Cooling Techniques: Characterizing *Escherichia coli* Population Changes in Low-Sodium Marinara Sauce

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The Center of Excellence for FOOD SAFETY RESEARCH IN CHILD NUTRITION PROGRAMS

Abstract

Introduction: The United States Food and Drug Administration has identified improper ("slow") cooling as a factor that contributes to foodborne illness outbreaks. School nutrition programs often cool leftover food products for reuse in future meals. Thus, research that characterizes the impact of a variety of cooling methods on foodborne pathogen populations is important for protecting public health.

***Purpose*:** Characterizing *Escherichia coli* population changes in low-sodium marinara sauce subjected to cooling methods commonly used in school foodservice was the purpose of this research.

Methods: Canned, low-sodium marinara sauce was heated to 165°F, poured to 2 and 3 inch depths into commercial serving pans and then cooled to 135-140°F before inoculation with *E. coli* (target concentration of 10^4 CFU/g) as a surrogate for Shiga Toxin-producing *E. coli*. All pans were stored uncovered or covered, with or without an air gap, in a commercial walk-in freezer (-20°C), or placed in ice water baths in a commercial walk-in refrigerator (4°C). MacConkey agar was used to enumerate *E. coli* populations at 0, 4, 8, 12, and 24 hours.

Results: Product depth ($p<0.0001$) and time ($p=0.0312$) were statistically significant. A difference of 0.40 $\log_{10}$ CFU/g *E. coli* was observed between 2-inch (4.20 $\log_{10}$ CFU/g) and 3-inch (3.79 $\log_{10}$ CFU/g) product depths. In regards to time, the largest increase in *E. coli* populations occurred between the 0- and 8-hour time points, with a difference of 0.21 $\log_{10}$ CFU/g.

Significance: Although significant, a marginal increase of 0.21 $\log_{10}$ CFU/g was more likely due to natural variation caused by inoculating and sampling large quantities of food rather than cooling failure. This combined with the lack of additional significant variables (i.e. cover), suggests that all cooling method combinations were effective at controlling *E. coli* populations in low-sodium marinara sauce.

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). Low sodium marinara sauce is commonly served in schools and daycares (5) and may become contaminated with foodborne pathogens such as *E. coli* O157:H7 because of improper hygiene and cross contamination after cooking; infectious food handlers are often implicated in outbreaks of gastrointestinal foodborne illness in school settings (6). This study was conducted to investigate *E. coli* populations during 24 hours of cooling low sodium marinara sauce utilizing different cover methods, depths, and storage temperatures.

Figure 1: Food preparation, inoculation, and initial 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.

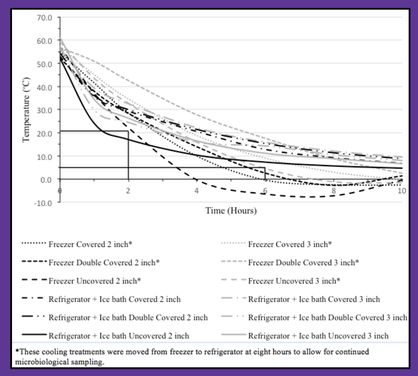

Figure 2: Cooling curves for all cooling technique combinations tested for low sodium marinara sauce.
Black lines represent the two FDA Food Code time and temperature criteria .

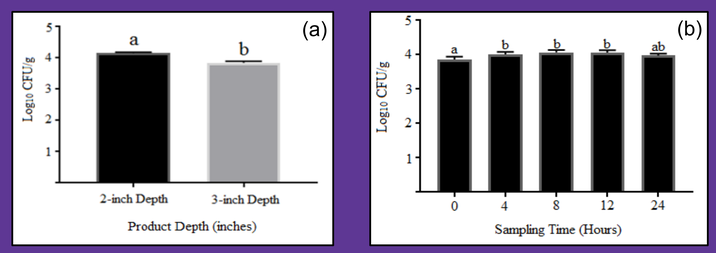

Figure 3: Surrogate *Escherichia coli* populations (Log_{10} CFU/g) in low sodium marinara sauce analyzed by (a) product depth, and (b) time.
[abc] Different superscripts indicate statistically significant differences.
Error bars represent the standard error of the mean.

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.Venuto, M., & Garcia, K. (2015). Analyses of the contributing factors associated with foodborne outbreaks in school settings (2000–2010). J. Environ. Health, 77:16-20.

Methods

***Experimental Design*:** This study was developed to test the efficacy of cooling techniques used by school nutrition programs on controlling microbial growth, such as *Escherichia coli* (*E. coli*). In this study, four ATCC strains of *E. coli* were combined in a cocktail to a target population of 10^4 CFU/g in order to accurately simulate survivability of the foodborne pathogen *E. coli* O157:H7 low sodium marinara sauce.

