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Survival of *Salmonella* Tennessee, *Salmonella* Typhimurium DT104, and *Enterococcus faecium* in Peanut Paste Formulations at Two Different Levels of Water Activity and Fat

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ABSTRACT

Long-term survival of heat-stressed *Salmonella* Tennessee, *Salmonella* Typhimurium DT104, and *Enterococcus faecium* was evaluated in four model peanut paste formulations with a combination of two water activity (a_w) levels (0.3 and 0.6) and two fat levels (47 and 56%) over 12 months at 20 ± 1°C. Prior to storage, the inoculated peanut paste formulations were heat treated at 75°C for up to 50 min to obtain an approximately 1.0-log reduction of each organism. The cell population of each organism in each formulation was monitored with tryptic soy agar plate counts, immediately after heat treatment, at 2 weeks for the first month, and then monthly for up to 1 year. The log reductions (log CFU per gram) following 12 months of storage were between 1.3 and 2.4 for *Salmonella* Tennessee, 1.8 and 2.8 for *Salmonella* Typhimurium, and 1.1 and 2.1 for *E. faecium* in four types of model peanut paste formulations. Enhanced survivability was observed in pastes with lower a_w for all organisms, compared with those with higher a_w ($P < 0.05$). In contrast, the effect of fat level (47 and 56%) on survival of all organisms was not statistically significant ($P > 0.05$). Whereas survivability of *Salmonella* Tennessee and Typhimurium DT104 did not differ significantly ($P > 0.05$), *E. faecium* demonstrated higher survivability than *Salmonella* ($P < 0.05$). *Salmonella* survived in the model peanut pastes well over 12 months, which is longer than the expected shelf life for peanut butter products. The information from this study can be used to design safer food processing and food safety plans for peanut butter processing.

Salmonella is a major cause of foodborne disease in the United States and worldwide. The U.S. Centers for Disease Control and Prevention estimates that *Salmonella* causes 11% of domestically acquired foodborne illnesses annually, second to norovirus (58%); it was also the leading cause of hospitalization (35%) and death (28%) among 31 pathogens monitored by surveillance systems *(50)*. This may be due to the ubiquitous nature of *Salmonella* in the environment *(48, 55)*. Although *Salmonella* is known to be associated with eggs, poultry, and meat *(18, 54)*, recent foodborne outbreaks with low-moisture foods such as milk powder, nuts, spices, cereals, chocolate, and peanut butter products *(8–17)* reveal that these food commodities are emerging vehicles of salmonellosis and that controlling *Salmonella* in low-moisture foods is a food safety priority.

One of the challenges facing processors of low-moisture foods is that *Salmonella* can survive in food processing facilities *(38, 46, 55, 57)* and in low-moisture food matrices for extended periods of time *(6, 20, 23, 26, 31, 47, 56)*. Although *Salmonella* does not grow in low–water activity (a_w) environments (e.g., $a_w < 0.85$) *(5)*, a very small number of *Salmonella* cells is sufficient to cause illnesses and outbreaks. For example, investigations of an outbreak associated with chocolate products revealed that *Salmonella* was recovered from implicated products at levels between 2 and 23 CFU/g after approximately 7 months of production and that the organism was still isolated after 12 months of production *(23)*.

In low-moisture foods, *Salmonella*'s survivability has been shown to increase as the a_w decreased *(5, 6, 44)*. Other factors such as ingredient composition (e.g., fat, sugar, and other solutes) may contribute to long-term survival of *Salmonella* in low-a_w conditions (synergistic effect) *(25, 26, 38, 44)*. For example, Hiramatsu et al. *(26)* demonstrated that survival rates of *Salmonella* increased by 10 to 79 times in paper disks (a desiccation model system) as the concentration of sucrose increased. The study also suggested that vegetable oil in a cocoa drink could contribute to the enhanced survival of *Salmonella* in their model system *(26)*. In a study by Barrile et al. *(4)*, the heat resistance of *Salmonella* in milk chocolate increased as the concentration of cocoa butter was increased. Other researchers have demonstrated a possible synergistic effect of high fat and low a_w on *Salmonella*'s resistance to adverse conditions such as heat *(25, 51)*, disinfectants *(24)*, and resistance to gastric stress *(3)* in peanut butter products.

