Evaluating the Impact of Cooling Techniques on *Bacillus cereus* Populations in Brown Rice

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Abstract

Introduction: In institutional settings, large quantities of food may be cooked, cooled, and stored for later service. Improper, or "slow," cooling has been identified by the United States Food and Drug Administration as a contributing factor in foodborne illness outbreaks. Therefore, validating cooling methods that are feasible and effective at preventing pathogen growth is critical for public health.

Purpose: This study was designed to test the efficacy of cooling technique combinations on controlling *Bacillus cereus spore* outgrowth within brown rice.

Methods: Brown rice was prepared according to product label instructions and then cooled to 135-140°F before inoculation with spores (10^4 spores/g) of *B. cereus*. All pans were stored in a commercial walk-in freezer (-20° C) or placed in ice water baths stored inside a commercial walk-in refrigerator (4° C), either uncovered or covered with one or two layers of aluminum foil. Samples were obtained at 0, 4, 8, 12, and 24 hours, plated onto Mannitol Egg Yolk with Polymyxin B agar, and incubated for 24-48 hours to enumerate *B. cereus* populations.

Results: Treatment*time (P=0.0026) and product depth*time (P=0.0268) were statistically significant for *B. cereus* populations within the brown rice product during cooling. *B. cereus* populations decreased by 0.37 $\log_{10}$ CFU/g between 0 and 24 hours when stored in the freezer, whereas populations decreased by 0.09 $\log_{10}$ CFU/g between 0 and 24 hours when stored in the refrigerator. *B. cereus* populations decreased in both 2 and 3-inch product depths between 0 and 24 hours by 0.21 $\log_{10}$ CFU/g and 0.25 $\log_{10}$ CFU/g, respectively.

***Significance*:** The slight decrease in *B. cereus* populations observed over the 24-hour cooling period, combined with no significant difference (P>0.05) in *B. cereus* population observed for the cover (two layers, one layer, uncovered) variable, indicate that all cooling techniques were effective at controlling *B. cereus* population outgrowth from spores in prepared rice.

Introduction

School nutrition programs provide more than 31 million children with meals in over 100,000 schools across the United States (1). In school settings, certain foods may be cooked, cooled, and stored for later service making proper food preparation practices critical to preventing outbreaks of foodborne illness, especially among young children who are a high-risk population for severe illness and complications. The US Food and Drug Administration has consistently identified time/temperature control, specifically cold holding, as a major factor contributing to the incidence of foodborne illness (2, 3). Improper cooling can lead to time and temperature parameters conducive for foodborne pathogen growth (2, 3). Schools in particular may struggle with this critical control point for several reasons including: limited cooling capacity in freezers or refrigerators, a lack of funding for more effective cooling equipment, or the limitations that come with a short workday for school lunch program employees 4, 5). Fried rice, a dish commonly served in schools and daycares, has been implicated in several outbreaks of emetic-type *B. cereus* food poisoning in United States schools due to improper cooling practices after preparation (6, 7). This study was conducted to investigate outgrowth potential of *B. cereus* spores in brown rice during 24 hours of cooling utilizing different cover methods, depths, and storage temperatures.

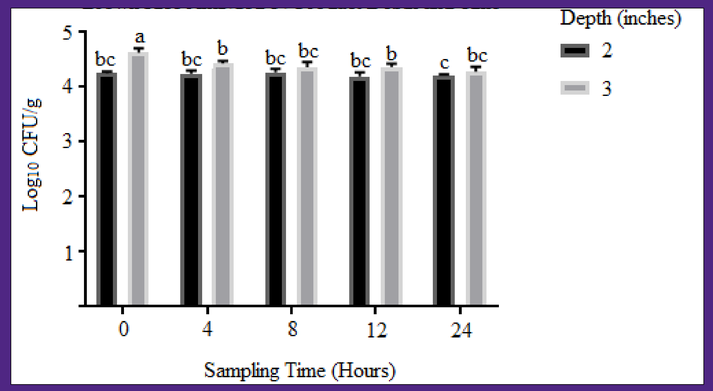

Figure 1: *Bacillus cereus* populations (Log_{10} CFU/g) in brown rice when analyzed by product depth and chilling time.
[abc] Different superscripts indicate statistically significant differences.
Error bars represent the standard error of the mean.

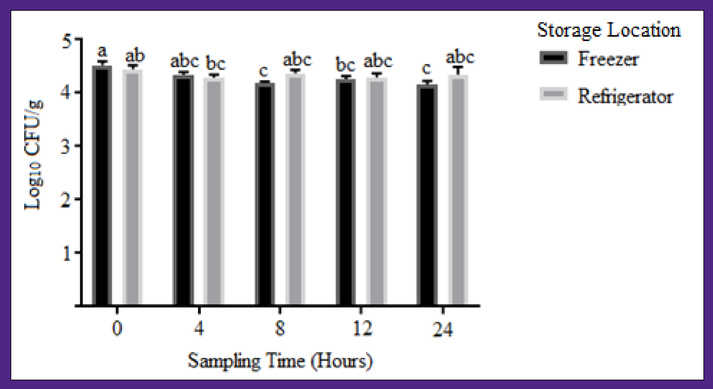

Figure 2: *Bacillus cereus* populations (Log_{10} CFU/g) in brown rice when analyzed by storage location and time.
[abc] Different superscripts indicate statistically significant differences.
Error bars represent the standard error of the mean.

