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PEER-REVIEWED ARTICLE

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Control of *Bacillus cereus* Populations in Brown Rice by Use of Common Foodservice Cooling Methods

ABSTRACT

Large quantities of food are often prepared, cooled, and stored for future food service in schools and other foodservice settings. The United States Food and Drug Administration indicates that inadequate (slow) cooling contributes to outbreaks of foodborne illness. Brown rice is commonly prepared in school settings and, when subjected to slow cooling, presents a risk for *Bacillus cereus* growth. To investigate this risk, brown rice was portioned to 2- and 3-inch depths in pans before inoculation with heat-shocked *B. cereus* spores (10^4–10^5 spores/g). All pans were stored, either uncovered or covered with single or double layers of aluminum foil, in a 20°C commercial walk-in freezer or were situated in ice water baths inside a 4°C commercial walk-in refrigerator. *B. cereus* populations were enumerated at 0, 4, 8, 12, and 24 hours. Treatment*time ($P = 0.0026$) and product depth*time ($P = 0.0268$) were significant. Between 0 and 24 hours, *B. cereus* populations declined during storage in the freezer and refrigerator and at both depths of brown rice. Temperature data indicate four cooling treatment combinations satisfied FDA Food Code cooling criteria. The lack of cover type ($P > 0.05$) significance, combined with *B. cereus* population declines during cooling, indicates that each cooling technique controlled *B. cereus* outgrowth in brown rice.

INTRODUCTION

Foodborne illness has been reported for a variety of foodservice settings, but schools are most often associated with outbreaks and illnesses. Schools are responsible for almost half of illnesses (44%) and outbreaks (48%) compared with outbreak data from camps, prisons, cafeterias, and daycares. Furthermore, the median size of outbreaks occurring in schools rank second behind those associated with jails and prisons *(13)*. Considering that the National School Lunch Program prepares meals for nearly 31 million United States children daily, these school-associated outbreaks may be directly related to the large number of meals provided to school children *(32)*. Increased severity of foodborne illness and the occurrence of complications among young children are well documented, which makes

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foodborne illness outbreaks particularly concerning for this population *(4, 29, 39)*. The low body weights and underdeveloped immune systems of young children are contributing factors to their increased susceptibility to foodborne illness *(4)*. Although proper food preparation practices are important in all settings, it is particularly critical in schools, where a large number of children, who constitute an at-risk population, are served meals.

Previous research indicates that 78% of school foodservice managers cool leftover food with the intention of reheating it for a future meal; thus, cooling food is a fundamental food preparation technique used by school nutrition programs *(20)*. The Centers for Disease Control and Prevention (CDC) considers improperly cooled food to be at risk for microbial growth, which includes growth of pathogens *(8)*. Similarly, according to the United States Food and Drug Administration (FDA), time/temperature control of food is a critical control point for foodborne illness prevention *(33–36)*. Furthermore, foodborne outbreaks in schools have been associated with improper cooling as a contributing factor *(25, 38)*. Because slow cooling has been recognized as a risk to public health, the FDA Food Code was updated in 2009 to specifically address this concern by requiring food to be cooled to 21.1°C within 2 hours and to 5°C within a total of 6 hours *(37)*. Previously published research has evaluated cooling techniques commonly employed by school nutrition programs for a variety of food products, and the general consensus from these studies is that very few cooling techniques satisfy the cooling requirements outlined in the FDA Food Code *(20, 23, 24, 26)*.

Bacillus cereus causes more than 63,000 illnesses annually, 100% of which foodborne in origin *(27)*. Compared with other pathogens, *B. cereus* generally causes mild and self-limiting symptoms of diarrhea or vomiting, contributing to it being underreported as a cause of foodborne illness *(3, 15, 16, 28)*. Between the years of 1973 and 1997, six outbreaks of *B. cereus* in schools were reported *(10)*, and the Centers for Disease Control and Prevention Foodborne Outbreak Online Database (FOOD Tool) indicates that 11 *B. cereus* outbreaks occurred in schools/universities/colleges and childcare/daycare centers from 1998 to 2016 *(7)*. Therefore, *B. cereus* outbreaks are occurring as a result of consumption of food prepared by foodservice operations.