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TABLE 1. *Microbial organisms used in this study*

Serotype/strain name	Source	Isolate ID no.	Description	GMA ref no.
Salmonella Tennessee	FDA	5010 H	2007 peanut butter outbreak isolate	NN-4157
Salmonella Tennessee	Washington University	*S*13952 (782)	Used in other survival study	NN-4159
Salmonella Tennessee	Washington University	*S*13972 (783)	(Park et al. 2008 *(47)*)	NN-4160
Salmonella Tennessee	Washington University	*S*13999 (784)		NN-4161
Salmonella Tennessee	Cornell University	FSL R8-5221	Peanut isolate	NN-4162
Salmonella Typhimurium DT-104	Cornell University	W1-030	—	NN-4163
Enterococcus faecium	U.S. Department of Agriculture	NRRL B-2354	—	NN-4164

Peanut butter products are high-fat foods with a_w typically <0.5 *(6, 25, 42, 51)*. They have been associated with outbreaks of salmonellosis recently in the United States *(11, 14, 17)*. Peanut butter products are made of peanuts (meal), oil, and other ingredients (sugar, salt, and stabilizing agents). The total fat content should be less than 55% (vol/vol) *(58)* and may vary from 6.25 to ~54% (vol/vol) depending on the type of product *(19, 25, 27, 34)*. Peanut butter or spread may become contaminated with *Salmonella* from raw ingredients due to inappropriate peanut roasting, cross-contamination, or postprocessing contamination *(11, 31)*. In commercial peanut butter processing, raw peanuts are air roasted at >180°C, which is considered to be a *Salmonella* inactivation step *(14, 22, 31, 39)*. Then, roasted peanuts undergo a grinding process for ~20 min that results in product temperatures of 71 to 77°C, and peanut butter is pasteurized at 70 to 75°C for ~20 min before packaging *(39, 42, 51)*. The temperature and time of the grinding and pasteurization steps may not be sufficient to kill *Salmonella* *(14, 31, 42, 51)*. If the peanut butter is contaminated with *Salmonella* after the roasting step, organisms may be carried into the final products and may even survive throughout the products' shelf life (6 to 9 months) *(59)*. Burnett et al. *(6)* demonstrated that a five-serotype mixture of *Salmonella* survived up to 24 weeks in five commercial peanut butter products and two commercial peanut spreads stored at 5 and 21°C when inoculated with a high cell density. However, there is a lack of information on how fat content itself influences the survivability of *Salmonella* in low-a_w and high-fat food matrices such as peanut butter products over time. Furthermore, considering the steps in peanut butter processing, it is important to investigate the behavior of heat-stressed *Salmonella* in the product to better control this organism. The purpose of this study was to evaluate the impact of a_w and fat content on long-term survival of thermally stressed *Salmonella,* using model peanut paste formulations with four combinations of a_w and fat level. This was conducted by comparing *Salmonella* Tennessee, *Salmonella* Typhimurium DT104, and *Enterococcus faecium* NRRL B-2354.

MATERIALS AND METHODS

Bacterial strains. *Salmonella enterica* serotypes Tennessee and Typhimurium DT104 were selected for this study based upon their associations with peanut butter outbreaks *(11),* a previously published study on *Salmonella* survival in peanut butter products *(47),* and significance from a food safety and public health standpoint *(35, 43)*. *E. faecium* (*Pediococcus* NRRL B-2354) was also included because this organism has been used as a surrogate for *Salmonella* in challenge studies for thermal processing in low-moisture foods *(2)*. The sources of cultures are listed in Table 1, together with strain information. Each working culture was made from a −80°C stock culture in tryptic soy broth (TSB; Difco, BD, Sparks, MD) supplemented with 20% glycerol and maintained on tryptic soy agar (TSA; Difco, BD) slants stored at 4 ± 1°C. The working cultures were transferred monthly for up to 3 months.

