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# Survival of *Salmonella* in Cookie and Cracker Sandwiches Containing Inoculated, Low–Water Activity Fillings

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## ABSTRACT

A study was done to determine the rate of inactivation of *Salmonella* in cookie and cracker snack sandwiches. Two cookie bases (chocolate and vanilla) and cheese crackers, along with high-sugar chocolate and peanut butter–based crème cookie fillings and peanut butter– and cheese-based cracker fillings, were obtained from commercial sources. Fillings and sandwiches containing fillings that had been dry- or wet-inoculated with *Salmonella* were stored at 25°C for 1, 6, 21, 35, 70, 112, and 182 days (6 months). At initial populations of 3.4 and 3.6 log CFU/g of cookie sandwiches containing chocolate crème and peanut butter crème fillings, respectively, *Salmonella* survived for at least 182 days; initially at 0.36 log CFU/g, the pathogen survived for at least 35 and 70 days. Initially at 2.9 and 3.4 log CFU/g of cracker sandwiches containing peanut butter– and cheese-based fillings, respectively, *Salmonella* survived for at least 182 and 112 days; initially at 0.53 log CFU/g, the pathogen survived for at least 6 and 35 days. Inactivation of *Salmonella* was more rapid in wet-inoculated peanut butter crème cookie filling than in dry-inoculated filling but was less affected by type of inoculum in peanut butter–based cracker filling. Chocolate cookie base (water activity [$a_w$] 0.39) and chocolate crème filling ($a_w$ 0.30) components of sandwiches equilibrated to $a_w$ 0.38 within 15 days at 25°C; vanilla cookie base ($a_w$ 0.21) and peanut butter–based crème filling ($a_w$ 0.27) equilibrated to $a_w$ 0.24 between 50 and 80 days. Cheese cracker ($a_w$ 0.14) and peanut butter–based filling ($a_w$ 0.31) or cheese-based filling ($a_w$ 0.33) components of sandwiches equilibrated to $a_w$ 0.33 in 80 days. The ability of *Salmonella* to survive for at least 182 days in fillings of cookie and cracker sandwiches demonstrates a need to assure that filling ingredients do not contain the pathogen and that contamination does not occur during manufacture.

Outbreaks of foodborne illness associated with consumption of foods with low water activity ($a_w$) have increased in frequency in recent years. Powdered infant formula, cereal, chocolate, spices, seasonings, nuts and nut products, and dried meats have been among the vehicles responsible for these outbreaks *(3, 21)*. Although foodborne pathogenic bacteria cannot grow at $a_w$ below 0.83, they can often survive for many months, even years, in low-$a_w$ environments. Rates of inactivation at a given $a_w$ depend on the type and form of the pathogen, i.e., vegetative cell or spore, as well as storage temperature and pH, composition, and other factors unique to various food matrices. Reduced temperature, pH near neutrality, and nonionic solutes tend to protect pathogens against inactivation.

High-sugar products such as dried fruits and fruit preserves, syrups, and candies are not generally thought to pose a microbiological hazard. It has been hypothesized that osmotic shock resulting from high concentrations of sugars is sufficient to quickly inactivate vegetative cells of foodborne pathogens. Detection of *Salmonella* in prunes *(26)* and halva (halvah, helva), a high-sugar, sesame seed–based product *(7, 10, 25),* and *Staphylococcus* in raisins *(26)* at the retail level, coupled with international outbreaks of salmonellosis associated with consumption of halva *(1, 10)* and a marshmallow confectionery *(17),* however, have raised interest in knowing more about the ability of foodborne pathogens to survive in high-sugar, low-$a_w$ foods and food ingredients.

Tysset and Durand *(24),* as cited by Snowdon and Cliver *(23),* reported that some *Salmonella* serotypes can survive for up to 28 months in honey stored at 10°C. Inactivation occurred within 26 to 34 days at 20°C. *Staphylococcus* was found in 3 of 25 samples of honey. *Salmonella enterica* ser. Enteritidis has been reported to survive in halva ($a_w$ 0.17, 49.5% sugar) for at least 8 months at 18 to 20°C *(16)*. Nummer et al. *(19)* recovered *S. enterica* ser. Typhimurium from a high-sugar, peanut butter–flavored candy fondant ($a_w$ 0.65 to 0.69) stored for 12 months at room temperature. *S. enterica* ser. Typhimurium has been reported to survive longer at 13°C in whole egg powder supplemented with corn syrup solids and salt, compared with several other types of egg powders *(14)*. More recently, *Salmonella* has been shown to survive for up to 3 months on 11 of 12 types of dried fruits stored at 4 and 10°C *(6)* and on dried cranberries and raisins and in date paste stored at 4°C for at least 8 months *(4)*.

Recognizing that various components in multicomponent foods may have dissimilar physicochemical properties that may result in different local microenvironments, Li et al.

