Research Note

Survival of *Salmonella* during Baking of Peanut Butter Cookies

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ABSTRACT

Peanuts and peanut-based products have been the source of recent *Salmonella* outbreaks worldwide. Because peanut butter is commonly used as an ingredient in baked goods, such as cookies, the potential risk of *Salmonella* remaining in these products after baking needs to be assessed. This research examines the potential hazard of *Salmonella* in peanut butter cookies when it is introduced via the peanut-derived ingredient. The survival of *Salmonella* during the baking of peanut butter cookies was determined. Commercial, creamy-style peanut butter was artificially inoculated with a five-strain *Salmonella* cocktail at a target concentration of 10⁵ CFU/g. The inoculated peanut butter was then used to prepare peanut butter cookie dough following a standard recipe. Cookies were baked at 350°F (177°C) and were sampled after 10, 11, 12, 13, 14, and 15 min. Temperature profiles of the oven and cookies were monitored during baking. The water activity and pH of the inoculated and uninoculated peanut butter, raw dough, and baked cookies were measured. Immediately after baking, cookies were cooled, and the survival of *Salmonella* was determined by direct plating or enrichment. After baking cookies for 10 min, the minimum reduction of *Salmonella* observed was 4.8 log. In cookies baked for 13 and 14 min, *Salmonella* was only detectable by enrichment reflecting a *Salmonella* reduction in the range of 5.2 to 6.2 log. Cookies baked for 15 min had no detectable *Salmonella*. Results of this study showed that proper baking will reduce *Salmonella* in peanut butter cookies by 5 log or more.

Researchers have investigated the survival and thermal tolerance of *Salmonella* in peanut butter (4, 13, 16, 18, 21). Burnett et al. (4) showed that *Salmonella* can survive for up to 24 weeks in a variety of peanut butters and spreads. Two thermal inactivation studies using different strains showed that to achieve a 7-log reduction of *Salmonella* in peanut butter a process of 90°C for 60 to 120 min would be needed (16, 21). The lengthy persistence and high heat resistance of *Salmonella* in peanut butter raises concerns about its use as an ingredient, even in products that are cooked prior to sale.

The presence of *Salmonella* in bakery products has been a concern for years. Outbreaks have been associated with custards, trifles, apple pie, cakes, bread, and cake mixes (2, 3, 11, 26). Although bakery ingredients such as flour, chocolate, peanut butter, and dairy can all be potential sources of *Salmonella*, the majority of outbreaks involving baked goods were either associated or suspected to be associated with raw eggs and, in most cases, were the result of cross-contamination or undercooking. To address these concerns, both the American Bakers Association and NSF International established guidance documents for pathogen challenge studies in baked goods (1, 17). However, neither of these guidance documents considers the potential of microbial contamination occurring via a low-moisture ingredient or the effect of the baking process on the contaminating microorganism.

The fate of *Salmonella* in bakery products when it is introduced via a low-moisture ingredient has not been thoroughly examined. It has been assumed that baking will eliminate vegetative pathogens due to the temperatures that

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products typically reach during cooking (22). Previous research indicated that low numbers of *Salmonella* (10 CFU/g) are destroyed by the baking process in pumpkin pie and cheesecake (12, 25). In other bakery products, this is likely the case; however, low-moisture ingredients that are also high in fat, such as peanut butter, may not be as easily incorporated into the mix and could create a low-water activity (a<sub>w</sub>) microenvironment. Moreover, if the peanut butter is contaminated with *Salmonella*, cells may remain in this high-fat, low-a<sub>w</sub> microenvironment, which could result in increased heat resistance.

Because of recent *Salmonella* outbreaks involving peanut butter, the risk of using contaminated peanut butter needs to be assessed in baked goods. This research examines the potential hazard of *Salmonella* in peanut butter cookies when it is introduced via the peanut-derived ingredient. The objective was to determine the survival of *Salmonella* during the baking process.

**MATERIALS AND METHODS**

*Salmonella* strains. Five serovars of *Salmonella enterica* were used in the study: *Salmonella* Tennessee (FSL-R8-5221, peanut), *Salmonella* Tornow (FSL-R8-5222, peanut), *Salmonella* Hartford (FSL-R8-5223, peanut), *Salmonella* Agona (FSL-SS-517, human), and *Salmonella* Typhimurium DT-104 (FSL-WI-030, human) were obtained from the International Life Sciences Institute, North America (Cornell University, Ithaca, NY). All cultures were maintained on tryptic soy agar (Remel, Lenexa, KS) at 4°C. Cultures were prepared by transferring a loopful of each organism into 10 ml of tryptic soy broth (BD, Sparks, MD) and incubating at 35°C for 24 h. This was done for three consecutive 24-h intervals prior to being used as an inoculum. After incubation, equal volumes of each strain were pooled to form a cocktail. The cocktail was centrifuged (1,800 g × 15 min), washed twice with 0.1% peptone water, and then suspended in 0.1% peptone water to achieve the targeted population.

