., 2000;

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Lee et al., 2010; Rangel et al. 2005). This research takes into consideration that, although food preparation in many school settings may not include handling raw meat products, it is still necessary to evaluate pathogens introduced in food products through cross contamination or poor hand hygiene, which may occur following cooking.

Developing and Assessing Acid Tolerance:

The first repetition with the marinara sauce product revealed lower than expected survival rates of surrogate *E. coli*; something not observed with the other two food products (taco meat and chili con carne). A 5-$\log_{10}$ CFU/g inoculum of *E. coli* surrogate cocktail was prepared using methods described in the Inoculation Procedure section of this paper, however, colony enumeration from time point testing revealed inconsistent results over a 24-hour period ranging from poor to no surrogate survival from the first experimental repetition of the marinara sauce product. Therefore, a hypothesis was developed: if the *E. coli* surrogates could not survive as effectively at low pH like *E. coli* O157:H7 then there would be a lower than expected rate of survival in the marinara sauce product. The level of acidity was hypothesized to have a negative impact on surrogate survival in the food product. The first step to assess this hypothesis involved utilizing a benchtop pH meter (Education pH meter; Fisher Scientific, Lenexa, KS) to accurately measure the acidity of the marinara sauce. Once the meter was calibrated, the pH of the uninoculated, room temperature sauce product measured 4.18. To further evaluate these hypotheses, preliminary testing was performed by conducting a small study to compare the survival of three microorganisms in the marinara sauce product: *E. coli* surrogate cocktail, *Salmonella enterica subsp. enterica* serovar Typhimurium (ATCC® 14028), and *Escherichia coli* O157:H7. Each microorganism was prepared in two different growth mediums for inoculum: TSB + 1% glucose and Buffered Peptone Water (BPW; BD Difco™ Fischer Scientific, Franklin

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Lakes, NJ). It was hypothesized that the TSB + 1% glucose (Fisher Scientific, Lenexa, KS) would foster an increased acid tolerance after incubation for 18 hours, a hypothesis previously tested by Buchanan, et. al. in 1996.

Acid Habituation Preliminary Study Results

Salmonella enterica subsp. enterica serovar Typhimurium (ATCC® 14028), *E. coli* O157:H7, and the cocktail of four ATCC® *E. coli* surrogates were each grown for 24 hours at 37°C (98.6°F). Each microorganism was grown in both TSB + 1% glucose and BPW. Six 500 - mL glass bottles were each filled with 100 mL of marinara sauce after it had been heated to 73.8°C (165°F) in a commercial tilt skillet (Cleveland Tilt Skillet). The sauce was allowed to cool to 60°C ± 5°C (140°F ± 5°F), at which time, 1 mL of inoculum was added to each bottle of sauce to achieve a 5-$\log_{10}$ CFU/g distribution of each pathogen grown in each medium. Samples were obtained at time points 0, 4, and 8 hours. 25 gram samples were diluted with 225 mL BPW and serially diluted and plated on MacConkey Agar (MAC; Remel, Lenexa, KS). MAC plates were incubated at 37°C (98.6°F) for 18-24 hours at which point colonies were enumerated.

The pH of the ATCC® *E. coli* surrogate cocktail grown in TSB + 1% glucose was 4.68. During the 8 hour cooling period, the ATCC® *E. coli* surrogate cocktail inoculum grown in TSB + 1% glucose provided increased population survival of 0.23 $\log_{10}$ CFU/g over the ATCC® *E. coli* surrogate cocktail grown in BPW. *Salmonella* serovar Typhimurium survival was improved when grown in TSB + 1% glucose by an average of 1.56 $\log_{10}$ CFU/g compared to survival when grown in BPW. *Escherichia coli* O157:H7 survival was nearly identical when grown in TSB + 1% glucose as in BPW, with only 0.07 $\log_{10}$ CFU/g difference. This acid habituation method was chosen to prepare inoculum for the marinara sauce product because of the moderately improved survival of the ATCC® *Escherichia coli* surrogate cocktail when grown in TSB + 1% glucose.

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Bacillus cereus

Two *B. cereus* strains of BSL I status were utilized in a cocktail including ATCC® 11778 and ATCC®14579. Both isolates were originally obtained from air samples taken within a cow shed (Frankland & Frankland, 1887). *B. cereus* was selected for evaluation in brown rice because it is commonly associated with starchy foods and has been implicated in outbreaks of foodborne illness in schools. Although vegetative cells should be killed during the cooking process at a temperature of 73.89°C (165°F), spores can survive and then germinate and outgrow under improper cooling conditions. Therefore, *B. cereus* spores were harvested for the inoculum of the cooked brown rice product to mimic the death of vegetative cells and the survival of spores after the cooking process.

As discussed in the Background section, this research study was unable to model another sporeformer, *C. perfringens*, due to the lack of evidence in the literature regarding a proper surrogate and inaccessibility to cooking and cooling facilities with a BSL II designation.

Harvesting Bacillus cereus Spores

In order to harvest *B. cereus* spores for inoculum preparation, a procedure outlined by Grande et al. (2006) was performed. Briefly, each ATCC® strain (11778 and 14579) was grown in Brain Heart Infusion Broth (BHI; Fisher Scientific, Lenexa, KS) at 30°C (86°F) for 24 hours, plated on Nutrient Agar supplemented with 0.05 g/l Manganese sulfate ($MnSO_4$), and incubated for four days at 37°C (98.6°F) in order to obtain spores from 90-95% of cells (Grande et al., 2006). Spores and vegetative cells were harvested from the plates using a sterile, cotton-tipped applicator pre-moistened with sterile distilled water, deposited in sterile distilled water to create

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spore and vegetative cell suspensions that were dispensed into 5 mL amounts in 15 mL conical tubes (MIDSCI, St. Louis, MO), and stored at -20°C until later use.

Preliminary Testing with Bacillus cereus Spores

Enumerating Vegetative Cells and *Bacillus cereus* Spores: The procedure for preparing the spore inoculum on the day of inoculation included a 10-minute sub-lethal heat shock of 80°C (176°F) in order to inactivate vegetative cells. Spores, however, would still be active; generally, temperatures greater than 100°C (212°F) are considered lethal or necessary to inactivate spores (Leguérinel, Spegagne, Couvert, Gaillard, & Mafart, 2005; Byrne, Dunne, & Bolton, 2006; El-Nour & Hammad, 2013). This process is designed to mimic the cooking process of foods at 73.89°C (165°F) and the subsequent survival of spores that may germinate and outgrow under improper cooling conditions. Therefore, to verify the population of the vegetative cells and spores harvested for use in this research study, preliminary enumeration testing was performed. A frozen spore and vegetative cell suspension in distilled water was thawed to room temperature (20°C or 68°F). A 1 mL aliquot was serially diluted in buffered peptone water (BPW; Fisher Scientific, Lenexa, KS) and plated on Mannitol Egg Yolk Polymyxin (MYP; Remel, Lenexa, KS) agar plates.

The suspension was then heat shocked at 80°C (176°F) for 10 minutes to simulate inoculum preparation, which was designed to mimic the cooking process. A 1 mL aliquot of the heat-shocked suspension was then serially diluted in BPW and plated on MYP agar plates. All MYP agar plates were then incubated at 30°C (86°F) for 24-48 hours. The population pre-heat shock was determined to be 8.06 $\log_{10}$ CFU/mL. The population post-heat shock was determined to be 8.63 $\log_{10}$ CFU/mL. This was considered to be primarily spores, as vegetative cells are

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sensitive to heat treatment. The lack of immediate cooling in this study may have allowed for spore germination and outgrowth to occur, thus contributing to an increase in population post-heat shock. The immediate cooling of heated suspensions on ice has been suggested in some research in order to stabilize spores and prevent germination (Grande et al., 2006; Stalheim & Granum, 2001).

A phase contrast microscope was utilized to visually confirm vegetative cell and spore changes pre- and post-heat shock. Under 100x magnification of the phase contrast microscope, large populations of both vegetative cells and spores were observed pre-heat shock. Post-heat shock, few vegetative cells were observed, but a large population of spores was visually apparent (Figure 1).
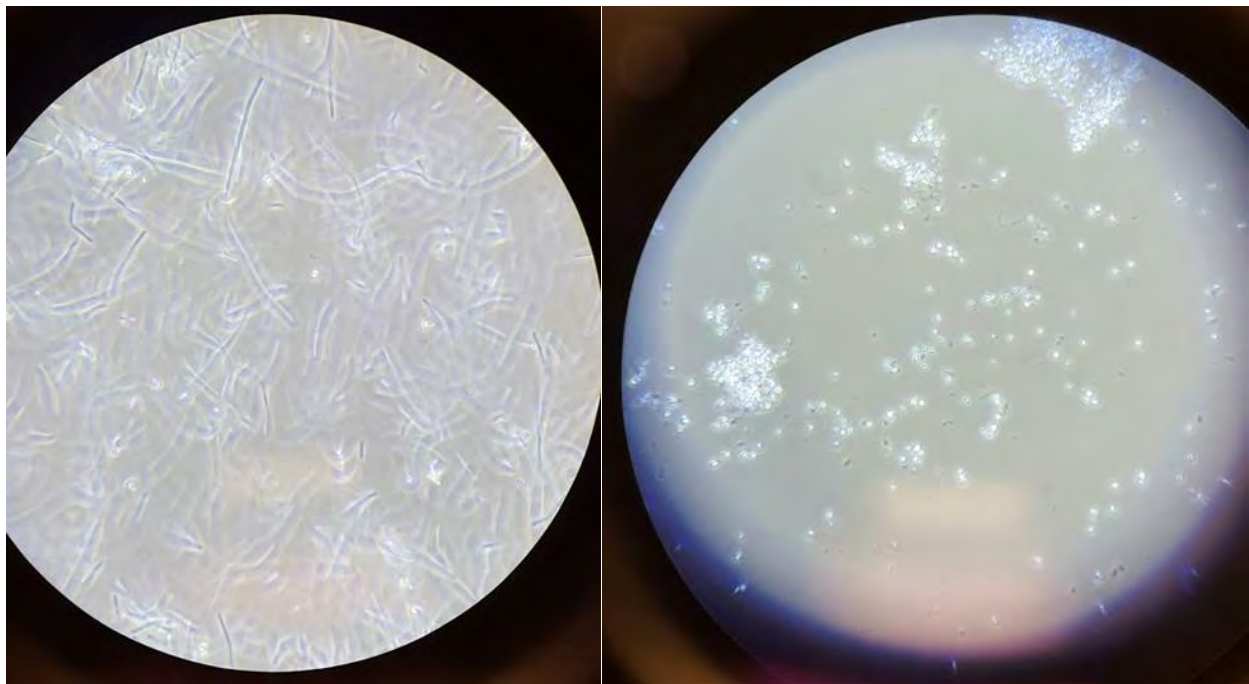
Left to right: Pre-heat shock suspension containing spores and numerous vegetative cells (rods), post-heat shock suspension contains numerous spores (cocci).

Figure 1: *Bacillus cereus* **Spores and Vegetative Cells Pre- and Post-Heat Shock Viewed Under 100x Magnification of a Phase Contrast Microscope**

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Verifying Bacillus cereus Spore Heat Shock and Subsequent Outgrowth in Brown Rice: The methods described herein are designed to simulate the behavior of spores subjected to heat treatment during the cooking process and subsequent cooling of a brown rice product. As product inoculation occurred following cooking, it became critical to demonstrate that the heat shocking procedure effectively simulated the cooking process with regards to *B. cereus* spore germination and outgrowth. Therefore, a preliminary test was performed to monitor the population of spores vs. vegetative cells within the brown rice product. A 2- and 3-inch product depth of brown rice was prepared according to the procedures outlined in the Product Preparation section, allowed to cool to 60°C ±5°C (140 ± 9°F), and then inoculated with a post-heat shock inoculum (prepared as described in the section below) of 10^5 CFU/mL spores.

Over a 3-hour period, the 2- and 3-inch product depths of brown rice were stored in a walk-in refrigerator (4°C or 39.2°F) and 25 g samples were collected each hour. Sampling procedures were identical to those in the Sampling section, with dilutions plated on MYP agar that were then incubated at 30°C (86°F) for 24-48 hours. Enumeration from MYP agar plates revealed no substantial population changes over the 3-hour cooling period for both product depths. However, from the BPW of diluted rice samples at each hour, an endospore stain was performed using the Schaeffer-Fulton method (Hussey & Zayaitz, 2007) to visualize the ratio of spores to vegetative cells during the 3-hour period.

Briefly, a slide was prepared by air drying and heat fixing a loopful or smear of suspension, and an initial stain with malachite green (Acros Organics™ from Fisher Scientific, Lenexa, KS) was applied and the slide was heated for 5 minutes. The slide was then rinsed and counterstained with safranin (Fisher Scientific, Lenexa, KS) for 30 seconds and then rinsed for a final time. Endospores appeared green and vegetative cells appeared red. The resulting

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endospore stains were observed under 100x magnification of a compound light microscope. The endospore stain from time 0 hour revealed a large spore population and few vegetative cells. The endospore stains from time 1, 2, and 3 hours revealed a decreasing spore population and a slight increase in vegetative cell population (data not shown).

The results from this preliminary testing indicate the sub-lethal heat shock of 80°C (176°F) in the laboratory successfully mimicked the cooking process to 73.89°C (165°F). Based on these results, it was concluded that the target population of 10^5 CFU/mL of heat-shocked *B. cereus* spores was achieved on the day the brown rice would be inoculated. This spore population would then have the ability to germinate during the cooling process and outgrow if cooling was ineffective.

Inoculum Preparation

The day prior to inoculation of pre-cooked taco meat and chili products, a microcentrifuge tube of each frozen ATCC® *Escherichia coli* strain (BAA 1427, BAA 1429, BAA 1430, BAA 1431) was thawed to room temperature (20°C or 68°F) and grown separately in four 50 mL centrifuge tubes with 25 mL of BPW. These cultures were incubated at 37°C (98.6°F) for 18-24 hours. For the marinara sauce product, each ATCC® *E. coli* surrogate was grown separately at 37°C (98.6°F) for 18-24 hours in 25 mL of TSB with 1% glucose in order to prepare acid-adapted cultures, per Buchanan and Edelson (1996). The following day, the 25mL culture tubes were centrifuged at 5,000 x *gram* for 15 minutes at 4°C (39.2°F). The supernatant was discarded, and the pellets were then 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 100 mL of cocktail. This 100 mL cocktail in PW was

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estimated to be 10^9 CFU/mL and was utilized to prepare inoculum for all samples. To achieve a target concentration of 10^4 CFU/g, inoculum for each pan was prepared 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 (National Advisory Committee on Microbiological Criteria for Foods, 2010).

On the day of inoculation of the brown rice food product, six conical tubes of 5 mL frozen spore suspension were removed and allowed to completely thaw for 45-60 minutes at room temperature (20°C or 68°F). The thawed tubes were then placed in an 80°C (176°F) bead bath and heat shocked for 10 minutes in order to simulate the cooking process and subsequent sub-lethal, heat-induced germination of spores. After the spore suspensions were allowed to cool to room temperature, tubes were thoroughly vortexed and inoculum was prepared from these tubes of 10^5-10^6 spores/mL suspensions. Inoculum for each pan was prepared by diluting the spore suspensions in 0.1% PW—based on the weight of food product in each pan—to achieve 10^4-10^5 spores/g at inoculation, such that the inoculum comprised no more than 1% of the total food product (National Advisory Committee on Microbiological Criteria for Foods, 2010).

All food products were inoculated with a 10^4-10^5 CFU/g concentration of *E. coli* vegetative cells or *B. cereus* heat-shocked spores. This concentration was chosen based on parameters set in a publication delineating parameters for microbial challenge studies (National Advisory Committee on Microbiological Criteria for Foods, 2010). As a study to monitor potential growth, a 10^4-10^5 CFU/g concentration was chosen to reflect a pre-stationary phase population. The inoculum concentration was higher than the suggested concentration of 10^2-10^3 $\log_{10}$ CFU/g to ensure the bacterial populations would remain detectable using the enumeration methods described herein. More specifically, this safeguards against 1) possible population

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

Food Product Preparation

All food products met the nutritional standards for Child Nutrition Programs (Appendices B-E). Food products and ingredients were ordered from a foodservice distributor. Pre-cooked, frozen taco meat was stored in a commercial refrigerator at 4°C (39.2°F) for several days prior to an experimental replication in order to thaw properly for reheating. On day 0 of the study, five-pound bags were placed in 2 ¼-inch steam table pans and heated in commercial steamers (Electrolux Air-o-Steam Touchline Combi Oven and Cleveland SteamChef Electric Countertop Steamer) to 18.33°C (165°F). Canned, low sodium marinara sauce was cooked to 18.33°C (165°F) in a commercial tilt skillet (Cleveland Tilt Skillet). Chili was prepared according to a recipe used by a school nutrition program in the Kansas City area (Appendix F) and was cooked to 18.33°C (165°F) in the same commercial tilt skillet. For the brown rice product, water was heated to 87.77°C (190°F) in the commercial tilt skillet and was then added to uncooked brown rice measured in 2 ½- and 4-inch counter pans. The ratio of water to uncooked rice was based upon product label instructions. Pans were then covered with a layer of plastic wrap and a layer of aluminum foil and placed in a commercial-grade convection oven (Garland Master 200) at 176.66°C (350°F) for 35 minutes. After the food products were reheated or cooked, they were then portioned to 2- and 3-inch food product depths in 2½- and 4-inch deep counter pans. The product was stirred and allowed to cool to 60°C ± 5°C (140°F ± 9°F) for inoculation.

Food Product Inoculation

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The temperature of all products was monitored using a Taylor 9842FDA waterproof digital thermometer and all food products were stirred and allowed to cool to 60°C ± 5°C (140°F ± 9°F) prior to inoculation. After liquid inoculum was added to each pan (Figure 2), food was stirred approximately 2 minutes per pan to obtain an even distribution of bacterial cells or heat-shocked spores. Inoculation times were recorded for each pan upon completion of stirring and time points of 0, 4, 8, 12, and 24 hours were set accordingly. Directly after inoculation, a time point 0-hour composite sample was collected before the pans were covered. Additional details regarding sampling can be found in the section entitled Sampling.

Top left, following arrows: Food products were prepared with convection ovens, steamers, or tilt skillet; food products were then portioned to 2 and 3-inch product depths and allowed to cool to 60°C ± 5°C (140°F ± 5°F); pans were then inoculated and stirred thoroughly for ~2 minutes; time point 0-hour composite samples were collected.

