Development of a Dry Inoculation Method for Thermal Challenge Studies in Low-Moisture Foods by Using Talc as a Carrier for *Salmonella* and a Surrogate (*Enterococcus faecium*)

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ABSTRACT

The objective of this study was to obtain dry inocula of *Salmonella* Tennessee and *Enterococcus faecium,* a surrogate for thermal inactivation of *Salmonella* in low-moisture foods, and to compare their thermal resistance and stability over time in terms of survival. Two methods of cell growth were compared: cells harvested from a lawn on tryptic soy agar (TSA-cells) and from tryptic soy broth (TSB-cells). Concentrated cultures of each organism were inoculated onto talc powder, incubated at 35°C for 24 h, and dried for additional 24 h at room temperature (23 $\pm$ 2°C) to achieve a final water activity of ≤0.55 before sieving. Cell reductions of *Salmonella* and *E. faecium* during the drying process were between 0.14 and 0.96 log CFU/g, depending on growth method used. There was no difference between microbial counts at days 1 and 30. Heat resistance of the dry inoculum on talc inoculated into a model peanut paste (50% fat and 0.6 water activity) was determined after 1 and 30 days of preparation, using thermal death time tests conducted at 85°C. For *Salmonella,* there was no significant difference between the thermal resistance ($D_{85°C}$) for the TSB-cells and TSA-cells (e.g. day 1 cells $D_{85°C}$ = 1.05 and 1.07 min, respectively), and there was no significant difference in $D_{85°C}$ between dry inocula on talc used either 1 or 30 days after preparation ($P > 0.05$). However, the use the dry inocula of *E. faecium* yielded different results: the TSB-grown cells had a significantly ($P < 0.05$) greater heat resistance than TSA-grown cells (e.g. $D_{85°C}$ for TSB-cells = 3.42 min versus 2.60 min for TSA-cells). *E. faecium* had significantly ($P < 0.05$) greater heat resistance than *Salmonella* Tennessee regardless what cell type was used for dry inoculum preparation; therefore, it proved to be a conservative but appropriate surrogate for thermal inactivation of *Salmonella* in low-moisture food matrices under the tested conditions.

Reduced water activity (a_w) has been widely used for food preservation, resulting in shelf-stable dried, low-moisture foods (also called low a_w foods); these types of product have historically been considered low risk as a vehicle for foodborne illness as they do not support bacterial growth. Although the a_w value used to define low-moisture foods can vary, many references, including the Codex Alimentarius Commission, use the value of <0.85 *(10)*. Although *Salmonella* cannot grow in these low-moisture foods, foodborne outbreaks and scientific studies have demonstrated that *Salmonella* can survive for lengthy periods of time *(13, 24)*. *Salmonella* outbreaks have been associated with a range of low-moisture food products such as spices, chocolate, and peanut butter, where cell levels may have been as low as one to three cells per gram *(11, 12, 18, 37)*. The European Food Safety Authority estimated that worldwide there were 279 outbreaks associated with low-moisture food products between 2004 and 2009 and that *Salmonella* was the causative agent in ~85% of these outbreaks *(15, 28)*. Furthermore, 119 recalls of low-

moisture food products, including, peanut butter, dry nuts, spices, powdered infant formula, dry milk, seeds, and pet food, were conducted in the United States between 2008 and 2012 *(16)*. In response to the *Salmonella* outbreaks associated with low-moisture foods, the Grocery Manufacturers Association formed a *Salmonella* Control Task Force that has developed industry guidance to assist food manufacturers in controlling *Salmonella* in processing facilities and to enhance microbial safety in this type of food *(8, 9, 19, 35, 36)*.

