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Consumer Storage Period and Temperature for Peanut Butter and Their Effects on Survival of *Salmonella* and *Escherichia coli* O157:H7

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ABSTRACT

Recent recurrence of *Salmonella* contamination of peanut butter has become a serious food safety concern for consumers. A study was conducted to identify storage periods and temperature conditions of peanut butter in domestic kitchens and to determine the effects of those storage periods and conditions on survival of *Salmonella* and *Escherichia coli* O157:H7. Surveys assessed consumer storage periods of peanut butter in 150 households in Middle Tennessee. To simulate consumers' peanut butter storage conditions, *Salmonella* and *E. coli* O157:H7 were inoculated in peanut butter and held at 4 and 25°C for up to 15 weeks. Initial populations of *Salmonella* and *E. coli* O157:H7 in peanut butter were 4.78 CFU/g and 5.56 CFU/g, respectively. After 15 weeks of storage at 4°C, *Salmonella* and *E. coli* O157:H7 populations had decreased to 3.72 and 2.73 log CFU/g, respectively. A significantly higher reduction ($P < 0.05$) of *Salmonella* and *E. coli* O157:H7 was observed in peanut butter stored at 25°C than in that stored at 4°C for the same duration. Our results indicate that post-process contamination of peanut butter with *Salmonella* and *E. coli* O157:H7 may result in survival of these pathogens during their shelf life, posing health risks to consumers.

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INTRODUCTION

Foodborne pathogens have a significant impact on the food processing industry, consumers, and regulatory agencies. In the past, most outbreaks of *Salmonella* and *Escherichia coli* O157:H7 have been linked to consumption of animal products such as meat, poultry, and eggs *(17)*. However, the presence of *Salmonella* and *E. coli* O157:H7 in non-animal products has emerged as a serious food safety concern. Foods low in water activity, such as chocolate and cheese, have been implicated in *Salmonella* outbreaks *(5)*. Several reports have suggested that *Salmonella* in foods with low water activity and high lipid content tend to have increased resistance to heat *(8, 9, 11)*.

In 1996, an outbreak of *Salmonella* Mbandaka infection in Australia was associated with peanut butter, a food of low water activity *(16)*. *Salmonella* Agona infection has also been linked to consumption of peanut butter-coated savory in England and Israel *(10, 18)*. In 2007, a multistate outbreak of *Salmonella* Tennessee associated with peanut butter consumption was reported in 47 states *(3)*. This was the first reported outbreak of

TABLE 1. Percentage of consumers with different education levels who store peanut butter < 2 to > 24 weeks

	Percentage of consumers who store peanut butter for:				
Education level	< 2 weeks	2–4 weeks	5–12 weeks	13–24 weeks	> 24 weeks
< High school	1.7	3.4	5.0	1.7	0.8
High school	5.9	9.2	5.0	2.5	3.4
Some college	5.0	5.9	13.4	0.8	3.4
Bachelors or higher	1.7	10.1	11.8	5.0	4.2

foodborne illness caused by peanut butter consumption in the United States, with at least 625 cases. Another major *Salmonella* Typhimurium outbreak associated with peanut butter occurred in 2008–2009, with at least 486 people involved in 44 states *(4, 15)*. Therefore, *Salmonella* contamination of peanut butter continues to be a challenge in the United States, as suggested by these recent outbreaks.

Peanuts are the main ingredient in peanut butter, and contamination of peanuts with *Salmonella* or other foodborne pathogens is possible during growth, harvest, transportation, and even storage *(11)*. Thermal processing of peanut butter might not always eliminate *Salmonella (17)*, and post-process contamination during repackaging may lead to its presence at the point of consumption *(2)*. Because of the frequency of outbreaks of *Salmonella* associated with peanut butter and the associated substantial economic burden on society, additional studies on consumers' peanut butter storage conditions are needed. In addition, the survival of *E. coli* O157: H7 in peanut butter has not been evaluated. *E. coli* O157:H7 has a low infective dose *(19)* and is one of the most serious foodborne known pathogens *(1, 12)*. Therefore, this study recruited participants from the general public to gain a better understanding of preferred duration and storage temperatures of peanut butter in consumers' domestic kitchens, and of how these conditions affect the survival of *Salmonella* and *E. coli* O157:H7.