***Sample Preparation*:** Canned low sodium marinara sauce was cooked to 74°C (165°F) in a commercial tilt skillet, and then prepared at two and three inch depths in steam table pans. The product was allowed to cool to 140°F and then inoculated with the *E. coli* surrogate cocktail.

***Treatments*:** Six treatments were evaluated to determine if there was an effect on the rate of cooling and subsequent *E. coli* growth. Steam table pans (2- and 3-inch depth) were portioned and either left uncovered, 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 cooling techniques.

***Microbiological Analysis*:** A composite sample of sauce 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 MacConkey agar. Plates were incubated for 18-24 hrs and pink colonies were counted and recorded.

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

Conclusion and Significance

Product depth ($P<0.0001$) and time ($P=0.0312$) were statistically significant for low sodium marinara sauce. The difference in *E. coli* populations between 2-inch (4.20 $\log_{10}$ CFU/g) and 3-inch (3.79 $\log_{10}$ CFU/g) product depths overall was 0.41 $\log_{10}$ CFU/g (Figure 6). In regards to time, 0.21 $\log_{10}$ CFU/g was the largest increase in populations, occurring between the 0- and 8-hour time points (Figure 7). 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 (data not shown), and no interaction combinations tested were significant. Overall, these results indicate all cooling method variables suppressed growth to the same degree, suggesting all cooling methods evaluated were effective at controlling *E. coli* populations in marinara sauce.

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.

Acknowledgements

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

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The Center of Excellence for FOOD SAFETY RESEARCH IN CHILD NUTRITION PROGRAMS

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.

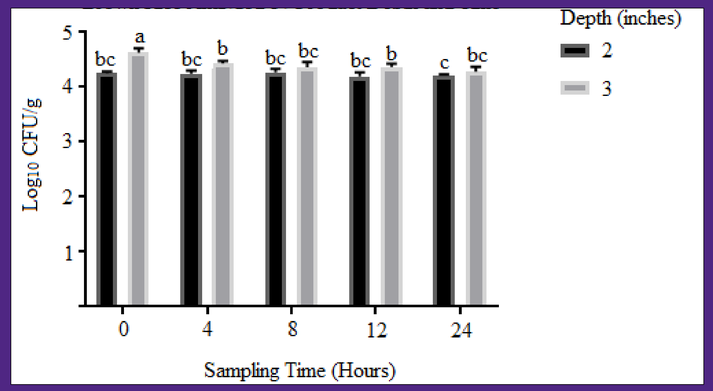

Figure 1: *Bacillus cereus* populations (Log_{10} CFU/g) in brown rice when analyzed by product depth and chilling time.
[abc] Different superscripts indicate statistically significant differences.
Error bars represent the standard error of the mean.

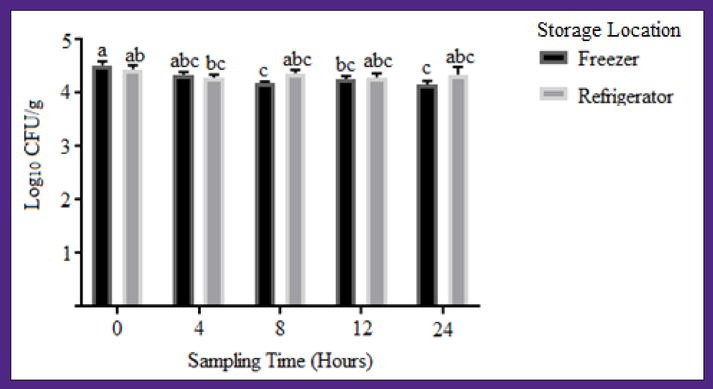

Figure 2: *Bacillus cereus* populations (Log_{10} CFU/g) in brown rice when analyzed by storage location and time.
[abc] Different superscripts indicate statistically significant differences.
Error bars represent the standard error of the mean.

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.

Acknowledgements

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Evaluating the Impact of School Foodservice Cooling Techniques on *Escherichia coli* Populations in Recipe Prepared Chili Con Carne with Beans

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

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Abstract

Introduction & Purpose: In preventing foodborne illness outbreaks, proper food preparation practices are especially critical in institutional settings where food products are prepared in large quantities. The third leading factor in outbreaks of school associated foodborne illness is improper or "slow" cooling. Therefore, conducting research regarding cooling methods that are both effective and feasible for preventing pathogen growth is critical to public health. The purpose of this study was to evaluate combinations of cooling techniques and their impact on *Escherichia coli* populations in a recipe prepared chili con carne with beans.

Methods: Chili was prepared according to a school nutrition program recipe and heated to 165°F, poured into steam table pans to 2 and 3 inch depths, then cooled to 135-140°F before inoculation with *E. coli* (target concentration of 10^4 CFU/g). Pans were stored in a commercial walk-in freezer (-20˚ C) or placed in ice water baths in a commercial walk-in refrigerator (4˚ C). All pans were stored uncovered or covered with one or two layers of aluminum foil. Samples were plated onto MacConkey agar at 0, 4, 8, 12, and 24 hours, and incubated for 18-24 hours to enumerate *E. coli* populations.