Bacillus cereus cells and spores are ubiquitous in the environment and can be present at low populations (< 2 log CFU/g) in food products such as uncooked rice and raw vegetables *(5)*. Cooking protocols generally kill vegetative cells of *B. cereus*; however, spores can survive the cooking process. Optimum growth occurs between 30°C and 40°C *(17)*, and during product cooling, heat-shocked spores may germinate and outgrow in the temperature "danger zone" of between 4.4°C and 60°C (40°F and 140°F) *(6, 12, 21)*. Brown rice, one of the grain options that can be used to meet the nutritional standards for schools and childcare centers, is frequently used as an ingredient for some of the proposed USDA recipes for schools and childcare centers *(30)*. Rice is also commonly served in other foodservice operations, such as in jails *(9)*, and is frequently cooled, either as a leftover or for later service *(20)*.

As a continuation of previous research conducted by The Center for Food Safety in Child Nutrition Programs, this study assessed the impact of cooling techniques frequently used in school nutrition programs on *B. cereus* populations in brown rice throughout a 24-hour cooling period. Although the research protocol was based on practices followed in school nutrition programs, it is important to consider that because rice is served in other types of foodservice operations, results from this study will be useful for any foodservice operation serving rice.

MATERIALS AND METHODS

Bacillus cereus strains

Two mesophilic *B. cereus* strains of Biosafety Level I status were utilized in a cocktail (ATCC® 11778 and ATCC® 14579) for brown rice inoculation. The ATCC®14579 strain is capable of enterotoxin production *(19)* and designated for food testing *(1)*, while the ATCC® 11778 strain (FDA strain PCI 213) is designated as a quality control product for food testing *(2)* and does not produce enterotoxins *(19)*. The two ATCC® isolates were rehydrated in Nutrient Broth (BD Difco™ from Fisher Scientific, Frankland Lakes, NJ) and incubated separately at 30°C for 24 hours. They were then dispensed in 1-ml portions to microcentrifuge tubes with 10% glycerol added and stored at -80°C until later use.

Harvesting of *B. cereus* spores

Spore preparation and harvesting for inoculum preparation were based on the procedure of Grande et al. *(14)*. A frozen microcentrifuge tube of each *B. cereus* strain was thawed, and 1 ml was added to separate test tubes containing 9 ml Brain Heart Infusion Broth (BHI; Remel, Lenexa, KS). BHI tubes were incubated at 30°C for 24 hours, after which 100 µl of each strain was spread plated onto Nutrient Agar (Remel, Lenexa, KS) supplemented with 0.05 g/l manganese sulfate (Acros Organics™, Geel, Belgium). Plates were incubated for four days at 37°C to obtain spores from an estimated 90% to 95% of cells *(14)*. Spores and vegetative cells were directly harvested from the plates with sterile loops. The spores and cells harvested from the sterile loop were then deposited directly into a test tube containing 3 ml of distilled water. The sterile loop was agitated to release the spores and cells into the water. This procedure was repeated for each plate. These 3 ml spore + vegetative cell suspensions were vortexed and then pipetted into a 25 ml centrifuge tube and centrifuged at 5,000 × *g* for 15 minutes at 4°C. The resulting pellet was washed with sterile distilled water and re-suspended for a second, identical centrifugation and washing. The final pellet was re-suspended in 25 ml of sterile distilled water.

Aliquots (5 ml) of this suspension, each with a concentration of 10^5–10^6 spores/ml, were transferred to 15 ml conical tubes (MIDSCI, St. Louis, MO), stored at -20°C, and used for this study within three months. Each *B. cereus* strain was harvested and frozen separately.

***Bacillus cereus* inoculum preparation**

On the day of pre-cooked brown rice inoculation, six conical tubes of frozen spore suspension (5 ml each; three tubes representing each *B. cereus* strain) were removed from storage and allowed to thaw for 45 to 60 minutes at room temperature (20°C). The tubes were then immersed in an 80°C bead bath and heat shocked for 10 minutes (total exposure time) to simulate the cooking process and subsequent sub-lethal, heat-induced germination of spores. After the spore suspensions had cooled to room temperature, tubes were thoroughly vortexed and inoculum was prepared by combining the contents of six tubes (30 ml total of heat-shocked spores) and diluting the spore suspensions in 0.1% PW to achieve a target concentration of 10^4 to 10^5 spores/g of brown rice, such that the inoculum comprised no more than 1% of the total food product *(22)*. Briefly, volumes of 75 ml and 100 ml were used to inoculate 2- and 3-inch depths, respectively.