Dry inoculum preparation. A talc dry inoculum was prepared for inoculation of the peanut paste formulations to ensure uniform distribution of the bacterial cells in the matrix and to maintain a_w at a given level. The initial transfer was made from a working culture of each strain of *Salmonella* or *E. faecium* into 10 ml of TSB (pH 7.0 ± 0.1), which was incubated overnight (~20 h) at 35 ± 1°C to reach the stationary phase of growth. From this culture broth, a second transfer was made into fresh TSB in centrifuge tubes (Fisher Scientific, Pittsburgh, PA) for each strain (six tubes, 40 ml in each), and cultures were grown overnight (~20 h) at 35 ± 1°C. These cultures were centrifuged at 3,500 rpm for 20 min (RC-5B Plus centrifuge, Sorvall, Newtown, CT), the supernatant was discarded, and the contents from the six tubes were combined for a resulting volume of approximately 17 ml. Sterile talc (25 g; Spectrum Chemical Mfg. Corp., Gardena, CA) that had been sterilized at 140°C for 4 h *(52)* was inoculated in a sterile glass crystallizing dish with the approximately 17 ml of culture broth. Each serovar of *Salmonella* or *E. faecium* was inoculated into a separate 25-g portion of talc. For *Salmonella* Tennessee, a five-strain composite was used for the preparation of dry inoculum by combining an equal amount of each strain. Each strain was enumerated on TSA prior to compositing to ensure approximately equal cell numbers. The inoculated talc was dried at 35 ± 1°C overnight (~20 h) and was held at room temperature (22 ± 1°C) for an additional ~20 h before sieving through a sterile fine mesh strainer to generate a fine powdered inoculum. After the preparation, the dry talc inocula were stored in sterile air-tight plastic bottles at room temperature. The cell density in the dry inocula was evaluated immediately after the sieving process and was measured periodically using TSA plate count. When assessing the cell population, maximum recovery diluent (Oxoid, Ltd., Hampshire, England) was used for the first dilution. Subsequent 10-fold dilutions were made in 0.1% peptone water (pH 7.0, Fisher Scientific, Fair Lawn, NJ) and were plated onto TSA plates. Following incubation at 35 ± 1°C for 24 to 48 h, plates were counted using a Q counter (Spiral Biotech, Norwood, MA). The initial counts of talc dry inocula were approximately 10^8 CFU/g for *Salmonella* and 10^9 CFU/g for *E. faecium*.

Preparation of model peanut butter formulations. Four model peanut formulations with combinations of two different

TABLE 2. *Peanut paste formulations and characteristics*

Peanut paste formulation (F)	Fat level (%)	Water activity	Deionized water added to 100 g of peanut paste (ml)[a]	Talc added to 100 g of paste (g)	Paste characteristics
F_1	47	0.3	0.0	8.0	Close to or slightly thicker than regular peanut butter product
F_4	47	0.6	4.4	0.0	Thicker than F_1 paste
F_{13}	56	0.3	0.0	20.0	Softer than F_1 paste
F_{16}	56	0.6	3.5	20.0	Close to or slightly softer than regular peanut butter product

[a] The amount of deionized water added to each paste varied slightly from sample to sample.

levels of a_w (0.3 and 0.6) and fat (47 and 56%) were prepared. To prepare the model food matrix (peanut paste), 12% fat peanut flour (medium roast) and peanut oil without additives (Golden Premium) were obtained from Golden Peanut Company (Blakely, GA). Aerobic plate counts were performed for the peanut flour and oil samples to evaluate the level of background microflora in each sample. A volume of 225 ml of 0.1% peptone water (pH 7.0; Fisher Scientific) containing 1% Tween 80 (Acros Organics, Morris Plains, NJ) was added to 25 g of oil; the same amount of 0.1% peptone water, without Tween, was added to a 25-g sample of peanut flour. Each sample was stomached for 2 min, serially diluted (decimal dilutions), and plated onto TSA plates. Plates were incubated at 35 ± 1°C for 48 h. The a_ws of the peanut flour and various peanut pastes were measured with an AquaLab series 4TEV water activity meter (Decagon Devices, Inc., Pullman, WA). Peanut pastes were made with peanut flour and peanut oil by mixing different amounts to obtain two desired fat levels (47 and 56%). Peanut flour and oil were weighed and mixed well in a sterile stainless steel mixing bowl with a sterile spoon. Sterile deionized water was used to adjust the a_w (0.3 or 0.6) of the peanut paste formulations (F). Characteristics of each peanut paste formulation (F_1: 0.3 a_w, 47% fat; F_4: 0.6 a_w, 47% fat; F_{13}: 0.3 a_w, 56% fat; and F_{16}: 0.6 a_w, 56% fat) are presented in Table 2, along with amounts of talc and sterile deionized water added into each paste formulation. The actual amount of sterile deionized water may have been adjusted slightly for each peanut formulation, depending upon the a_w level of peanut flour.

Inoculation of peanut paste formulations. Dry talc inoculum (4 g) was added to 200 g of a paste formulation, by thorough mixing using a sterile spoon for several minutes, to achieve 10^6 to 10^7 CFU/g of each organism in each paste formulation. Inoculated paste was held at 20 ± 1°C overnight (~20 h) to allow the bacteria to adapt to sample conditions, and the cell density in each paste was evaluated using TSA plate counts. A sample (1 g) of inoculated peanut paste formulation was weighed into a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI) and mixed thoroughly by hand with 9.0 ml of maximum recovery diluent containing 1% Tween 80 for the first dilution to minimize the cells' osmotic shock and to better emulsify the sample with high fat content. Further decimal dilutions of the sample were made using 0.1% peptone water. After appropriate dilution, TSA plates were incubated at 35 ± 1°C for 24 to 48 h and counted with the Q-counter.