MATERIAL AND METHODS

Survey of consumer storage of peanut butter

A total of 150 households in Middle Tennessee participated in this study. Participants were recruited through posted flyers at senior housing communities, churches, and community organizations. Researchers contacted the subjects and used a script/screener to determine eligibility. In each household, the person mainly responsible for food purchase, storage, and preparation, and at least 18 years old, was interviewed. To mirror the general population, the participants were in the following categories: less than high school, (13.3%), high school diploma (26%), bachelor's degree or higher (32%), and some college (28.7). Most respondents had incomes between $15,000 and $75,000 a year. The survey questionnaire inquired about consumers' peanut butter purchasing, storage conditions, and storage period. Consumers were also questioned whether they ever threw away peanut butter after a certain period of storage and if so, why.

Laboratory simulation of consumer peanut butter storage conditions

Storage periods and temperatures of peanut butter in domestic kitchens and their effects on the survival of *Salmonella* and *E. coli* O157:H7 were evaluated in a laboratory setting. Peanut butter was contaminated with *Salmonella* and *E. coli* O157:H7 and thereafter stored either at room or refrigeration temperature to simulate consumers' storage conditions.

Preparation of bacterial cell suspension

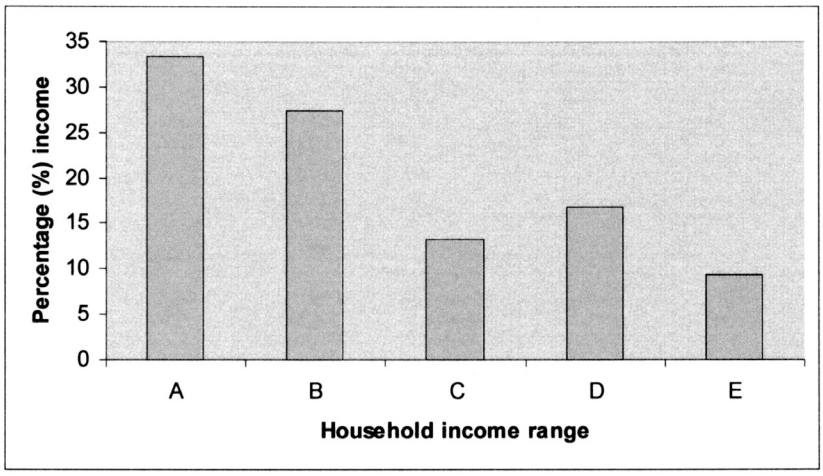
S. Mission (isolated from rectal swabs), *S.* Typhimurium (associated with peanut butter), *S.* Enteritidis (isolated from human feces), *E. coli* O157:H7 204P (pork isolate), *E. coli* O157:H7 301C (chicken isolate), and *E. coli* O157:H7 505B (beef isolate) were used in this study. These bacterial strains were obtained from Auburn University (Department of Nutrition and Food Science, Auburn, Alabama, USA) and have been linked to foodborne illnesses in the past. Information on the survival of these organisms in peanut butter is lacking. To test for ability to maintain genes associated with antibiotic resistance, antibiotic-resistant *Salmonella* and *E. coli* O157:H7 strains were grown in a series of broth-to-agar media inoculated with the respective antibiotics (*Salmonella*, 100 ppm nalidixic acid; *E. coli* O157:H7, 200 ppm nalidixic acid and 0.025 ppm novobiocin; Sigma, St. Louis, MO).

Bacterial cell cultures were maintained on Tryptic Soy Agar (TSA, Difco, Lawrence, Kansas) plates and subjected to two successive transfers into 10 ml Tryptic Soy Broth (TSB) and incubation at 37°C for 20 h. Cells were harvested by centrifugation (3,500 × g, 15 min) at 4°C and washed three times in Butterfield's phosphate buffer (BPB). Bacterial cell pellets were resuspended in 5 ml of sterile BPB and combined to form a three serotype cocktail for each bacterium. Concentration levels of each cocktail were quantified by spread plating 100 µl onto TSA plates inoculated with the appropriate antibiotics for *Salmonella* and *E. coli* selection. To facilitate recovery and eliminate background flora, antibiotic-resistant strains of *Salmonella* (100 ppm nalidixic acid) and *E. coli* O157:H7 (200 ppm nalidixic acid; 0.025 ppm novobiocin) were used.