Preliminary research demonstrated that this heat-shocking technique was effective at inducing spore germination. Briefly, over a 3-hour period, 2- and 3-inch product depths of brown rice, inoculated with heat-shocked *B. cereus* spores as described in the Brown rice inoculation section to follow, were stored in a 4°C walk-in refrigerator, and 25 g samples were collected each hour. Sampling procedures were as will be described in the Sampling section. Using the sample homogenate, an endospore stain was performed using the Schaeffer-Fulton method *(18)* to visualize the ratio of spores to vegetative cells during the 3-hour period.

A slide was prepared by air-drying and heat fixing a loopful or smear of suspension; 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, 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 endospore stains were observed under 100× magnification of a compound light microscope (Fisher. Scientific, Frankland Lakes, NJ) 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).

Brown rice preparation

The Uncle Ben's Whole Grain Brown Rice (Mars Incorporated, McLean, VA) used in this study met the nutritional standards outlined by Child Nutrition Programs *(31)* and was ordered from a foodservice distributor. The brown rice product was prepared using a 2:1 ratio of water to uncooked rice. Water was heated to 88°C in a commercial tilt skillet (Cleveland Tilt Skillet) and then added to uncooked brown rice measured in 2½- and 4-inch stainless steel steam table pans. Pans were then covered with a layer of plastic food wrap (Sysco, Houston, TX) and a layer of aluminum foil (Sysco, Houston, TX) before being placed in a commercial-grade convection oven (Garland Master 200) at 177°C for 35 minutes. Following cooking, the brown rice was distributed into 2½- and 4-inch deep stainless steel steam table pans at depths of 2- and 3-inches, respectively, in accordance with 2013 FDA Food Code recommended cooling methods, which was the version of the FDA Food Code in effect at the time this study was completed. Therefore, a gap of ½- and 1-inch remained between the top of the product and the top of the pan for the 2- and 3-inch depths, respectively.

Brown rice inoculation

A Taylor 9842FDA waterproof digital thermometer (Taylor Precision Products, Oak Brook, IL) was used to monitor temperature of the brown rice product. Prior to inoculation with heat-shocked *B. cereus* spores, the product was stirred to facilitate cooling to 60°C ± 5°C and distribute the heat, minimizing the occurrence of random pockets of elevated temperature that may have existed throughout the product. The calculated inoculum volume was then added to each pan, and the brown rice was stirred manually for approximately 2 minutes to distribute the inoculum. The time of inoculation was immediately recorded upon completion of stirring, and this time was used to determine the 0-, 4-, 8-, 12-, and 24-hour sampling time points.

Sampling

At each sampling time point, a composite sample was collected from each pan by sampling the product at four to five randomly selected sub-surface locations from each pan. Random sampling was conducted to account for the possibility that pockets of elevated temperature may have remained after the rice had been stirred. Sub-surface samples were obtained in an effort to test what was likely the warmest brown rice product, which would also be representative of the product most at risk for *B. cereus* growth. Each composite sample was hand mixed, from which one 25-g sub-sample was collected and homogenized in 225 ml of BPW for one minute at 230 rpm (Stomacher® 400 Circulator; Seward, Bohemia, NY). The homogenized sample was serially diluted in BPW and then spread plated on MYP agar. MYP plates were incubated at 30°C for 24 to 48 hours, after which colonies representative of *B. cereus* were counted.