Heat treatment and storage of peanut pastes. Inoculated pastes were placed in sterile Whirl-Pak bags and vacuum sealed (VacMaster VP-321 commercial vacuum packaging machine, Pleasant Hill Grain, Hampton, NE). Each sample bag was flattened to approximately 1.0-cm thickness to achieve a uniform heat treatment. The sample bags were completely submerged in a thermostatically controlled water bath (model W45, Thermo Haake, Karlsruhe, Germany) and heated at 75°C for 25 to 50 min to obtain approximately 1.0-log reduction of each organism, to result in thermally stressed cells. The heating time was determined based upon preliminary analysis. One uninoculated sample with a thermocouple (model HH506RA, Omega Engineering, Inc., Stamford, CT) was included with the heating treatment to monitor the temperature of the peanut paste formulation. Also, one negative control was included to evaluate potential background microflora. Following the heat treatment, bags were submerged in an ice-water bath for 30 to 40 s. The sample bags were opened aseptically, and the pastes were transferred into sterile air-tight plastic containers and stored at 20 ± 1°C for 12 months. The cell population in the pastes was evaluated with TSA plate counts immediately after heating, at 2 weeks, and then monthly as described above. Samples were mixed weekly with a sterile spoon to ensure uniformity during the storage. The amount of dilutions plated onto TSA was adjusted from 0.1 ml to 1.0 ml depending on an expected cell density in each sample formulation (detection limit, 10 to 100 CFU/g). For samples with lower counts, 3.0 ml of maximum recovery diluent with 1% Tween 80 was added to 1.0 g of sample, and 1.0 ml of the solution was plated onto TSA (detection limit, 4 CFU/g). If colonies were not present on the TSA plate, a 1.0-g portion of a sample was transferred to a sterile Whirl-Pak bag, and 10 ml of TSB was added and thoroughly mixed by hand. This sample mixture was incubated at 35 ± 1°C overnight (~20 h) for enrichment, and a loopful of sample mixture was inoculated onto a TSA plate and incubated at 35 ± 1°C for 24 to 48 h. Selected colonies from TSA plates were streaked on xylose lysine deoxycholate (Sigma-Aldrich, St. Louis, MO) agar plates and were incubated at 35 ± 1°C for 24 h to ascertain whether *Salmonella* was still present. In addition, the a_w of each peanut paste formulation was monitored in tandem with the plate counts.

Statistical analysis. At least two individual experiments were conducted for each organism for each peanut formulation. Cell counts were log transformed, and log reductions over time were calculated. Statistical data analysis was conducted using Minitab release 14 software (Minitab, Inc., State College, PA). The response variable in the statistical model was the log reduction (log CFU per gram) at each point. The effects of fat content and a_w were determined by analysis of variance. Differences between mean values were considered significant at $P < 0.05$. For the data points under the detection limit (4 CFU/g), the log of the limit of detection (0.6 log CFU/g) was used as a set value in order to calculate log reductions.

RESULTS

Heat treatment and initial inoculum levels. The initial inoculum levels of the peanut pastes ranged from 5.8

TABLE 3. *Initial inoculum levels and log reductions in each sample paste after heat treatment at 75°C for* Salmonella *and* Enterococcus faecium[a]

	Peanut paste formulation (fat%, a_w)	Heating time (min)	Initial inoculum level (log CFU/g)	Inoculum level after heat treatment (log CFU/g)	Log reduction after heat treatment (log CFU/g)
Salmonella Tennessee	F_1 (47, 0.3)[b]	30	7.0	4.7	2.3
	F_4 (47, 0.6)		5.8	3.1	2.7
	F_{13} (56, 0.3)[c]		6.8	5.3	1.5
	F_{16} (56, 0.6)		5.9	4.1	1.8
Salmonella Typhimurium DT104	F_1 (47, 0.3)	30	6.5	5.0	1.5
	F_4 (47, 0.6)		6.7	4.2	2.5
	F_{13} (56, 0.3)		6.8	5.1	1.7
	F_{16} (56, 0.6)		6.8	3.8	3.0
Enterococcus faecium	F_1 (47, 0.3)	50	6.2	5.8	0.4
	F_4 (47, 0.6)		7.0	5.8	1.2
	F_{13} (56, 0.3)		7.0	6.6	0.4
	F_{16} (56, 0.6)		7.1	5.7	1.4

[a] Data are expressed as the average value calculated from enumeration of survivors from two independent experiments, unless otherwise specified.
[b] Heat treatment duration for one of the experiments was 25 min; for the other, 30 min.
[c] Three experiments were averaged.