Results & Conclusions: No statistically significant difference (P>0.05) in *E. coli* population was observed for cover (two layers, one layer, uncovered), treatment (refrigerator vs. freezer), or depth variables. However, time (P=.0015) and a two way interaction, depth by time (P=.0197), were significant for this product. Although time was statistically significant, the largest recorded change in *E. coli* population (-0.1755 log10 CFU/g) between time points 4 and 12 may not be considered microbiologically significant. Depth by time was also statistically significant, with the largest population change (-0.2777 log10 CFU/g) recorded for three inch food depths between time point 0 and time point 4. For two inch depths, the largest change in *E. coli* population (-0.1534 log10 CFU/g) occurred between time point 0 and 12. This data indicates that most cooling treatments evaluated were effective at controlling *E. coli* populations in commercially prepared chili product.

Introduction

Schools are associated with the largest number of outbreaks (286) and illnesses (17,266) when compared with other institutions like daycares, workplace cafeterias, and prisons or jails (1). The National School Lunch Program serves over 31 million children each day, contributing to the significant size of outbreaks and number of illnesses (2). Improper or "slow" cooling is a considerable risk for schools and other institutional settings and has been identified as the third leading factor in school associated foodborne illness (3). When food is prepared in large quantities, the risk to public health lies in the cooling process when food is stored until later service. Therefore, the Food and Drug Administration issued a food code in 2009 that requires food products to be cooled to 70°F within two hours of cooking and down to 41°F within a total of six hours. Several studies have evaluated cooling techniques for food products commonly used in school nutrition programs (4,5) and have concluded that very few techniques meet the 2009 FDA food code standards. This study was conducted to evaluate surrogate *E. coli* survival during 24 hours of cooling as a follow up to these studies.

Figure 1: Pan preparation and cooking equipment. Upper left: Steam table pans with data loggers and appropriate treatment. Bottom left: Device used to keep temperature probe in the center of the pan. Right: Commercial grade tilt skillet used to prepare the chili con carne with beans.

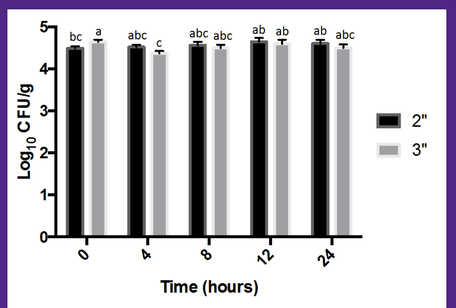

Figure 2: Data were analyzed by depth and time because of the two way interaction significance. Error bars represent standard error of the mean.

Data are shown according to significance detected. However, because the largest difference was .2777 log10 CFU/g, differences in depth by time values are not indicated with superscripts.

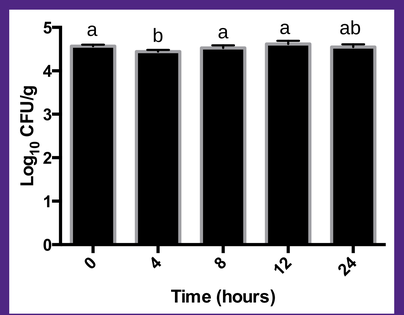

Figure 3: Data were analyzed by time alone because time was a single significant factor. Error bars represent standard error of the mean.

[ab] Different superscripts indicate statistically significant difference (P<0.05)

Selected References

1. Gould HL, Walsh KA, Vieira AR, Herman K, Williams IT, Hall AJ, Cole D. 2013. Surveillance for foodborne disease outbreaks — united states, 1998–2008. MMWR Morb Mortal Wkly Rep 62(2):1-34.
2. Pogostin L, et al. 2008. School-associated foodborne outbreaks in the united states-1998-2006. poster session presented at the 4th annual OutbreakNet conference, june, 6, 2008, denver, CO.
3. 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 U.S. Department of Agriculture, Food Safety and Inspection Service.
4. Olds DA and Sneed J. 2005. Cooling rates of chili using refrigerator, blast chiller, and chill stick methods. J of Child Nutr Manag 29(1):1-6.
5. Roberts KR, Olds DA, Shanklin CW, Sauer KL, Sneed J. 2013. Cooling of foods in retail foodservice operations. Food Prot Trends 33(1):27-31.

Methods

***Experimental Design*:** This study was developed to test the efficacy of cooling techniques used by school nutrition programs on controlling microbial growth, such as *Escherichia coli* (*E. coli*). In this study, four ATCC strains of *E. coli* were combined in a cocktail to a target population of 10^4 CFU/g in order to accurately simulate survivability of the foodborne pathogen *E. coli* O157:H7 in chili con carne with beans.