Treatments and cooling

The cooling treatment variables selected were based upon FDA recommended practices highlighted in the 2017 FDA

Food Code *(37)* and/or those described in the literature *(20, 23, 24, 26)*. Following brown rice inoculation and retrieval of the time point 0 composite sample, all pans were equipped with a temperature data logger (Lascar EL-USB-2- LCD USB; Lascar Electronics, Erie, PA) in the center of the pan, as previously described by Olds et al. *(23)*, to record brown rice temperature every minute throughout the 24-hour cooling period. Next, pans were either covered with aluminum foil in a single layer across the top of the pan, covered with a double layer of aluminum foil to restrict exposure to air, or left uncovered. When covered with a single layer, an air gap of ½- and 1-inch remained for 2- and 3-inch pans, respectively. Double-covered pans were prepared by placing a single aluminum foil layer in direct contact with the brown rice surface and a second aluminum foil layer across the top of the pan, such that an air space was present between the two layers. Both 2- and 3-inch pans were subjected to each cover type, and prepared in duplicate so that each combination of cover type and brown rice depth could be stored in either a 4°C (Average: 4.4°C + 0.2) walk-in cooler or a -20°C (Average: -22.1°C + 2.2) walk-in freezer. All pans assigned to storage in the refrigerator were first placed into ice baths as recommended by the 2017 FDA Food Code *(37)*. Accordingly, 4- and 6-inch stainless steel steam table pans were filled ¾ full with ice, and pans containing 2- and 3-inch depths of brown rice were then placed inside the ice baths, respectively. This resulted in the storage of six pans of inoculated brown rice in the refrigerator and of the remaining six pans in the freezer. The brown rice product began to freeze approximately 8 hours into cooling, which interfered with product sampling. To avoid this, pans originally stored in the freezer were removed and placed on a shelf in the refrigerator immediately following the 8-hour sampling point.

Statistical analyses

All experimental procedures were replicated three times. *Bacillus cereus* population data and brown rice temperature data were analyzed with linear mixed models using the PROC MIXED procedure of Statistical Analysis Software 9.4 (SAS; Cary, NC), combined with a compound symmetry covariance structure, a compound symmetry with heterogeneous time variances structure, or an unstructured covariance matrix. The covariance structures were chosen on the basis of Akaike's information criterion, which allowed for the best covariance structure for *B. cereus* population data to be obtained. This was analyzed as a repeated-measures experiment with four factors. A Type III test for fixed effects was also conducted.

The LSMEANS statement in SAS was used to obtain Least Square Means (LSMEANS) of *B. cereus* populations. All variables and their interactions were analyzed to determine significance at a threshold of $P \leq 0.05$. Averaging five values near each time point reduced variability within the temperature data. A threshold of $P \leq 0.05$ was also used to determine significance of the temperature data variables and their interactions.

RESULTS

Temperature data analysis

All main effect variables (cover type, storage location, and depth variables), as well as interactions of these variables, were statistically analyzed to determine significance. To perform the cooling study, temperatures were recorded as they declined throughout the cooling process. As a result, all main effects and their interactions were analyzed at six specific time points (0, 2, 4, 8, 12, and 24 hours) and time was not included in the statistical analyses as a main effect. Temperature data will be incorporated into the following discussion of microbiological data, whereas this section will highlight the ability of each combination of cooling techniques to satisfy cooling criteria described in the 2017 FDA Food Code *(37)*. At time point 4 hours of cooling, product depth and cover type were significant, and 3-inch product depths were significantly higher in temperature. Products in 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.

Storage location and product depth by cover type were significant at the 24-hour time point for brown rice. 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.

Temperature data and FDA Food Code criteria

Brown rice temperature data indicate that four cooling technique combinations achieved the 2017 FDA Food Code criteria *(37)* *(Table 1; Fig. 1)*. At time point 0 hours, product depth and storage location by cover type were significant, as 3-inch product depths were significantly higher in temperature than 2-inch product depths. 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 product depths were cooler than 3-inch product depths, and uncovered products were lowest in temperature.