Inoculation of peanut butter with *Salmonella* and *E. coli* O157:H7

Commercially processed jars of peanut butter were purchased at a local grocery store. Creamy peanut butter (Kroger Co., Cincinnati, OH) listed ingredients were: roasted peanuts, sugar, 2% molasses, fully hydrogenated vegetable oils (rapeseed, cottonseed, and soybean) and salt. Peanut butter sam-

FIGURE 1. Percentage by income of participants who purchased peanut butter. A, less than $15,000; B, 15,000 – 34,000; C, 35,000 – 49,000; D, 50,000 – 79,000; E, $75,000 and above



ples (100 g) were placed in sterile 500-ml glass beakers and kept in a heated water bath at 44°C. Warm water resulted in less viscous peanut butter and therefore minimized large pockets of inoculum in the peanut butter. Each bacterial cocktail (1 ml) was added separately to different batches of peanut butter and mixed with sterile spatula. Four 100-g portions of contaminated peanut butter were pooled into sterile blenders to form six 400-g peanut butter samples, each contaminated with *Salmonella* and *E. coli* O157:H7. To ensure uniform distribution of the inoculums, the pooled peanut butter samples were stirred for 4 minutes. The achieved concentrations of *Salmonella* and *E. coli* O157:H7 in the peanut butter samples were 4.78 and 5.56 log CFU/g, respectively. Another set of 400-g peanut butter samples were contaminated with antibiotic-sensitive *Salmonella* (4.74 log CFU/g) and *E. coli* O157:H7 (5.05 log CFU/g) to compare their survival capacity with that of antibiotic-resistant mutants. All samples were aseptically transferred to sterile jars and stored at either 25 (room temperature) or 4°C (refrigeration temperature) for up to 15 weeks.

Microbial analysis

Jars of contaminated peanut butter were opened every week and analyzed for detectable *Salmonella* and *E. coli* O157:H7. Approximately 25-g samples of peanut butter were placed in sterile stomacher bags and 225 ml of BPB was added. To achieve homogeneous suspensions, samples were pummeled at 230 rpm for 2 minutes. Aliquots (1 ml) of the homogeneous samples were plated (pour plate) onto TSA plates that contained the appropriate antibiotic. The plates were incubated at 37°C for 20 h. *Salmonella* was confirmed by plating typical colonies on xylose-lysine-tergitol 4 agar plates and by using the Reveal for *Salmonella* complete System- SC (Neogen, Lansing, MI). The MacConkey agar plates and Reveal for *E. coli* O157:H7 20 h complete systems were used to confirm *E. coli* O157:H7.

Statistical analysis

All experiments were performed in triplicate. Means were analyzed by one-way ANOVA, followed by the Tukey test. Significance implies $P < 0.05$ unless stated otherwise.

RESULTS AND DISCUSSION

Consumer survey of peanut butter storage

Peanut butter, which is found in about 75% of American homes, is considered by many to be a staple like bread and milk. Peanut butter is spread on a slice of bread, is melted into a soup, and finds its way into everything from breakfast to dessert. In our study, 80% of the participants consumed peanut butter in their households; most surveyed were female (76%) rather than male (24%). The survey targeted the person mainly responsible for food purchase, storage, and preparation in each household, and for the most part, this person tended to be female. Most respondents had incomes between $15,000 and $75,000 a year (Fig. 1). Findings from this study indicate that a *Salmonella* or *E. coli* O157:H7 outbreak associated with peanut butter consumption could affect consumers regardless of income levels.

In this study, 1.3% of the participants had children under 2 years of age, and 20% were adults over 60 years of age. Persons affected by the recent *Salmonella* Typhimurium outbreak associated with peanut butter ranged in age from < 1 to 98 years *(4)*. Participants in this study were within this age range; it must be borne in mind that immunocompromised individuals, as well as the old and young, are at increased risk for foodborne illness. Previous reports have shown that *Salmonella* infections can lead to severe and potentially fatal conditions such as bacteremia, septic arthritis, meningitis, and pneumonia, especially in infants and immunocompromised hosts *(6)*.

Our results suggest that 87% and 13% of the householders stored peanut butter at room temperature and at refrigeration temperatures, respectively. Consumers' commonly used areas for storage of peanut butter included: cabinets (80%), inside refrigerators (13%), top of refrigerators (4%), counter tops (1%), ledge of a window (1%) and on dinner or breakfast table (1%). The duration of consumers' peanut butter storage ranged from less than 2 weeks to about 6 months. The storage period of peanut butter was independent of education level and age group; there was no association between education level and storage period or between age and storage period (Tables 1, 2). During those storage times, some consumers ate all the peanut butter purchased while others threw away part of it for specific reasons. Some prominent reasons why consumers discarded peanut butter were: (1) the peanut butter "smelled funny" (5%); (2) there was a peanut butter recall (7.5%) especially due to a *Salmonella* Typhimurium outbreak, and (3) the peanut butter was too old to eat (16%). Commercial peanut butter requires no refrigeration and can be kept up to six months after opening. Unopened jars can be stored up to one year in a cool, dark location.

