A Study of Consumer Storage of Commercially Available Peanut Butter with Analysis of Variance

Abstract

Outbreaks of *Salmonella* contamination of peanut butter have become a serious food safety concern for consumers. Based on the recent studies on peanut butter storage periods and storage methods, we aim to investigate how temperature and light affect the bacteria growth on generic brands of peanut butter. Our results indicate strong temperature and light effects on bacteria growth and the two main effects are independent. In addition, the effectiveness of each storage method depends on the brand. We hope the study could help improve the dining services, and at the same time in Grinnell, draw consumers' attention to potential food safety risks.

Background and Significance

Most outbreaks of *E.coli* are linked to consumption of animal products such as meat, poultry and eggs. Recent outbreaks in non-animal products have drawn significant public concerns. Peanut butter is a non-animal food and very low in water activity, which is usually considered safe and a relatively bacteria-free food. However, this beloved component of American breakfast leads to potential health risks. Referenced in the Centers for Disease Control and Prevention (CDC) report in 2012, multi-state outbreak of *Salmonella*, a species of *E.coli*, infections were linked to peanut butter, and drew the attention of public health officials in several states (CDC, 2012).

Based on our own experience in the introductory biology course and the research presented above we know that *E. Coli* can grow quite well on peanut butter. With the high rates of peanut butter consumption on campus, we thought it best to explore the various ways we see our Dining Hall store the peanut butter students consume, factoring into account the temperature of the storage and whether the peanut butter is exposed to light by covering the samples in petri dishes with aluminum foil.

Methods

Preparation of inoculum

The stereotype of *E.coli* used in this project was *Serratia marcescene* HY wild type (ATCC 8195). The culture was maintained on tryptic soy agar slants at 5 degrees Celsius and activated by transferring loop inocula into 10mL tryptic soy broth and incubating at 37 degrees Celsius for 24hrs (He, Y. et al., 2011). Then, the culture was centrifuged at room temperature and cells were collected. Pellets were re-suspended in 9mL of sterile 0.05mol potassium phosphate buffer (pH=7.2). One milliliter of this high-population inoculum suspension was serially diluted 10^{-4} in buffer to make a standard-population inoculum.

Data Collection

We randomly selected three generic brands of peanut butter and we used one jar per brand for treatments. We mixed 5.0g of peanut butter of each brand with 5.0mL *E.coli* inoculum and equally divided the mixture into two standard sized petri dishes by mass. We prepared two sets of peanut butter mixtures stored in 12 standard sized petri dishes. We randomly covered four samples from each brand with aluminum foil, and put two samples at room temperature and two samples at 4 degrees Celsius. The treatments lasted for seven days and then were ready for plating. We took 1.0g of the mixture diluted with 4.0mL of physiological saline (pH=7.60) and pipetted 100uL on the standard sized agar plate. The plates were placed in an incubator at 37 degrees Celsius and check after 72 hours (Burnett, S.L. et al., 2000 and Kilonzo-Nthenge, A. et al., 2009)

Analytical Methods

We used a complete randomized block design, utilizing the brands of peanut as blocks. We performed an ANOVA test to examine the two-way interaction between our two treatment factors, and our treatment factors with our blocks. The main effects are temperature (4C or 25C)

and coverage (light or dark). The response variable measured the number of colonies grown in the petri dish.

Results and Discussion

Main Experimental Effects

The results of our ANOVA shown in Table 1 show significant differences among our main treatment effects. There is a difference of 6.08 colonies on average when the peanut butter is stored at room temperature versus in a refrigerator at 4 degrees Celsius, with a statistical significance of $p<.0001$. There is also a difference of 2.92 colonies on average for storage without aluminum covering, also at a significant p-value of .0006. Our treatments effectively reduce *E. coli* growth in three randomly chosen brands of peanut butter. We also found that there is no significant interaction between these two main factors (p=0.2727). As we alter the temperature, the effect of a lower or higher temperature is not exacerbated or dampened by the level of light (Figure 1).

Brands as Blocks

The brands exhibit very distinct average of colonies. The F-value of 17.69 and a p-value equal to 0.0001 at α level 0.05 indicate strong evidence that there is a significant difference in colony production for each brand. This actually misrepresents the value of blocking in many ways. Especially as these brands, Hyvee, Essential, and Peter Pan, were selected randomly from the grocery store shelf. The relative efficiency indicator for the blocks should be done to examine the advantage of block design over a completely randomized design (CRD). Our model of block design proved to be 4.24 times more efficient than the CRD.