One of the greatest challenges for low-moisture food processors is that, if present, *Salmonella* can persist in the processing environment for lengthy periods of time *(35, 37)*. A study by Janning et al. *(22)* examined the survival of 18 bacterial strains (including 9 strains of *Salmonella*) under dry conditions by using anhydrous silica gel (a_w = 0.2) at 22°C. They showed that after an initial decrease in cell numbers, *Salmonella* persisted with a ≤1-log reduction of viable cells for up to 1,351 days. In general, stability can be defined as the degree of change in viable cell numbers over time. In the current study, a dry inoculum was considered stable when a ≤1-log reduction occurred over a 30-day period. *Salmonella* also demonstrates greater heat resistance

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TABLE 1. *Bacterial strains used in this study*

Serotype/strain	Source	Isolate no.	Description/reference	GMA reference no.[a]
Salmonella Tennessee	U.S. Food and Drug Administration	5010 H[b]	Related to 2007 peanut butter outbreak	NN-4157
Salmonella Tennessee	Washington University	S13952 (782)	Park et al., 2008 (33)	NN-4159
Salmonella Tennessee	Washington University	S13972 (783)	Park et al., 2008 (33)	NN-4160
Salmonella Tennessee	Washington University	S13999 (784)	Park et al., 2008 (33)	NN-4161
Salmonella Tennessee	Cornell University	FSL R8-5221	Peanut isolate	NN-4162
E. faecium	U.S. Department of Agriculture	NRRL B-2354	ABC, 2007 (1)	NN-4164

[a] GMA, Grocery Manufacturers Association.
[b] Originally from Minnesota Department of Health (original identification no. E2007000304).

in low-moisture foods, compared with heat resistance in high-moisture foods (20, 22). The increased heat resistance of *Salmonella* in low-moisture food products is affected by many factors, including conditions before and during heating, such as a_w; medium composition (presence of sugars, salts, fats); pH; food matrix; *Salmonella* strains used; and the physiological state of the cells (i.e. stationary versus logarithmic growth phase) (23, 35, 37). A study by Li et al. (28) demonstrated that local microenvironment in multi-ingredient foods (a_w of 0.55 and 0.8) consistently affected *Salmonella* survival and thermal inactivation.

There is increased interest in how food manufacturers validate their processes, including thermal inactivation parameters. To accomplish this task, it is desirable to identify a surrogate to conduct in-plant process validation, and to identify an adequate inoculation procedure. A dry inoculum is useful due to ease of inoculation, more uniform distribution in low a_w matrices (such as flours, grains, water in oil emulsions), and the elimination of the drying time for the inoculated product. Furthermore, the same batch can be used for multiple challenge studies at different times, different facilities, or both (13). Therefore, the general focus of this study was to identify a successful method to obtain dry inocula of *Salmonella* Tennessee and *E. faecium* (a surrogate) and to demonstrate the suitability of this procedure for use in challenge studies conducted with low-moisture foods. The specific objectives were to determine the (i) stability of cell concentration over time, which was measured by evaluating the change in cell numbers after 1 and 30 days of preparation and storage at room temperature; (ii) thermal resistance ($D_{85°C}$) after 1 and 30 days of preparation and storage at room temperature; and (iii) influence of the cell growth method (tryptic soy broth [TSB] or tryptic soy agar [TSA] lawn) on either the stability or the heat resistance of the dry inocula.

MATERIALS AND METHODS

Test microorganisms. Five strains of *Salmonella enterica* serovar Tennessee and one strain of *E. faecium* were used (Table 1). Working cultures were made from frozen ($-80°C$) TSB with 20% glycerol stocks and maintained on TSA slants stored at $4 \pm 1°C$; slants were transferred monthly for up to 3 months. TSA and TSB were purchased from Difco, BD, Sparks, MD.