Microbiological data analysis

Time ($P < 0.0001$) and product depth ($P = 0.0235$) were significant for the brown rice product. 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 (SEM: 0.08 $\log_{10}$ CFU/g). The ice bath in the refrigerator

TABLE 1. Brown rice cooling technique combinations that achieved 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	
		Lower	Upper		Lower	Upper
2-inch						
Refrigerated ice bath	13.65°C ✓	6.37°C	20.93°C	6.18°C	-0.77°C	13.09°C
Single cover	(56.57°F)	(43.47°F)	(69.67°F)	(43.12°F)	(30.61°F)	(55.57°F)
2-inch						
Refrigerated ice bath	20.94°C ✓	13.67°C	28.22°C	8.43°C	1.51°C	15.33°C
Double cover	(69.69°F)	(56.61°F)	(82.80°F)	(47.17°F)	(34.72°F)	(59.60°F)
2-inch						
Refrigerated ice bath	9.46°C ✓	2.18°C	16.74°C	4.06°C ✓	-2.86°C	10.96°C
Uncovered*	(49.03°F)	(35.92°F)	(62.13°F)	(39.31°F)	(26.86°F)	(51.74°F)
3-inch						
Refrigerated ice bath	20.02°C ✓	12.74°C	27.29°C	9.06°C	2.14°C	15.97°C
Single cover	(68.04°F)	(54.93°F)	(81.12°F)	(48.31°F)	(35.86°F)	(60.74°F)
3-inch						
Refrigerated ice bath	24.20°C	16.92°C	31.48°C	9.74°C	2.82°C	16.56°C
Double cover	(75.56°F)	(62.46°F)	(88.66°F)	(49.53°F)	(37.08°F)	(61.81°F)
3-inch						
Refrigerated ice bath	8.94°C ✓	1.66°C	16.22°C	1.76°C ✓	-5.16°C	8.67°C
Uncovered*	(48.09°F)	(34.99°F)	(61.20°F)	(35.17°F)	(22.72°F)	(47.61°F)
2-inch, freezer	20.32°C ✓	13.03°C	27.59°C	1.37°C ✓	-5.54°C	8.26°C
Single cover*	(68.58°F)	(55.45°F)	(81.66°F)	(34.47°F)	(22.02°F)	(46.87°F)
2-inch, freezer	28.86°C	19.94°C	37.77°C	13.21°C	4.94°C	21.53°C
Double cover	(83.95°F)	(67.89°F)	(99.97°F)	(55.78°F)	(40.89°F)	(70.67°F)
2-inch, freezer	10.68°C ✓	3.41°C	17.96°C	0.96°C ✓	-5.95°C	7.87°C
Uncovered*	(51.23°F)	(38.13°F)	(64.33°F)	(33.73°F)	(21.29°F)	(46.17°F)
3-inch, freezer	30.22°C	22.94°C	37.50°C	4.72°C ✓	-2.19°C	11.63°C
Single cover	(86.40°F)	(73.29°F)	(99.50°F)	(40.50°F)	(28.05°F)	(52.94°F)
3-inch, freezer	30.98°C	23.70°C	38.26°C	6.76°C	-0.16°C	13.67°C
Double cover	(87.77°F)	(74.66°F)	(100.87°F)	(44.17°F)	(31.72°F)	(56.61°F)
3-inch, freezer	28.33°C	21.16°C	35.61°C	1.04°C ✓	-5.88°C	7.95°C
Uncovered	(83.00°F)	(70.08°F)	(96.10°F)	(33.87°F)	(21.42°F)	(46.31°F)

*Indicates cooling method achieved both FDA Food Code criteria.