Figure 2. Food Preparation, Inoculation, and Initial Sampling

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Sampling

   After inoculation, samples were collected from each pan at five time points: 0, 4, 8, 12, and 24 hours. At each time point, a representative composite sample was obtained by using a spoon to gather food from four to five different areas within each pan. These sampling points were randomly selected and the food was taken from under the food surface in order to collect from the interior of the pan where the food was likely the warmest and, therefore, most at risk for microbial growth. This composite sample was homogenized by hand mixing, after which a 25 g sample was removed and deposited in a sterile stomacher bag for further testing. This 25 g sample was then diluted 1:10 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, after which -2, -3, and -4 dilutions (Figure 3) were spread plated onto MacConkey (MAC; Remel, Lenexa, KS) agar and MYP to enumerate *E. coli* and *B. cereus* populations, respectively. The MAC plates were incubated at 37°C (98.6°F) for 18-24 hours while the MYP plates were incubated at 30°C (86°F) for 24-48 hours. After respective incubation times, colonies on plates were counted, multiplied by the appropriate dilution factor, and log transformed to generate a $\log_{10}$ CFU/g value for each sample at each time point.

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 in the center of the pan to track the temperature of the food product every 60 seconds for the next 24 hours. To ensure the probe of the data logger was centered, a placement system consisting of a ruler, binder clips, and a straw was used (Figure 4).

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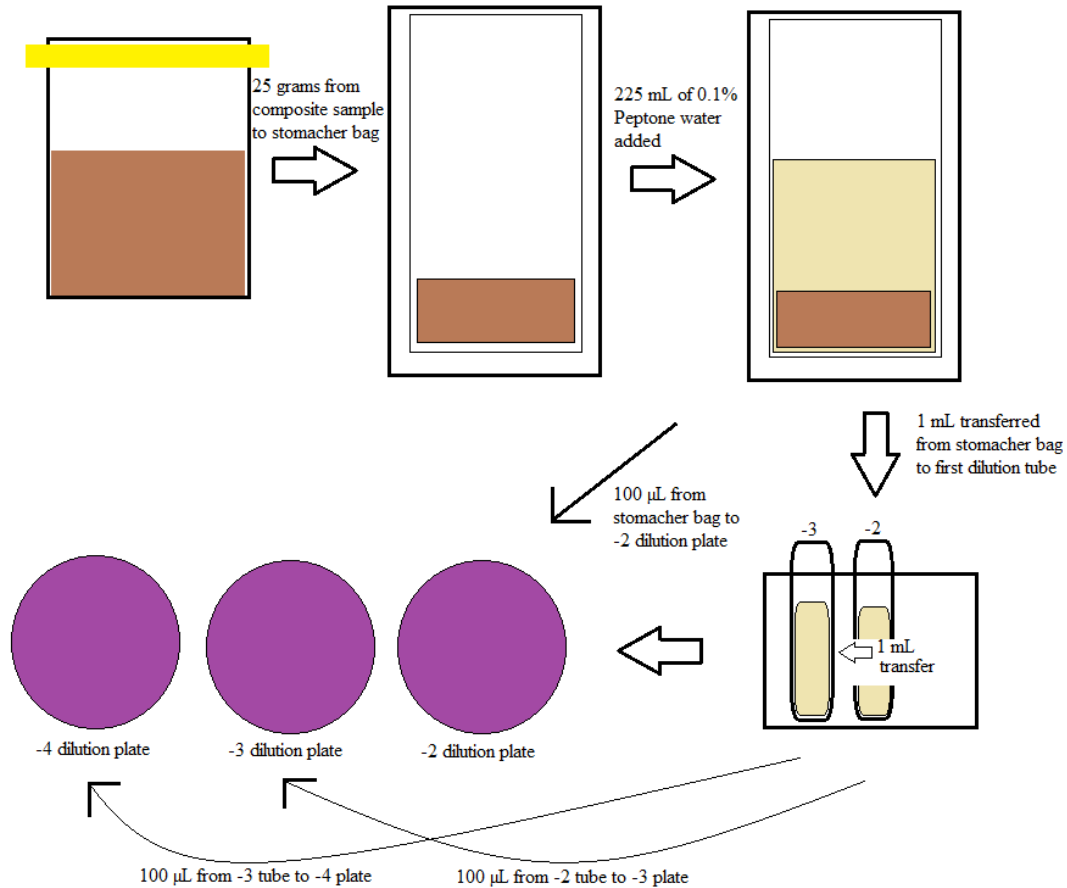
Top left, following arrows: 25 g from composite sample transferred to a stomacher bag, 225 mL of 0.1% Peptone water added to the stomacher bag and homogenized, 1 mL transferred from stomacher bag to first dilution tube, 1 mL transferred from first dilution tube to second, 100 µL from stomacher bag plated as -2 dilution plate, 100 µL from -2 dilution tube is plated on -3 dilution plate, and 100 µL from -3 dilution tube is plated on -4 dilution plate.

Figure 3. Sample Dilution and Spread Plating

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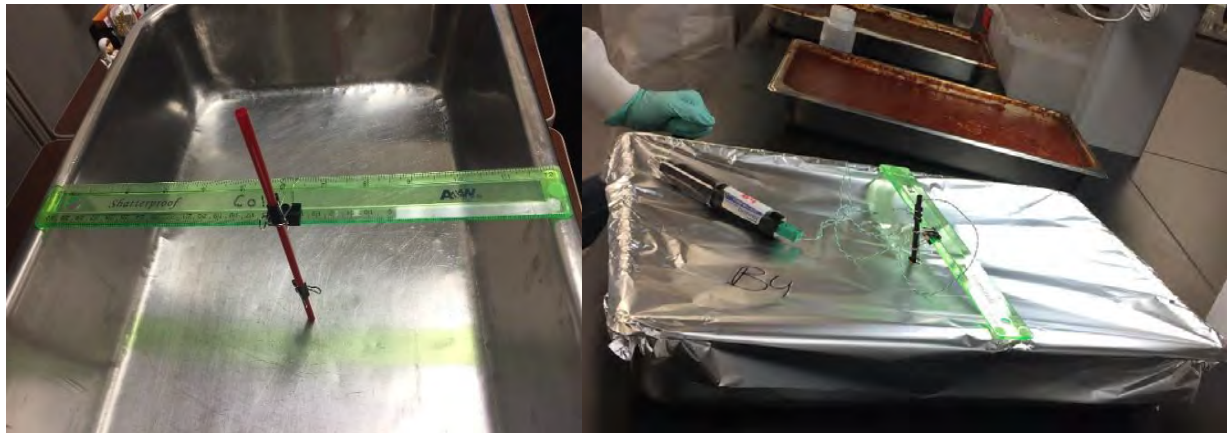
From left to right: The data logger placement system created from a straw, binder clips, and ruler to ensure the probe measured temperature at the center of the food product; the placement system shown on a finished pan.

Figure 4. Data Logger Placement System

Pans were then prepared with three cover types: uncovered, covered with a single layer of aluminum foil over the top of the pan to allow for air exposure, or double covered to restrict air exposure. Double-covered pans had one layer of plastic wrap (for marinara sauce product due to acidity) or aluminum foil (pre-cooked taco meat, brown rice, and chili con carne with beans) directly over the top of the food product and another layer of aluminum foil over the top of the pan. Each cover type was applied to both a 2- and 3-inch food product depth pan and prepared in duplicate such that one pan would be stored at 4°C (39.2°F; refrigerator) and the duplicate pan would be stored at -20°C (-4°F; freezer) (Figure 5). Pans in the refrigerator were also situated in ice baths as suggested in FDA Food Code (U.S. FDA, 2013). The ice baths were prepared by filling 3- and 6-inch steam table pans ¾ full with ice (for use with the 2- and 3-inch food depth pans, respectively). Thus, six pans were stored for cooling and sampling in each of the storage locations (refrigerator or freezer). To avoid food products becoming completely

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frozen and unable to be sampled, pans in the freezer were transferred to the refrigerator

immediately after the 8-hour time point.
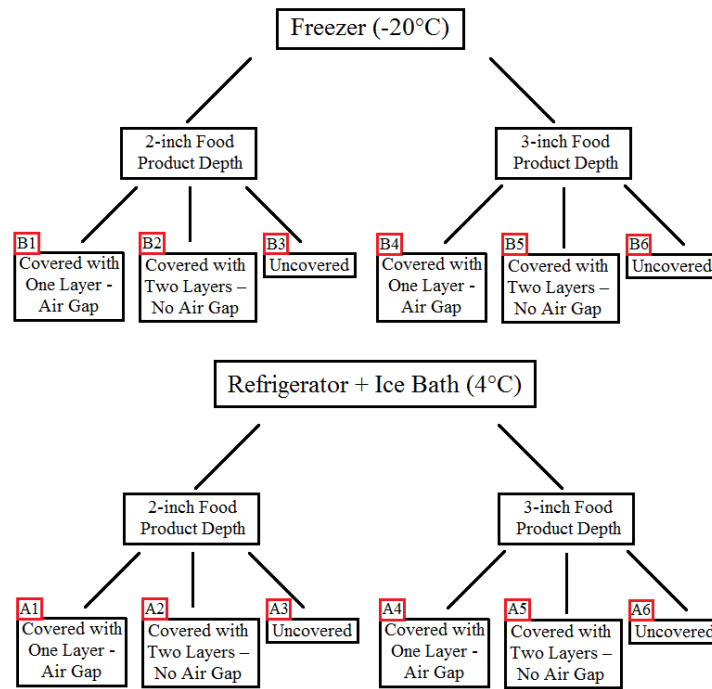
Diagram delineating storage location (freezer or refrigerator with ice bath), depth, and cover variables.

Figure 5. Diagram of Cooling Technique Combinations

Statistical Analysis

E. coli and *B. cereus* population data and temperature data were analyzed using 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 based on Akaike's information criterion (AIC) to obtain the best covariance

structure for microbial population data from each product. This was considered a four-factor,

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repeated-measures experiment and it was analyzed accordingly. A Type III test for fixed effects was also conducted.

Least square means (LSMEANS) of microbial populations were calculated using the LSMEANS statement in SAS and were used to compare the significance of variables and variable interactions at a significance threshold of $p \leq 0.05$. For the cooling curves, the average of five temperature values near each time point was utilized in order to reduce variability. The significance of variables and variable interactions for temperature data were also observed at a significance threshold of $p \leq 0.05$.

Results and Discussion

Temperature Data Analysis

Temperature data for the pre-cooked taco meat, chili con carne with beans, low sodium marinara sauce, and brown rice products were similar to previously published findings (Roberts et al., 2013; Olds et al., 2005; Olds et al., 2013). Discussed for each product in the sections below is the significance of cover type, storage location, and depth variables (main effects), as well as variable interactions and their effect on the cooling process at six time points (0, 2, 4, 8, 12, and 24 hours). In this section, if a variable or variable interaction is described as significant, it is implied that $p \leq 0.05$.

Pre-Cooked Taco Meat

No variable was significant at time point 0 hours. At the 2-hour time point, cover type and storage location by product depth were significant. Uncovered pans were significantly cooler than single-covered or double-covered pans. The 3-inch product depth in the freezer was

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significantly cooler than 2-inch product depth in the freezer or both depths in the refrigerator with an ice bath.

At 4- and 8-hour time points, storage location, storage location by product depth, and cover type were significant. The freezer cooled 2-inch product depths more rapidly than 2-inch product depths in the refrigerator during the first 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, 3-inch product depths were at lower temperatures in the freezer. Also during the first 8 hours of cooling, the 2-inch product depth in the refrigerator cooled less rapidly than the 3-inch product depth in the refrigerator. The significance of cover type was observed for uncovered pans, which cooled more rapidly than single-covered or double-covered pans during the first 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 (as discussed in the Treatments and Cooling Section) continued to remain at a lower temperature than those stored in the refrigerator with an ice bath.

Olds et al. (2013), previously concluded the only method that met FDA Food Code criteria for cooling beef taco meat was storing the product at 2-inch product depths in the freezer; the results of the current study, however, indicate uncovered, 3-inch depth product stored in ice baths in the refrigerator also meet FDA Food Code criteria (Table 1). However, it is important to consider that the starting cooling temperature for this study was lower than on the previous study due to inoculation.

Figures 6-8 are cooling curve graphs illustrating the effects of storage location, depth, and cover type variables on the cooling of the pre-cooked taco meat product. Black lines represent the two FDA Food Code time and temperature criteria, and cooling technique

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combinations are referenced by color patterns shown in the Cooling Technique key at the bottom of each graph. Figure 6 represents all 12 cooling technique combinations, Figure 7 represents each storage location and product depth combination tested, and Figure 8 represents each cover type tested. Two cooling technique combinations met FDA Food Code criteria in Figure 6. The curve representing the freezer-cooled, uncovered, 3-inch depth product was also close to meeting FDA Food Code criteria. Table 1 shows this cooling technique combination did not meet the first criteria by 3.06°C (5.30°F).

*These cooling treatments were moved from freezer to refrigerator at eight hours to allow for continued microbiological sampling.
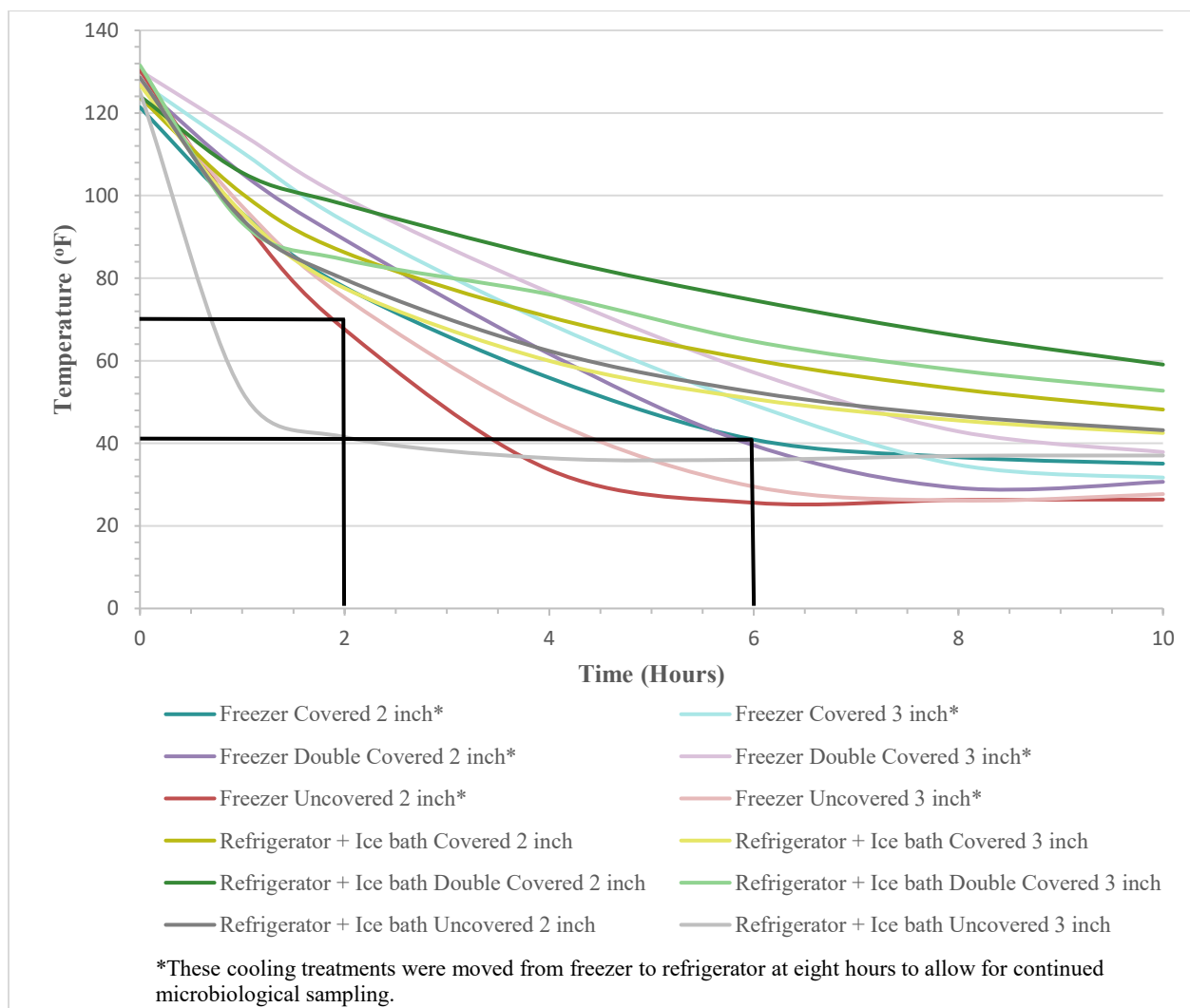
Figure 6. Cooling Curves for All Cooling Technique Combinations Tested for Pre-Cooked Taco Meat

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Table 1. Pre-Cooked Taco Meat Cooling Technique Combinations that Met FDA Food Code Criteria

Cooling Technique Combination	57°C to 21°C (135°F to 70°F) 2 hours	Limits		57°C to 5°C (135°F to 41°F) 6 hours	Limits		Both Criteria
		Lower	Upper		Lower	Upper	
2-inch Refrigerated ice bath Single cover	30.12°C (86.22°F)	23.81°C (74.86°F)	36.45°C (97.61°F)	15.65°C (60.17°F)	9.78°C (49.60°F)	21.52°C (70.74°F)	
2-inch Refrigerated ice bath Double cover	36.57°C (97.83°F)	30.26°C (86.47°F)	42.89°C (109.20°F)	23.68°C (74.62°F)	17.82°C (64.08°F)	29.56°C (85.21°F)	
2-inch Refrigerated ice bath Uncovered	26.51°C (79.72°F)	20.20°C (68.36°F)	32.83°C (91.09°F)	11.33°C (52.39°F)	5.46°C (41.83°F)	17.20°C (62.96°F)	
3-inch Refrigerated ice bath Single cover	25.32°C (77.58°F)	18.99°C (66.18°F)	31.63°C (88.93°F)	10.41°C (50.74°F)	4.54°C (40.17°F)	16.28°C (61.30°F)	
3-inch Refrigerated ice bath Double cover	29.15°C (84.47°F)	22.83°C (73.09°F)	35.47°C (95.85°F)	18.15°C (64.67°F)	12.28°C (51.10°F)	24.02°C (75.24°F)	
3-inch Refrigerated ice bath Uncovered*	5.28°C ✓ (41.50°F)	-1.04°C (31.13°F)	11.59°C (52.86°F)	2.24°C ✓ (36.03°F)	-4.86°C (23.25°F)	9.34°C (48.81°F)	✓
2-inch, freezer Single cover	25.46°C (77.83°F)	19.14°C (66.45°F)	31.78°C (89.20°F)	4.94°C ✓ (40.89°F)	-0.93°C (30.33°F)	10.82°C (51.48°F)	
2-inch, freezer Double cover	31.85°C (89.33°F)	25.53°C (77.95°F)	38.17°C (100.71°F)	4.17°C ✓ (39.51°F)	-1.71°C (28.92°F)	10.04°C (50.07°F)	
2-inch, freezer Uncovered*	19.78°C ✓ (67.60°F)	13.46°C (56.23°F)	26.09°C (78.96°F)	-3.56°C ✓ (25.59°F)	2.32°C (36.17°F)	-9.43°C (48.97°F)	✓
3-inch, freezer Single cover	34.32°C (93.78°F)	27.99°C (82.38°F)	40.63°C (105.13°F)	9.61°C (49.30°F)	3.74°C (38.73°F)	15.48°C (59.86°F)	
3-inch, freezer Double cover	37.48°C (99.46°F)	31.16°C (88.09°F)	43.80°C (110.84°F)	13.98°C (57.16°F)	8.11°C (46.60°F)	19.85°C (67.73°F)	
3-inch, freezer Uncovered	24.06°C (75.31°F)	17.73°C (63.91°F)	30.37°C (86.67°F)	-1.39°C ✓ (29.50°F)	-7.26°C (18.93°F)	4.48°C (40.06°F)	