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*(18)* studied the survival of *Salmonella* in an intermediate-moisture, multi-ingredient model food ($a_w$ 0.55). Inactivation rates varied in a homogenous mixture of ingredients, depending on which ingredient initially contained the inoculum. Hills et al. *(12)* examined the survival of *Salmonella* Typhimurium in randomly packed beds of glass beads, microporous silica particles, and Sephadex microspheres. A decrease in cell recovery in beds with reduced water content was not correlated with $a_w$ but rather with the osmotic shock induced by sudden redistribution of water and air among the microscopic pores in the matrix surrounding cells. Survival at $a_w$ 0.94 was later shown to depend on the microstructure of the particulate matrix and microscopic water distribution *(11)*. We are not aware of studies describing the inactivation of *Salmonella* in low-$a_w$ multicomponent foods in which two or more separate components with distinctly different composition and initial $a_w$ are assembled to constitute a whole. Examples of such foods include filled cookie and cracker sandwiches.

The objective of the study reported here was to determine the rate of inactivation of *Salmonella* in crème and noncrème types of fillings in cookie and cracker snack sandwiches, respectively. To mimic potential ways in which fillings might become contaminated before and during production of sandwiches, survival of *Salmonella* in dry- and wet-inoculated fillings was studied. Initially at two inoculum levels, the presence (by enrichment) and populations of *Salmonella* in filled sandwiches and fillings stored at 25°C for up to 182 days (6 months) were determined. The time required for equilibration of $a_w$ of fillings and cookie or cracker components of sandwiches was determined.

## MATERIALS AND METHODS

**Cookies, crackers, and fillings.** Cookie bases (chocolate and vanilla), cheese crackers, and fillings were obtained from commercial cookie and cracker manufacturers. Filled sandwiches consisting of two chocolate cookies and chocolate crème filling, two vanilla cookies and peanut butter–based crème filling, two cheese crackers and peanut butter–based filling, and two cheese crackers and cheese-based filling were prepared. Fillings were inoculated with a five-serotype mixture of *Salmonella* before assembling sandwiches.

**Measurement of $a_w$, moisture, and pH.** The cookie and cracker components of sandwiches were pulverized using a mortar and pestle. The $a_w$ of 5-g samples of each type of cookie, cracker, and filling was determined using an AquaLab model CX2 water activity meter (Decagon Devices, Inc., Pullman, WA).

The moisture content of 5-g samples was determined with a Mettler Toledo moisture analyzer (model HB43-S, Mettler Toledo, Greifensee, Switzerland). Cookie and cracker samples were dried at 100°C for 4 to 5 min. Fillings were dried at 160°C for 3 min. Weight loss was attributed to removal of water during drying. The percent moisture in cookies, crackers, and fillings was calculated.

Cookie and cracker slurries were prepared by combining 10 g of pulverized material with 10 ml of deionized water. The pH of slurries and undiluted fillings was measured using an Accumet pH meter (model AB15, Fisher Scientific, Pittsburgh, PA).

**Salmonellae used.** Inocula consisted of a mixture of five serotypes of *Salmonella enterica*: Agona, strain F5567, isolated from a dry cereal manufacturing plant; Enteritidis, strain 2415 (ATCC BAA-1045), from raw almonds; Montevideo, strain G4639, clinical isolate from patient in a tomato-associated outbreak of salmonellosis; Tennessee, strain K4643, clinical isolate from a patient in an outbreak of salmonellosis associated with consumption of peanut butter; and Typhimurium DT104 (source unknown). All strains were preserved at –20°C in tryptic soy broth (TSB; BD, Sparks, MD) supplemented with 15% glycerol.

**Preparation of inocula.** Fillings were inoculated using two methods: mixing with sand on which a five-serotype mixture of salmonellae had been dried (dry inoculum) and misting (atomizing) with an aqueous suspension of a five-serotype mixture (wet inoculum). These methods mimic contamination of filling ingredients and fillings resulting from cross-contamination by contact with surfaces, dust, or soil and from contact with water containing *Salmonella,* respectively.

To adapt the five *Salmonella* serotypes to reduced pH and nalidixic acid, they were grown in 10 ml of TSB supplemented with 1% glucose and 50 μg/ml nalidixic acid for 22 to 24 h at 37°C. After two consecutive 24-h transfers, 1 ml of culture (pH 4.7) was spread on tryptic soy agar (TSA) supplemented with 1% glucose and 50 μg/ml nalidixic acid (TSAGN) in large petri dishes (150 by 15 mm) and was incubated for 24 h at 37°C. Three plates were inoculated for each serotype. Cells were harvested by depositing 5 ml of sterile 0.1% peptone on the surface of each lawn and gently rubbing with a sterile bent glass rod. Suspensions were pooled to give approximately 15 ml of each serotype, combined, and thoroughly mixed to give approximately 75 ml of a five-serotype mixture.

To prepare the dry (sand) inoculum, 70 ml of cell suspension was combined with 350 g of sterile sand (40 to 400 mesh; Argos Organics, Geel, Belgium) and thoroughly mixed. After 30 min at 22°C, the excess suspension was decanted. Sand was spread (approximately 0.5 cm thick) on filter paper and placed in a biosafety cabinet for 20 to 24 h at 22°C to reduce the $a_w$ to 0.40. The sand on which salmonellae had dried was sealed in a glass jar and stored at 4°C for 3 to 4 weeks to facilitate stabilization of the number of viable cells before use as an inoculum for fillings. To prepare cell suspensions used for mist inoculation, salmonellae were grown on TSAGN and were harvested in 0.1% peptone as described above for preparation of inoculum for sand. Five-serotype suspensions diluted in sterile deionized water were used to inoculate fillings.