***Sample Preparation*:** Chili was prepared according to a school nutrition program recipe, heated in a commercial tilt skillet to 165°F , and then prepared at two and three inch depths in steam table pans. The product was allowed to cool to 140°F and then inoculated with the *E. coli* surrogate cocktail.

***Treatments*:** Six different treatments were evaluated to determine if there was an effect on the rate of cooling and subsequent microbial growth. Two and three inch steam table pans 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 duplicated in a walk-in freezer and walk-in refrigerator. Pans in the walk-in refrigerator were situated in ice baths to model common food cooling techniques.

***Microbiological Analysis*:** A composite sample of chili was collected from various locations in each pan at sampling time points of 0, 4, 8, 12, and 24 hours. 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 MacConkey agar (MAC). MAC plates were incubated for 18-24hrs and lactose fermenting colonies were enumerated and recorded.

Data Analysis: Data were analyzed as using a mixed procedure, repeated measures model in SAS.

Conclusion and Significance

Time was statistically significant for this food product (P=.0015). The slight decrease in *E. coli* population over time indicates an effective control for the cooling methods evaluated. The time by depth interaction was significant for this food product as well (P=.0197). The decrease in *E. coli* population over time for the two and three inch food depths also demonstrates an effective control for the cooling methods evaluated. Although these effects were statistically significant, it should be noted that the variation observed in population was well under 0.5 Log_{10} CFU/g. It is possible that this small degree of difference is the result of natural variation in populations throughout the food. These results, along with the lack of statistical differences among cover and treatment variables, indicate that a majority of foodservice cooling methods evaluated were effective at controlling *E. coli* populations in chili con carne with beans.

Acknowledgements

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Evaluating the Effectiveness of Cooling Techniques in Chili Con Carne with Beans

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The Center of Excellence for FOOD SAFETY RESEARCH IN CHILD NUTRITION PROGRAMS

Abstract

Introduction & Purpose: Large quantity recipes are commonly used in child nutrition programs. Improper cooling of food is a major contributing factor to foodborne illnesses. Requirements in the FDA's Food Code 2013 state food should cool from 135°F to 70°F (57.2°C to 21.1°C) within two hours and from 135°F to 41°F (57.2°C to 5°C) within a total of six hours. Cooling foods within these time and temperature parameters is essential to prevent foodborne illness outbreaks, especially for vulnerable populations like children. Identifying cooling methods that are effective and feasible is an important component to reduce public health risks. This research is a continuation of previous studies to determine the effectiveness of cooling methods used in school nutrition programs by identifying which procedures best meet cooling requirements in the Food Code.

Methods: Chili was cooked to 165°F, portioned to 5.1 cm (2-inch) or 7.6 cm (3-inch) depths in stainless steel steam table pans, and cooled to 140-135°F. Pans were covered with a single layer of foil, two layers of foil, or left uncovered; and cooled in a walk-in freezer (-4°F, -20°C) or on an ice bath placed in a walk-in refrigerator (39°F, 4°C). Temperatures were monitored every 60 seconds for 8 hours. ***Results:*** At 2 hours, a significant difference was found between the freezer and ice bath (p=<.0001); the depths of the pans (p=0.0082), and the pan covering method (p=<.0001). Three cooling methods reached the 70°F (21.1°C) as recommended by FDA. By 6 hours, a significant difference was found between the depths of the pans (p=0.0083) and the pan covering method (p=0.0020). Five cooling methods reached 41°F (5°C).

Conclusions: Three cooling methods met Food Code requirements: uncovered 2-inch and 3-inch pans in the ice bath and uncovered 2-inch pan in the freezer. This study provides information about best practices for cooling large quantities of food following the Food Code 2013 guidelines using commercial kitchen equipment. Using the most effective practices to cool food can strengthen food safety practices in schools by preventing the growth of potential pathogens and, therefore, protecting students from foodborne illnesses.

Statement of Purpose

The purpose of this study was to determine if common cooling methods used in child nutrition programs for met the Food Code 2013 cooling guidelines.
Methods examined included:

- Ice bath in walk-in cooler
- Walk- in freezer
- Pan coverings
- Product depth

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This project has been funded at least in part with Federal funds from the U.S. Department of Agriculture. The contents of this publication do not necessarily reflect the view or policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government."