*Indicates cooling method achieved both FDA Food Code criteria

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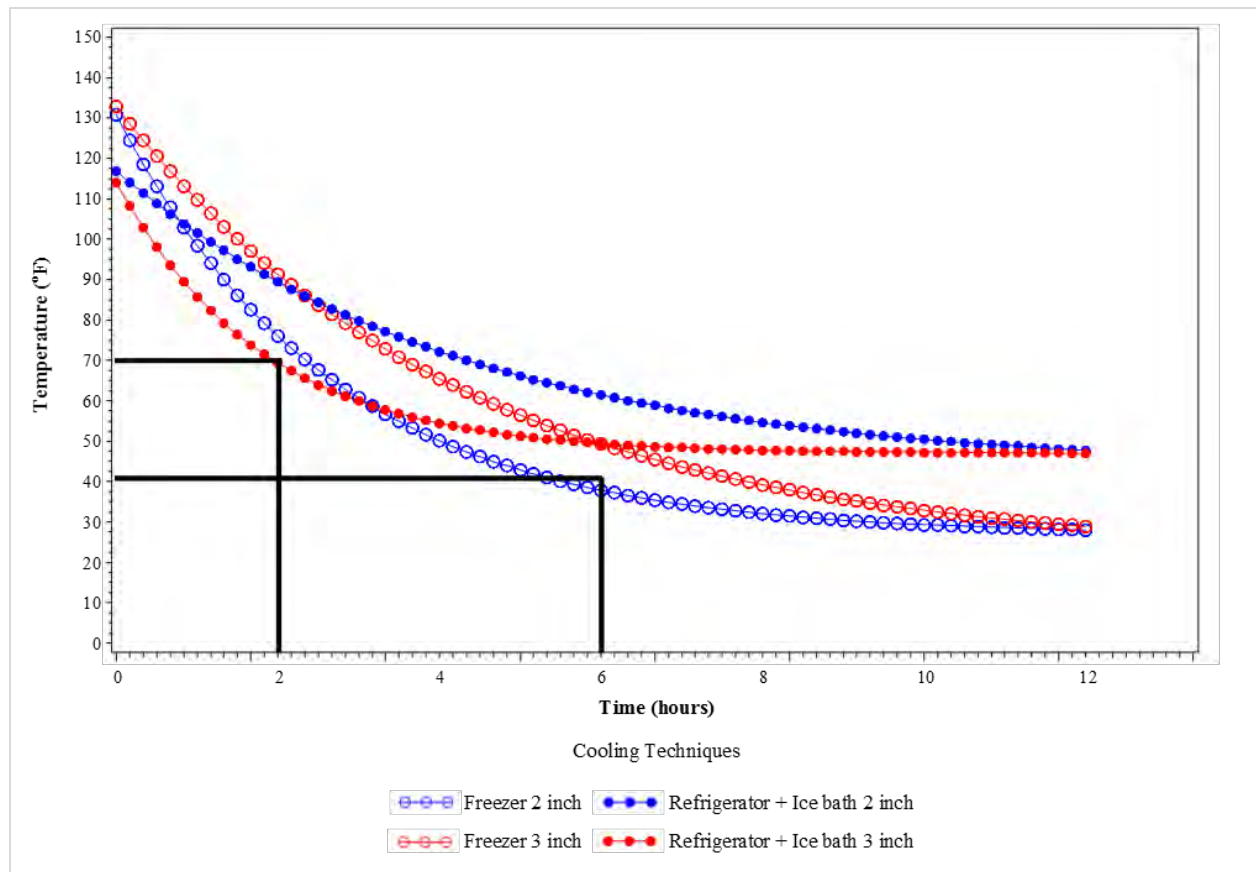
Figure 7. Fitted Model of Cooling Curves for Storage Location and Product Depth Combinations Tested for Pre-Cooked Taco Meat

Chili Con Carne with Beans

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

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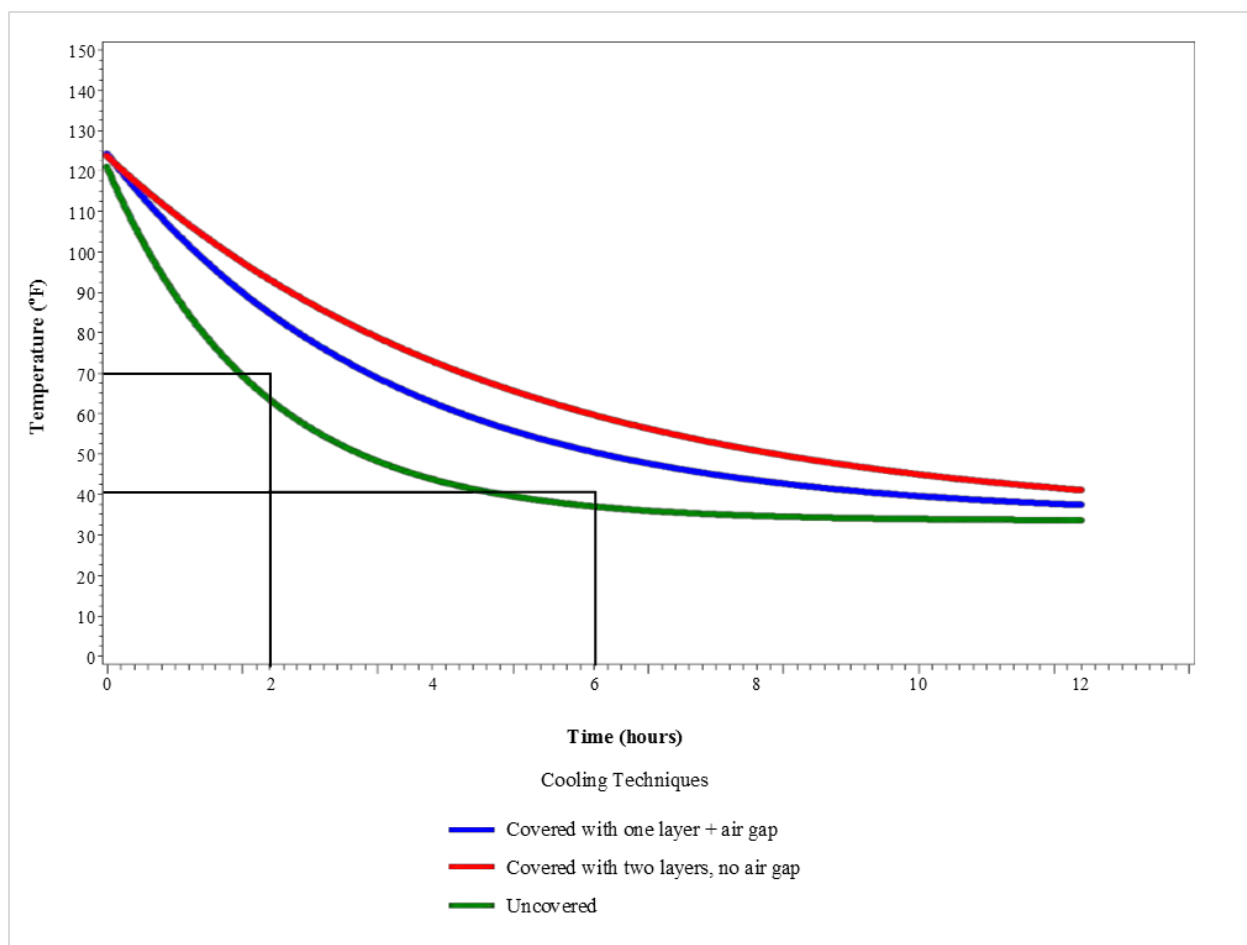
Figure 8. Fitted Model of Cooling Curves for Cover Type Variables Tested for Pre-Cooked Taco Meat

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

At the 8- and 12-hour time point, storage location, storage location by depth, and cover type were significant. During these hours, the 2-inch product depth in the freezer cooled most

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rapidly, while the 2-inch product depth in the refrigerator cooled at a slower rate than the 3-inch product depth in the refrigerator.

At the 24-hour time point, storage location and depth by cover type were significant. Pans in the refrigerator at the 24-hour time point were cooler by a small, but statistically significant, amount. The 3-inch product depths in the refrigerator were recorded as the lowest in temperature at the 24-hour time point, demonstrating a depth by cover type significance.

Olds et al. (2005), concluded the blast chiller was the only cooling method that met both FDA Food Code criteria. Roberts et al. (2013), concluded only 2-inch product depths cooled in the freezer met both FDA Food Code criteria for this product. Results from this study indicate three cooling methods met both FDA Food Code criteria as shown in Table 2.

Figures 9-11 are cooling curve graphs illustrating the effects of storage location, depth, and cover type variables on the cooling of the chili con carne with beans product. Black lines represent the two FDA Food Code time and temperature criteria, and cooling technique combinations are referenced by color patterns shown in the Cooling Technique key at the bottom of each graph. Figure 9 represents all 12 cooling technique combinations, Figure 10 represents each storage location and product depth combination tested, and Figure 11 represents each cover method tested. Three cooling technique combinations met both FDA Food Code criteria. Figure 9 additionally shows the chili con carne with beans product, refrigerated with an ice bath and covered at 2-inch depth, was close to meeting FDA Food Code criteria but narrowly missed meeting the second criteria by 0.65°C (1.17°F) (Table 2).

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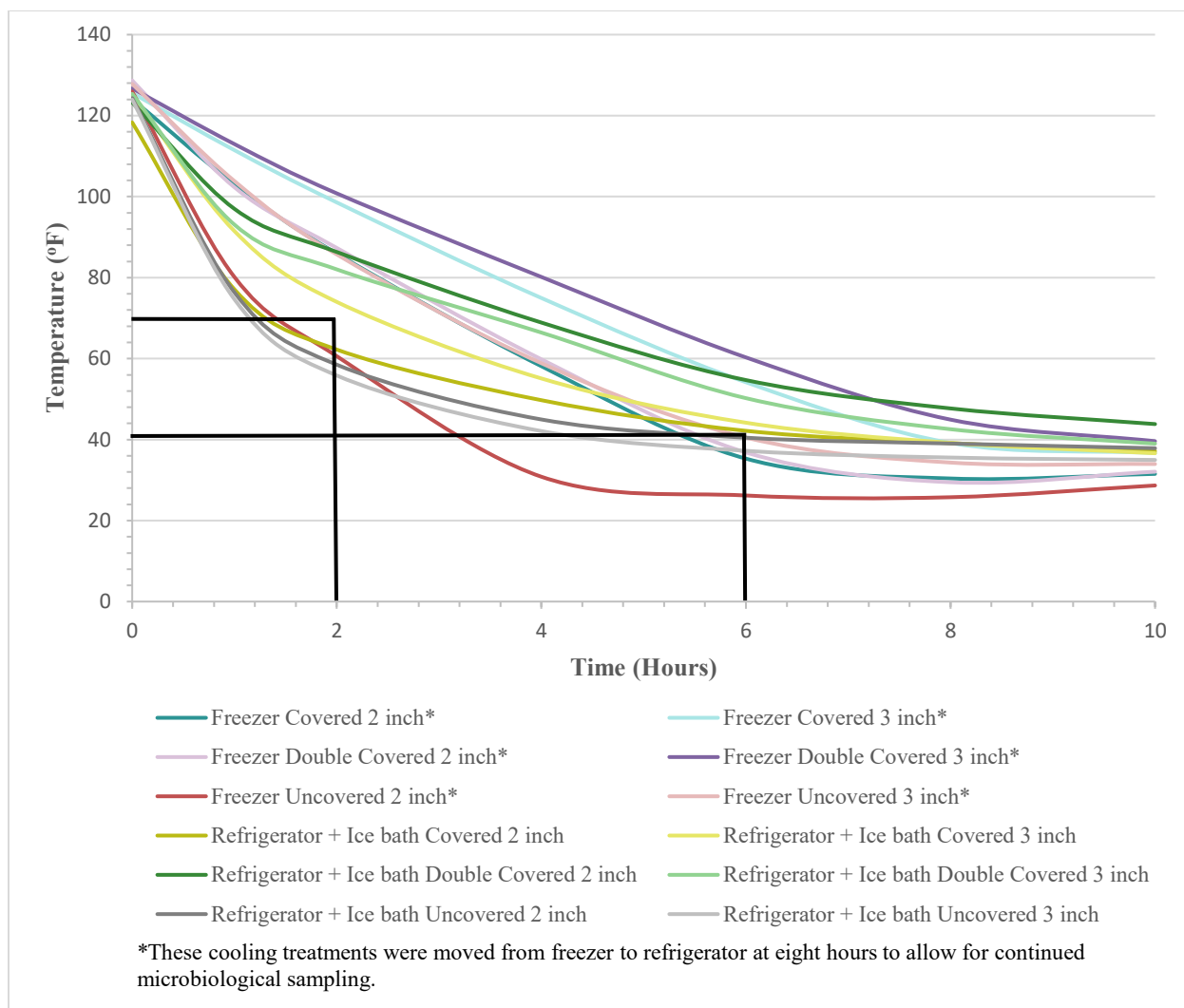
Figure 9. Cooling Curves for All Cooling Technique Combinations Tested for Chili Con Carne with Beans

Low Sodium Marinara Sauce

At time point 0, product depth was significant as 3-inch product depths were observed at a significantly higher temperature than 2-inch product depths. At 2 or 4 hours of cooling, there were no significant variables for cooling. Storage location and depth were significant at the 8-hour time point. Freezer-cooled pans fell to lower temperatures at this time point, and 3-inch product depths continued to be significantly higher in temperature than 2-inch product depths.

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Table 2. Chili Con Carne with Beans Cooling Technique Combinations that Met FDA Food Code Criteria

Cooling Technique Combination	57°C to 21°C (135°F to 70°F) 2 hours	Limits		57°C to 5°C (135°F to 41°F) 6 hours	Limits		Both Criteria
		Lower	Upper		Lower	Upper	
2-inch Refrigerated ice bath Single cover	16.79°C ✔ (62.22°F)	10.39°C (50.70°F)	23.20°C (73.76°F)	5.65°C (42.17°F)	-0.77°C (30.61°F)	12.07°C (53.73°F)	
2-inch Refrigerated ice bath Double cover	30.18°C (86.32°F)	23.78°C (74.80°F)	36.59°C (97.86°F)	12.61°C (54.70°F)	6.19°C (43.14°F)	19.03°C (66.25°F)	
2-inch Refrigerated ice bath Uncovered*	14.72°C ✔ (58.50°F)	8.32°C (46.98°F)	21.13°C (70.03°F)	4.70°C ✔ (40.46°F)	-1.72°C (28.90°F)	11.12°C (52.02°F)	✔
3-inch Refrigerated ice bath Single cover	23.33°C (73.99°F)	16.93°C (62.47°F)	29.74°C (85.53°F)	6.76°C (44.17°F)	0.34°C (32.61°F)	13.18°C (55.72°F)	
3-inch Refrigerated ice bath Double cover	27.79°C (82.02°F)	21.39°C (70.50°F)	34.20°C (93.56°F)	10.13°C (50.23°F)	3.70°C (38.66°F)	16.56°C (61.81°F)	
3-inch Refrigerated ice bath Uncovered*	13.24°C ✔ (55.83°F)	6.83°C (44.29°F)	19.64°C (67.35°F)	2.90°C ✔ (37.22°F)	-3.52°C (25.66°F)	9.33°C (48.79°F)	✔
2-inch, freezer Single cover	29.96°C (85.93°F)	23.56°C (74.41°F)	36.37°C (97.46°F)	1.83°C ✔ (35.29°F)	-4.59°C (23.74°F)	8.26°C (46.87°F)	
2-inch, freezer Double cover	30.74°C (87.33°F)	24.33°C (75.79°F)	37.14°C (98.85°F)	2.68°C ✔ (36.82°F)	-3.74°C (25.27°F)	9.10°C (48.38°F)	
2-inch, freezer Uncovered*	15.89°C ✔ (60.60°F)	9.48°C (49.06°F)	22.29°C (72.12°F)	-3.22°C ✔ (26.20°F)	-9.64°C (14.65°F)	3.20°C (37.76°F)	✔
3-inch, freezer Single cover	36.98°C (98.56°F)	30.58°C (87.04°F)	43.39°C (110.10°F)	12.32°C (54.18°F)	5.89°C (42.60°F)	18.74°C (65.73°F)	
3-inch, freezer Double cover	38.22°C (100.80°F)	31.82°C (89.28°F)	44.63°C (112.33°F)	15.72°C (60.30°F)	9.30°C (48.74°F)	22.14°C (71.85°F)	
3-inch, freezer Uncovered	29.85°C (85.73°F)	23.44°C (74.19°F)	36.26°C (97.27°F)	4.72°C ✔ (40.50°F)	-1.70°C (28.94°F)	11.14°C (52.05°F)	