Culture and dry inoculum preparation. Dry inocula on talc powder were prepared containing either a five-strain composite of *Salmonella* Tennessee or the single strain of *E. faecium*. Talc powder was heated at 140°C for 4 h before inoculation, after which absence of microbial contamination was verified by plating decimal dilutions of talc in 0.1% sterile peptone water (PW, pH 7; Fisher Scientific, Fair Lawn, NJ) onto TSA. Two methods of cell growth were used for *Salmonella* Tennessee and *E. faecium*: cells were harvested from TSB (TSB-cells) and from a lawn grown on TSA (TSA-cells). Before the inoculation of talc, each strain was transferred into 10 ml of TSB at pH 7.0 ± 0.1 and grown aerobically overnight (~20 h) at 35°C to reach stationary phase. From these individually grown cultures, a second transfer was made either into TSB or onto TSA. For the TSB-grown cells, the second transfer was made into centrifuge tubes containing 40 ml of TSB (six tubes for each strain) and incubated at 35°C overnight (~20 h). After incubation, the tubes were centrifuged at 3,500 rpm for 20 min (SORVALL RC-5B Plus centrifuge, Sorvall, Newtown, CT). Initially, each individual strain of *Salmonella* grown in TSB was enumerated on TSA to confirm that each strain grew to a similar level; all strains yielded a level of 8.8 to 9.1 CFU/ml/ml. Therefore, equal volumes of pellet (~4.0 to 5.0 ml per strain) of the five concentrated cultures were pooled to generate a five-strain composite, with a final volume of ~20 to 25 ml. The TSA-grown cells were prepared as described by the Almond Board of California (1). Five plates of each *Salmonella* strain or *E. faecium* were incubated at 35°C for 24 h. After incubation, 5 to 6 ml of sterile PW was added to each plate, and the bacterial lawn was loosened with a sterile spreader. The five-strain composite of *Salmonella* was made by mixing equal amounts of concentrated cells in PW yielded on TSA plates. Approximately 18 ml of the concentrated culture broth, or TSA-grown cells suspended in PW, was used to inoculate 25 g of talc powder. Talc powder was placed into sterile Pyrex crystallizing glass dish (diam 125 mm), the culture was added and mixed thoroughly using a sterile stainless steel spoon, and then the mixture was loosely covered with sterile gauze. The cell concentration at this point (inoculated talc before drying) was 9.2 and 8.9 log CFU/g for *Salmonella* TSA-cells and TSB-cells, respectively, and 9.7 and 9.4 CFU for *E. faecium* TSA-cells and TSB-cells, respectively. The inoculated talc was evenly spread in a thin layer (3 to 5 mm) covering the entire surface of the crystallizing dish bottom, incubated at 35°C for 24 h, and then removed from the incubator and held at room temperature for additional 24 h to dry to a final a_w of 0.44 to 0.46. After drying the inoculated talc was sieved through a sterile stainless steel fine mesh (0.7 by 0.7 mm) strainer to break up small clumps. The inocula were stored in sterile plastic bottles with the lid tightly closed, and the lid seam was covered with parafilm and held at room temperature for the duration of the study (13, 14, 24). This point, after sieving, completes the preparation of the dry inocula and is considered day 1, and the means and standard deviations for inoculum levels and a_w are presented in Table 2. To evaluate the

TABLE 2. $D_{85°C}$ and minimum calculated time for 5-log reduction (5D) of Salmonella Tennessee and Enterococcus faecium dry inoculum on talc (cells grown on TSB or TSA) in a model peanut paste formulation (50% fat and 0.6 a_w) on days 1 and 30 of preparation[a]

Organism	Cells harvested from	Dry inoculum (a_w)	Day 1				Day 30			
			Inoculum level (log CFU/g)	$D_{85°C}$ (min)	5D Regression	5D Weibull	Inoculum level (log CFU/g)	$D_{85°C}$ (min)	5D Regression	5D Weibull
Salmonella Tennessee	TSB	0.46 ± 0.03	8.7 ± 0.34	1.05 ± 0.01	5.22 ± 0.06	5.36 ± 0.17	8.6 ± 0.23	0.89 ± 0.08	4.45 ± 0.47	4.46 ± 0.59
Salmonella Tennessee	TSA	0.43 ± 0.04	8.3 ± 0.12	1.07 ± 0.16	5.35 ± 0.81	5.59 ± 1.10	8.1 ± 0.13	0.98 ± 0.12	4.92 ± 0.59	4.96 ± 0.75
Enterococcus faecium	TSB	0.45 ± 0.05	9.1 ± 0.18	3.42 ± 0.10	17.10 ± 0.50	17.30 ± 0.52	8.8 ± 0.05	3.43 ± 0.20	17.13 ± 1.03	17.46 ± 0.94
E. faecium	TSA	0.43 ± 0.03	9.6 ± 0.10	2.54 ± 0.12	12.72 ± 0.62	13.13 ± 0.48	9.4 ± 0.08	2.74 ± 0.04	13.70 ± 0.20	14.56 ± 0.15