TABLE 2. Percentage of consumers at different age groups who store peanut butter for < 2 to > 24 weeks

	Percentage of consumers who store peanut butter for:				
Age (yrs)	< 2 weeks	2–4 weeks	5–12 weeks	13–24 weeks	> 24 weeks
18–29	1.7	5.9	10.1	0.8	2.5
30–44	5.9	8.4	5.9	5.0	3.4
45–59	6.7	10.1	11.8	4.2	4.2
60–69	0.0	1.7	3.4	0.0	0.8
70+	0.0	2.5	4.2	0.0	0.8

TABLE 3. Populations of *Salmonella* and *E. coli* O157:H7 in peanut butter stored at 4 and 25°C for up to 15 weeks

	Population (log CFU/g)[a] over storage time (weeks) of:						
Storage Temperature	1	2	3	6	9	12	15
Salmonella							
4°C	4.72^{ax}	4.61^{ax}	4.53^{ax}	4.17^{ay}	3.90^{ayw}	3.83^{aw}	3.72^{aw}
25°C	3.83^{ax}	3.55^{bx}	3.23^{bx}	2.67^{by}	1.81^{bz}	ND^{bw}	ND^{bw}
E. coli O157:H7							
4°C	3.47^{axy}	3.53^{ax}	3.15^{axy}	3.12^{ayz}	2.93^{az}	2.83^{az}	2.73^{az}
25°C	2.82^{ax}	2.61^{bx}	2.23^{bx}	1.30^{by}	1.10^{by}	ND^{bz}	ND^{bz}

[a]Initial population of *Salmonella* was 4.78 log CFU/g; initial population of *E. coli* O157:H7 was 5.56 log CFU/g.

[a,b]Mean values (log CFU/g) in the same column within pathogen that are not followed by the same letter(s) are significantly different ($P < 0.05$).

[x,y]Mean values in the same row within pathogen that are not followed by the same letter are significantly different ($P < 0.05$).

The results of this study raise concerns in that peanut butter recalled because of a *Salmonella* Typhimurium outbreak was mentioned by about 7.5% of consumers surveyed in our study. Our results obviously indicate that extended periods of storage time of contaminated peanut butter pose risks of foodborne disease to consumers.

Viability of *Salmonella* and *E. coli* O157:H7 under simulated domestic kitchen conditions

Viable *Salmonella* and *E. coli* O157:H7 cells recovered were entirely attributed to the inoculated peanut butter; no traces of *Salmonella* or *E. coli* O157:H7 were detected in uncontaminated peanut butter (control). Populations of *Salmonella* and *E. coli* O157:H7 in inoculated peanut butter stored at either 4 or 25°C is shown in Tables 3. The initial populations of *Salmonella* and *E. coli* O157:H7 in peanut butter were 4.78 CFU/g and 5.56 CFU/g, respectively. All reductions were tabulated in reference to the initial concentrations of tested pathogens.

There was no significant ($P < 0.05$) difference in *Salmonella* reduction within weeks 1, 2 and 3 of peanut butter storage at 4°C (Table 3). However, *Salmonella* reductions of approximately 0.95 to 4.00 log CFU/g of tested peanut butter samples were observed during storage at room temperature (25°C). At 9, 12, and 15 weeks of peanut butter storage at 4°C, *Salmonella* populations were significantly ($P < 0.05$) lower than populations noted during the first 6 weeks of storage of the peanut butter. When the Reveal for *Salmonella* complete System kit was used, the presence of *Salmonella* was confirmed in peanut butter at weeks 12 and 15 (Table 3). Storage of peanut butter at 4°C resulted in the least reduction of *Salmonella*, which ranged from 0.06 to 1.06 log CFU/g, compared with the peanut butter stored at 25°C. Generally, reductions of *Salmonella* were significantly ($P < 0.05$) higher at 25°C than at 4°C for up to 15 weeks (Table 3). These results are in agreement with the report of Burnett et al. *(2)* that *Salmonella* deaths were more prevalent in butters

and spreads stored at 21°C than in those stored at 5°C.