*Indicates cooling method achieved both FDA Food Code criteria

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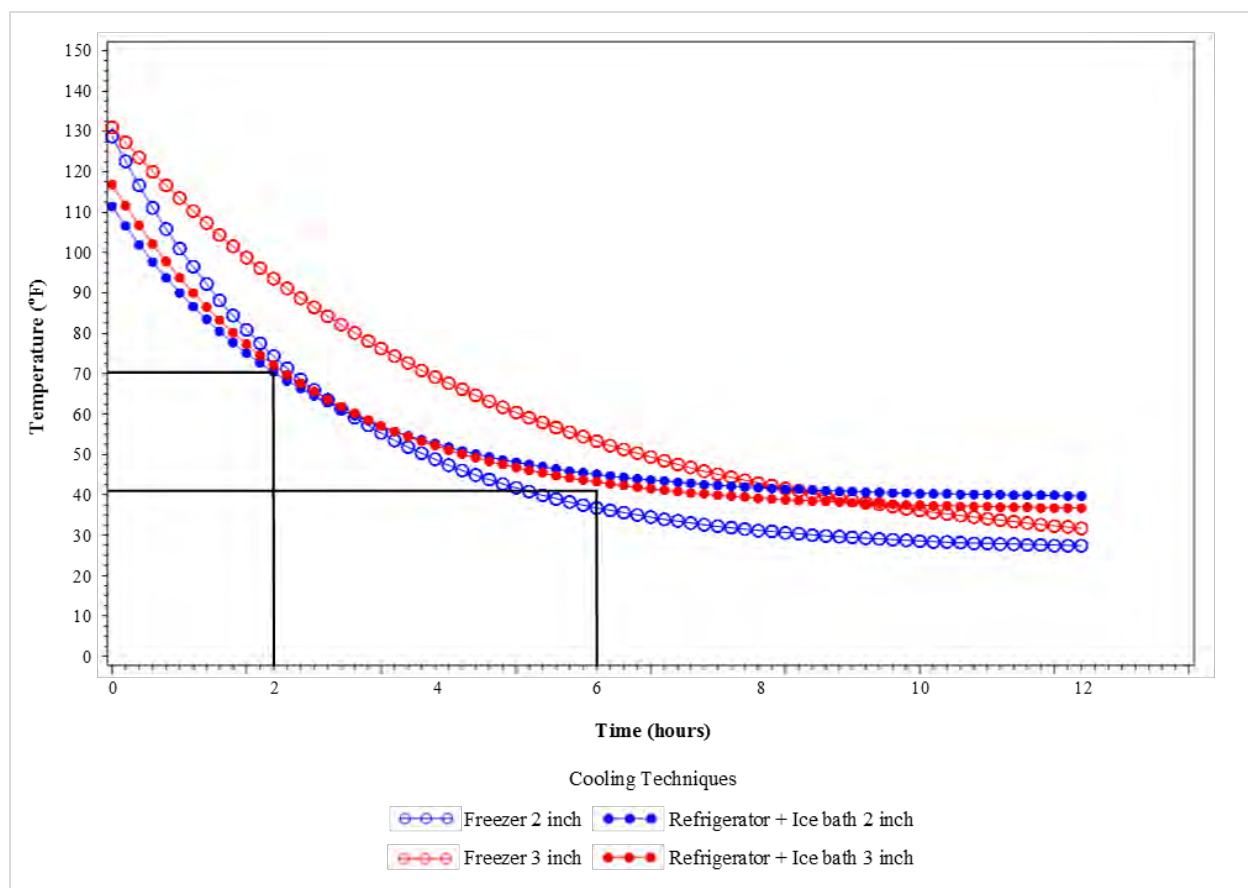
Figure 10. Fitted Model of Cooling Curves for Storage Location and Product Depth Combinations Tested for Chili Con Carne with Beans

Storage location was significant for the 12- and 24-hour time point, with freezer-cooled pans at lower temperatures than refrigerated pans. None of the cooling methods tested met either FDA Food Code criteria for this food product (Table 3). These data are not consistent with a previous Roberts et al. study, which concluded that 2-inch product depths of tomato sauce cooled in the freezer met both FDA Food Code criteria (Roberts et al., 2013); however, it is important to note that marinara sauce and tomato sauce are slightly different products.

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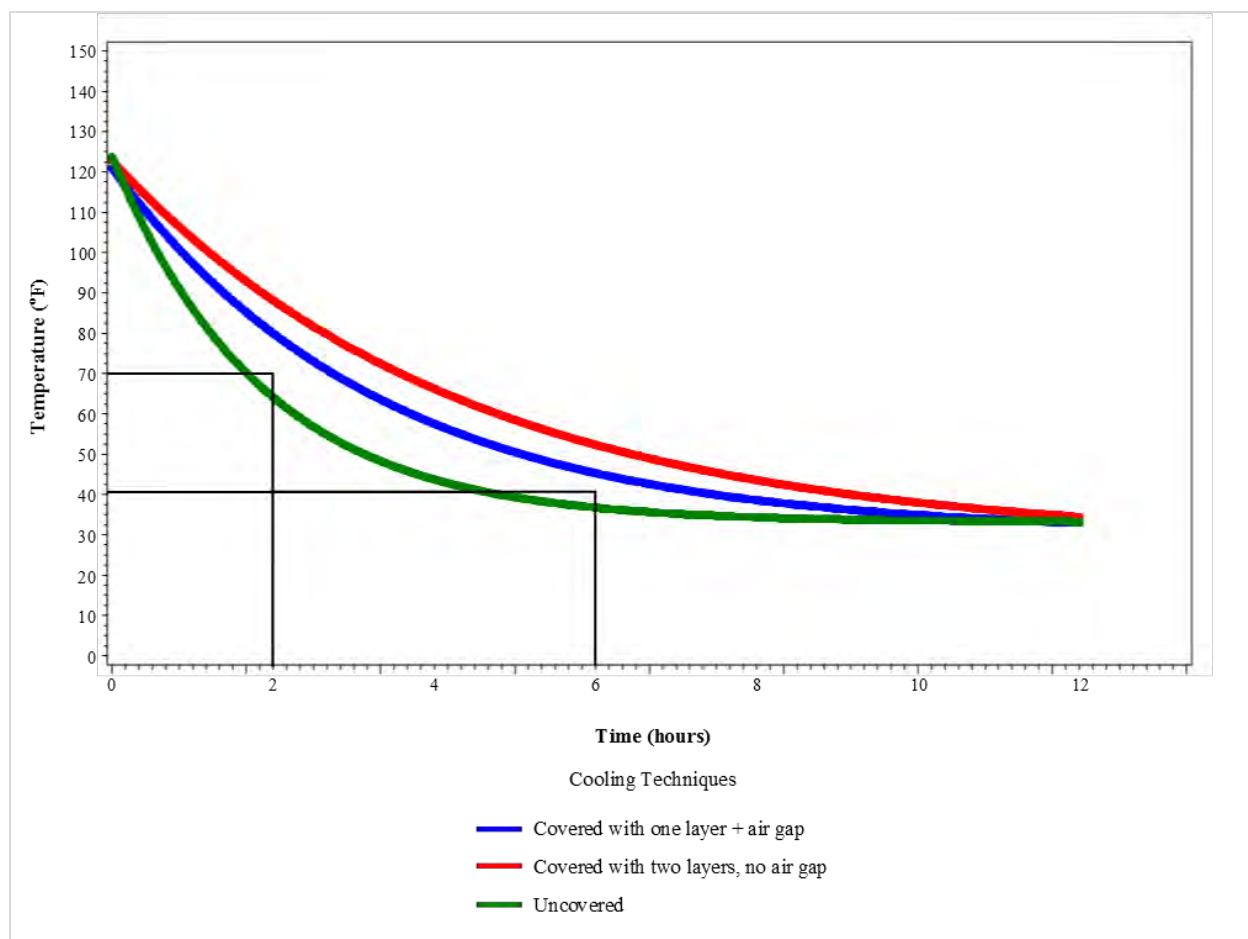
Figure 11. Fitted Model of Cooling Curves for Cover Type Variables Tested for Chili Con Carne with Beans

Figures 12-14 are cooling curve graphs illustrating the effects of storage location, depth, and cover type variables on the cooling of the low sodium marinara sauce product. Black lines represent the two FDA Food Code time and temperature criteria, and cooling technique combinations are referenced by color patterns shown in the Cooling Technique key at the bottom of each graph. Figure 12 represents all 12 cooling technique combinations and Figure 13 represents each storage location and product depth combination tested. Outlier data points for 2 inch product depths in the freezer were excluded from analysis. Figure 14 represents each cover method tested. No cooling technique combinations shown in Figures 12-14 meet both FDA Food

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Code criteria; two cooling technique combinations, however, come close to meeting both FDA Food Code criteria (Figure 12). The curve representing marinara product cooled in the freezer, uncovered, at 2-inch depth missed meeting the first step of the FDA Food Code criteria by 1.11°C (1.80°F), while successfully meeting the second time and temperature criteria. The curve representing marinara product in the refrigerator with ice bath, uncovered, and at 2-inch depth missed meeting the second criteria by 2.33°C (4.19°F) (Table 3).
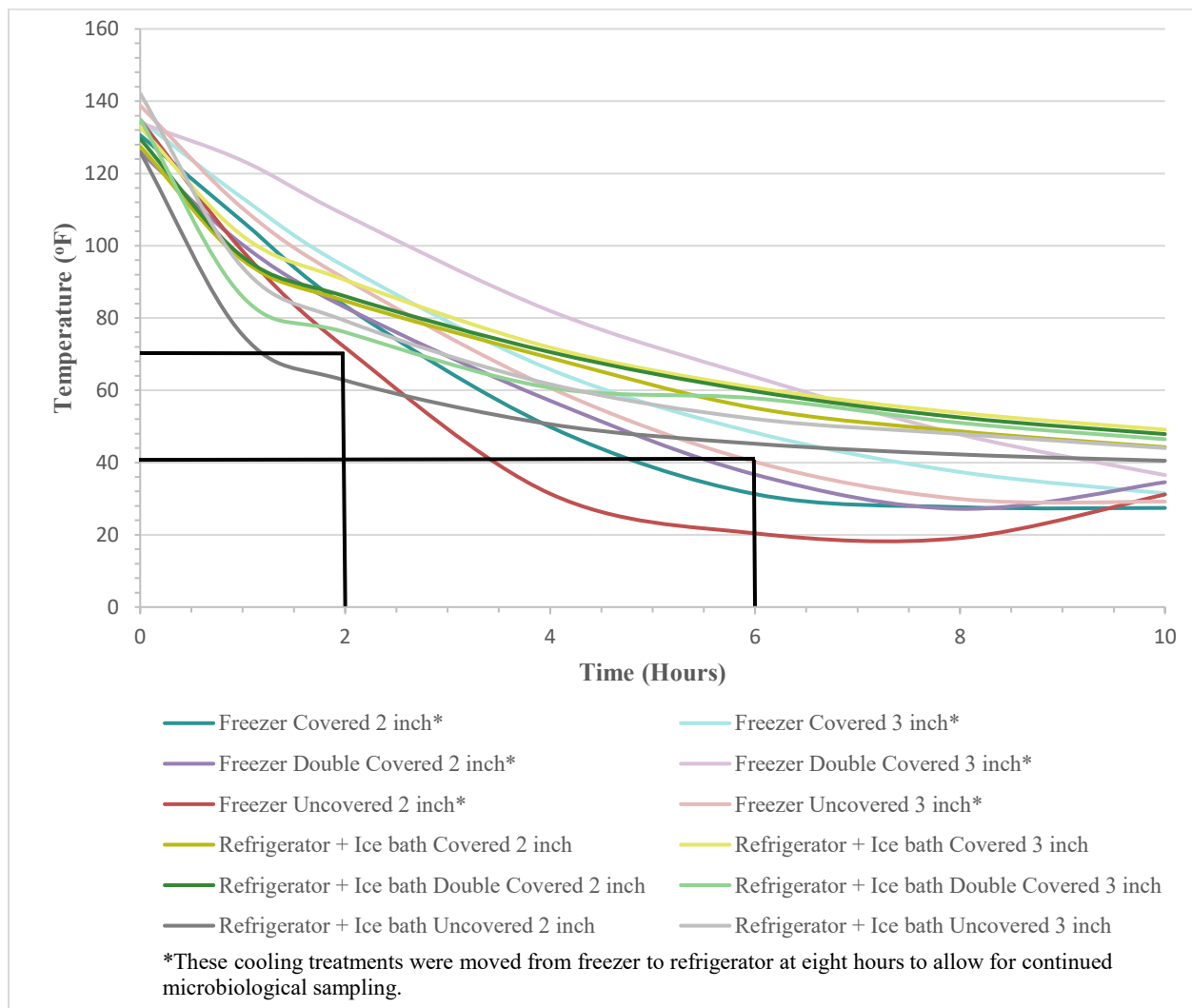
Figure 12. Cooling Curves for all Cooling Technique combinations Tested for Low Sodium Marinara Sauce

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Table 3. Low Sodium Marinara Sauce Cooling Technique Combinations that Met FDA Food Code Criteria

Cooling Technique Combination	57°C to 1°C (135°F to 70°F) 2 hours	Limits		57°C to 5°C (135°F to 41°F) 6 hours	Limits		Both Criteria
		Lower	Upper		Lower	Upper	
2-inch Refrigerated ice bath Single cover	29.29°C (84.72°F)	17.78°C (64.00°F)	40.80°C (105.44°F)	12.82°C (55.08°F)	3.23°C (37.81°F)	22.39°C (72.30°F)	
2-inch Refrigerated ice bath Double cover	30.00°C (86.00°F)	18.49°C (65.28°F)	41.51°C (106.72°F)	15.39°C (59.70°F)	5.81°C (42.46°F)	24.97°C (76.95°F)	
2-inch Refrigerated ice bath Uncovered	17.07°C ✔ (62.76°F)	5.56°C (42.01°F)	28.58°C (83.44°F)	7.33°C (45.19°F)	-2.24°C (27.97°F)	16.91°C (62.44°F)	
3-inch Refrigerated ice bath Single cover	32.52°C (90.54°F)	21.01°C (69.82°F)	44.03°C (111.25°F)	15.94°C (60.69°F)	6.36°C (43.45°F)	25.52°C (77.94°F)	
3-inch Refrigerated ice bath Double cover	24.48°C (76.06°F)	12.97°C (55.37°F)	35.99°C (96.78°F)	14.32°C (57.78°F)	2.64°C (36.75°F)	25.99°C (78.78°F)	
3-inch Refrigerated ice bath Uncovered	26.24°C (79.23°F)	14.73°C (58.51°F)	37.76°C (99.97°F)	11.14°C (52.05°F)	1.57°C (34.83°F)	20.73°C (69.31°F)	
2-inch, freezer Single cover	28.54°C (83.37°F)	17.03°C (62.65°F)	40.04°C (104.07°F)	-0.41°C ✔ (31.26°F)	-9.98°C (14.03°F)	9.17°C (48.51°F)	
2-inch, freezer Double cover	28.30°C (82.94°F)	14.20°C (57.56°F)	42.40°C (108.32°F)	2.59°C ✔ (36.66°F)	-9.08°C (15.66°F)	14.28°C (57.70°F)	
2-inch, freezer Uncovered	22.11°C (71.80°F)	10.60°C (51.08°F)	33.62°C (92.52°F)	-6.44°C ✔ (20.41°F)	-1.02°C (30.16°F)	3.13°C (37.63°F)	
3-inch, freezer Single cover	34.57°C (94.23°F)	23.07°C (73.53°F)	46.08°C (114.94°F)	9.03°C (48.25°F)	-0.54°C (31.03°F)	18.62°C (65.52°F)	
3-inch, freezer Double cover	42.50°C (108.50°F)	30.99°C (190.00°F)	54.01°C (129.22°F)	17.54°C (63.57°F)	7.96°C (46.32°F)	27.14°C (80.85°F)	
3-inch, freezer Uncovered	32.67°C (90.81°F)	21.16°C (70.08°F)	44.18°C (111.52°F)	4.53°C ✔ (40.15°F)	-5.04°C (22.93°F)	14.12°C (57.42°F)	

*Indicates cooling method achieved both FDA Food Code criteria

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Figure 13. Fitted Model of Cooling Curves for Storage Location and Product Depth Combinations Tested for Low Sodium Marinara Sauce

Brown Rice

At time point 0 hours, product depth and storage location by cover type was significant. At this time point, 3-inch product depths were significantly higher in temperature than 2-inch product depths. Concerning the significance of storage location by cover type, uncovered pans situated in ice water baths were the lowest in temperature at this time point. At 2 hours of cooling, storage location, product depth, and cover type were significant. Product stored in the refrigerator with an ice bath was significantly cooler than product stored in the freezer, 2-inch

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product depths were cooler than 3-inch product depths, and uncovered products were lowest in temperature.
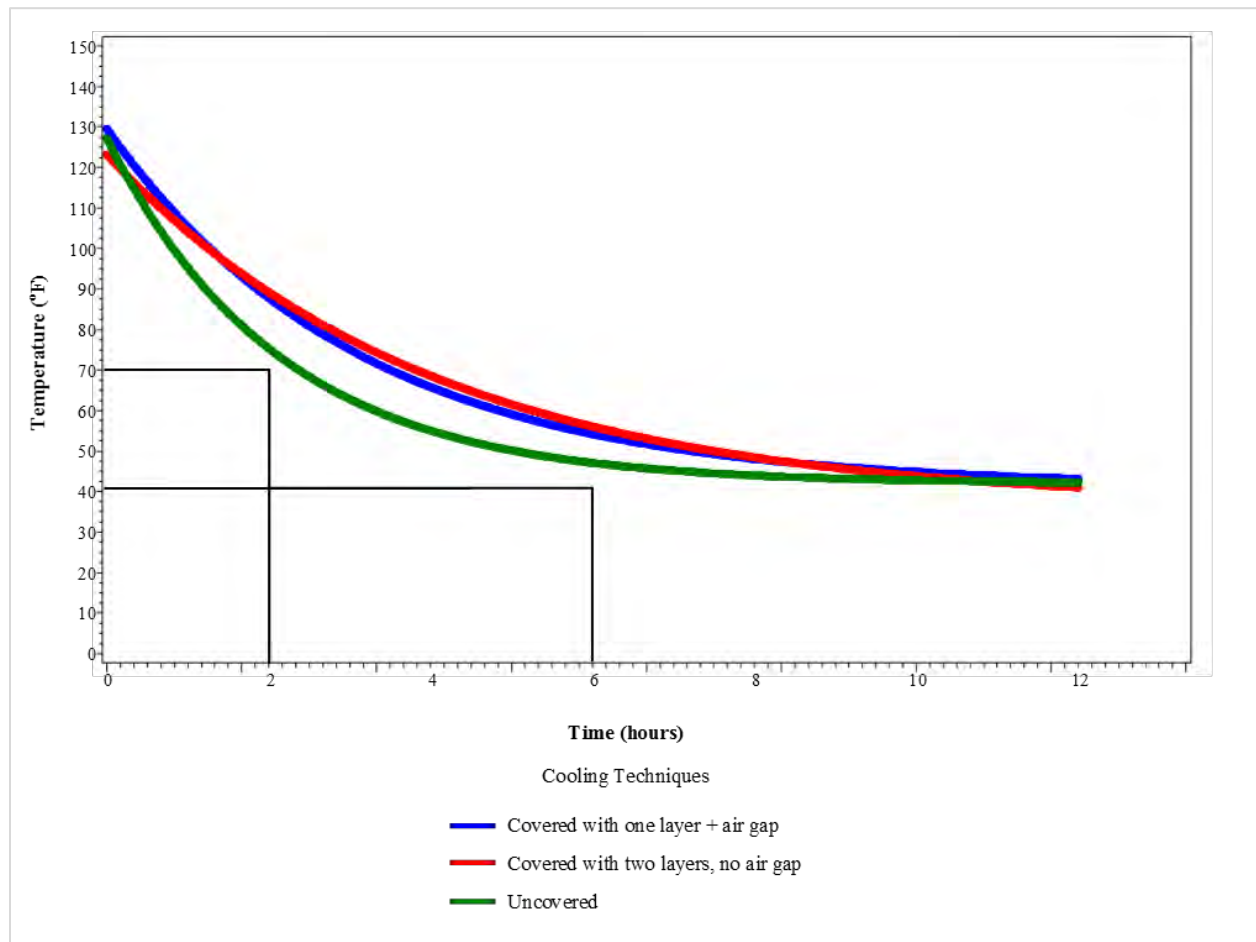
Figure 14. Fitted Model of Cooling Curves for Cover Type Variables Tested for Low Sodium Marinara Sauce

At time point 4 hours, product depth and cover type were significant and 3-inch product depths were significantly higher in temperature. Uncovered pans were significantly lower in temperature at this time point. At time point 8 and 12 hours, cover type was significant and uncovered pans were lowest in temperature.