**Procedure for dry inoculation of fillings and preparation of cookie and cracker sandwiches.** Chocolate crème filling and peanut butter–based crème filling were held at 30°C for 16 to 18 h before inoculation with salmonellae. Sand (8 g) on which salmonellae (5.92 log CFU/g) had been dried was combined with 800 g of softened chocolate crème or peanut butter–based crème fillings to give a calculated high inoculum level (3.92 log CFU/g of filling). The inoculated sand was diluted ($10^{-3}$) in uninoculated sand, and then 8 g was combined with 800 g of crème fillings to give a calculated low inoculum level (0.92 log CFU/g of filling). After thorough mixing, inoculated chocolate crème and peanut butter–based crème fillings (4.8 g) were deposited between two chocolate or two vanilla cookie bases (6.1 g each), respectively, and were distributed on the inside surfaces of the cookies by firmly pressing the outside surfaces of the sandwiches. Samples consisting of two filled cookie sandwiches (34 ± 0.2 g) were placed in 16-oz (454-g) Snap n' Seal freezer bags (Kroger Co., Cincinnati, OH) and sealed; multiple samples were double-sealed in 1-gal (3.63-kg) freezer bags, placed in plastic tubs (60 by 43 by

15 cm), hermetically sealed, and stored at 25°C for 0 (within 30 min after preparing cookie sandwiches), 1, 6, 21, 35, 70, 112, and 182 days (6 months) before analysis for the presence (by enrichment) and number of surviving *Salmonella*. Inoculated crème fillings (25-g samples) were also stored at 25°C and were monitored for the presence and number of *Salmonella* for up to 182 days.

The procedure for dry-inoculating peanut butter–based and cheese-based fillings for cheese crackers was similar to that followed for inoculating crème fillings for cookie sandwiches. Sand (4 g) on which salmonellae (6.19 log CFU/g) had been dried was combined with 400 g of peanut butter–based filling or cheese-based filling to give fillings with a calculated high inoculum level (4.19 log CFU/g of filling). To give a calculated low inoculum level (1.19 log CFU/g of filling), inoculated sand (5 g) was combined with 45 g of uninoculated sand; next, 1 g of this was combined with 99 g of uninoculated sand; and, finally, 4 g of this was combined with 400 g of uninoculated sand. After a thorough mixing, inoculated fillings (1.6 g) were deposited between two cheese crackers (2.4 g each) and were distributed on the inside surfaces of crackers by firmly pressing the outside surfaces of the sandwiches. Samples consisting of two filled cracker sandwiches (12.8 ± 0.2 g) were placed in Snap n' Seal freezer bags, sealed, doubled-sealed in 1-gal (3.63-kg) freezer bags, placed in plastic tubs, hermetically sealed, and stored at 25°C. Samples stored for up to 182 days were analyzed for *Salmonella,* as described for crème-filled cookie sandwiches. Inoculated peanut butter–based filling and cheese-based filling (25-g samples) were stored at 25°C and were analyzed for the presence and number of *Salmonella* for up to 182 days.

**Procedure for wet inoculation of fillings.** The five-serotype *Salmonella* suspension harvested from TSAGN was diluted in sterile deionized water to give 7.90 log CFU/ml (high inoculum level) and 5.33 log CFU/ml (low inoculum level). Peanut butter–based crème filling (425 g) and peanut butter–based filling (425 g) were spread in layers approximately 1 cm thick on sterile stainless steel trays. Inoculum (4.25 ml) was applied on the surface of each filling using a Misty 2.5 Personal Mister (model 10025, www.misty.com) and was allowed to dry for 1 h before thorough mixing. Calculated high and low numbers of *Salmonella* in the fillings were 5.90 and 3.33 log CFU/g, respectively. Inoculated peanut butter crème filling and peanut butter–based fillings (25-g samples) were stored at 25°C and were analyzed for the presence and number of *Salmonella* for up to 182 days.

**Procedure for determining equilibration of $a_w$ in filled sandwiches.** Uninoculated chocolate crème filling (4.8 g) and peanut butter–based crème filling (4.8 g) were deposited between two chocolate or two vanilla cookie bases (6.1 g each), respectively, and were distributed on the inside surfaces by firmly pressing the outside surfaces of the sandwiches. Uninoculated peanut butter–based filling (1.6 g) and cheese-based filling (1.6 g) were deposited between two cheese cracker bases (2.4 g each) and were distributed on the inside surfaces by firmly pressing the outside surfaces of the sandwiches. Cookie and cracker sandwiches were separately placed in freezer bags, hermetically sealed, and stored at 25°C as described above for preparation of cookie and cracker sandwiches containing fillings inoculated with *Salmonella*. The $a_w$ of the cookie base, cracker base, and filling components of sandwiches stored at 25°C for 0 (within 30 min after preparing sandwiches), 1, 2, 4, 9, 15, 22, 30, 50, and 80 days was measured. The crème filling was removed from duplicate cookie sandwiches, and the $a_w$ was analyzed separately from the $a_w$ of the pulverized cookie base. For cracker sandwiches, fillings from two sandwiches were combined to form one sample; the $a_w$ of duplicate samples of fillings and the pulverized cracker base was measured.