Methods

- Chili recipe met nutritional standards for the National School Lunch Program
- Chili was cooked to 165°F, cooled to 140-135°F before temperature monitoring
- Twelve treatments (n=3) tested all combinations of three factors:
  - Cooling method: walk in freezer (10% capacity) and ice bath in walk-in cooler (10% capacity)
  - Chili depth: 2 and 3-inch depth (commercial full-size stainless steel steam table pans)
  - Pan coverings: uncovered, single foil layer, double foil layer (standard weight food service aluminum foil)
- Chili temperatures were monitored every 60s for 8h (EasyLog thermocouple USB data logger, Lascar)

Results

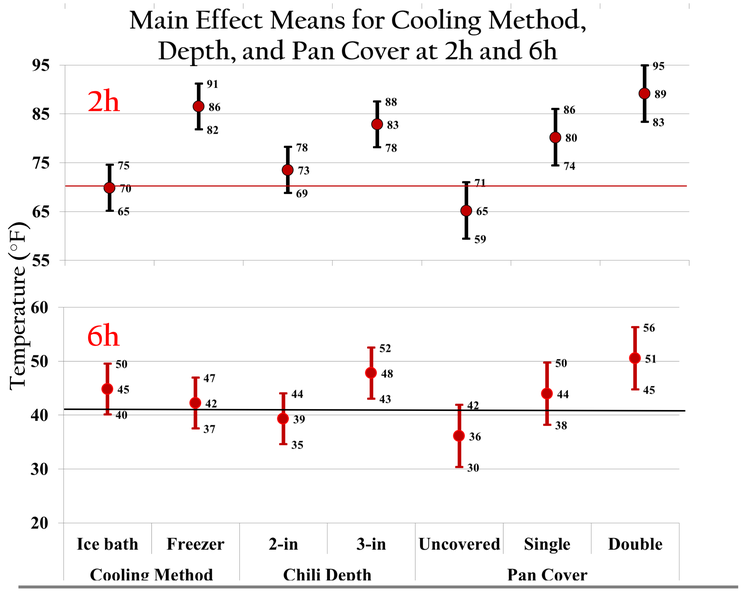

Figure 1. Main effect means for cooling method, chili depth and pan covering. Mean temperature of uncovered pans was below 70°F at 2h and below 41°F at 6h. Ice bath means reached 70°F at 2h. Mean for 2-in chili depth was below 41°F at 6h.

Results, Continued

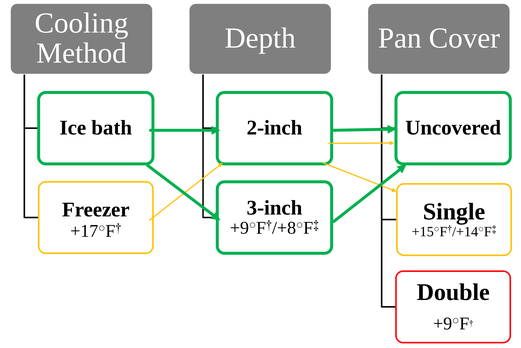

Figure 2. Factor combinations meeting Food Code 2013. Cooling factors in green met standards when combined. Factors in yellow met a part, but not all, of the standard. Factors in red did not meet standards.

†Significant temperature difference for the main effect at 2h. ‡Significant temperature difference for the main effect at 6h.

Three Way Treatments	2h	Lower	Upper	6h	Lower	Upper
Ice bath 2-in Uncovered†	56°F	48°F	64°F	38°F	31°F	46°F
Ice bath 3-in Uncovered†	58°F	49°F	65°F	36°F	29°F	44°F
Freezer 2-in Uncovered	65°F	57°F	73°F	25°F	18°F	33°F
Ice bath 2-in Single	71°F	63°F	79°F	33°F	26°F	41°F

Table 1. Estimated 3-way treatment means and intervals for the best combined cooling treatments. Ice bath cooled, uncovered pans with 2 and 3-in chili depths met Food Code 2013 standards. †Estimated temperature upper limit for the treatment below 70°F at 2h, and 41°F at 6h.

Implications

- Many cooling methods commonly used in child nutrition programs do not meet Food Code 2013 cooling standards.
- None of the cooling methods with covered pans met standards.

Evaluating the Impact of School Foodservice Cooling Techniques on Escherichia coli Populations in a Commercially Available Marinara Sauce Product

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

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The Center of Excellence for FOOD SAFETY RESEARCH IN CHILD NUTRITION PROGRAMS

Abstract

Introduction: Proper commercial food preparation practices are critical for preventing outbreaks of foodborne illness. Improper or "slow" cooling is the third leading factor in school associated foodborne illness outbreaks. Thus, research to scientifically characterize and validate cooling methods that are both effective and feasible for preventing pathogen growth in meals prepared in school nutrition program settings is critical to public health.

Methods: Marinara sauce was purchased from a local foodservice distributor and heated to 165°F in a commercial tilt skillet, measured to 2 and 3 inch depths in steam table pans and allowed to cool to 135-140°F before inoculation with *Escherichia coli* (*E. coli*; target population of 10^4 CFU/g). Pans were placed in a commercial walk-in freezer (-20 ° C) or situated in ice water baths in a commercial walk-in refrigerator (4 ° C). All pans were either uncovered or covered, with one or two layers of aluminum foil to allow for air exposure. At 0, 4, 8, 12, and 24 hours, samples were serially diluted and plated onto MacConkey agar. Plates were then incubated for 18-24 hours to quantify *E. coli* populations.