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Storage location and product depth by cover type were significant at the 24-hour time point. Pans in the refrigerator were lower in temperature than pans in the freezer. Uncovered, 3-inch product depths were lowest in temperature at this time point.

Previously, Olds et al. (2013), concluded the only cooling method that would meet both FDA Food Code criteria for steamed rice was 2-inch product depths cooled in a refrigerator with an ice water bath. Study results now indicate four cooling methods meet both FDA Food Code criteria for this food product (Table 4).

Figure 15-17 are cooling curve graphs illustrating the effects of storage location, depth, and cover type variables on the cooling of the brown rice product. Black lines represent the two FDA Food Code time and temperature criteria, and cooling technique combinations are referenced by color patterns shown in the Cooling Technique key at the bottom of each graph. Figure 15 represents all 12 cooling technique combinations. Four cooling technique combinations met both FDA Food Code criteria. Figure 16 represents each storage location and product depth combination tested, and Figure 17 represents each cover method tested.

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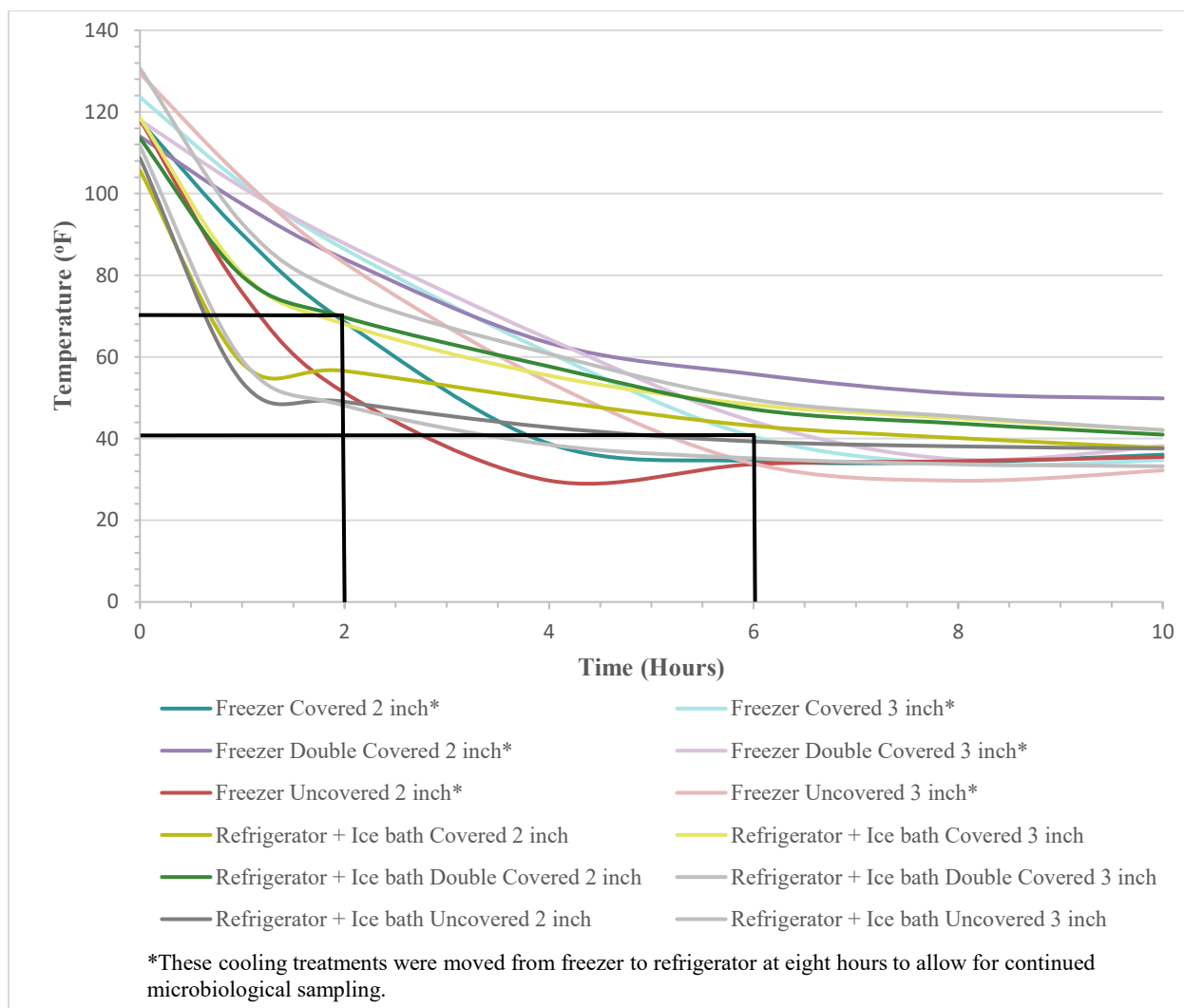
Figure 15. Cooling Curves for all Cooling Technique Combinations Tested for Brown Rice

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Table 4. Brown Rice Cooling Technique Combinations that Met FDA Food Code Criteria

Cooling Technique Combination	57°C to 21°C (135°F to 70°F) 2 hours	Limits Lower	Upper	57°C to 5°C (135°F to 41°F) 6 hours	Limits Lower	Upper	Both Criteria
2-inch Refrigerated ice bath Single cover	13.65°C ✔ (56.57°F)	6.37°C (43.47°F)	20.93°C (69.67°F)	6.18°C (43.12°F)	-0.77°C (30.61°F)	13.09°C (55.57°F)	
2-inch Refrigerated ice bath Double cover	20.94°C ✔ (69.69°F)	13.67°C (56.61°F)	28.22°C (82.80°F)	8.43°C (47.17°F)	1.51°C (34.72°F)	15.33°C (59.60°F)	
2-inch Refrigerated ice bath Uncovered*	9.46°C ✔ (49.03°F)	2.18°C (35.92°F)	16.74°C (62.13°F)	4.06°C ✔ (39.31°F)	-2.86°C (26.86°F)	10.96°C (51.74°F)	✔
3-inch Refrigerated ice bath Single cover	20.02°C ✔ (68.04°F)	12.74°C (54.93°F)	27.29°C (81.12°F)	9.06°C (48.31°F)	2.14°C (35.86°F)	15.97°C (60.74°F)	
3-inch Refrigerated ice bath Double cover	24.20°C (75.56°F)	16.92°C (62.46°F)	31.48°C (88.66°F)	9.74°C (49.53°F)	2.82°C (37.08°F)	16.56°C (61.81°F)	
3-inch Refrigerated ice bath Uncovered*	8.94°C ✔ (48.09°F)	1.66°C (34.99°F)	16.22°C (61.20°F)	1.76°C ✔ (35.17°F)	-5.16°C (22.72°F)	8.67°C (47.61°F)	✔
2-inch, freezer Single cover*	20.32°C ✔ (68.58°F)	13.03°C (55.45°F)	27.59°C (81.66°F)	1.37°C ✔ (34.47°F)	-5.54°C (22.02°F)	8.26°C (46.87°F)	✔
2-inch, freezer Double cover	28.86°C (83.95°F)	19.94°C (67.89°F)	37.77°C (99.97°F)	13.21°C (55.78°F)	4.94°C (40.89°F)	21.53°C (70.67°F)	
2-inch, freezer Uncovered*	10.68°C ✔ (51.23°F)	3.41°C (38.13°F)	17.96°C (64.33°F)	0.96°C ✔ (33.73°F)	-5.95°C (21.29°F)	7.87°C (46.17°F)	✔
3-inch, freezer Single cover	30.22°C (86.40°F)	22.94°C (73.29°F)	37.50°C (99.50°F)	4.72°C ✔ (40.50°F)	-2.19°C (28.05°F)	11.63°C (52.94°F)	
3-inch, freezer Double cover	30.98°C (87.77°F)	23.70°C (74.66°F)	38.26°C (100.87°F)	6.76°C (44.17°F)	-0.16°C (31.72°F)	13.67°C (56.61°F)	
3-inch, freezer Uncovered	28.33°C (83.00°F)	21.16°C (70.08°F)	35.61°C (96.10°F)	1.04°C ✔ (33.87°F)	-5.88°C (21.42°F)	7.95°C (46.31°F)	

*Indicates cooling method achieved both FDA Food Code criteria

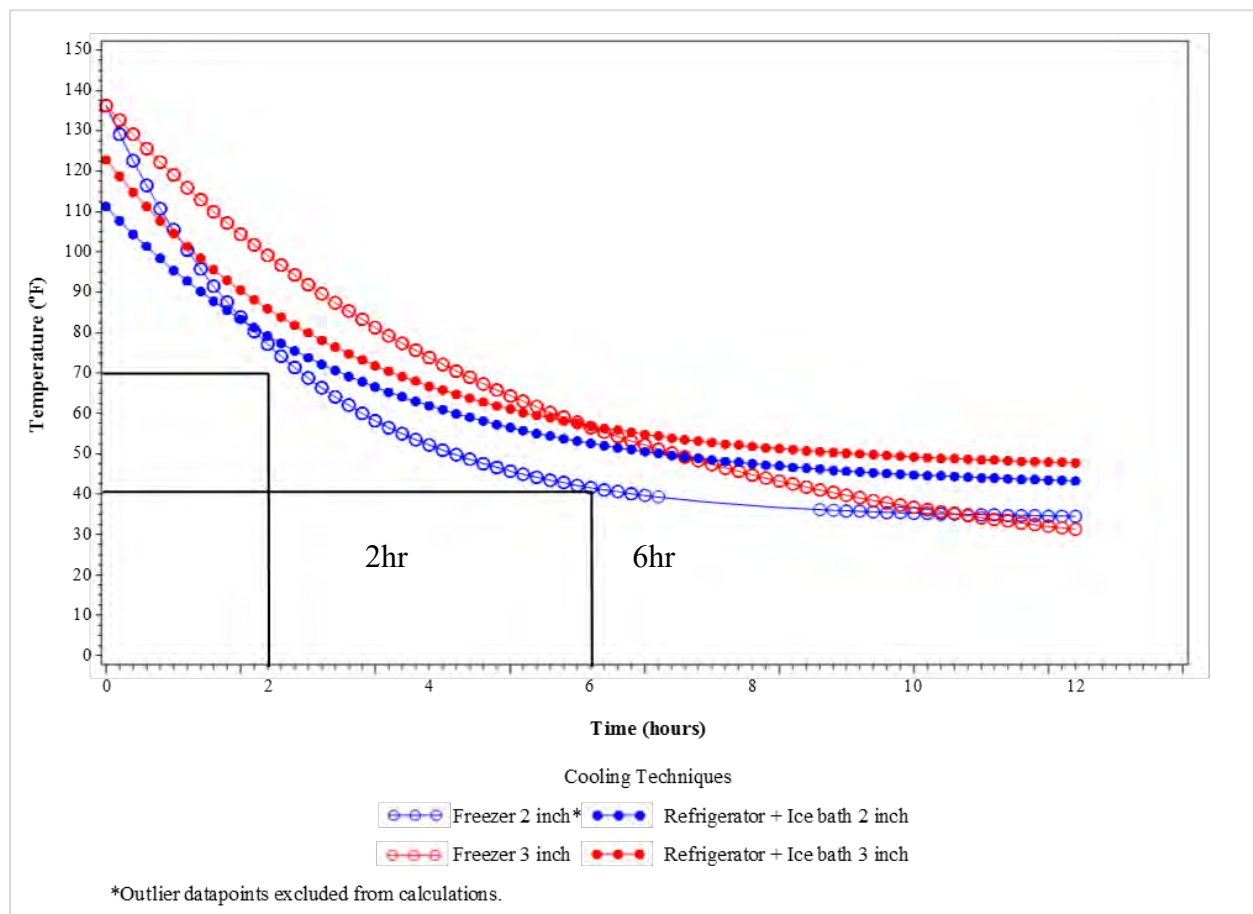Evaluating the Impact of Cooling Techniques on Biosafety Level I *Escherichia coli* and *Bacillus cereus* Populations in Four Food Products

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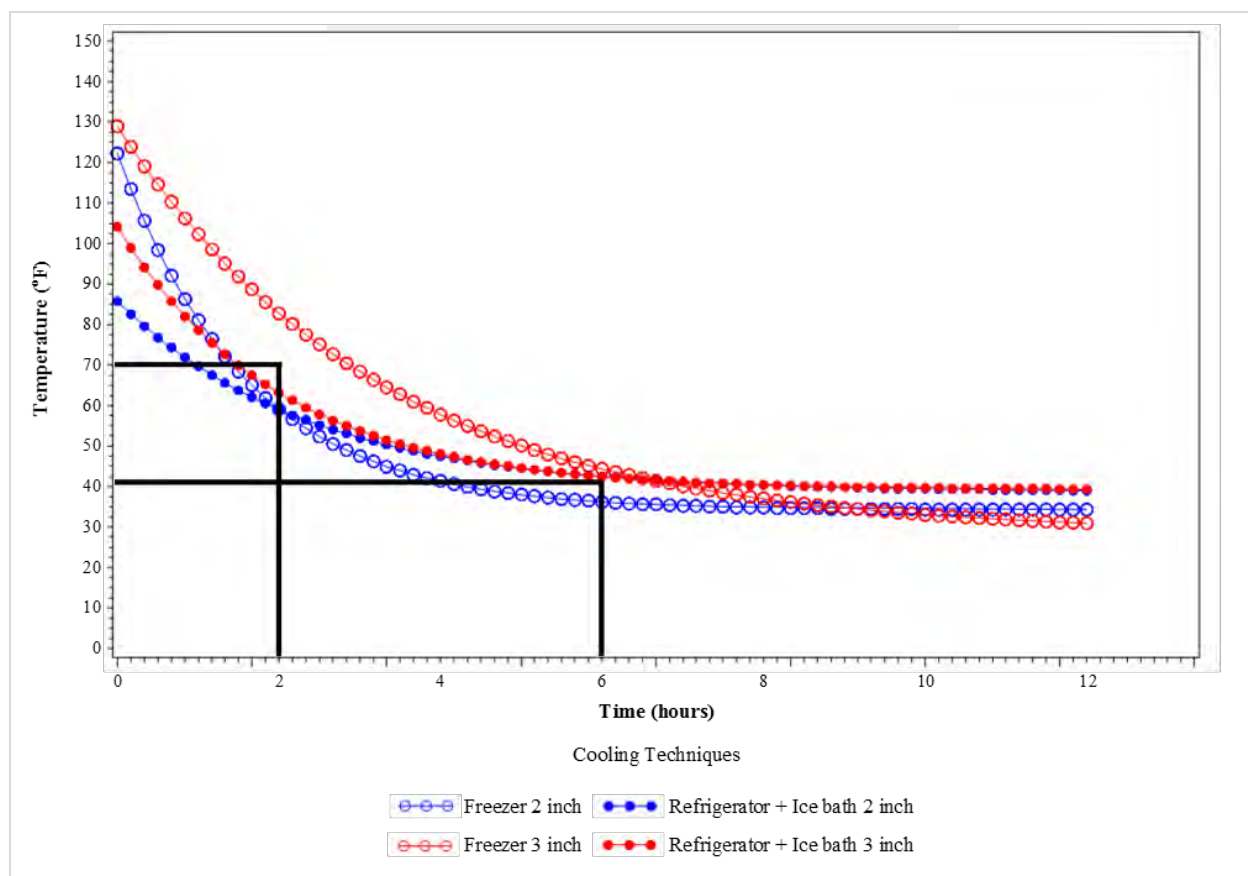
Figure 16. Fitted Model of Cooling Curves for Storage Location and Product Depth Combinations Tested for Brown Rice

Summary of Temperature Data Findings

Storage location, product depth, storage location by product depth, and cover type were often significant in 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 not statistically significant, it is noteworthy that in all four products, the 3-inch product depths stored in the freezer cooled less effectively in the first four hours than 3-inch product depths in the refrigerator with an ice bath.