to 7.1 log CFU/g before heating and from 3.1 to 6.6 log CFU/g after the heat treatment at 75°C for all test organisms (Table 3). The log reduction following heat treatment was between 0.4 to 3.0 log CFU/g, depending on the paste formulation and organism. For the initial heat treatment, lower reductions were observed in the formulations with a low a_w (0.3). A longer heating time (50 min) was needed to achieve the target log reduction for *E. faecium* than for *Salmonella* (~30 min). *E. faecium* is known to be generally more resistant to heat in low-moisture foods than *Salmonella,* and it is recognized as an appropriate surrogate for *Salmonella* inactivation in this type of food (e.g., almonds) *(2).*

Survival of *Salmonella* Tennessee, *Salmonella* Typhymurium DT104, and *E. faecium* in peanut paste formulations during 12 months of storage. The cell populations of all organisms in all peanut paste formulations declined gradually over 12 months of storage at 20 ± 1°C (Table 4A through 4C). Enumeration of surviving cells was still possible at the end of storage, except in the case of *Salmonella* Tennessee in the F_4 paste, for which the cell density dropped under the detection limit (<0.6 log CFU/g) at 10 months (Table 4A).

Survival of *Salmonella* Tennessee was highest in the F_1 paste (1.3-log reduction) and lowest in F_4 (2.5-log reduction) over 12 months. Differences in log reductions among the four pastes were less than 1.2 log CFU/g at 12 months (Table 4A). Similarly, the highest survival of *Salmonella* Typhimurium occurred in the F_1 paste (1.8-log reduction), with the lowest in the F_4 paste (2.8-log reduction) over a year (Table 4B). Whereas the survivability of *Salmonella* Tennessee appeared to be slightly higher in 47% pastes (>0.3 to 0.5 log CFU/g) than *Salmonella* Typhimurium, no significant difference ($P > 0.05$) was observed in the survival trend of the two organisms over 12 months (Fig. 1).

E. faecium demonstrated its highest survival in F_1 (1.1-log reduction) and its lowest in F_4 (2.1-log reduction) during 12 months of storage (Table 4C). Greater survival of *E. faecium* occurred in all model formulations over a year compared with *Salmonella,* and the difference was statistically significant ($P < 0.05$) (Fig. 1).

Survival of *Salmonella* and *E. faecium* was enhanced in the lower-a_w formulations (0.3: F_1 and F_{13}) compared with the higher-a_w formulations (0.6: F_4 and F_{16}), regardless of the fat levels (47 or 56%). The difference between the two a_w levels was statistically significant ($P < 0.05$) for the survivability of all organisms during 12 months of storage. However, fat level did not affect survivability for any of the organisms in the present conditions.

The a_w level of peanut paste formulations was measured concurrently with the plate counts; levels for all formulations generally decreased from each target a_w level by the end of the 12-month storage period. Peanut paste formulations with initial a_w of 0.3 decreased to 0.2 by the end of storage, with minor fluctuations over time. In formulations starting at a_w of 0.6, there was a noticeable decrease, starting at approximately the 10th month (i.e., $a_w = 0.4$).

DISCUSSION

Overall, the log reduction (log CFU per gram) in the study (12-month storage at 20 ± 1°C) was between 1.3 and 2.5 for *Salmonella* Tennessee, 1.8 and 2.8 for *Salmonella* Typhimurium, and 1.1 and 2.1 for *E. faecium* in four types of model peanut paste formulations. All organisms exhibited high survivability in these peanut paste formulations. As expected, a lower a_w (0.3) was correlated with greater survivability of *Salmonella* and *E. faecium* than higher a_w (0.6). This result is in agreement with previous research *(5, 6, 28, 36, 44).* The two fat levels, 47 and 56%, were chosen to represent peanut butter products with higher and lower fat percentages and were expected to illustrate the synergistic effect of fat and low a_w on long-term survival of *Salmonella* (i.e., the highest survival of *Salmonella* was expected in F_{13}, the peanut formulation with higher fat and lower a_w).