Selected References

1.U.S. Department of Agriculture, Food and Nutrition Service. 2012. National School Lunch Program. Available at: http://www.fns.usda.gov/cnd/lunch/AboutLunch/NSLP FactSheet.pdf
2.United States Food and Drug Administration. 2004. FDA Report on the Occurrence of Foodborne Illness Risk Factors in Selected Institutional Foodservice, Restaurant, and Retail Food Store Facility Types (2004). Availaible at: http://www.fda.gov/downloads/Food/FoodSafety/RetailFoodProtection/FoodborneIllnessandRiskFactorReduction/RetailFoodRiskFactorStudies/UCM224682.pdf
3.United States Food and Drug Administration. 2009. FDA Report on the Occurrence of Foodborne Illness Risk Factors in Selected Institutional Foodservice, Restaurant, and Retail Food Store Facility Types (2009). Available at: http://www.fda.gov/downloads/Food/GuidanceRegulation/RetailFoodProtection/FoodborneIllnessRiskFactorReduction/UCM224682.pdf
4.Roberts, K. R., D. A. Olds, C. W. Shanklin, K. L. Sauer, and J. Sneed. 2013. Cooling of Foods in Retail Foodservice Operations. Food Prot. Trends 33:27-31.
5.Krishnamurthy, K., and J. Sneed. 2011. Cooling practices used in school foodservice. Food Prot. Trends 31:828–833
6.Terranova, W., P. A. Blake. 1978. Bacillus cereus food poisoning. N. Engl. J. Med. 298:143-144
7.CDC. 1994. Bacillus cereus Food Poisoning Associated with Fried Rice at Two Child Day Care Centers — Virginia, 1993. MMWR Morb. Mortal. Wkly. Rep. 43:177-196

Methods

***Experimental Design*:** This study was designed to test the efficacy of cooling techniques which could be used by school nutrition programs on controlling microbial growth, such as *B. cereus*. Two strains of *B. cereus* (ATCC® 11778 and 14579) were combined in a cocktail, heat-shocked (80° C for 10 min) and inoculated into rice to provide a target population of 10^4 spores/g.

***Sample Preparation*:** Water was added to uncooked brown rice according to product label instructions and cooked in 2- and 3-inch steam table pans using commercial-grade convection ovens. The product was allowed to cool to 135-140°F and then inoculated with the heat-shocked *B. cereus*.

***Treatments*:** Six treatments were evaluated to determine if there was an effect on the rate of cooling and subsequent *B. cereus* outgrowth/growth. Steam table pans (2- and 3-inch depth) were portioned and either left uncovered and exposed to air, covered with one layer of aluminum foil that allowed a gap for air exposure, or covered with two layers of aluminum foil without a gap for air exposure between the foil and food product. These treatments were carried out in duplicate to evaluate the effects of walk-in freezer (-20° C) and walk-in refrigerator (4° C) storage scenarios. Pans in the walk-in refrigerator were situated in ice baths to model common food cooling techniques.

***Microbiological Analysis*:** A composite sample of brown rice was collected from various locations in each pan at 0, 4, 8, 12, and 24 hours of chilling. Composite samples were mixed by hand, measured to 25 gram samples and stomached for one minute with 225 mL of buffered peptone water (BPW). Samples were then serially diluted in BPW and dilutions were spread-plated onto Mannitol Egg Yolk with Polymyxin agar. Plates were incubated for 18-24 hrs and flat, pink colonies with an opaque zone were counted and recorded.

Data Analysis: Data were analyzed using the mixed procedure with repeated measures modeling in SAS.

Conclusion and Significance

A storage location*time interaction was observed. Between 0 and 24 hours of cooling, brown rice stored in the freezer demonstrated a *B. cereus* population decrease of 0.37 $\log_{10}$ CFU/g. Between time points 0 through 24 hours, the ice bath stored in the refrigerator was responsible for a *B. cereus* population decrease of 0.09 $\log_{10}$ CFU/g. A product depth*time interaction was also observed. *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 and 0.25 $\log_{10}$ CFU/g, respectively). The small but statistically significant decreases in *B. cereus* populations from the two significant variable interactions demonstrate that all twelve cooling techniques investigated were effective at controlling *B. cereus* populations.

Young children are an at-risk population for severe illness and life-threatening complications from foodborne pathogens. Therefore, it is necessary to conduct research to discover and evaluate cooling methods that are effective at controlling foodborne pathogens in school lunch programs and to translate these data into educational materials and trainings for both school nutrition program personnel and other commercial food service personnel.

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