**Microbiological analysis.** Triplicate samples of inoculated sand (5 g) were separately combined with 50 ml of sterile 0.1% peptone solution in a Stomacher 400 bag and were pummeled in a stomacher (Seward Medical Ltd., London, UK) for 1 min at normal speed. After resting for 10 min, the mixture was pummeled again for 1 min. The peptone wash was serially diluted in sterile 0.1% peptone, and 0.1-ml samples were surface plated in duplicate on TSA supplemented with nalidixic acid (50 μg/ml) (TSAN). The *Salmonella* suspension used to wet-inoculate fillings was likewise serially diluted in 0.1% peptone and plated on TSAN. Plates were incubated at 37°C for 24 h before colonies were counted.

Triplicate samples, each consisting of two cookie sandwiches (34 ± 0.2 g) or two cracker sandwiches (12.8 ± 0.2 g) containing dry-inoculated filling and stored at 25°C for up to 182 days, were analyzed for presence (by enrichment) and number of *Salmonella*. Each sample was placed in a Stomacher 400 bag containing 225 ml of lactose broth (BD) supplemented with 50 μg/ml nalidixic acid (LBN). The mixture was pummeled in a stomacher for 1 min at normal speed, allowed to set for 1 min, and pummeled again for 1 min. Undiluted homogenized samples (quadruplicate 0.25-ml samples and duplicate 0.1-ml samples) and samples (0.1 ml, in duplicate) serially diluted in sterile 0.1% peptone were surface plated on TSAN. *Salmonella*-presumptive colonies formed on TSAN within 24 h at 37°C were counted. Selected colonies were subjected to confirmation tests using BBL Enterotube II (BD) and API 20E (bioMérieux Vitek, Hazelwood, MO) assays and a *Salmonella* latex agglutination test (Oxoid, Basingstoke, UK). Bags containing homogenates of filled cookie or cracker sandwiches and LBN were incubated for 24 h at 37°C. The preenriched homogenate was streaked on bismuth sulfite agar (BD), and plates were incubated for 48 h at 37°C before examination for *Salmonella*-presumptive colonies and confirmation of randomly selected isolates. For samples anticipated to contain low numbers of *Salmonella,* 1 ml of preenriched LBN homogenate was added to 10 ml of tetrathionate broth (BD) and 0.1 ml was added to 10 ml of Rappaport-Vassiliadis broth (BD). Broths were incubated for 24 h at 37 and 42°C, respectively, before streaking on bismuth sulfite agar. *Salmonella*-presumptive colonies that formed on bismuth sulfite agar within 48 h at 37°C were randomly selected for confirmation.

Duplicate samples of dry- or wet-inoculated fillings that had been stored at 25°C for up to 182 days were analyzed for *Salmonella*. Samples (25 g) were combined with 225 ml of LBN. Subsequent steps in the analysis were the same as those used to analyze filled cookie and cracker sandwiches.

**Statistical analysis.** All experiments were repeated twice. Triplicate samples, each consisting of two filled cookie sandwiches or two filled cracker sandwiches, and two samples of fillings were analyzed for each test parameter combination at each sampling time in each replicate trail. Values were analyzed with a general linear model using SAS software (version 9.1, SAS Institute, Cary, NC). The least significant difference test was used to determine significant difference ($P \leq 0.05$) in mean values.

## RESULTS AND DISCUSSION

Weights and dimensions of cookie bases, cracker bases, and fillings, as well as $a_w$ and pH values, are shown in Table 1. The $a_w$ of fillings was 0.27 to 0.33, and the pH was 4.80

TABLE 1. *Physical characteristics and $a_w$, moisture content, and pH of cookies, crackers, and fillings*

| Sandwich base cookie/cracker | Filling | Weight (g) | | Base dimensions (cm) | Moisture (%) | | $a_w$ | | pH | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Base | Filling | | Base | Filling | Base | Filling | Base | Filling |
| Chocolate cookie | Chocolate crème | 6.1 | 4.8 | 5 (diam) by 0.64 | 5.71 | 0.24 | 0.39 | 0.30 | 7.38 | 4.80 |
| Vanilla cookie | Peanut butter–based crème | 6.1 | 4.8 | 5 (diam) by 0.64 | 4.81 | 0.68 | 0.21 | 0.27 | 7.43 | 5.10 |
| Cheese cracker | Peanut butter–based | 2.4 | 1.6 | 4 by 4 by 0.46 | 4.38 | 4.99 | 0.14 | 0.31 | 7.10 | 6.01 |
| Cheese cracker | Cheese-based | 2.4 | 1.6 | 4 by 4 by 0.46 | 4.38 | 6.53 | 0.14 | 0.33 | 7.10 | 5.75 |

to 6.01. Each sandwich consisted of two cookie or cracker bases and filling. The ratio of cookie:filling in cookie sandwiches was 2.5:1 (wt/wt), and the ratio of cracker:filling in cracker sandwiches was 3:1 (wt/wt). These ratios are typical of those used in commercially manufactured cookie and cracker sandwiches.