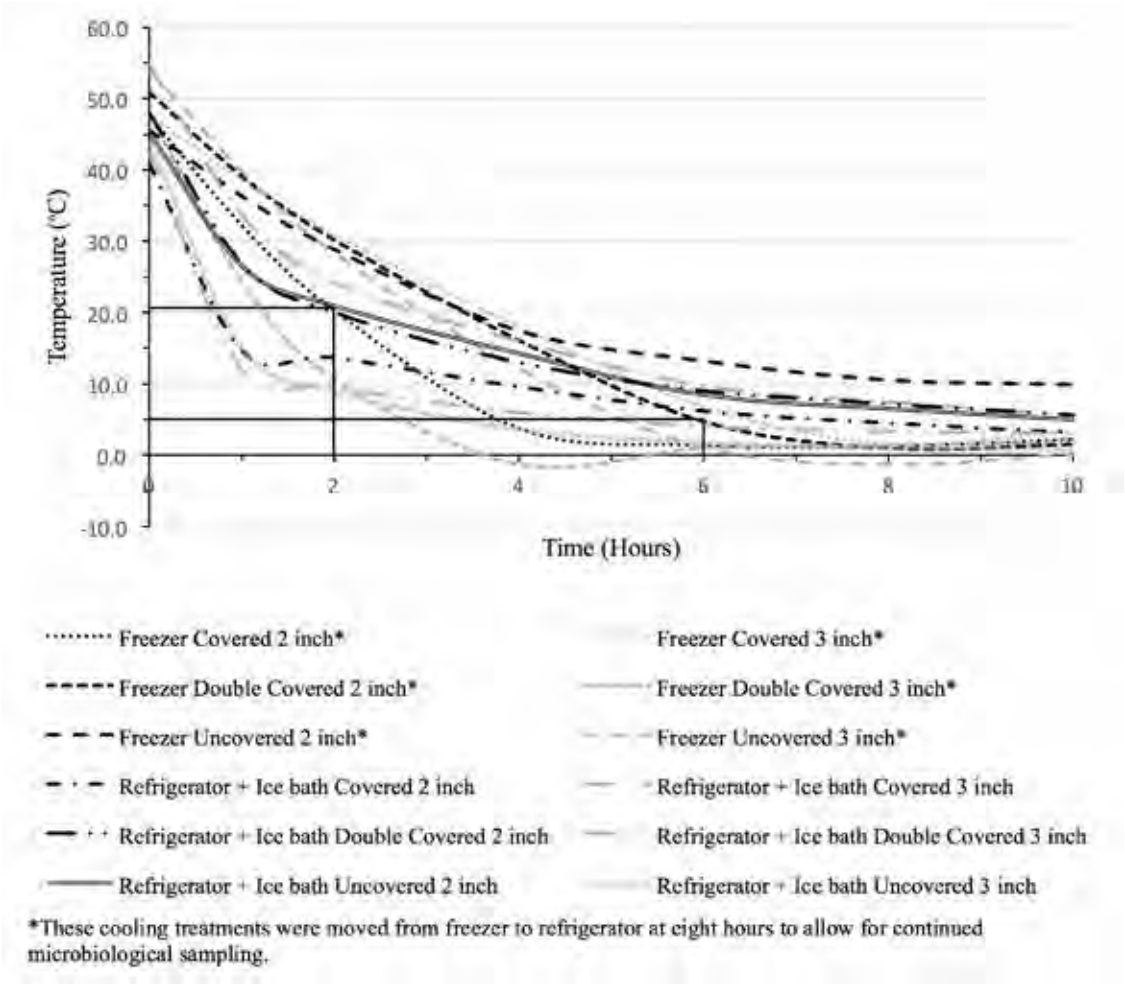

Figure 1. Cooling curves for all cooling technique combinations tested for brown rice. Black lines represent the two FDA Food Code time and temperature criteria.

resulted in a *B. cereus* population decrease of just 0.09 $\log_{10}$ CFU/g (SEM: 0.08 $\log_{10}$ CFU/g) between time points 0 through 24 hours *(Fig. 2)*.

Bacillus cereus 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 [SEM: 0.08 $\log_{10}$ CFU/g] and 0.25 $\log_{10}$ CFU/g [SEM: 0.08 $\log_{10}$ CFU/g], respectively). *Bacillus cereus* populations at time 0 were significantly different between the 2- and 3-inch pans, although the difference was small; the 3-inch product depths were observed to harbor a 0.30 $\log_{10}$ CFU/g (SEM: 0.07 $\log_{10}$ CFU/g) higher population than the 2-inch product depths at inoculation *(Fig. 3)*.

No statistically significant difference ($P > 0.05$) in *B. cereus* populations was observed for cover type (two layers, one layer, uncovered); therefore these data are not shown. Although statistically significant, *B. cereus* $\log_{10}$ CFU/g population data are not presented by time alone or by depth alone, because of the time variable and depth variable being included in the product depth by time interaction.