Inoculation of peanut butter. A commercial, creamy-style peanut butter purchased from a local retailer was used for the study. Peanut butter was inoculated as described by Ma et al. (16). Briefly, 240 g of peanut butter was aseptically weighed into a Whirl-Pak bag (Nasco, Fort Atkinson, WI). The peanut butter was then melted by heating to 50°C in a circulating water bath and then cooled to 37°C prior to inoculation. After cooling, 2.4 ml of the *Salmonella* cocktail at a concentration 10<sup>10</sup> CFU/ml was added and mixed by hand for 5 min to evenly distribute the bacterial cells and achieve a final population of approximately 10<sup>8</sup> CFU/g of peanut butter.

Preparation of peanut butter cookie dough. All ingredients (Table 1) were purchased from a local retailer and kept at refrigeration or ambient temperature as appropriate until used. Prior to cookie dough preparation, peanut butter was softened at room temperature (approximately 30 min). The cookie dough was prepared by creaming softened butter in a mixer (model Classic Plus, KitchenAid, St. Joseph, MI) for 2 min. Sugar and brown sugar were added and then mixed on medium speed for 5 min. Two eggs were individually added and were mixed well after each addition. Flour, vanilla extract, salt, baking soda, and baking powder were then added, one at a time, with continuous mixing, until well blended. Inoculated peanut butter was added to the dough last and blended for 5 min. To ensure all samples started at the same temperature, dough was weighed into 28-g portions and held at 68°F (20°C) prior to baking.

**Baking of peanut butter cookies.** Samples of dough were placed on a cookie sheet (11 by 17 in.) in one of three locations, the center, the corner, or the side, and baked in an oven (OV702G, Fischer Scientific, Dubuque, IA) set to 350°F (177°C) for 10, 11, 12, 13, 14, or 15 min. Immediately after baking, cookies were placed into a Whirl-Pak bag and cooled in an ice bath. The temperature of the oven was recorded at the beginning and every 1 min during baking using a data logger (DI-1000-TC, DATQA Instruments Inc., Akron, OH) with a type T thermocouple (Omega Scientific, Stamford, CT).

Three replicate trials of cookie temperature data were collected independent of the inoculated cookie trials. Cookie temperature was monitored by placing a thermocouple (type K, Omega Scientific) near the center of the cookie at each location on the sheet. During baking, the initial temperature and every 1 min thereafter were recorded using a data logger (DI-1000-TC). Oven temperatures were also recorded, as previously described.

**Microbiological analysis.** After cooling, baked cookies (25 g) were diluted with 225 ml of 0.85% saline solution containing 0.1% Tween 80 and blended in a stomacher for 2 min. The samples were serially diluted (1:10) in 0.1% peptone water and plated in duplicate onto tryptic soy agar yeast extract supplemented with ferric ammonium citrate (0.05%) and sodium thiosulfate (0.03%). The plates were incubated at 35°C for 48 h, and *Salmonella* colonies (identified by a black precipitate in the center) were counted. In anticipation of counts below the limit of detection by direct plating, sample homogenates were incubated at 35°C overnight prior to streaking on xylose lysine deoxycholate agar (Neogen, Lansing, MI) to determine the presence or absence of *Salmonella*. Samples negative by direct plating and positive by enrichment should contain between >1 and <10 CFU/g *Salmonella*, and for log-reduction calculations of these samples, a range was calculated (16).

**a<sub>w</sub> and pH analysis.** The a<sub>w</sub> and pH of the peanut butter before and after inoculation, the cookie dough, and the baked cookies were determined with a a<sub>w</sub> meter (Aqualab 4TE, Decagon Devices, Pullman, WA) and a pH meter (PC510; Oakton Instruments, Vernon Hills, IL), respectively.