Shown in Figure 1 are results of studies designed to determine the rates of inactivation of *Salmonella* in dry-inoculated, crème-filled cookie sandwiches and crème fillings stored at 25°C for up to 182 days (26 weeks). At initial populations of 3.4 and 3.6 log CFU/g of chocolate crème and peanut butter crème sandwiches, respectively, *Salmonella* decreased to less than 1 log CFU/g between 70 and 112 days and between 112 and 182 days, respectively. The pathogen was detected in all sandwich samples stored for 182 days. In chocolate crème and peanut butter crème fillings containing the same initial populations of *Salmonella* but not used to prepare sandwiches, the pathogen decreased to 1 and 1.9 log CFU/g, respectively, in 182 days.

At an initial population of 80 CFU per two-sandwich sample (2.3 CFU/g or 0.36 log CFU/g of sandwich), *Salmonella* was detected in dry-inoculated chocolate crème cookie sandwiches stored for 35 days, but not 70 days, and in peanut butter crème cookie sandwiches stored for 70 days, but not 112 days (Table 2). Chocolate crème and peanut butter crème fillings containing an initial population of 8.3 CFU/g, but not incorporated into sandwiches, were positive for the pathogen in 25-g samples at 6 and 35 days, respectively, but were negative after 21 and 70 days.

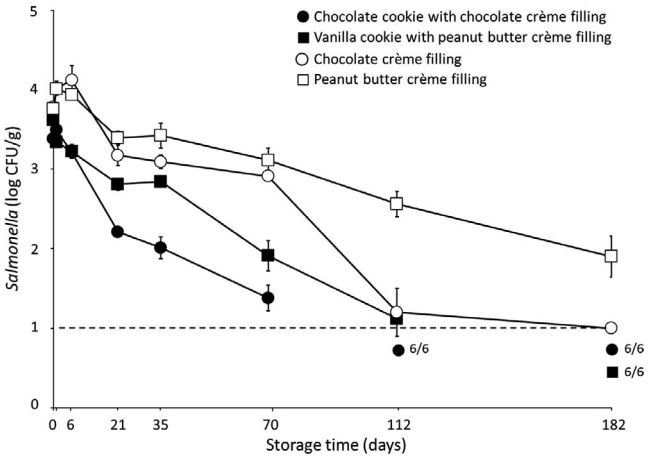


FIGURE 1. *Inactivation of* Salmonella *in dry-inoculated, crème-filled cookie sandwiches and crème fillings stored at 25°C for up to 182 days (26 weeks). Values shown below the detection limit (dashed line) by direct plating (1 log CFU/g) indicate the number of enriched samples positive for* Salmonella*/number of samples analyzed. Values are shown only for the number of cookie sandwich samples positive for* Salmonella *by enrichment out of the number of samples that were not positive by direct plating.*

Regardless of inoculum level, *Salmonella* survived longer in peanut butter crème filling, and in cookie sandwiches containing peanut butter crème filling, than in chocolate crème filling and sandwiches containing chocolate crème filling. This is attributed, in part, to the lower $a_w$ of the vanilla cookie base and peanut butter crème filling compared with the chocolate cookie base and chocolate crème filling. The higher pH of peanut butter crème filling (5.10) compared with chocolate crème filling (4.80) was also more likely to favor survival of *Salmonella*. The presence of antimicrobials in cocoa, an ingredient in chocolate cookie bases and chocolate crème filling, may have contributed to a more rapid reduction of *Salmonella*. Cocoa is known to be inhibitory to salmonellae *(9, 27)*.

*Salmonella* has been reported by others to survive in high-sugar confectionery products. *Salmonella* Typhimurium can survive in peanut butter fondant candy for at least 12 months *(19),* and *S. enterica* ser. Enteritidis can survive in halva ($a_w$ 0.18) for at least 8 months *(16)*. Baylis et al. *(2)* reported that verotoxin-producing *Escherichia coli* survived in biscuit (cookie) cream ($a_w$ 0.75) for 2 days at 38°C, 42 days at 22°C, and 58 days at 10°C, and in mallow ($a_w$ 0.40) stored for 113 days at 22°C and 273 days at 10°C. Survival of bacterial cells is favored by reduced $a_w$ and temperature

TABLE 2. *Number of samples of dry-inoculated, crème-filled cookie sandwiches and fillings positive for* Salmonella *after storage for 25°C for up to 182 days (26 weeks)*[a]

| Cookie | Filling | Storage time (days): 0 | 1 | 6 | 21 | 35 | 70 | 112 | 182 |
|---|---|---|---|---|---|---|---|---|---|
| Chocolate | Chocolate crème | 3/6 | 3/5 | 2/6 | 2/6 | 1/6 | **0/6** | **0/6** | **0/6** |
| Vanilla | Peanut butter crème | 5/6 | 6/6 | 2/6 | 4/6 | 2/6 | 1/6 | **0/6** | **0/6** |
| None | Chocolate crème | 4/4 | 2/2 | 2/4 | **0/4** | **0/4** | **0/4** | **0/4** | **0/4** |
| None | Peanut butter crème | 4/4 | 2/3 | 3/4 | 1/4 | 2/4 | **0/4** | **0/4** | **0/4** |

[a] Initial population of *Salmonella* was 2.3 CFU/g of sandwich (80 CFU per two-sandwich sample) and 8.3 CFU/g of filling. Number of enriched samples positive for *Salmonella*/number of samples analyzed. Values are shown only for the number of cookie sandwich samples (0 to 6) out of triplicate samples analyzed in two replicate trials ($n = 6$) and number of filling samples (0 to 4) out of duplicate samples analyzed in two replicate trials ($n = 4$) that were not positive by direct plating. Values shown in bold print indicate that all samples were negative for *Salmonella*.