TABLE 4. *Log reduction of* Salmonella *Tennessee,* Salmonella *Typhimurium DT104, and* Enteroccocus faecium *in peanut paste formulations at different water activities and fat percentages*[a]

Peanut paste formulation (fat%, a_w)	Inoculum level after heat treatment (log CFU/g)	Log reduction in peanut paste formulations over time (mo): 0.5	1	2	3	4	5	6	7	8	9	10	11	12
A. *Salmonella* Tennessee														
F_1 (47, 0.3)	4.7	0.1	0.5	0.2	0.4	0.2	0.6	0.7	0.7	0.8	1.0	1.4	1.4	1.3
F_4 (47, 0.6)	3.1	0.9	1.1	1.3	1.3	1.1	1.3	1.8	1.4[b]	2.5	2.3	≥2.5[b]	≥2.5[b]	≥2.5[b]
F_{13} (56, 0.3)[c]	5.3	0.6	0.6	0.5	0.2	1.1	0.7	1.1	1.9[d]	1.6	2.0	2.2	2.6	2.4
F_{16} (56, 0.6)	4.1	0.3	0.3	0.9	1.2	1.5	1.8	1.9	1.7	2.1	2.2	2.3	2.2	2.4
B. *Salmonella* Typhimurium DT104														
F_1 (47, 0.3)	5.0	−0.1	0.2	0.4	0.0	0.7	1.2	0.7	0.7	0.8	1.0	1.5	1.4[e]	1.8
F_4 (47, 0.6)	4.2	0.4	0.7	1.2	1.4	2.5	1.2	2.1	2.5	2.2	2.2	2.5	2.8	2.8
F_{13} (56, 0.3)	5.1	0.3	0.5	0.1	0.3	0.3	0.6	1.0	0.9	1.2	1.2	1.7	1.7	2.1
F_{16} (56, 0.6)	3.8	0.5	0.6	1.2	1.4	1.5	1.4	1.6	2.1	1.9	2.0	2.3	2.7	2.4
C. *Enterococcus faecium*														
F_1 (47, 0.3)	5.8	0.1	0.0	0.0	0.2	0.2	0.6	0.4	0.7	0.7	1.0	0.9	1.1	1.1
F_4 (47, 0.6)	5.8	0.2	0.3	1.6	0.7	1.1	1.0	1.2	1.2	0.9	1.4	1.9	2.1	2.1
F_{13} (56, 0.3)	6.6	0.2	0.2	0.1	0.4	0.3	0.4	0.6	0.7	0.8	1.0	1.2	1.5	1.4
F_{16} (56, 0.6)	5.7	0.2	0.2	0.8	0.9	0.8	1.1	1.1	1.2	1.2	1.5	1.7	1.6	1.8

[a] Peanut paste formulations, at two a_ws (0.3 or 0.6) and two fat content levels (47 or 56%), were stored at 20 ± 1°C for a year. Data are expressed as the average value calculated from enumeration of survivors from two independent experiments, unless indicated otherwise.
[b] No colony was observed with one-quarter dilution with maximum recovery diluent on TSA plate (detection limit, <0.6 log CFU/g), although *Salmonella* was recovered with TSB enrichment and was confirmed on xylose lysine deoxycholate agar.
[c] Average of three experiments.
[d] Average of two experiments.
[e] Only one experiment was recorded (no average between experiments).

However, our results did not support this hypothesis; one possible reason might be that fat levels >47% may have maximized the protective effect of fat on the survivability of bacterial cells and, thus, addition of even more fat may not increase survivability to any greater extent. Most reduced-fat products have levels <33.3% *(19, 34)*. Attempts to generate model peanut pastes at fat levels <47%, using only peanut oil and peanut flour, were unsuccessful because the desired texture (similar to peanut butter) could not be obtained. In the preliminary study, peanut paste of the various peanut flour and oil combinations were formulated within a range of 30 to 60% fat content. All combinations of peanut flour and oil at fat levels ≥30% and <47% resulted in a grainy product, not a paste (data not shown). Aviles et al. *(3)* were able to create similar model peanut pastes with two fat levels (low fat, 19%; high fat, 65%) and low a_w (0.27 to 0.28) and high a_w (0.96) to demonstrate the cross-protective effect of fat and a_w for *Salmonella,* using a model digestive system. In contrast to the current study, the consistency of the pastes may not have been an important factor in their experimentation. Pastes with lower fat levels should be created for the investigation of fat in peanut butter samples in future studies. However, the combination of high fat (>47%) and low a_w in the present study may have contributed to the organisms' survival over a year at 20 ± 1°C. As mentioned in the introduction, a previous study has shown that fat contributes to survival of *Salmonella* in a low-a_w model system *(26)*. After 16 months at 20 ± 1°C, *Salmonella* and *E. faecium* still survived, with no major log reduction (<0.5).