DISCUSSION

Temperature data

In general, 2-inch pans cooled more rapidly than did 3-inch pans of brown rice. The 3-inch brown rice depth cooled less effectively in the first four hours when stored in the freezer than when stored with an ice bath in the refrigerator. However, approximately 4 to 5 hours into cooling, product situated within an ice bath in the refrigerator began to maintain a fairly steady temperature. It could be hypothesized that this reduced cooling was the result of melting of the ice within the ice bath which would reduce the transfer of cold from the ice bath to the brown rice. In contrast, brown rice stored in the freezer continued to cool at a steady rate and

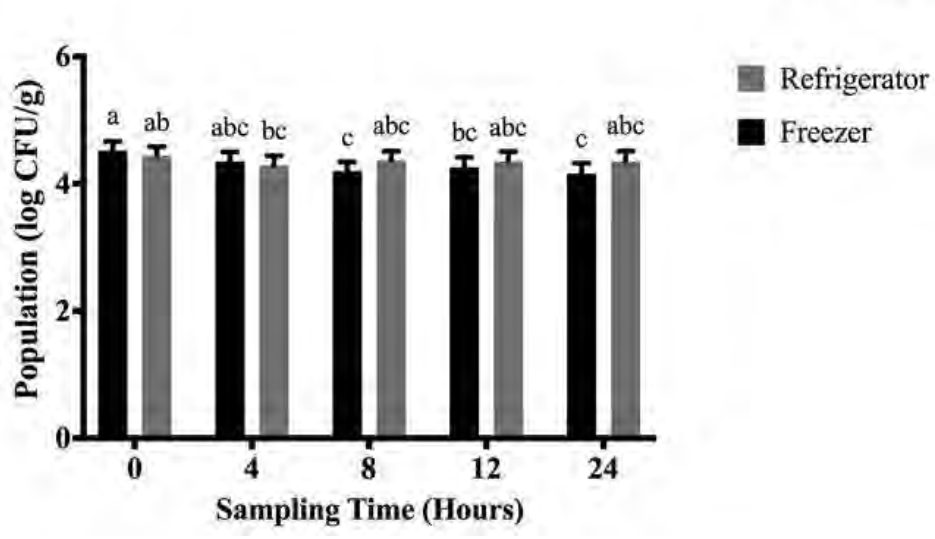

Figure 2. ***Bacillus cereus*** **populations (log_{10} CFU/g) in brown rice analyzed by storage location and time. The storage location × time interaction was significant ($P = 0.0026$) and did not include cover type or depth. Therefore, data associated with all cover types and depths are displayed as storage location and time.**

[a,b,c]Different superscripts indicate statistically significant differences.

Error bars represent the standard error of the mean.

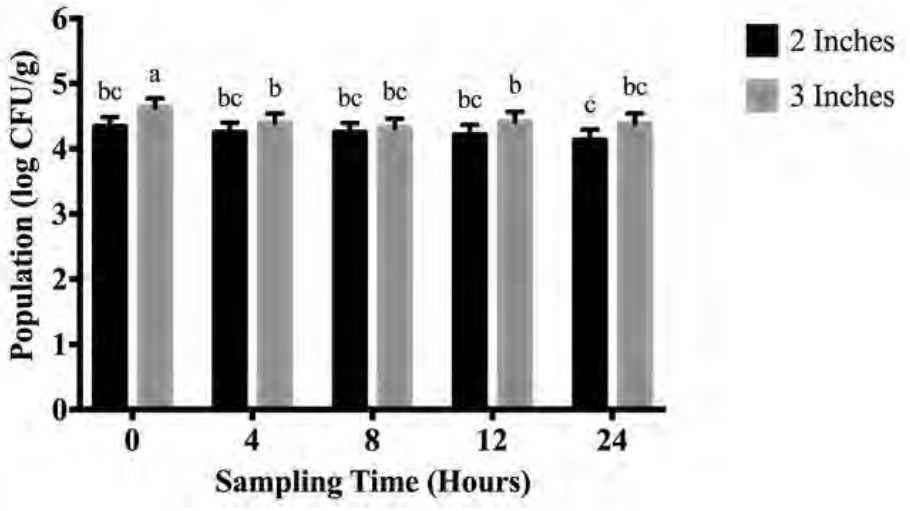

Figure 3. ***Bacillus cereus*** **populations (log_{10} CFU/g) in brown rice analyzed by product depth and time. The storage product depth × time interaction was significant ($P = 0.0268$) and did not include cover type or storage location. Therefore, data associated with all cover types and storage locations are displayed as product depth and time. Product depth was a significant variable ($P = 0.0235$), but data are not presented by depth alone because of the depth × time interaction.**

[a,b,c]Different superscripts indicate statistically significant differences.