**Statistical analysis.** For each experiment three independent trials were conducted. Average data from inoculated peanut butter, raw cookie dough, and baked cookies were averaged and converted

**TABLE 1. Ingredients and quantity used in the production of peanut butter cookies**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour</td>
<td>300 g</td>
</tr>
<tr>
<td>Butter</td>
<td>226 g</td>
</tr>
<tr>
<td>Sugar</td>
<td>200 g</td>
</tr>
<tr>
<td>Peanut butter</td>
<td>180 g</td>
</tr>
<tr>
<td>Brown sugar</td>
<td>180 g</td>
</tr>
<tr>
<td>Eggs</td>
<td>2 large</td>
</tr>
<tr>
<td>Vanilla extract</td>
<td>10 ml</td>
</tr>
<tr>
<td>Salt</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Baking soda</td>
<td>2.1 g</td>
</tr>
<tr>
<td>Baking powder</td>
<td>1.9 g</td>
</tr>
</tbody>
</table>
to log values. Total positive enrichments by location and temperature data were compared using analysis of variance (JMP, SAS Institute, Cary, NC) to determine significant differences at a 95% confidence interval ($P < 0.05$).

**RESULTS AND DISCUSSION**

Peanut butter $a_w$ and pH values increased after inoculation (Table 2). The pH of the cookie dough made with inoculated peanut butter was also higher than dough made with uninoculated peanut butter; however, the $a_w$ was lower in cookie dough made with inoculated peanut butter. As expected, the $a_w$ of the cookies decreased over time during baking from approximately 0.82 to 0.70, 0.62, 0.60, 0.53, 0.54, and 0.51 after 10, 11, 12, 13, 14, and 15 min, respectively. Immediately after baking, cookies were placed into a Whirl-Pak bag and cooled in an ice bath instead of being cooled on an open rack, so this value is higher than typical literature values for cookies (0.40 to 0.30). Because of this, the reported values more closely reflect the $a_w$ in the oven during the time intervals tested.

The temperature profiles of the cookies during baking are illustrated in Figure 1. Consistently monitoring cookie temperature was challenging. As the cookies baked, the thermocouples often slipped out, and it was not possible to determine or keep the thermocouple in the cookie’s cold spot. Because of this, temperature profiles were measured independent of inoculated cookie trials, and results are an estimate of the typical temperature profiles achieved during baking. For all baking tests, the oven temperature reached $350 \pm 2^\circ F$ ($177 \pm 1^\circ C$) prior to placing cookies in the oven. Opening the oven door caused the oven temperature to decrease nearly 50 $^\circ F$ ($28^\circ C$), and by the end of the 15 min baking period, the average oven temperature was $347^\circ F$ ($175^\circ C$). The temperature of the cookies increased from an initial average value of 69 $^\circ F$ to a final average value of 197 $^\circ F$ (92 $^\circ C$), 198 $^\circ F$ (92 $^\circ C$), 217 $^\circ F$ (103 $^\circ C$), 207 $^\circ F$ (97), 228 $^\circ F$ (109 $^\circ C$), and 229 $^\circ F$ (109 $^\circ C$) after baking for 10, 11, 12, 13, 14, and 15 min, respectively. Depending on the location of the cookie on the cookie sheet, cookies reached a temperature of 160 $^\circ F$ ($71^\circ C$) after baking between 7 to 8 min. The temperature of the cookie was significantly different ($P < 0.05$), depending on the location when controlling for the time of baking, with the center cookie being 5.3 $^\circ F$ warmer than the average, the side cookie being 0.9 $^\circ F$ cooler than the average, and the corner cookie being 4.4 $^\circ F$ cooler than the average. The differences in cookie location temperature correlate to the total number of positive enrichments at each location; the center, side, and corner locations had 10, 11, and 13 total positive samples, respectively (Table 3).

The average initial *Salmonella* load in the inoculated peanut butter was 8.22 log CFU/g and when this was used to make cookie dough, the *Salmonella* concentration in the dough averaged 6.21 log CFU/g. *Salmonella* survived during the baking of peanut butter cookies when the bake times were 14 min or less (Table 3). Cookies baked for 10, 11, or 12 min appeared underbaked, while cookies baked for 14 or 15 min appeared done. The 13-min bake time produced cookies that could potentially be perceived as underbaked or done, depending on individual preference. The 10-min bake time produced two samples with detectable populations of *Salmonella* by direct plating, and in the cookie with the highest count, a 4.8-log reduction was achieved. Less than 50% of the cookies were enrichment positive for *Salmonella* after 13 and 14 min of baking, resulting in a minimum observed log reduction in the range of 5.2 to 6.2. *Salmonella* was undetectable in cookies baked for 15 min.