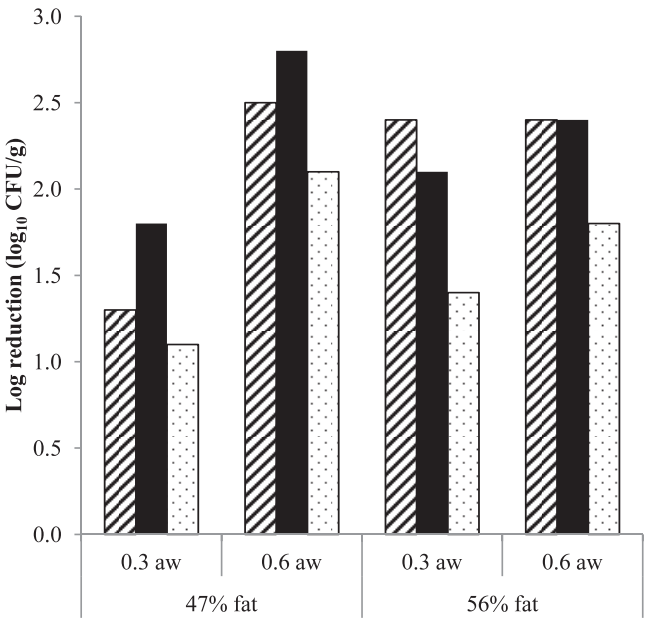

FIGURE 1. *Log reduction of* Salmonella *Tennessee (▨), Salmonella Typhimurium DT104 (■), and* E. faecium *(⬚) in model peanut butter formulations with combinations of two levels of water activity (a_w) and two levels of fat content at 20 ± 1°C after a year.*

Burnett et al. *(6)* reported higher log reductions of *Salmonella* in a natural peanut butter product (similar to the formulations used in this study) than in other products such as regular, low-fat, or low-sugar products at 5 or 21°C for 24 weeks (4.3 and 4.5 log CFU/g, respectively). Low-fat products showed less inactivation of *Salmonella* during the

first 2 weeks, although differences in log reduction were minor compared with regular products at the end of storage *(6)*. He et al. *(25)* also observed a similar trend of a greater log reduction in natural peanut butter products (fat, 50%; carbohydrate, 21.88%) and the least log reduction of *Salmonella* in a regular peanut butter product (fat, 33.33%; carbohydrate, 41.67%) after 30 days of storage at 4 or 25°C. These results may be due to the difference in ingredients used. These natural products have fewer additional ingredients (usually salt or sugar only) than regular or low-fat products that are formulated with different types of sugar and other ingredients. It was demonstrated that addition of sugar to the experimental medium and/or food matrix resulted in decreased a_w, enhancing *Salmonella*'s resistance to adverse environment conditions such as heat *(44)* and desiccation *(26)*. Furthermore, bacterial cells are thought to aggregate in or around water droplets in peanut butter products, making a colloidal suspension of lipids and water in peanut meal *(6, 44, 51)*. The survivability of *Salmonella* may be affected by the size of water droplets and nutrient conditions in a matrix *(6, 47, 51)*. Without stabilizers, peanut butter tends to separate over time, influencing the colloidal property of peanut paste and, therefore, the sizes of water droplets within the mixture. This may be one of the reasons for a higher inactivation of *Salmonella* in natural peanut products observed in other studies *(6, 25)*. In the present study, sample peanut formulations were stirred weekly to avoid the separation and to keep pastes uniform, which may have contributed to the higher survivability of organisms observed in our study. Log reductions of test organisms in the present peanut paste formulations were much lower than those observed in the Burnett et al. study, which had a similar storage temperature for a shorter storage period (4.5-log reductions for the natural product for 24 weeks at 21°C) *(6)*. The difference in log reductions between the two studies might also be due to many factors, including the strains tested, medium composition (e.g., presence of sugars and/or salts), inoculum (e.g., wet versus dry inoculum), inoculation procedure, and heat stress before storage, etc.

Mechanisms for the long-term survival of *Salmonella* in reduced-a_w environments have been investigated by many researchers. The formation of filaments *(37, 43)*, accumulations of osmolytes such as betaine (*N,N,N*-trimethyl glycine) *(1)* or proline *(30)*, modification of the outer membrane *(49)*, functions of σ^E- and σ^S-regulated genes *(45)*, and entrance of cells into the viable but nonculturable state *(7, 40)* have been suggested as mechanisms to increase the survival of *Salmonella* in a low-a_w environment. However, these theories appear to be based upon studies with a_w above 0.85; thus, further investigations with a lower a_w level (<0.85) are needed to elucidate mechanisms of the long-term survivability of *Salmonella* in a low-a_w matrix.