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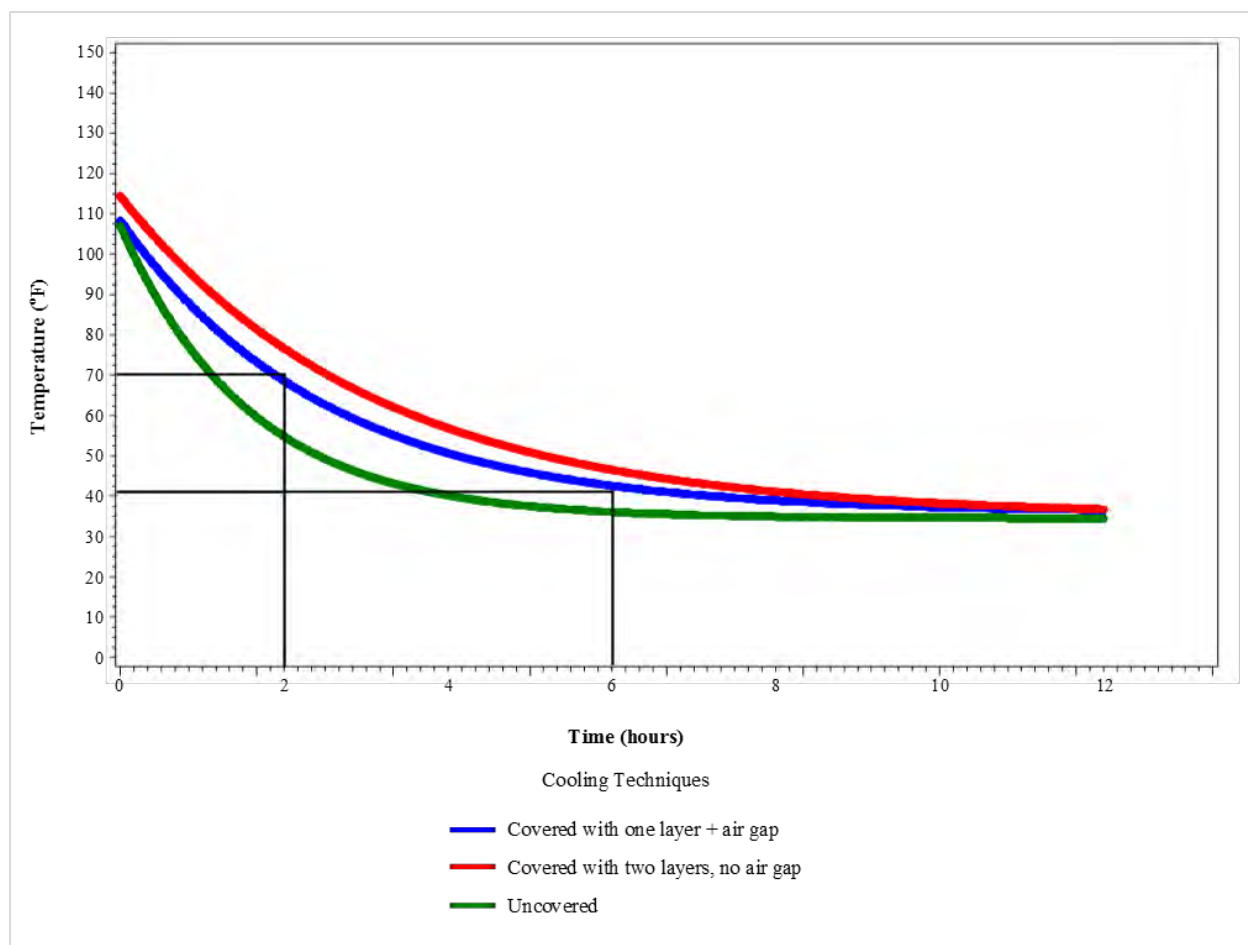
Figure 17. Fitted Model of Cooling Curves for Cover Type Treatments Tested For Brown Rice

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. 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 similar conclusions to previously published research (Olds et al., 2005; Olds et al., 2013; Roberts et al., 2013). This study identified several refrigerator and ice bath cooling combinations that achieved FDA Food Code criteria which

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previous studies did not identify for pre-cooked taco meat, chili con carne with beans, and brown rice (Olds et al., 2005; Olds et al., 2013; Roberts et al., 2013). Previous researchers hypothesized that chili and taco meat products may be too dense for refrigerator and ice bath methods to effectively cool to FDA Food Code criteria (Olds et al., 2005; Olds et al., 2013; Roberts et al., 2013). This may be due to the composition of the ice water baths, as this study utilized pans filled ¾ full of ice with no water added. Conversely, Roberts et al. (2013), concluded that 2-inch product depths of tomato sauce cooled in the freezer met both FDA Food Code criteria (U.S. FDA, 2013), which was not consistent with the findings presented herein. In the present study, the freezer-cooled, uncovered, 2-inch product depth missed achieving the first step of the FDA Food Code criteria by 1.8°F; however, this technique successfully met the second time and temperature criteria.

Temperature differences such as these may be attributed to several variations between studies, including the cooling of food products to 60°C ± 5°C (140°F ± 5°F) before placement in the freezer or refrigerator, how often the refrigerator or freezer door is opened during the cooling period, the model and current product capacity of the coolers and freezers, and whether water is used as an additive in the ice bath method. The three main studies compared in this report evaluated the cooling of chili, meatless tomato sauce, beef taco meat, and steamed rice; however, in these studies, the freezer and refrigerator were not opened once the cooling process had begun (Olds et al., 2005; Olds et al., 2013; Roberts et al., 2013). In order to access the food products for microbiological sampling at the five time points for this study, the -20°C (-4°F) walk-in freezer and 4°C (39.2°F) 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, whereas this study facilitated the cooling of food products to 60°C ± 5°C

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(140°F ± 9°F) before placement in the -20°C (-4°F) walk-in freezer and 4°C (39.2°F) walk-in

refrigerator (Olds et al., 2013; Roberts et al., 2013). The four food products were also left

uncovered in the previous cooling studies (Olds et al., 2013; Roberts et al., 2013), which, based

upon the data presented herein, likely influenced cooling and the differences between cooling

results

Microbiological Data Analysis

Pre-cooked Taco Meat

Time ($p=0.0022$) was the only significant factor in the cooling of pre-cooked taco meat.

The most significant decrease in *E. coli* population 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 (Figure 18). This population decrease is marginal and may have been due

to variations of *E. coli* populations within the food product itself rather than an effect of the

cooling procedure. No statistically significant difference ($p>0.05$) in *E. coli* populations was

observed for cover type (two layers, one layer, uncovered), storage location (refrigerator vs.

freezer), or product depth (2-inch vs 3-inch) variables (Figure 19), and there were no significant

variable interactions. The lack of these effects combined with the slight, but statistically

significant, decrease in *E. coli* populations over time demonstrates effective control of *E. coli*

populations for the cooling methods evaluated.

Chili con carne with beans

Microbiological data revealed no statistically significant difference ($p>0.05$) in *E. coli*

populations for cover type (two layers, one layer, uncovered), storage location (refrigerator vs.

freezer), or product depth (2-inch vs 3-inch) variables. However, time ($p=0.0015$) and the

product depth by time interaction ($p=0.0197$) were significant for this product. Populations

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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) (Figure 20).

Surrogate *E. coli* Populations ($\log_{10}$ CFU/g) in Pre-cooked Taco Meat Analyzed by Time

[abc] Different superscripts indicate statistically significant differences

Error bars represent the standard error of the mean
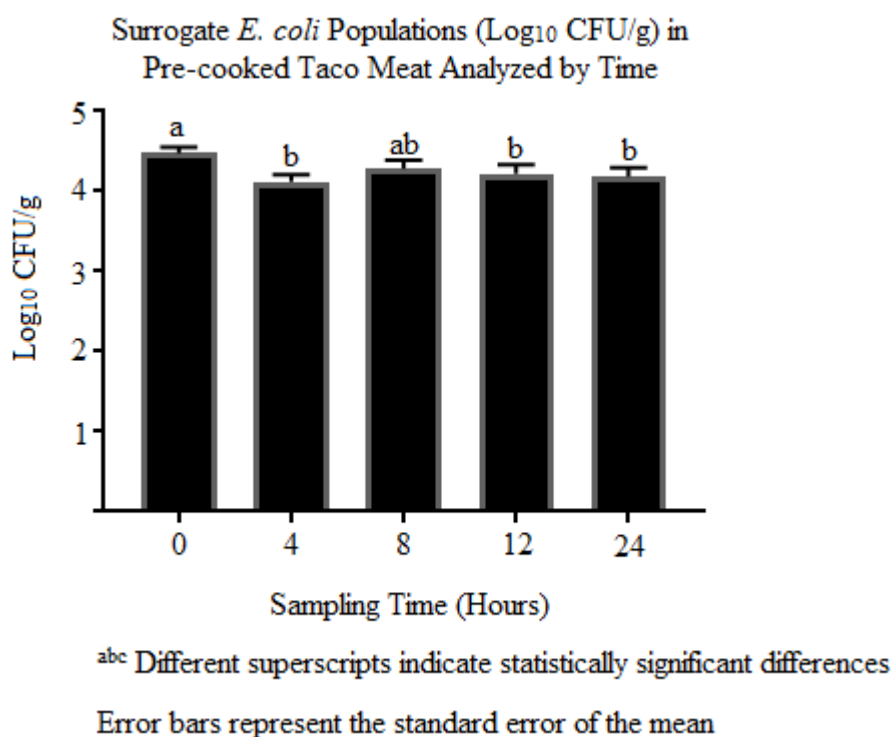
Figure 18. Surrogate *E. coli* Populations ($\log_{10}$ CFU/g) in Pre-Cooked Taco Meat Analyzed by Time

Temperature data 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 may have resulted in pockets of lethal (73.89°C, 165°F) temperature, which led to a

small, but significant, population decline (0.28 $\log_{10}$ CFU/g) during the first 4 hours of cooling.

Therefore, the *E. coli* population in 3-inch product depths at time point 4 hours was interpreted

as statistically different in comparison to populations in 2- or 3-inch product depths at other time

points. However, these population differences were well under 0.5 $\log_{10}$ CFU/g and it is possible

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that a difference in population of this magnitude was the result of natural variation in populations throughout the food product.
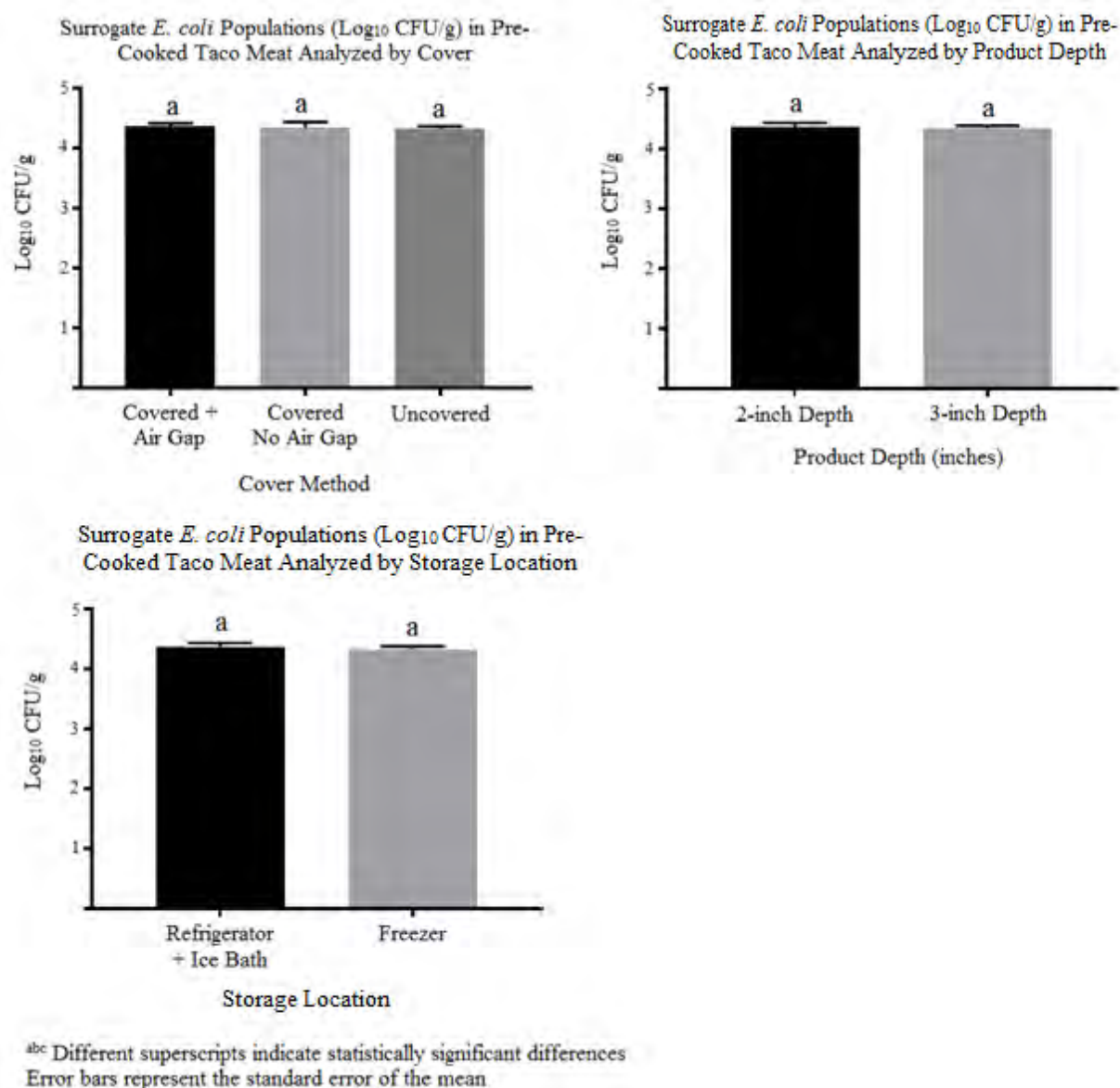
Figure 19. Graphs of Non-Significant (*p*>0.05) Main Effects for Surrogate *E. coli* Populations (Log$_{10}$ CFU/g) in Pre-Cooked Taco Meat

These results, along with the lack of statistical differences among cover type and storage location variables (Figure 21), indicate that the cooling methods evaluated were effective at

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controlling *E. coli* populations in chili con carne with beans. Although statistically significant (Figure 21), *E. coli* $\log_{10}$ CFU/g population data was not discussed by time alone due to the time variable being included in the product depth by time interaction.
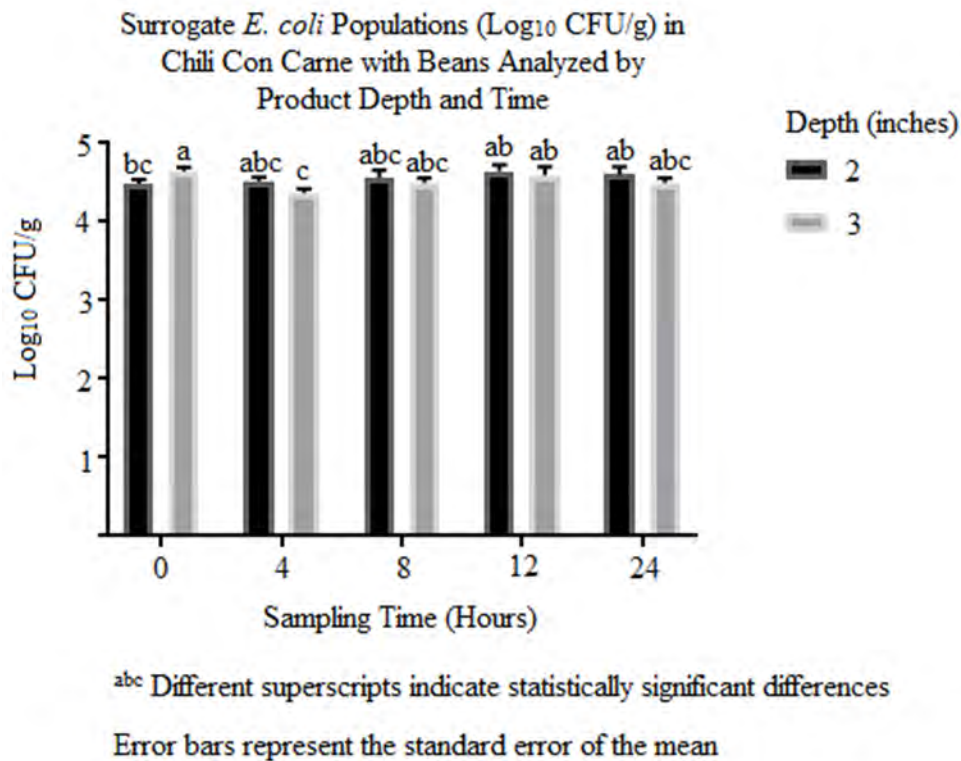
Figure 20. Surrogate *E. coli* Populations (Log$_{10}$ CFU/g) in Chili Con Carne with Beans Analyzed by Product Depth and Time

Low Sodium Marinara sauce

Product depth ($p<0.0001$) and time ($p=0.0312$) were statistically significant for 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 overall was 0.40 $\log_{10}$ CFU/g (Figure 22). Therefore, the *E. coli* population in 3-inch product depths was considered significantly less than 2-inch product depths.

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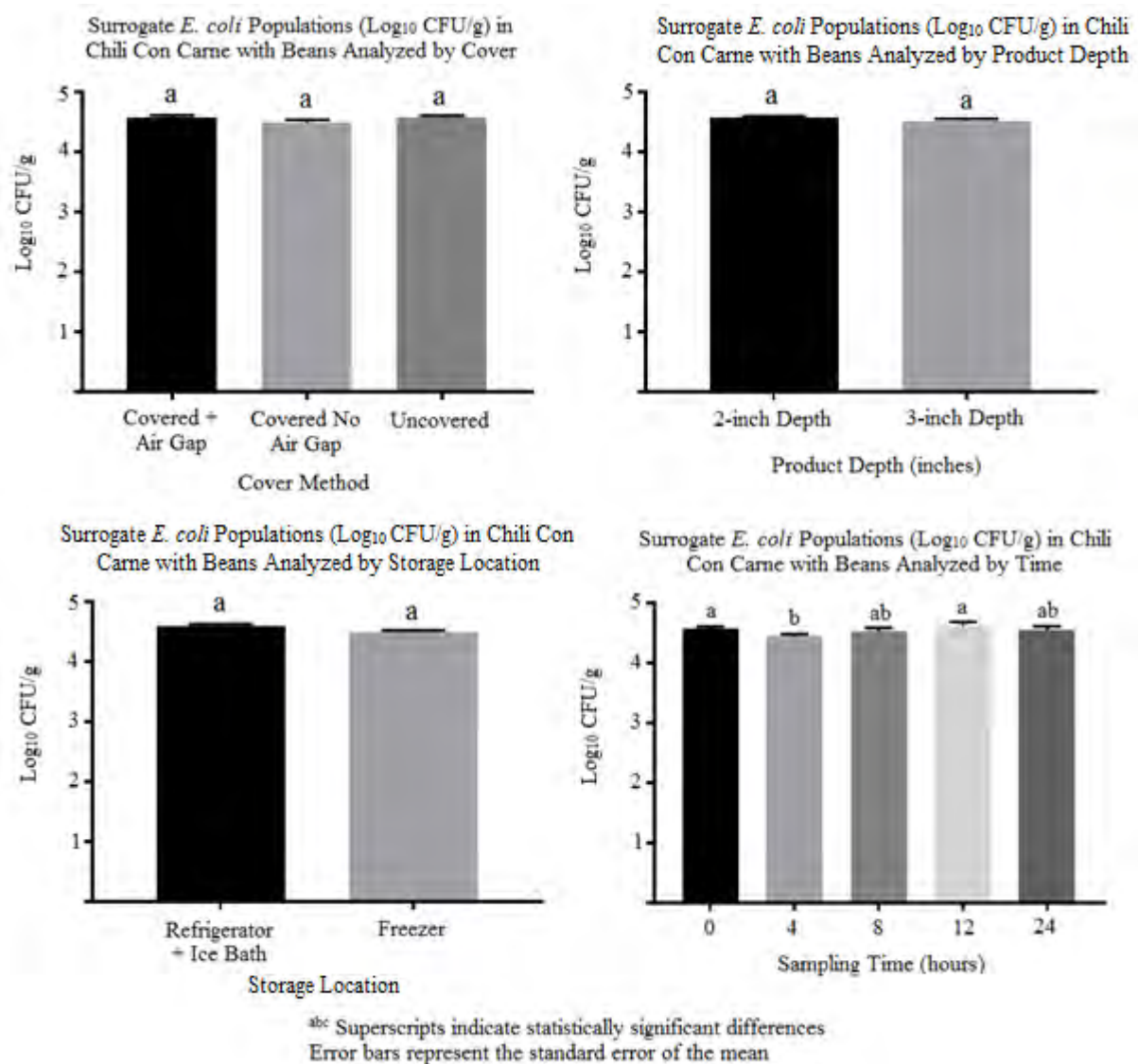
Figure 21. Graphs of Main Effects for Surrogate *E. coli* Populations (Log$_{10}$ CFU/g) in Chili Con Carne with Beans

Temperature data also suggests product depth was significant within the first four hours of cooling. It is possible the significance of depth was influenced by 3-inch product depths that may have facilitated the retention of pockets of lethal (73.89°C, 165°F) temperature, which may have reduced some of the bacterial population at inoculation. More specifically, the heat combined with the acidity (pH 4.18; Education pH meter; Fisher Scientific, Lenexa, KS) may

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have injured the cells, causing them to lag initially and then recover over time. It should also be noted that temperature data indicates depth was significant at inoculation, or time 0 hour, with 3-inch product depths being significantly higher in temperature than 2-inch product depths. It is possible that even though stirring took place to cool the product and again for approximately 2 minutes to distribute inoculum, the product did not cool evenly.

