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

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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.

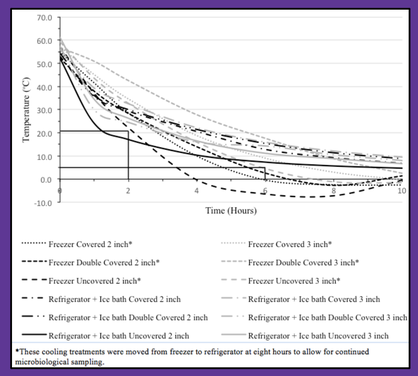

Figure 2: Cooling curves for all cooling technique combinations tested for low sodium marinara sauce.
Black lines represent the two FDA Food Code time and temperature criteria .

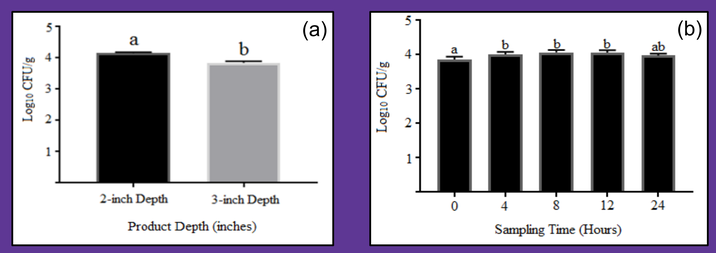

Figure 3: Surrogate *Escherichia coli* populations (Log_{10} CFU/g) in low sodium marinara sauce analyzed by (a) product depth, and (b) time.
[abc] Different superscripts indicate statistically significant differences.
Error bars represent the standard error of the mean.

Selected References

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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.

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