**TABLE 2. pH and $a_w$ of uninoculated and inoculated peanut butter and raw cookie dough**

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>$a_w$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated peanut butter</td>
<td>6.07 ± 0.04</td>
<td>0.3930 ± 0.0018</td>
</tr>
<tr>
<td>Inoculated peanut butter</td>
<td>6.52 ± 0.08</td>
<td>0.4735 ± 0.0253</td>
</tr>
<tr>
<td>Uninoculated raw cookie dough</td>
<td>6.73 ± 0.08</td>
<td>0.8274 ± 0.0044</td>
</tr>
<tr>
<td>Inoculated raw cookie dough</td>
<td>6.85 ± 0.05</td>
<td>0.8223 ± 0.0071</td>
</tr>
</tbody>
</table>

**TABLE 3. Survival of *Salmonella* in peanut butter cookies baked at 350 $^\circ F$ when prepared with contaminated peanut butter**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Center</th>
<th>Side</th>
<th>Corner</th>
<th>Total (% positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>3/3$^b$</td>
<td>3/3$^c$</td>
<td>3/3</td>
<td>9/9 (100)</td>
</tr>
<tr>
<td>11</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>9/9 (100)</td>
</tr>
<tr>
<td>12</td>
<td>3/3</td>
<td>3/3$^c$</td>
<td>3/3</td>
<td>9/9 (100)</td>
</tr>
<tr>
<td>13</td>
<td>1/3</td>
<td>1/3</td>
<td>2/3</td>
<td>4/9 (44)</td>
</tr>
<tr>
<td>14</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/9 (0)</td>
</tr>
</tbody>
</table>

$^a$ Peanut butter was inoculated with the bacterium at an average concentration of 8.22 log CFU/g; the average initial *Salmonella* load in the cookie dough was 6.21 log CFU/g.

$^b$ One sample had an estimated plate count of 1.4 log CFU/g.

$^c$ One sample had an estimated plate count of 0.7 log CFU/g.
Several properties, such as pH, a_w, and the composition of the food affect microorganisms’ heat resistance. In addition to the production composition, experimental factors, including strain and serotypes tested, culture growth and storage conditions, and microorganism recovery procedures can also affect heat resistance. In general, *Salmonella* heat resistance increases with decreasing a_w (19). Because the cookie dough started at an intermediate a_w value of 0.82 and gradually decreased during baking, it was expected that the heat resistance would be greater than what has been observed in high-a_w foods but less than low-a_w foods. While no D-values were determined in this study, results indicate that the heat resistance of *Salmonella* in the dough was greater than what is typically observed in high-a_w foods. Doyle and Mazzotta (10) reviewed literature values of *Salmonella* thermal resistance in a variety of broth and high-moisture food systems and determined that at 160°F (71°C), 1.2 s would be required to inactivate 1 log of *Salmonella*. If this was the case in the cookie dough, no *Salmonella* would have been detected in samples baked for 10 min or longer, because the cookies reached 160°F (71°C) in 8 min or less. Instead, all samples after 10 min of baking were positive for *Salmonella* via enrichment, and some samples had detectable populations by direct plating, resulting in the inactivation of only 4.8 log of *Salmonella*. While *Salmonella* appears to be relatively heat resistant in the peanut butter cookie dough, it does not appear to be as resistant as in peanut butter alone. Ma et al. (16) reported that in peanut butter at 194°F (90°C), a minimum of 9 min was needed to inactivate 3 log of *Salmonella*. Shachar and Yaron (21) observed similar *Salmonella* heat resistance, reporting a 2.5-log reduction of *Salmonella* in peanut butter exposed to heat for 5 min at 194°F (90°C). In a third study, *Salmonella* D-values at 194°F (90°C) in peanut butter were reported to be approximately 4 to 5.5 min (13). In this study, the cookie dough did not reach 194°F (90°C) until approximately 10 min of baking, and at this time interval, a minimum log reduction of 4.8 was observed.

Results of this study indicate that *Salmonella* can survive temperatures encountered during baking of peanut butter cookies, and even under some conditions when cookies appeared fully baked, *Salmonella* was detected. However, in this study, peanut butter was inoculated with a high level of *Salmonella*, which is very unlikely to occur naturally. Because of this and because a >5-log reduction was observed after baking for 13 min or longer, results show proper baking will reduce the risk of *Salmonella* in peanut butter cookies.

**ACKNOWLEDGMENTS**

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**REFERENCES**