Though time was statistically significant, 0.21 $\log_{10}$ CFU/g was the largest increase in populations occurring between the 0- and 8-hour time points, which is likely due to natural variation within the product rather than a result of the cooling procedure itself (Figure 23). It is also possible that *E. coli* populations that were initially injured due to heat and/or acidity were able to make a slight recovery during the first 8 hours of cooling. The recovery of *E. coli* O157:H7 cells after sub-lethal heat treatment has been well documented (Stringer, George, & Peck, 2000). The cells experience periods of recovery, regaining their ability to grow and divide during the first 9 hours after being subjected to sub-lethal heat conditions (Stringer et al., 2000). Therefore, rather than the result of a cooling failure or risk, it is possible that the slight increase in *E. coli* populations occurred because the *E. coli* cells recovered from injury imposed by the acidic nature of the marinara sauce combined with heat at inoculation. No statistically significant difference ($p>0.05$) in populations was observed for cover (covered two layers, covered one layer, uncovered) or storage location (refrigerator vs. freezer) variables (Figure 24), and no interaction combinations tested were significant. These results indicate all cooling method variables suppressed growth to the same degree, suggesting all the cooling methods evaluated were effective at controlling *E. coli* populations in marinara sauce. Figure 22 represents $\log_{10}$ CFU/g population data analyzed by depth. Figure 23 represents $\log_{10}$ CFU/g population data

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analyzed by time and Figure 24 represents $\log_{10}$ CFU/g population data for the two other main

effect variables.

Surrogate *E. coli* Populations ($\log_{10}$ CFU/g) in Low Sodium Marinara Sauce Analyzed by Product Depth

abc Different superscripts indicate statistically significant differences
Error bars represent the standard error of the mean
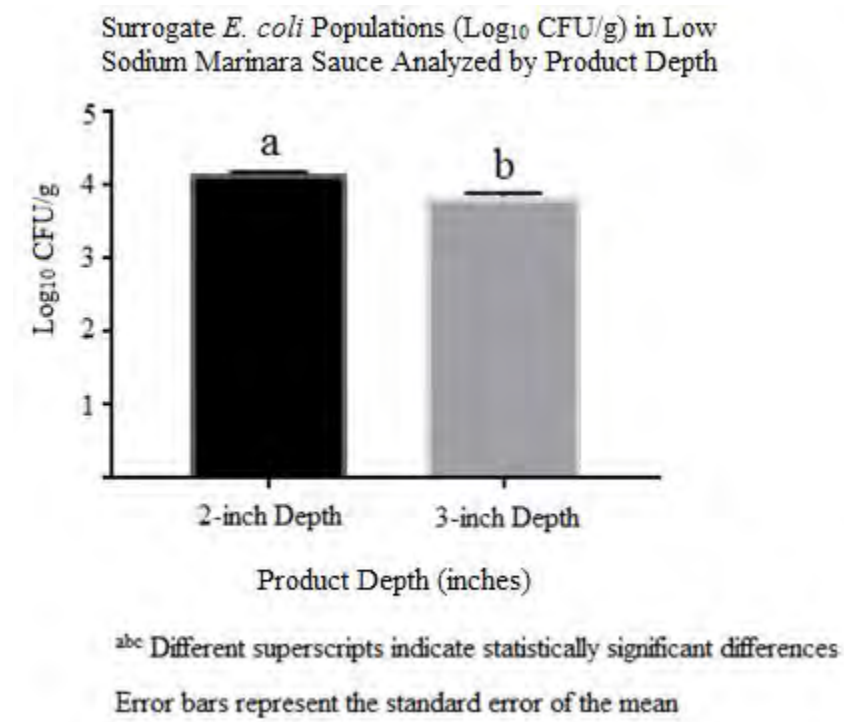
Figure 22. Surrogate *E. coli* Populations ($\log_{10}$ CFU/g) in Low Sodium Marinara Sauce Analyzed by Product Depth

Brown Rice

Microbiological data revealed two factors were significant for the brown rice product,

including time ($p<0.0001$) and product depth ($p=0.0235$). Significant two-way variable

interactions include storage location by time ($p=0.0026$) and product depth by time ($p=0.0268$).

Between 0 and 24 hours of cooling, product stored in the freezer demonstrated a population

decrease of 0.37 $\log_{10}$ CFU/g. The ice bath in the refrigerator resulted in a *B. cereus* population

decrease of just 0.09 $\log_{10}$ CFU/g between time points 0 through 24 hours (Figure 25).

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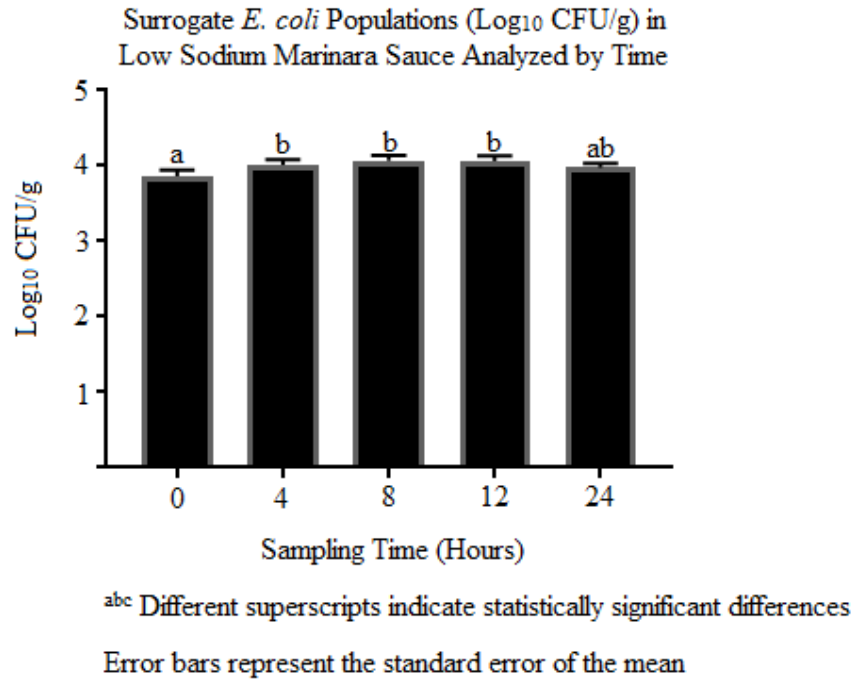
Figure 23. Surrogate *E. coli* Populations (Log$_{10}$ CFU/g) in Low Sodium Marinara Sauce Analyzed by Time
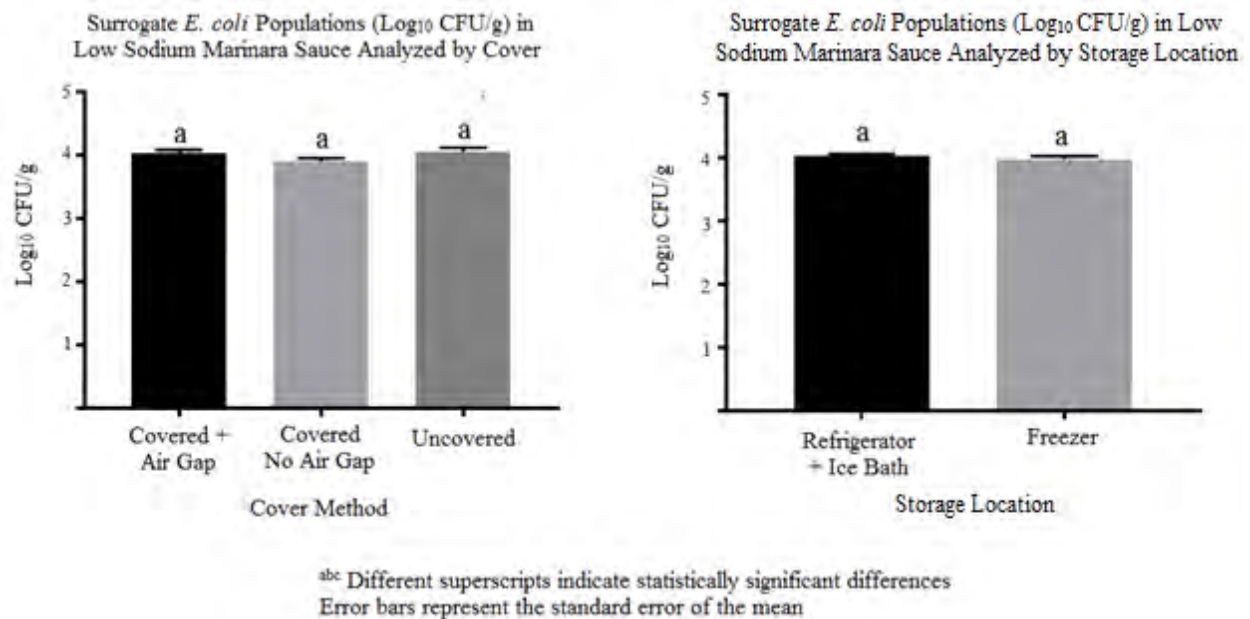
Figure 24. Graphs of Non-Significant (*p*>0.05) Main Effects for Surrogate *E. coli* Populations (Log$_{10}$ CFU/g) in Low Sodium Marinara Sauce

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Product depth by time significance was observed because populations did decrease overall in both 2- and 3-inch product depths between time points 0 and 24 hours (0.21 $\log_{10}$ CFU/g and 0.25 $\log_{10}$ CFU/g, respectively). *Bacillus cereus* populations at time 0 were slightly, but significantly, different; the 3-inch product depths were observed to harbor a 0.30 $\log_{10}$ CFU/g higher population than the 2-inch product depths at inoculation (Figure 26). This difference in population could be due to uneven distribution of inoculum. More specifically, brown rice is absorbent, and it is possible that the product absorbed the inoculum immediately upon introduction, which would lessen the efficacy of subsequent stirring efforts. *Bacillus cereus* populations in 3-inch product depths were interpreted as statistically different in comparison to populations in 2-inch product depths, or even 3-inch product depths, at other time points. No statistically significant difference ($p > 0.05$) in *B. cereus* populations was observed for cover type (two layers, one layer, uncovered) or storage location (Figure 27), and the slight decrease in *B. cereus* populations from the two significant variables demonstrates that cooling techniques tested were effective at controlling *B. cereus* populations. Although statistically significant (Figure 27), *B. cereus* $\log_{10}$ CFU/g population data was not discussed by time alone or by depth alone, due to the time variable and depth variable being included in the product depth by time interaction.

Summary of Microbiology Data Findings

E. coli was evaluated in pre-cooked taco meat, low sodium marinara sauce, and chili con carne with beans; and B. cereus was evaluated in brown rice to serve several research purposes. The microbiological data suggests that all 12 cooling methods were effective at controlling microbial populations.

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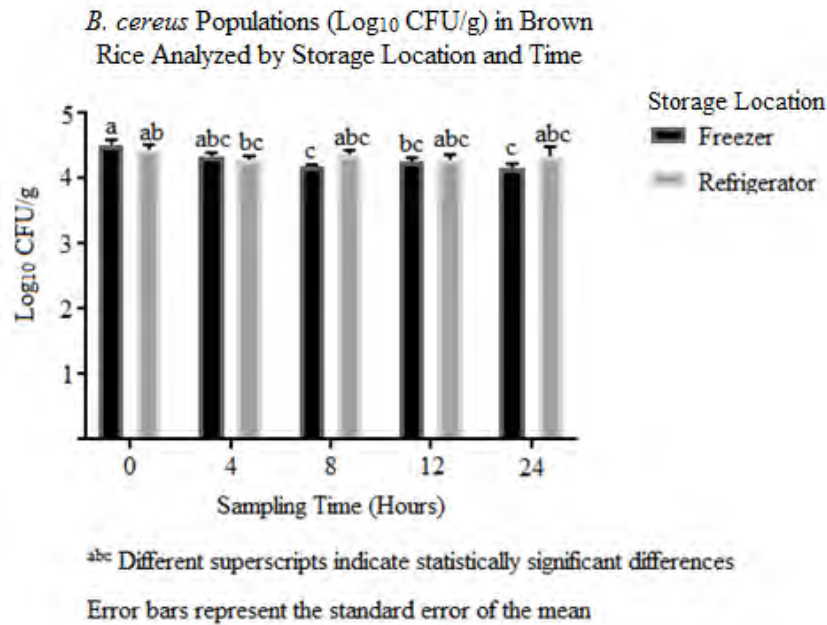
Figure 25. *B. cereus* **Populations (Log₁₀ CFU/g) in Brown Rice Analyzed by Treatment and Time**
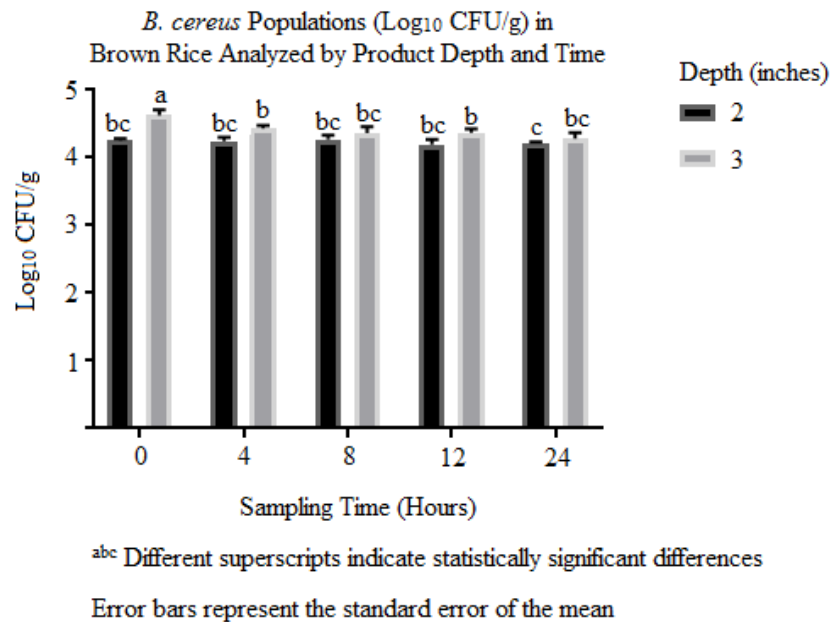
Figure 26. *B. cereus* **Populations (Log₁₀ CFU/g) in Brown Rice Analyzed by Product Depth and Time**

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B. cereus Populations (Log₁₀ CFU/g) in Brown Rice Analyzed by Cover

B. cereus Populations (Log₁₀ CFU/g) in Brown Rice Analyzed by Product Depth

B. cereus Populations (Log₁₀ CFU/g) in Brown Rice Analyzed by Storage Location

B. cereus Populations (Log₁₀ CFU/g) in Brown Rice Analyzed by Time

[abc] Superscripts indicate statistically significant differences
Error bars indicate standard error of the mean

Figure 27. Graphs of Main Effects for *B. cereus* Populations (Log₁₀ CFU/g) in Brown Rice

More specifically, the pre-cooked taco meat, chili con carne with beans, and brown rice products all exhibited a certain degree of population decline over the 24-hour cooling period. The small recovery of the microbial population in the low sodium marinara sauce product and 2-inch product depths of chili con carne with beans were less than 0.50 log10 CFU/g. It is also

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noteworthy that the variation in microbial populations for all of the cooling variable combinations tested was lower than 0.50 log10 CFU/g.

Annex 3, Section 3-501.19 of the 2013 FDA Food Code states that hot foods held without temperature control should meet the performance standard of no more than 1 $\log_{10}$ growth of *Clostridium perfringens* and *Bacillus cereus* (U.S. FDA, 2013). This is based on the knowledge that 10^5-10^7 CFU/g of vegetative cells within food products lead to a production of enterotoxin within the intestines; however, concentrations of spores in raw food products are relatively low at 10-1000 CFU/g (U.S. FDA, 2013). *Bacillus cereus* populations actually declined in this study; thus, with regards to microbiological data presented herein, all cooling techniques employed safely cooled the brown rice product.

The 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 (U.S. FDA, 2013). 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, only exhibited growth (0.21 $\log_{10}$ CFU/g) in one food product (low sodium marinara sauce). Not only is this growth marginal, but also it is well below the 1 $\log_{10}$ CFU/g of *Listeria monocytogenes* growth allowable in cold food products.

Prediction Modeling Programs for Foodborne Pathogens

There are several programs available to model specific pathogens, either within specified food products or under certain growth conditions including temperature, % NaCl, a_w, and pH. To evaluate the pathogens and temperature conditions modeled in this study, two online programs were utilized: the USDA Pathogen Modeling Program (PMP) and the ComBase Predictor

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modeling program (PMP, ComBase). Neither the PMP nor the ComBase programs had data from previous research regarding the specific surrogate or pathogen combined with the food products tested, or cooling conditions evaluated in this study. ComBase has a comprehensive database, but only broth cultures and foods that were not similar in % NaCl or pH were available at cold holding temperatures. In fact, only broth models were available for *B. cereus* at a cold holding temperature. Therefore, modeling was carried out based on conditions that were intrinsic to the food products tested including initial population, % NaCl or a_w, and pH as well as external conditions like holding temperature. Nutrition labels from the food products provided information to calculate % NaCl based on weight (See Appendices B - F).