Results: No statistically significant difference (P>0.05) in *E. coli* populations were observed for cover (covered two layers, covered one layer, uncovered) or treatment (refrigerator vs. freezer) variables and no interaction combinations tested were significant. However, product depth (P<0.0001) and time (P=.0312) were statistically significant for this product. The difference in *E. coli* populations between 2 inch (4.20 log10 CFU/g) and 3 inch (3.79 log10 CFU/g) food depths were 0.40 log10 CFU/g. Though time was statistically significant, .20 log10 CFU/g was the largest difference in populations over time which may or may not be microbiologically significant.

Significance: Depth and time were statistically significant for this product. However, the lack of statistical differences among cover and treatment variables and variable interactions indicates that a majority of foodservice cooling methods evaluated were effective at controlling *E. coli* populations in Marinara sauce.

Introduction

A 2013 Morbidity and Mortality Weekly Report by the CDC analyzed foodborne illness outbreak data gathered from 1998-2008 and attributed illnesses to food preparation locations. Results revealed that schools were associated with the largest number of outbreaks (286) and illnesses (17,266) when compared with other institutions like daycares, workplace cafeterias, and prisons or jails (1). The large number of outbreaks and illnesses may be due to the fact that The National School Lunch Program serves over 31 million children each day (2). A significant risk for schools and other institutional settings is improper or "slow" cooling, which has been identified as the third leading factor for school associated foodborne illness (3). The risk to public health lies in the preparation of large quantities of food that are cooked, cooled, and stored until later service. For this reason, the Food and Drug Administration issued a food code in 2009 requiring food products be cooled to 70°F within two hours of cooking and down to 41°F within a total of six hours. Studies have been carried out to evaluate effective cooling techniques for several food products (4,5) and have produced very few techniques that meet the 2009 FDA code standards. This study was conducted to evaluate pathogen survival and activity during cooling as a follow up to these studies.

Figure 3: Treatment preparation and cooling techniques. Far left: Commercial pans in walk-in refrigerator with data loggers and appropriate treatment. Top right: Sampling from multiple areas in the pan after inoculation. Bottom right: Ice baths prepared for refrigerator treatments.

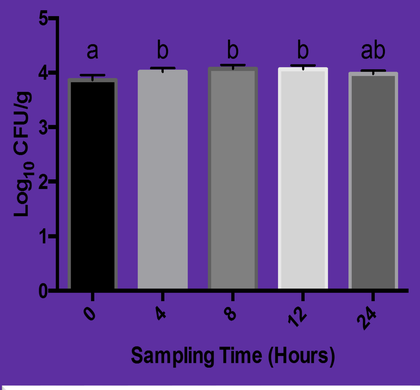

Figure 4: *E. coli* populations at each sampling point. Time was significant, therefore, data are displayed accordingly.

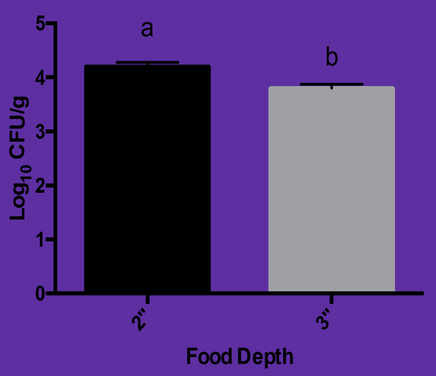

Figure 5: *E. coli* populations at 2 and 3 inch food depths. Food depth was significant, therefore, data are displayed accordingly.

[ab] Different superscripts indicate statistically significant difference (P<0.05)
Error bars represent standard error of the mean

Selected References

1. Gould, H. L., K. A. Walsh, A. R. Vieira, K. Herman, I. T. Williams, A. J. Hall, and D. Cole. 2013. Surveillance for Foodborne Disease Outbreaks — United States, 1998–2008. CDC MMWR 62:1-34. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/ss6202a1.htm
2. Pogostin, L., T. Ayers, S. Gray, T. Nguyen, M. Lynch, and I. Williams. 2008. School-associated foodborne outbreaks in the United States-1998-2006. Poster session presented at the 4th annual OutbreakNet Conference, June, 6, 2008, Denver, CO.
3. 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 U.S. Department of Agriculture, Food Safety and Inspection Service.
4. Olds, D. A., J. Sneed. 2005. Cooling Rates of Chili Using Refrigerator, Blast Chiller, and Chill Stick Methods. Journal of Child Nutrition Management 29:1-6.
5. Roberts, K. R., D. A. Olds, C. W. Shanklin, K. L. Sauer, and J. Sneed. 2013. Cooling of Foods in Retail Foodservice Operations. Food Protection Trends 33:27-31.

Methods

***Experimental Design*:** This study was developed to test the efficacy of school nutrition program cooling techniques on controlling microbial growth, such as *Escherichia coli* (*E. coli*). In this study, four ATCC strains of *E. coli* were combined in a cocktail to a target population of 10^4 CFU/g in order to simulate survivability of the foodborne pathogen *E. coli* O157:H7 in marinara sauce product.