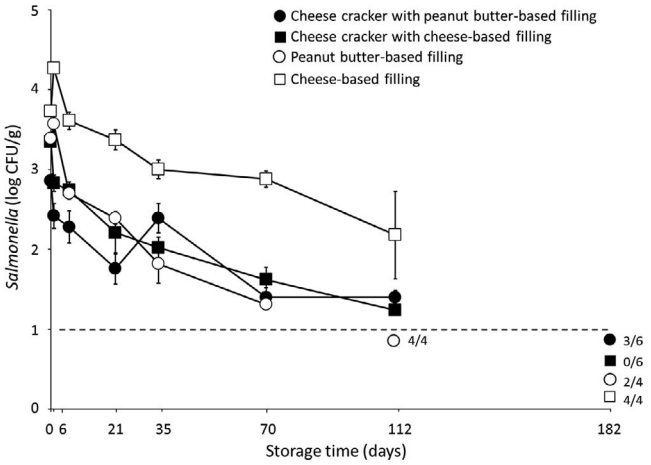


FIGURE 2. *Inactivation of* Salmonella *in dry-inoculated, filled cracker sandwiches and fillings stored at 25°C for up to 182 days (26 weeks). Values shown below the detection limit (dashed line) by direct plating (1 log CFU/g) indicate the number of enriched samples positive for* Salmonella*/number of samples analyzed. Values are shown only for the number of cracker sandwich samples and the number of filling samples positive for* Salmonella *by enrichment out of the number of samples that were not positive by direct plating.*

*(3),* but factors such as product composition and serotype may also influence the rate of inactivation *(22)*. Sucrose and sucrose-fat matrices are known to protect *Salmonella* against inactivation *(13, 20)*. Crème fillings in cookie sandwiches typically contain about 60% sugars and 30% fat, thereby likely favoring retention of viability of *Salmonella,* as observed in our study.

Figure 2 shows inactivation rates for *Salmonella* in cheese cracker sandwiches containing dry-inoculated, peanut butter–based and cheese-based fillings and in fillings not incorporated into sandwiches. Initially at 2.9 and 3.4 log CFU/g of cracker sandwich containing inoculated peanut butter– and cheese-based fillings, respectively, *Salmonella* numbers decrease to <1 log CFU/g between 112 and 182 days. The pathogen was detected in three of six cheese cracker sandwiches containing peanut butter–based filling after storage for 182 days; sandwiches containing inoculated cheese-based filling were negative for *Salmonella* after 182 days. *Salmonella* survived for 182 days in peanut-based and cheese-based fillings not incorporated into cracker sandwiches.

Shown in Table 3 are results of studies designed to determine inactivation rates of *Salmonella* in cracker sandwiches containing fillings dry-inoculated at a low level (3.4 or 0.53 log CFU/g of sandwich; 47.2 CFU per two-sandwich sample). Sandwiches containing peanut butter–based and cheese-based fillings were positive for *Salmonella* at 6 days but not 21 days and at 35 days but not 70 days, respectively. The pathogen was detected in 25-g samples of peanut butter–based and cheese-based fillings not incorporated into cracker sandwiches after storage for 6 days, but not 21 days, and after 112 days, but not 182 days, respectively.

Although we are not aware of studies focused on determining inactivation rates of *Salmonella* in peanut butter–based fillings in cracker sandwiches, work has been done to determine rates of inactivation of the pathogen in peanut paste and peanut butter that may be used as ingredients in fillings. Heat-stressed *Salmonella* can survive at least 12 months in peanut paste stored at 20 ± 1°C *(15)*. Survival was enhanced at $a_w$ 0.3 compared with $a_w$ 0.6 but appeared to be unaffected by fat content (47 and 56%). *Salmonella* has been reported to survive in peanut butter and peanut spread ($a_w$s 0.20 and 0.33) stored at 21°C for 24 weeks *(8)*. The composition of the peanut butter–based filling, and certainly the cheese-based filling, used in our study was different than that of peanut paste and peanut butter; however, observations of the behavior of *Salmonella* in these reduced-$a_w$ food matrices do indicate that the pathogen can survive for times exceeding the intended shelf life of filled cracker sandwiches.

TABLE 3. *Number of samples of dry-inoculated, filled cheese cracker sandwiches and fillings positive for* Salmonella *after storage for 25°C for up to 182 days (26 weeks)*[a]

| | | Storage time (days): | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Cracker | Filling | 0 | 1 | 6 | 21 | 35 | 70 | 112 | 182 |
| Cheese | Peanut butter–based | **0/6** | 1/6 | 1/6 | **0/6** | **0/6** | **0/6** | **0/6** | **0/6** |
| Cheese | Cheese-based | 3/6 | 3/6 | 3/6 | 3/6 | 3/6 | **0/6** | **0/6** | **0/6** |
| None | Peanut butter–based | 3/4 | **0/4** | 1/4 | **0/4** | **0/4** | **0/4** | **0/4** | **0/4** |
| None | Cheese-based | 4/4 | 1/3 | 2/4 | 2/4 | 2/4 | 2/4 | 2/4 | **0/4** |

[a] Initial population of *Salmonella* was 3.4 CFU/g of sandwich (47.2 CFU per two-sandwich sample) and 15.5 CFU/g of filling. Number of enriched samples positive for *Salmonella*/number of sample analyzed. Values are shown only for the number of cracker sandwich samples (0 to 6) out of triplicate samples analyzed in two replicate trials ($n = 6$) and number of filling samples (0 to 4) out of duplicate samples analyzed in two replicate trials ($n = 4$) that were not positive by direct plating. Values shown in bold print indicate that all samples were negative for *Salmonella*.