The ComBase Predictor offers a non-thermal survival prediction method, but this method only allows for modeling of *Listeria monoctyogenes* and *Salmonella* and not for modeling of the pathogens evaluated in this study. Therefore, the growth prediction method was chosen as the most appropriate predictor model. The dynamic version of the model correlates certain temperatures with time points during the "growth" process. This was an advantage over the static model, as temperatures changed frequently during the cooling process in this study. However, this model was not ideal as the temperature ranges for each pathogen were limited to growth conditions, for *B. cereus* 5-34°C (41-93°F) and *E. coli* 10-42°C (50-107°F). Therefore, temperature data gathered from this project fell out of range for modeling during a majority of the 24-hour cooling period. The dynamic model was carried out for food products stored in the refrigerator as more time points fell within modeling range than those for products in the freezer. The ComBase program was also limited as far as pH input with the lower limit being 4.5, so modeling for the marinara sauce product at a pH of 4.18 was not possible.

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As for the PMP, there were models for cooling conditions regarding *Clostridium botulinum* and *Clostridium perfringens*; however, there were no cooling models for the pathogens evaluated in this study. The PMP did not contain a bacteria specific model for the food products tested in this study, so a bacteria specific, broth-based model was chosen to evaluate the food products in this study with their surrogate or pathogen combination. The PMP allowed for a modeling scenario including initial level and pH but also had the advantage of allowing a lower temperature limit to be selected, 5°C (41°F), than the ComBase program. However, the PMP did not allow a dynamic model where temperatures could be input at certain time points and allows a lower limit of just 0.5 % NaCl. This lower limit resulted in limited prediction potential as the food products tested in this study were between 0-0.45% NaCl.

The following information was input in ComBase to run the modeling program for the pre-cooked taco meat product: initial level = 4.52-$\log_{10}$, temperatures of food product in the refrigerator from time points 2, 4, 6, and 8 hours of cooling that were within modeling limits, 0.45% NaCl, and two pH scenarios at 5 and 6. Two pH scenarios were run to model a worst-case scenario as the final pH of the pre-cooked taco meat product was unknown. At a pH of 5 and 6, *E. coli* was predicted by the ComBase dynamic model to grow by 0.11-$\log_{10}$ and 0.49-$\log_{10}$, respectively, over the 8 hour period. The PMP was also run to model growth in this product, with identical input information, but at a lower temperature of 5°C (41ºF) and at the lower limit of 0.5% NaCl. This model reported a 0.013 $\log_{10}$ (CFU/mL)/h growth rate in an aerobic, broth-based scenario. The model predicted that over a 24-hour period, the population would increase by 0.17-$\log_{10}$ and 0.26-$\log_{10}$ for pH 5 and 6, respectively. The results from the microbiological data for the pre-cooked taco meat in this study showed a decrease of 0.16 log10 CFU/g between time point 0 and 8 hours.

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For the low sodium marinara sauce product, there was limited prediction potential as the pH for the product (4.18) fell below the lower limit in ComBase (4.5). The following information was used as input for ComBase to run the modeling program for the low sodium marinara sauce product: initial level = 3.86-$\log_{10}$, temperatures of food product in the refrigerator from time points 1, 2, 4, and 6 hours of cooling that were within modeling limits, 0.16% NaCl, and pH = 4.5. Under these conditions, *E. coli* was predicted by the ComBase dynamic model to grow by 0.15-$\log_{10}$ over the 6 hour period. The PMP was also run to model growth in this product, but at a lower temperature of 5°C (41°F), at the lower limit of 0.5% NaCl, and at the lower limit of pH 4.5. This model predicted a 0.01 $\log_{10}$ (CFU/mL)/h growth rate. At 24-hours, the population was predicted to grow by 0.12-$\log_{10}$. The results from the microbiological data for the low sodium marinara sauce in this study showed an increase of 0.15 $\log_{10}$ CFU/g between time point 0 and 4 hours which was very similar to the ComBase and PMP predictions.

The following information was input in ComBase to run the modeling program for the chili con carne with beans product: initial level = 4.56-$\log_{10}$, temperatures of food product in the refrigerator from time points 1, 2, and 4 hours of cooling that were within modeling limits, 0.17% NaCl, and two pH scenarios at 5 and 6. Two pH scenarios were run to model a worst-case scenario as the final pH of the chili con carn with beans product was unknown. *E. coli* was predicted by the ComBase dynamic model to grow by 0.05-$\log_{10}$ and 0.09-$\log_{10}$, at a pH of 5 and 6 respectively. The PMP was also run to model growth in this product, but at a lower temperature of 5°C and at the lower limit of 0.5% NaCl. This model reported a 0.01 $\log_{10}$ (CFU/mL)/h growth rate. At 24-hours, the population was predicted to grow by 0.17-$\log_{10}$ and 0.26 $\log_{10}$ at a pH of 5 and 6, respectively. The results from the microbiological data for the chili

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con carne with beans product in this study showed a decrease of 0.12 log10 CFU/g between time point 0 and 4 hours.

The following information was input in ComBase to run the modeling program for the brown rice product: initial level = 4.48-$\log_{10}$, temperatures of food product in the refrigerator from time points 1, 2, 4, and 6 hours of cooling that were within modeling limits, 0% NaCl, and pH= 6. *B. cereus* was predicted by the ComBase dynamic model to remain at the same population over the 6 hour period. The PMP was also run to model growth in this product, but at a lower temperature of 5°C (41ºF) and at the lower limit of 0.5% NaCl. This model reported a 0.04-$\log_{10}$ (CFU/mL)/h growth rate. At 24-hours, the population was predicted to grow by 0.60-$\log_{10}$. The results from the microbiological data for the brown rice product in this study showed a decrease of 0.16 $\log_{10}$ CFU/g between time point 0 and 4 hours.

In summary, both programs were restricted in their ability to model our surrogate or pathogen survival in the four food products. The lack of cooling modeling for the pathogens tested in this study compounded with the lower limits of temperature and % NaCl result in only a nominal amount of reliability in the data predictions. Perhaps this research will add to databases and programs such as these to further the understanding of how surrogates and pathogens behave in food products during cooling.

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Conclusions and Recommendations

The data from this project build upon previously published studies that evaluated cooling methods used in school nutrition program settings and whether they met FDA Food Code criteria (Olds et al., 2005; Olds et al., 2013; Roberts et al., 2013). This study was designed to simulate cooling techniques that are utilized by school nutrition programs and determine their impact on food safety relative to changes in microbiological populations and product temperature. More specifically, the present study built on previous research by addressing the knowledge gap surrounding the behavior of microbial populations within food products during the cooling process.

In general, uncovered pans cooled most rapidly, followed by those with a single layer of foil for air exposure and, least rapidly, a double layer of foil and plastic wrap for no air exposure. Overall, 2-inch product depths cooled more rapidly than 3-inch product depths, and the freezer cooled more evenly over time, and to a lower temperature, than the refrigerator with ice bath method. Few cooling methods for pre-cooked taco meat, chili con carne with beans, and brown rice achieved both FDA Food Code criteria by cooling from 57ºC (135°F) to 21ºC (70°F) within two hours and 5ºC (41°F) or below after a total of six hours (U.S. FDA, 2013). For taco meat, 3-inch product depths uncovered in the refrigerator with an ice bath and 2-inch product depths uncovered in the freezer met FDA Food Code criteria (U.S. FDA, 2013). Regarding chili con carne, 2-inch and 3-inch product depths uncovered in the refrigerator with an ice bath and the 2-inch product depth uncovered in the freezer achieved FDA Food Code criteria (U.S. FDA, 2013). In reference to brown rice, 2-inch and 3-inch product depths uncovered in the refrigerator, 2-inch covered with one layer in the freezer, and the 2-inch product depth uncovered in the freezer met

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the FDA Food Code criteria (U.S. FDA, 2013). For the low sodium marinara sauce product, none of the cooling methods tested met both criteria.

Although cover method was statistically significant ($p \leq 0.05$) with regards to temperature of three of the four food products, this variable was not significant ($p > 0.05$) for the control of microbial populations in the food products evaluated. Storage in the freezer (-20°C, -4°F) or refrigerator (4°C, 39.2°F) with ice bath and product depth were also variables that were significant for temperature control of food products, but were not statistically significant with regards to microbial populations. The pre-cooked taco meat, chili con carne with beans, and brown rice products exhibited overall microbial population decline over the 24-hour cooling period, which indicates that microorganism populations were controlled by the cooling methods tested. The small recovery of the microbial population in the low sodium marinara sauce and 2-inch product depth of chili con carne with beans product was less than 0.50 $\log_{10}$ CFU/g. Both of these results suggest all 12 cooling treatment combinations tested were low risk and, therefore, effective at controlling microbial populations equally.

Recommendations

The Center proposes the following categorical recommendations:

Research Opportunities

1. Investigate the impacts of various cooling methods on *Clostridium perfringens* in proteinaceous foods, such as chili con carne and taco meat, as rapid cooling is imperative for controlling this microorganism.

    a. Validate *Clostridium sporogenes* as a suitable surrogate for *Clostridium perfringens* in taco meat and chili con carne in order to explore cooling

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methods under commercial conditions not certified for BSL II pathogenic research.

2. Explore the impacts of various cooling methods on *Bacillus cereus* populations in chili con carne and taco meat, as this sporeformer has also been implicated in outbreaks involving cooked meat products.

3. Examine the impacts of various cooling methods on *Staphylococcus aureus*, a microorganism that is associated with products that are handled extensively by humans and produces enterotoxin when foods are not cooled rapidly.

Education/Application Opportunities

1. Develop educational materials emphasizing specific recommendations for rapid cooling, including leaving pans uncovered when possible or covering with just one layer of aluminum foil or plastic food wrap, replacing or removing ice baths after several hours of cooling, and cooling foods at 2-inch product depths in the freezer whenever possible.

    a. More specifically, there is opportunity to enhance the Food Code language by specifying the following:

        i. "Shallow pans" could be updated to indicate 2- or 3-inch food depths, or that 2-inch depth is preferred.

        ii. "Using rapid cooling equipment" could be updated to identify a -20°C (-4°F) freezer as a preferred option for storage.

        iii. "Loosely covered, or uncovered if protected from overhead contamination" could be updated to reflect that uncovered is most effective and should be implemented whenever possible.

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2. Incorporate study findings and recommendations into manuscripts, conference abstracts/presentations, and other outreach efforts.

Research Community Opportunity

The methods used to cool food in this study provided equivalent microbiological control. These data suggest that all 12 cooling combinations tested can be utilized as an effective strategy for controlling microbial populations in these food products, despite the inability of some cooling methods to meet FDA Food Code criteria with regard to temperature. However, it must be noted that these data are limited in scope with regards to food products and microorganisms investigated, making these conclusions preliminary in nature. More research is necessary, as this study is not exhaustive with regard to potential foodborne pathogen and food product combinations, which should be explored in future experimentation. Thus, this research could be used as part of a greater body of evidence in order to inform time and temperature limits for cooling through the Conference of Food Protection and FDA.

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Appendix A

References to 3-501.14 and 3-501.15 sections of Chapter 3 of the FDA Food Code

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3-501.14   Cooling.

(A) Cooked TIME/TEMPERATURE CONTROL FOR SAFETY FOOD shall be cooled:

    (1) Within 2 hours from 57°C (135°F) to 21°C (70°F); [P] and

    (2) Within a total of 6 hours from 57°C (135°F) to 5°C (41°F) or less. [P]

(B) TIME/TEMPERATURE CONTROL FOR SAFETY FOOD shall be cooled within 4 hours to 5°C (41°F) or less if prepared from ingredients at ambient temperature, such as reconstituted FOODS and canned tuna. [P]

(C) Except as specified under ¶ (D) of this section, a TIME/TEMPERATURE CONTROL FOR SAFETY FOOD received in compliance with LAWS allowing a temperature above 5°C (41°F) during shipment from the supplier as specified in ¶ 3-202.11(B), shall be cooled within 4 hours to 5°C (41°F) or less. [P]

(D) Raw EGGS shall be received as specified under ¶ 3-202.11(C) and immediately placed in refrigerated EQUIPMENT that maintains an ambient air temperature of 7°C (45°F) or less. [P]

3-501.15   Cooling Methods.

(A) Cooling shall be accomplished in accordance with the time and temperature criteria specified under § 3-501.14 by using one or more of the following methods based on the type of FOOD being cooled:

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    (1) Placing the FOOD in shallow pans; [Pf]

    (2) Separating the FOOD into smaller or thinner portions; [Pf]

    (3) Using rapid cooling EQUIPMENT; [Pf]

    (4) Stirring the FOOD in a container placed in an ice water bath; [Pf]

    (5) Using containers that facilitate heat transfer; [Pf]

    (6) Adding ice as an ingredient; [Pf] or

    (7) Other effective methods. [Pf]

(B) When placed in cooling or cold holding EQUIPMENT, FOOD containers in which FOOD is being cooled shall be:

    (1) Arranged in the EQUIPMENT to provide maximum heat transfer through the container walls; and

    (2) Loosely covered, or uncovered if protected from overhead contamination as specified under Subparagraph 3-305.11(A)(2), during the cooling period to facilitate heat transfer from the surface of the FOOD.

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Appendix B

Nutrition label for the pre-cooked taco meat

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Casa Solana Pre-cooked Taco Meat

Nutrition Facts

Serving Size: 2 OZ (56g)
Servings Per Container: About 163

Amount Per Serving
Calories 110   Calories from Fat 60

                                        % Daily Value*

Total Fat 7g   11%
    Saturated Fat 2.5g   13%
    Trans Fat 0g
    Polyunsaturated Fat 0g
    Monounsaturated Fat 3g
Cholesterol 35mg   12%
Sodium 180mg   8%
Total Carbohydrate 3g   1%
    Dietary Fiber 0g   0%
    Sugars 1g
Protein 11g   22%

Vitamin A 6%   Vitamin C 0%
Calcium 0%   Iron 6%

* Percent daily values are based on a 2,000 calorie
diet. Your daily values may be higher or lower
depending on your calorie needs.

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Appendix C

Nutrition label for low sodium marinara sauce

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Nutrition Facts

Serving Size: 1/2 cup (125g)
Servings per Case:

Amount per Serving

Calories: 60   Calories from Fat: 10

	% Daily Value*
Total Fat: 1 g	2%
Saturated Fat: 0 g	0%
Trans Fat: 0 g	
Polyunsaturated Fat: 0 g	
Monounsaturated Fat: 0.5 g	
Cholesterol: 0 mg	0%
Sodium: 20 mg	1%
Potassium: 580 mg	17%
Total Carbohydrates: 10 g	3%
Dietary Fiber: 3 g	12%
Sugars: 6 g	
Protein: 2 g	
Calcium:	2%
Vitamin A:	10%
Vitamin C:	6%

*Percent Daily Values are based on a 2000 calorie diet. Your daily values may differ depending on your calorie needs.

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Appendix D

Nutrition label for the chili con carne with beans

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School Lunch Chili

Nutrition Facts

100 servings per container

Serving size   **1 cup (260.34g)**

Amount per serving
Calories   180

% Daily Value*

Total Fat 4.5g	**6%**
Saturated Fat 1.5g	**8%**
Trans Fat 0g	
Cholesterol 20mg	**7%**
Sodium 440mg	**19%**
Total Carbohydrate 25g	**9%**
Dietary Fiber 7g	**25%**
Total Sugars 8g	
Includes 2g of Added Sugars	**4%**
Protein 13g	**26%**
Vitamin D 0mcg	**0%**
Calcium 74mg	**6%**
Iron 4mg	**20%**
Potassium 720mg	**15%**

* The % Daily Values (DV) tells you how much a nutrient in a serving contributes to a daily diet. 2000 calories a day is used for general nutrition advice

Ingredients: Red Kidney Beans, Crushed Tomatoes, Water, Tomatoe Puree, 85/15 Ground Beef, Onion Flakes, Chili Powder, Sugar, Ground Cumin, Ground Black Pepper, Garlic Powder

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Appendix E

Nutrition label for the brown rice

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Uncle Ben's Brown Rice

Nutrition Facts

Serving Size 1/4 cup dry (48g)
(About 1 cup cooked)
Servings Per Container About 10

Amount Per Serving

Calories 180   Calories from Fat 15

% Daily Value*

Total Fat 1.5g	**2%**
Saturated Fat 0g	**0%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 0mg	**0%**
Potassium 105mg	**3%**
Total Carbohydrate 36g	**12%**
Dietary Fiber 2g	**8%**
Sugars 0g	
Protein 4g	

Vitamin A 0%	•	Vitamin C 0%
Calcium 0%	•	Iron 4%
Thiamin 10%	•	Niacin 15%
Folate 2%		

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Potassium			3,500mg	3,500mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

INGREDIENTS: WHOLE GRAIN PARBOILED BROWN RICE.

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Appendix F

Recipe utilized for the chili con carne with beans product

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RECISE   NAME: Chili   DATE: 8/1/15

INGREDIENTS	100 SERVINGS		SERVINGS	NOTES
Beef Crumbles, cooked, frozen	5 lb.			
Dry Onions	2 ½ cups			
Garlic, powder	1 ¼ tsp.			
Tomatoes, canned, crushed	2 - #10 cans			
Tomato Puree	1 - #10 can			
Water	1 gal.+1 qt.			
Chili Powder	1 ½ cups			
Cumin, ground	5 TBL			
Pepper, Black	5 tsp.			
Sugar	¾ cup			
Beans, chili (do not drain)	3 - #10 cans			

DIRECTIONS:
1. Thaw beef crumbles under refrigeration.
2. Combine beef crumbles, canned tomatoes, puree, beans, dry ingredients, and water. Do not use mixer.
3. Cook for approximately 45 – 60 minutes or until flavors are blended and internal temperature reaches 165 degrees.
4. CCP. Heat to 165 degrees for 15 seconds.
5. CCP. Hold for hot service 150 degrees or higher.
6. Process 2 same day cook or Process 3 if left over
Note: Frozen beef can be thawed by placing sealed bag in the steamer or boiling water for 10 – 15 minutes. Open bag carefully to avoid being burned. Immediately combine with other ingredients and continue the cooking process.

Serving Size: 8 oz. ladle	Provides: 1.75 oz. Meat/Meat Alternate	Yield: 100 servings
		(Weight or Volume)
		servings:
	(Component Requirements)	(Weight or Volume)

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