***Sample Preparation*:** Marinara sauce was heated in commercial tilt skillet to 165°F and prepared at two and three inch depths in commercial pans. The product was allowed to cool to 140°F and then inoculated with the *E. coli* surrogate cocktail.

***Treatments*:** Six different treatments were utilized to determine if there was an effect on the rate of cooling and subsequent microbial growth. Two and three inch steam table pans were portioned and either left uncovered and exposed to air, covered with one layer of aluminum foil and 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 duplicated in a walk-in freezer and walk-in refrigerator. Pans in the walk-in refrigerator were situated in ice baths to represent a common food cooling technique.

***Microbiological Analysis*:** A composite sample of the marinara sauce product was collected from various locations in each pan at sampling time points of 0, 4, 8, 12, and 24 hours. Composite samples were mixed by hand, measured to 25 gram samples and stomached for one minute with 225 mL of buffered peptone water (BPW). Following homogenization, samples were serially diluted in BPW and appropriate dilutions were spread-plated onto MacConkey agar (MAC). MAC plates were incubated for 18-24hrs and lactose fermenting colonies were enumerated and recorded.

Data Analysis: Data were analyzed as using a mixed procedure, repeated measures model in SAS.

Results and Conclusion

Depth was statistically significant for this food product (P<0.0001). It is possible that 3 inch pans facilitated the retention of pockets of high temperature within the food product that reduced some of the bacterial population at inoculation. The increased depth resulted in slower cooling, perhaps allowing pockets of high temperature to persist. Time was also significant for this product. The largest population fluctuations occurred between 0 and 8 hour time points, perhaps pointing to a time point risk where there is loss in pathogen population control for the cooling methods tested. However, the lack of statistical differences among cover and treatment variables and variable interactions indicates that a majority of foodservice cooling methods evaluated were effective at controlling *E. coli* populations in marinara sauce.

Scientific validation of cooling methods that are effective at controlling foodborne pathogens will continue to be a significant research area that directly benefits public health by reducing the risk of foodborne illness and allowing a degree of necessary flexibility for foodservice operations. This data could also be translated into educational materials and trainings that can be used to inform cooling protocols used in institutional or commercial foodservice settings.

Acknowledgements

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Evaluating the Impact of School Foodservice Cooling Techniques on Escherichia coli Populations in a Commercially Available Pre-Cooked Taco Meat Product

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The Center of Excellence for FOOD SAFETY RESEARCH IN CHILD NUTRITION PROGRAMS

Abstract

Introduction & Purpose: Proper commercial food preparation practices are critical for preventing outbreaks of foodborne illness. Improper or "slow" cooling is the third leading factor in school associated foodborne illness outbreaks. Thus, research to scientifically characterize and validate cooling methods that are both effective and feasible for preventing pathogen growth during school foodservice preparation is critical to public health.

Methods: Commercially available pre-cooked taco meat was re-heated to 165°F, measured to 2 and 3 inch depths in commercial serving pans and allowed to cool to 135-140°F before inoculation with *Escherichia coli* (target population of 10^4 CFU/g). Pans were placed in a commercial walk-in freezer (-20°C) or situated in ice water baths in a commercial walk-in refrigerator (4°C). All pans were either uncovered or covered with or without a gap to allow for air exposure. At 0, 4, 8, 12, and 24 hours, samples were plated onto MacConkey agar and incubated for 18-24 hours to quantify *E. coli* populations.

Results: No statistically significant difference (P=0.9335) in *E. coli* population level was observed for the cooling technique combinations evaluated in this study. However, sampling time was significant (P=0.0001). A time by cooling treatment interaction was not observed (P=0.1462); thus, data were evaluated by time alone. *E. coli* populations declined slightly from 4.5 $\log_{10}$ CFU/g at 0 hours to 4.2 $\log_{10}$ CFU/g at 24 hours.

Conclusion: The lack of a cooling treatment effect combined with a small but statistically significant decline in target microbial population indicates that all foodservice cooling treatments evaluated were effective at controlling *E. coli* populations in cooked taco meat.

Introduction

Between 1998 and 2008, the CDC received 13,405 reports of foodborne illness outbreaks (1). In fact, the number of reported illnesses related to school food preparation were second only to those reported for prisons or jails (1). Good food preparation practices are critical for preventing outbreaks of foodborne illness and improper or "slow" cooling poses a significant public health risk. It has been identified as the third leading factor in school associated foodborne illness outbreaks (2). Schools provide, on average, 32 million meals daily (3), which means focusing research on proper cooling techniques and microbiological consequences is crucial. As of 2009, the Food and Drug Administration issued a food code requiring food products be cooled to 70 degrees Fahrenheit within two hours and down to 41 degrees Fahrenheit within a total of six hours. Variability related to food preparation facilities and chilling capacity, as well as variability in specific chilling protocols, exist in school foodservice operations across the state. Researching and evaluating cooling methods that are both effective and feasible for school foodservice preparation environments is essential.