Error bars represent the standard error of the mean.

to a lower temperature. Use of an ice bath in the refrigerator was most effective within the first 4 hours of brown rice cooling. After 4 hours of cooling, the freezer reduced brown rice temperatures in a manner that was both steady and predictable, and ultimately resulted in lower temperatures throughout the remaining 20 hours of brown rice cooling.

All of the four cooling technique combinations that satisfied the 2017 FDA Food Code *(37)* were associated with cooling brown rice in ice baths within a refrigerator, which is not in agreement with temperature data collected in previously published studies *(23, 24, 26)*. Olds et al. *(24)* concluded that the 2-inch product depth cooled in a refrigerator with an ice water bath was the only cooling method that satisfied FDA Food Code cooling requirements for a steamed rice product. Studies published by Olds et al. *(24)* and Roberts et al. *(26)* investigated brown rice cooling by placing the brown rice product uncovered into refrigerated or freezing storage immediately after heating. In the present study, brown rice was cooled to 60°C ± 5°C prior to inoculation and then stored with various cover types, a notable difference that may have resulted in the discrepancy between studies.

Microbiological data

Published parameters for inoculation challenge studies suggest a target inoculation concentration of 10^2 to 10^3 $\log_{10}$ CFU/g *(22)*, which is less than the target inoculation concentration of 10^4 to 10^5 heat-shocked spores/g used in this study. Inoculation occurred when the brown rice had reached a target temperature of 60°C ± 5°C, and a study conducted by Desai and Varadaraj *(11)* reported that *B. cereus* vegetative cells demonstrate population decline at 60°C. Thus, in the event that the heat-shocked *B. cereus* spores had begun outgrowth, there was potential for a decline in the vegetative cell population upon inoculation at 60°C. Inoculating with a larger-than-recommended inoculation concentration ensured that *B. cereus* populations would remain above the limit of detection throughout the cooling period.

With the exception of *B. cereus* populations at inoculation (0 hours) in 3-inch product depths, populations in the 2- and 3-inch depths exhibited little variability throughout storage *(Fig. 3)*. Because brown rice is absorbent, it is possible that the product absorbed the inoculum immediately upon introduction, which would lessen the efficacy of subsequent stirring efforts, resulting in uneven distribution of the inoculum throughout the product. It is possible that this contributed to the difference in populations observed at 0 hours. However, the sampling method employed was not designed to detect this; thus, determining the extent to which this may have occurred was beyond the scope of this study.

Figures 2 and 3 illustrate that a slight decrease in *B. cereus* populations occurred during the 24 hour cooling period. While some of the *B. cereus* population reductions were statistically significant, it should be noted that all differences were less than 0.50 log CFU/g, and a population difference of this magnitude is not substantial from a biological sense. Overall, the data presented herein demonstrate that cooling techniques tested were effective at controlling *B. cereus* populations. Because mesophilic *B. cereus* strains were used in this study, one consideration for future research would be to investigate population changes of psychrotrophic or psychrotolerant *B. cereus* strains during cooling.

Annex 3, Section 3-501.19 of the 2017 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* *(37)*. *Bacillus cereus* populations actually declined in this study; thus, with regard to microbiological data presented herein, all cooling techniques employed safely cooled the brown rice product. As mentioned previously, the research protocol for this study was designed to simulate school nutrition program practices. School nutrition programs can implement the various cooling methods presented in this study as an effective cooling strategy for controlling microbiological populations in rice. Although microbiological data suggests that all methods control *B. cereus* populations, it is recommended that school nutrition programs and other foodservice operations preferentially use the four cooling techniques that satisfied 2017 FDA Food Code *(37)* cooling requirements. Considering the popularity of rice in the United States, results from this study will be useful for any foodservice operation serving rice.

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