Another reason for the enhanced survivability of *Salmonella* observed in the present study might be the heat treatment at 75°C before the incubation period. It is recognized that exposure to a single stressor appears to prompt a development of cross-tolerance to other stressors in bacterial cells *(1, 29)*. For example, Humphrey et al. *(29)* demonstrated that a mild heat shock treatment improved heat, acid, and hydrogen peroxide tolerance of *Salmonella* Typhimurium and its survival on surfaces. A study by Deng et al. *(21)* demonstrated that genes involved in heat and cold shock response, DNA protection, and regulatory functions play a role in survival of *Salmonella* in low-a_w conditions (i.e., peanut oil). This suggests that heat treatment in the present study may have contributed to *Salmonella* gaining enhanced survivability in a food matrix with a low a_w for an extended period of time. Therefore, if the peanut butter is contaminated with *Salmonella* after the killing step (peanut roasting) during the processing, then the pathogen may achieve enhanced survivability through a subsequent thermal process (grinding or pasteurization) and survive better in final products for the duration of the product shelf life, as demonstrated in our study. Thus, for food safety, it is crucial to eliminate any chance of contamination after the peanut roasting step during peanut butter processing.

Mattick et al. *(44)* demonstrated the importance of a habituated inoculum for a low-a_w matrix in a challenge study. *Salmonella* found in raw peanuts, processing environments, or peanut butter has been exposed to a low-a_w environment, which may contribute to an enhanced resistance of *Salmonella* to other stresses (heat, desiccation, and starvation) *(25, 48)*. Therefore, use of *Salmonella* cells acclimated to dry environmental conditions is ideal for challenge studies that evaluate the behavior of *Salmonella* in peanut butter products *(25, 44)*. In the present study, dry talc inocula were used to inoculate the peanut paste formulations. The bacterial cells in the dry inoculum were desiccated and, thus, acclimated to low a_w, which may have contributed to the enhanced survival in the experimental conditions. In a report by Burnett et al. *(6)*, washed cells concentrated in phosphate buffer were used to inoculate pastes. They observed a rapid decline of viable cells within a week, which might be due to the death of cells by osmotic pressure and starvation. Contrary to the findings of Burnett et al. *(6)*, in the first 2 weeks, minor, if any, log reduction was observed for either *Salmonella* or *E. faecium* in the present study, supporting the benefit of using habituated bacterial cells in a challenge study *(44)*. A similar trend was observed by Tamminga et al. *(53)* in their study of *Salmonella* survival on chocolate. A sharp decline of cell population was observed in milk chocolate samples inoculated with *Salmonella* broth culture at the beginning of storage, whereas a similar decline was not seen in milk chocolate samples inoculated with *Salmonella* dry milk culture *(53)*. Use of a dry inoculum method has additional advantages in conducting a challenge study for *Salmonella* in low-a_w foods; the dry inoculum does not affect the target a_w in samples, and it may insure a better distribution of inoculum in pastes or other dry matrices.

Use of a nonpathogenic surrogate is desirable in process validation so as to avoid pathogen introduction to food processing areas; selecting the proper surrogate is crucial for evaluating behaviors of a pathogen of concern. *E. faecium* NRRL B-2354 has been used in the food industry for heat resistance challenge studies and process validation studies as a surrogate for *Salmonella* and *Listeria monocytogenes*

(33, 41), especially for air roasting almonds for *Salmonella* inactivation *(2)*. In our study, the long-term survival behavior of *E. faecium* was compared with *Salmonella*. *E. faecium* demonstrated better survival (less inactivation) during a 12-month storage period at 20 ± 1°C compared with *Salmonella* Tennessee and *Salmonella* Typhimurium DT104 in our peanut butter formulations. Therefore, *E. faecium* could be used as a conservative surrogate to evaluate long-term survival of *Salmonella* in peanut butter products.

In conclusion, the present study demonstrated enhanced survivability of *Salmonella* Tennessee, *Salmonella* Typhimurium DT104, and *E. faecium* in four peanut paste formulations (four combinations of a_w [0.3 and 0.6] and fat [47 and 56%]) over a 12-month storage period at 20 ± 1°C. All of the organisms persisted during the storage period, resulting in lower log reductions than expected. The two serotypes of *Salmonella* showed similar long-term survival behaviors, whereas *E. faecium* had higher survivability. Peanut paste formulations with lower a_w resulted in greater survival for all of the organisms tested, but no difference in long-term survival between the two fat levels (47 and 56%) was observed. Future investigation with lower fat levels (<47%) should be conducted to elucidate the fat influence on long-term survival of *Salmonella* in low-a_w food. The heat treatment used in our study prior to storage may have also improved the survivability of all organisms, which further supports the importance of preventing *Salmonella* contamination from occurring after peanut roasting in peanut butter processing facilities. *E. faecium* may be a suitable surrogate for a long-term survival challenge study for *Salmonella* in peanut butter products. Findings from this study will help the food industry design appropriate processes and food safety plans to provide a safer food supply.

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