Inactivation curves for *Salmonella* wet inoculated into peanut butter crème filling used for cookie sandwiches and peanut butter–based filling used for cracker sandwiches are shown in Figure 3. Inactivation was more rapid in crème filling. An initial population of 5.4 log CFU/g decreased to less than 1 log CFU/g of peanut butter crème filling, as determined by direct plating, between 35 and 70 days (peanut butter–based filling between 70 and 112 days), but *Salmonella* was detected by enrichment (≥1 CFU/25 g) for at least 112 and 182 days, respectively. Table 4 shows counts in fillings that were wet inoculated with low numbers of *Salmonella* (1.2 to 1.5 log CFU/g). Peanut butter crème filling and peanut butter–based fillings were positive for *Salmonella* after storage for 35 and 70 days, respectively, but negative after 70 and 112 days.

The initial high number of *Salmonella* in dry-inoculated peanut butter crème filling (Fig. 1) and peanut butter–based filling (Fig. 2) was 3.6 to 3.9 log CFU/g; the initial high number in wet-inoculated fillings was 5.4 log CFU/g (Fig. 3). Reductions in dry- and wet-inoculated peanut butter crème fillings were, respectively, 1.7 log CFU/g after storage for 182 days and 4.1 log CFU/g after storage for 35 days.

TABLE 4. *Number of salmonellae recovered from wet-inoculated peanut butter crème and peanut butter–based fillings stored at 25°C for up to 182 days (26 weeks)*[a]

| Filling | Storage time (days): 0 | 1 | 6 | 21 | 35 | 70 | 112 | 182 |
|---|---|---|---|---|---|---|---|---|
| Peanut butter crème | 1.18 | 1.00 | 1.00 (3/3) | <1.00 (4/4) | <1.00 (1/4) | <1.00 (**0/4**) | <1.00 (**0/4**) | <1.00 (**0/4**) |
| Peanut butter–based | 1.54 | 1.35 | 1.48 (1/1) | <1.00 (4/4) | <1.00 (4/4) | <1.00 (4/4) | <1.00 (**0/4**) | <(1.00 (**0/4**) |

[a] *Salmonella* numbers are expressed as log CFU per gram. Initial populations of *Salmonella* were 15.1 CFU/g (1.18 log CFU/g) of peanut butter crème filling and 34.8 CFU/g (1.54 log CFU/g) of peanut butter–based filling. Values in parentheses indicate number of enriched samples positive for *Salmonella*/number of samples analyzed. Values are shown only for the number of filling samples (0 to 4) out of duplicate samples analyzed in two replicate trials ($n = 4$) that were not positive by direct plating. The detection limit by enrichment was 1 CFU/25 g. Values shown in bold print indicate that all samples were negative for *Salmonella*.

This clearly indicates that desiccated cells were more resistant to osmotic shock and perhaps other stresses imposed by exposure to the low $a_w$ of high-sugar crème filling than were cells not habituated to a low-$a_w$ environment. In contrast to the behavior of dry and wet inoculum in peanut butter crème filling, reductions were, respectively, 2.4 log CFU/g (Fig. 2) and 3.6 log CFU/g (Fig. 3) of peanut butter–based filling stored for 70 days. Desiccated cells were more resistant than cells not habituated to the low $a_w$ of peanut butter–based filling, but the magnitude of reduction in viability was not as great as that observed in peanut butter crème filling.

These results are not in agreement with a study showing that inactivation of *Salmonella* on dried fruits is unaffected by the type of inoculum, i.e., dry versus wet *(4)*. Inactivation was clearly affected, however, by the type of fruit. Blessington et al. *(5)* reported that rates of inactivation of *S. enterica* ser. Enteritidis on almonds and walnuts were unaffected by dry- or wet-inoculation methods. Differences in rates of inactivation of dry- and wet-inoculated *Salmonella* in cookie and cracker sandwich fillings, as well as in other low-$a_w$ foods and food ingredients with similar $a_w$, are attributed not only to the physiological state of cells in the inoculum, but also to the type of solute and other components of the food matrix. Injury of *Salmonella* caused by exposure to the high osmotic conditions imposed by the fillings may have resulted in increased sensitivity to nalidixic acid in LBN and TSAN. This may have resulted in an inability of cells to resuscitate and be detected or enumerated, thereby resulting in an underestimation of the presence and number of viable cells.