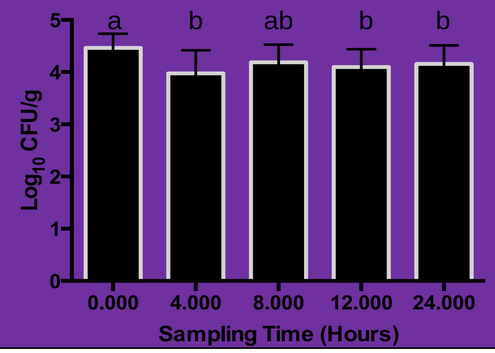

Figure 1: *Escherichia coli* populations at each sampling point. Because treatment and the time x treatment interaction were not statistically significant, data are displayed according to time.
[a,b] Time points with different subscripts differ statistically.

Figure 2: Treatment preparation and cooling techniques. Far left: Commercial pans in walk-in refrigerator with data loggers and appropriate treatment. Top right: Properly distributing *E. coli* inoculum with thorough stirring. Bottom right: *E. coli* cocktail matched with appropriate pans.

Selected References

1.Gould, Hannah L. "Surveillance for Foodborne Disease Outbreaks — United States, 1998–2008." *Centers for Disease Control and Prevention*. Centers for Disease Control and Prevention, 28 June 2013. Web. 22 Oct. 2015.
2.Pogostin, L., T. Ayers, S. Gray, T. Nguyen, M. Lynch, and I. Williams. 2008. School-associated foodborne outbreaks in the United States-1998-2006. Poster session presented at the 4th annual OutbreakNet Conference, June, 6, 2008, Denver, CO.
3.U.S. Department of Agriculture, Food and Nutrition Service. (2012). National School Lunch Program. Retrieved from http://www.fns.usda.gov/cnd/lunch/AboutLunch/NSLP FactSheet.pdf U.S. Department of Agriculture, Food Safety and Inspection Service.

Materials and Methods

Experimental Design: This study was designed to test the efficacy of school foodservice cooling techniques on controlling microbial growth, such as *Escherichia coli* (*E. coli*). In this study, four ATCC strains of *E. coli* were utilized at a target population of 10^4 CFU/g as surrogates to simulate survivability and growth of the foodborne pathogen *E. coli* O157:H7 in taco.

Sample Preparation: Taco meat was re-heated in commercial steamers to 165°F and prepared at two and three inch depths in commercial pans. The product was allowed to cool to 140°F and then inoculated with the *E. coli* surrogate cocktail.

Treatments: Six different treatments were utilized to determine if there was an effect on the rate of cooling and subsequent microbial growth. Two and three inch commercial pans were prepared and either left uncovered and exposed to air, covered with tin foil and allowed a gap for air exposure, or covered and without a gap for air exposure. These treatments were duplicated in a walk-in freezer and walk-in refrigerator. Pans in the walk-in refrigerator were situated in ice baths to represent a common food cooling technique.

Microbiological Analysis: A composite sample of the taco meat product was collected from various locations in each pan at sampling time points of 0, 4, 8, 12, and 24 hours. Composite samples were mixed by hand, measured to 25 gram samples and stomached for one minute with 225 mL of buffered peptone water (BPW). Following homogenization, samples were serially diluted in BPW and appropriate dilutions were spread-plated onto MacConkey agar (MAC). MAC plates were incubated for 18-24hrs and lactose fermenting colonies were enumerated and recorded.

Data Analysis: Data were analyzed as a two-way ANOVA with repeated measures modeling using GraphPad Prism 6 software.

Results and Conclusion

No significant difference was observed in *E. coli* populations (P=0.9335) for the cooling techniques evaluated in this study. However, sampling time alone was found to be significant (P=0.0001); therefore, data were analyzed on time alone. *Escherichia coli* populations declined slightly from 4.5 $\log_{10}$ CFU/g at 0 hours to 4.2 $\log_{10}$ CFU/g at 24 hours. The lack of a cooling treatment effect combined with a small, but statistically significant, decline in target microbial population indicates that all foodservice cooling treatments evaluated were effective at controlling *E. coli* populations in cooked taco meat.

Research into cooling methods that are effective at controlling foodborne pathogens in school lunch programs will continue to grow in significance, as it directly benefits public health by reducing the risk of foodborne illness and allowing a degree of necessary flexibility for foodservice operations. While this study is targeted specifically for school foodservice, translating these data into educational materials and trainings that can be used to inform cooling protocols used in other commercial foodservice settings is a benefit as well.

Acknowledgements

This research was conducted by Kansas State University and was funded, in part, with federal funds from the U.S. Department of Agriculture.