While the brands were chosen at random, they are of interest to Grinnell College students. Hyvee is a grocery store often visited by students because of the liberal politics so students often purchase their store brand peanut butter. Essential is an even lower cost peanut butter available to students concerned about the financial impact of their eating habits. As well, Peter Pan received a significant amount of press coverage regarding a 2007 Salmonella outbreak (Close, 2016). Thus, the interactions between brand and the two main effects are worth examining.

Both interactions prove to be significant. Brand and temperature show a significant F value of 7.35 and a p-value of .0066. While there is a less significant interaction between brand and coverage (F=4.55 and a p=0.0300) there is moderate evidence the effects of brand are affected by the lack of sunlight and variation in temperature. The most notable effect of the interaction of temperature and brand is how much more the colonies increase for Essential peanut butter when left at higher temperatures. While Hyvee and Peter Pan see an increase of 5.25 and 3.5 colonies on average, Essential increases by 9.5 colonies, seen in Figure 2. In contrast, we largely see a dampening effect for the interaction of coverage and brand. While Peter Pan and Essential exhibit relatively normal decreases in colonies with a lack of sunlight, there is almost no difference for Hyvee, which can be seen visually in Figure 3.

While we cannot explain precisely what in the different brands may cause this interaction, it does point to an area of future study that could include ANCOVA using the available nutrition and preservative information of each brand. Based on our results, to avoid *E.coli* growth in peanut butter, we would recommend purchasing Peter Pan and storing in the fridge or covered. Regardless we encourage consumers to be wary of how they store the food they consume.

References

1. Burnett, S.L. et al. (2000). Survival of Salmonella in peanut butter and peanut butter spread. Journal of Applied Microbiology 89(3). P.381-392.

2. CDC Annual Report 2012. https://www.cdc.gov/salmonella/bredeney-09-12/ [Accessed 05/4/2017].

3. Close K. (2016). Peter Pan's parent hit with $11 million penalty for selling contaminated peanut butter. Fortune. http://fortune.com/2016/12/14/conagra-grocery-products-peter-pan-fine-contaminated-peanut-butter-salmonella/ [Accessed 05/04/2017].

4. He, Y. et al. (2011). Survival and heat resistance of Salmonella enterica and Escherichia coli O157:H7 in peanut butter. Journal of Applied and Environmental Microbiology 77(23). P.8484-8438.

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Appendix

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Brand	2	91.5833333	45.7916667	17.69	0.0001
Temperature	1	222.0416667	222.0416667	85.75	<.0001
Coverage	1	51.0416667	51.0416667	19.71	0.0006
Brand*Temperature	2	38.0833333	19.0416667	7.35	0.0066
Brand*Coverage	2	23.5833333	11.7916667	4.55	0.0300
Temperature*Coverage	1	3.3750000	3.3750000	1.30	0.2727
Error	14	36.2500000	2.5892857		
Corrected Total	23	465.9583333			

Table 1. ANOVA Model Including Main Effects, Blocks, and Interactions.

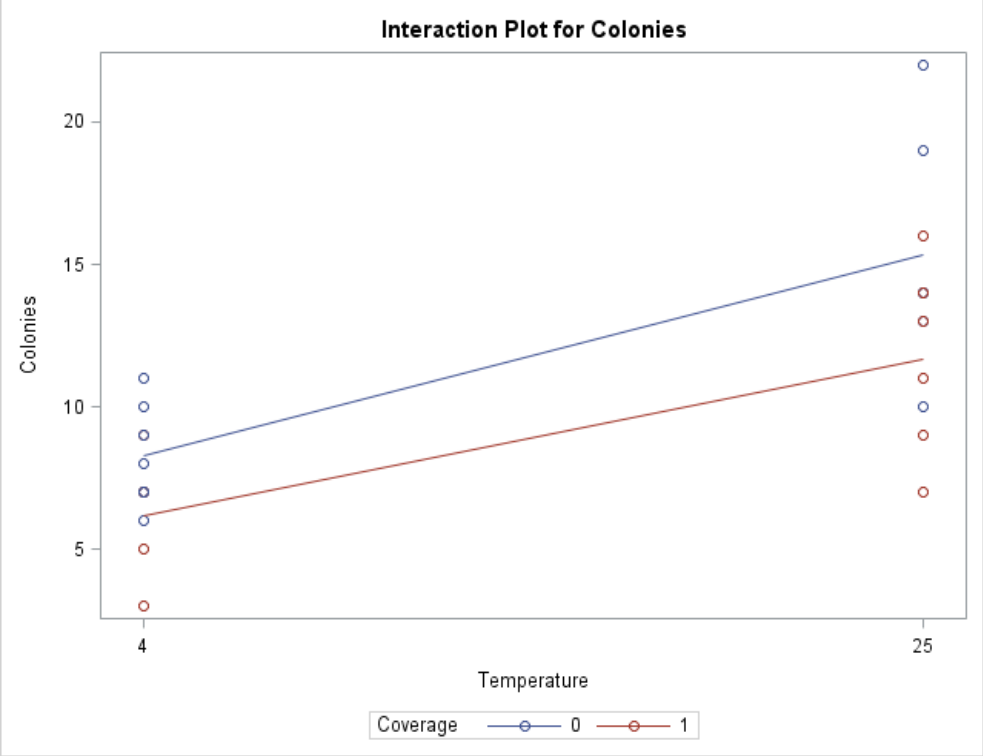

Figure 1. Interaction Plots for Temperature and Coverage.