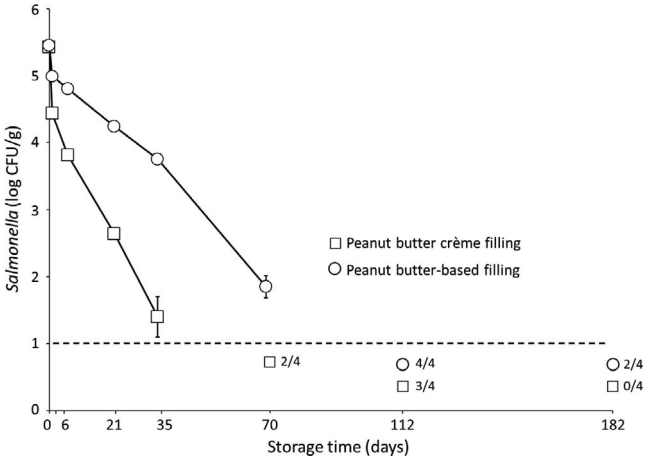


FIGURE 3. *Inactivation of* Salmonella *in wet-inoculated peanut butter crème filling and peanut butter–based filling stored at 25°C for up to 182 days (26 weeks). Values shown below the detection limit (dashed line) by direct plating (l log CFU/g) indicate the number of enriched samples positive for* Salmonella*/number of samples analyzed. Values are shown only for the number of filling samples positive for* Salmonella *by enrichment out of the number of samples that were not positive by direct plating.*

Equilibration of $a_w$ in cookie base and filling components of filled cookie sandwiches stored at 25°C for up to 80 days is shown in Figure 4. Chocolate cookie base ($a_w$ 0.39) and chocolate crème filling ($a_w$ 0.30) components of sandwiches equilibrated at $a_w$ 0.38 within 15 days; vanilla cookie base ($a_w$ 0.21) and peanut butter–based crème filling ($a_w$ 0.27) equilibrated to $a_w$ 0.24 between 50 and 80 days. Differences in the time required for equilibration of $a_w$ are attributed in part to differences in initial $a_w$ as well as composition of the two types of cookie bases and fillings. The rate of moisture migration between the cookie and filling components is apparently affected by sandwich components. Equilibration of $a_w$ in cracker base and filling components of filled cracker sandwiches is shown in Figure 5. Cheese cracker ($a_w$ 0.14) and peanut butter–based filling ($a_w$ 0.31) or cheese-based filling ($a_w$ 0.33) components of

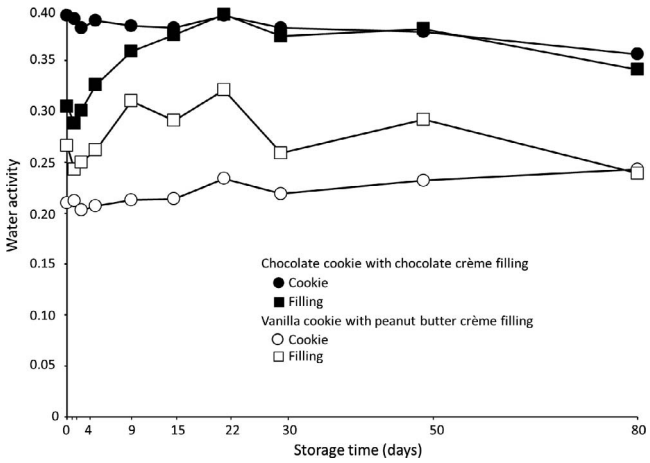


FIGURE 4. *Equilibration of $a_w$ in cookie base and filling components of filled cookie sandwiches stored at 25°C for up to 80 days.*

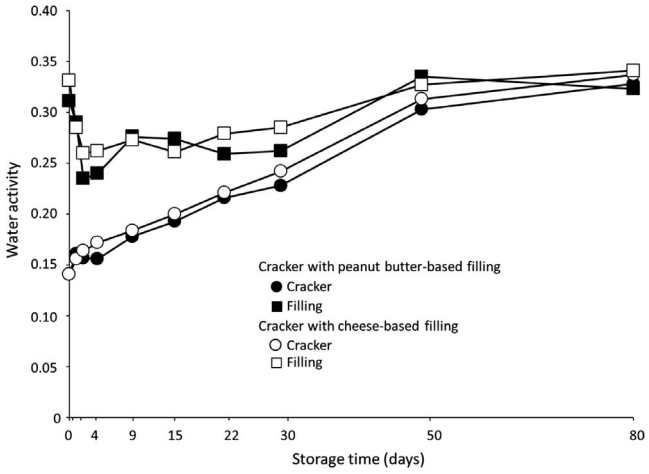


FIGURE 5. *Equilibration of $a_w$ in cracker base and filling components of filled cracker sandwiches stored at 25°C for up to 80 days.*

sandwiches equilibrated to $a_w$ 0.33 and 0.34, respectively, within 80 days.

In conclusion, results show that *Salmonella* can survive in low-$a_w$ fillings in cookie and cracker sandwiches stored under conditions typically found in commercial and home settings. Rates of inactivation are affected by filling composition and were more rapid if cells had not been exposed to a low-$a_w$ environment before they were exposed to low-$a_w$ filling matrices. The time needed for equilibration of $a_w$ between fillings and cookie or cracker bases depends on the differential between initial $a_w$ of the filling and base components and the composition of components. These findings highlight the need to assure that filling ingredients do not contain *Salmonella* and that contamination does not occur during manufacture of cookie and cracker sandwiches.

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