The pattern of *E. coli* O157:H7 reduction was generally similar to that of *Salmonella* (Table 3). The *E. coli* O157:H7 reductions in samples stored for 1, 2, 3, weeks at 25°C were 2.74, 2.95, and 3.33 log CFU/g, respectively (Table 3). When stored much longer, to 9 weeks at 25°C, *E. coli* O157:H7 reductions in the peanut butter were significantly higher ($P < 0.05$) than those of peanut butter stored at 25°C for 1, 2 and 3 weeks (4.46 log CFU/g vs 2.74, 2.95 and 3.33 log CFU/g, respectively).

Overall, *E. coli* O157:H7 cell count reductions of peanut butter stored at 4°C ranged from 2.73 to 3.53 log CFU/g (Table 3). The *E. coli* O157:H7 was confirmed by the Reveal for *E. coli* O157:H7 20 h complete system method at 12 and 15 weeks of peanut butter storage. *E. coli* O157:H7 reductions were significantly ($P < 0.05$) higher with storage at 25°C than at 4°C for up to 15 weeks of peanut butter storage. These observations are in agreement with previous reports *(20)* that *E. coli* O157:H7 reductions in mayonnaise were higher when storage was at room temperature (25°C) than when storage was at refrigeration temperature (4°C). Antibiotic-sensitive *Salmonella* cell counts in samples stored for 6 and 15 weeks at 25°C were 3.37 and 1.72 log CFU/g, respectively. After 6 and 15 weeks at 25°C, antibiotic-sensitive *E. coli* O157:H7 showed cell counts of 2.73 and 1.01 log CFU/g, respectively. Survival capacity of antibiotic-resistant mutant strains exhibited slower growth rates, compared with antibiotic-sensitive strains. These results suggest that the use of antibiotic resistance as a selective marker could present different growth rates in laboratory media and show different resistance to stresses. This possibly will result in overestimates of any treatment, such as heat, against antibiotic-sensitive *Salmonella* and *E. coli* O157:H7.

Findings in this report indicate that post-process contamination of peanut butter with *Salmonella* and *E. coli* O157:H7 may result in survival of these pathogens during their shelf life. This premise is in agreement with previous studies showing that *Salmonella* populations decreased more rapidly in peanut butter at 22°C than at 4°C storage *(13)*. Similar results were observed when *Salmonella* populations decreased more rapidly in a butter and margarine blend stored at 21°C, compared to 4°C *(7)*. It is most probable that at 25°C, the conditions are highly conducive to bacterial growth in the peanut butter, resulting in accelerated growth and hence attainment of a stationary phase sooner than when storage is at 4°C.

It is well documented that storage temperature of colloidal food products influence the availability of *Salmonella (13)*. In our study, *Salmonella* and *E. coli* O157:H7 were detected in the peanut butter throughout the storage period. These findings give cause for concern, because previous reports have shown that consumption of even very low numbers of *Salmonella* or *E. coli* O157:H7 can cause disease *(16)*. It has also been pointed out that during thermal processing of products such as peanut butter, foodborne pathogens are expected to be eliminated, but post-process contamination may take place during repackaging or with use of ingredients in other food products not subjected to conditions sufficient to kill the pathogens *(2)*. Personal hygiene as well as cross-contamination of finished products with raw materials and unsanitary equipment are significant fundamentals in controlling the contamination of food products with pathogens and spoilage microorganisms *(14)*.

CONCLUSIONS

Salmonella grows over a wide range of temperatures and will survive long periods of dehydration. As demonstrated in our results, *Salmonella* and *E. coli* O157:H7 can survive in contaminated peanut butter stored at room and refrigerated temperatures for long periods of time and therefore, can pose a health risk to consumers. To minimize or eliminate such risks in peanut butter, Food Safety Programs (FSP) should be imposed in peanut butter processing facilities. Such actions would eliminate *Salmonella*, *E. coli* O157:H7 or other foodborne pathogens. In addition, plant sanitation and verification of any heat processes are crucial and must be key components of an inclusive FSP to ensure food safety to the public. More research on the survival of foodborne pathogens in peanut butter will be of great importance to the food industry and will translate to fewer recalls of products, recapturing of lost prestige and improvement of the income potential of the food industry. Peanut butter processing facilities must have in place Food Safety Programs to eliminate and control foodborne pathogens in the product.

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