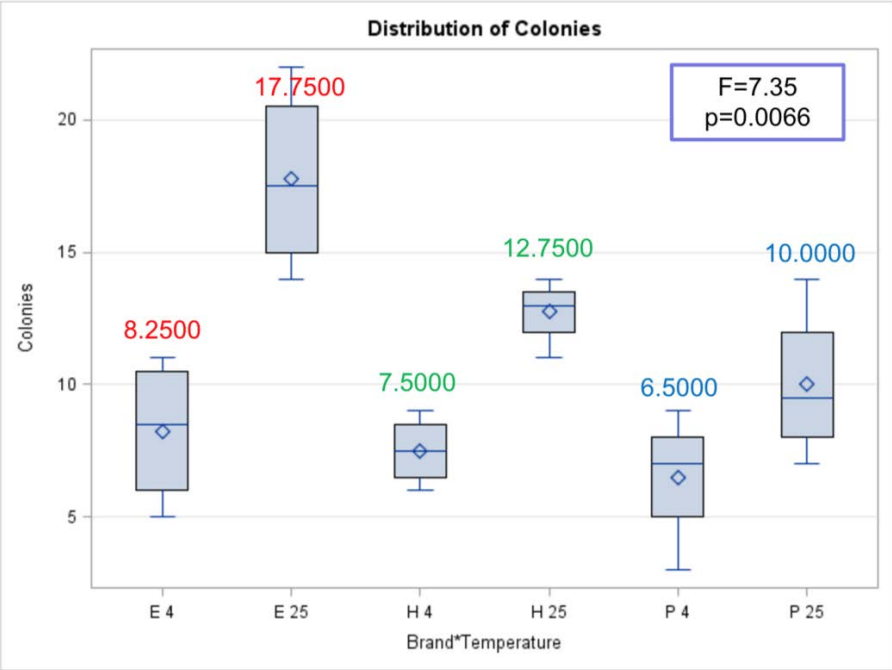

Figure 2. Boxplot Displaying the Interaction between Brand and Temperature.

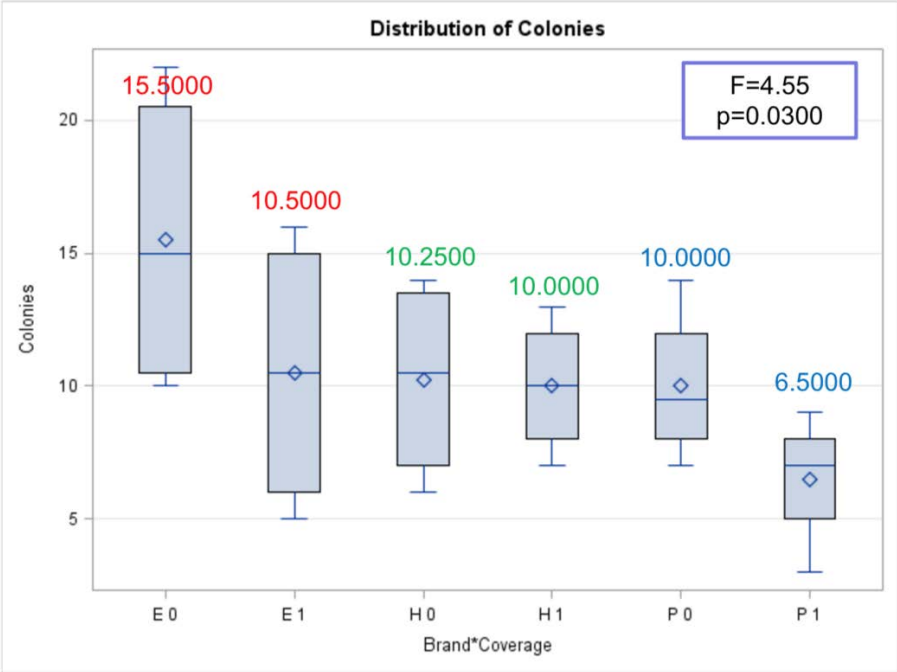

Figure 3. Boxplot Displaying the Interaction between Brand and Coverage.