[a] Values are means ± standard deviations and were calculated from enumeration data obtained from three independent experiments ($n = 3$) (each individual experiment was conducted in duplicate); for a_w measurements, $n = 5$.

talc inoculum concentration, maximum recovery diluent (0.8% sodium chloride, 0.1% peptone in sterile deionized water; Oxoid, Inc., Hampshire, England) was used for the first decimal dilution. Subsequent dilutions were made in 0.1% PW and then spiral plated (Autoplate 4000, Spiral Biotech, Norwood, MA) onto TSA. TSA plates were incubated at 35°C for 24 to 48 h and enumerated using a Q-Counter (Spiral Biotech, Norwood, MA).

Stability over time. For the evaluation of the dry inoculum stability over time, each dry inoculum was enumerated on TSA after 1 and 30 days from preparation. A dry inoculum on talc was considered stable for at least 30 days when a ≤1-log reduction in the microbial concentration (CFU per gram) occurred for the same organism grown under the same culture method (i.e. TSB- or TSA-cells).

Model food system. A model peanut paste formulation with 50% fat concentration and 0.6 a_w was used in the model food system experiment. The peanut paste was made by adding 43.2 g of peanut oil (golden premium, without additives) to 56.8 g of peanut flour (medium roast, 12% fat) in a sterile stainless steel bowl and then mixing thoroughly with a sterile stainless steel spoon. The a_w level in the model peanut paste was monitored and adjusted, by adding sterile deionized water, immediately after preparation and before inoculation. Absence of background microflora was confirmed through enumeration of decimal dilutions of the uninoculated peanut paste (negative control) onto TSA.

Thermal death time. Thermal death time tests for *Salmonella* Tennessee and *E. faecium* were conducted in the model peanut paste. For each experiment, a 100-g sample of peanut paste was inoculated with 2 g of dry inoculum on talc to achieve a concentration of at least 10^6 CFU/g of the test organism. The inoculated pastes were thoroughly homogenized by mixing with a sterile spoon for several minutes, and then they were incubated at 22 ± 1°C overnight (~20 h). After the overnight incubation, the a_w was measured again and when needed, the a_w of the peanut paste formulation was adjusted by adding a negligible amount (0 to 400 μl/100 g) of sterile deionized water. Five-hundred-milligram samples of inoculated peanut paste were placed into sterile 4-oz (118-ml) Whirl-Pak bags (Nasco Fort Atkinson, WI) and vacuum sealed with a Vacuum Sealer Chamber (VacMaster Commercial Vacuum Sealer VP-321, Pleasant Hill Grain, NE). Thermal death time tests were conducted using a thermostatically controlled water bath set at 85°C. Duplicate bags for each heating time were placed onto a magnetic copper plate (20 by 5 cm), and then another plate was added to sandwich the two sample bags between the two plates (Fig. 1A through 1D). The magnetic force was strong enough to secure the plates together and to compress the sample bag to give a very thin layer (≤1 mm in thickness) of peanut paste. The copper plates were completely immersed in the water bath for predetermined times (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 min for *Salmonella* Tennessee; 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 min for the surrogate). Precision fine wire thermocouples (diam/gauge 0.005 in. [0.13 mm]) from Omega Engineering, Inc. (Stamford, CT) were introduced in two bags containing uninoculated paste to monitor the temperature inside the paste during the heat treatment. The tested temperature as well as cooling temperature was reached within 10 s, due to the high thermal conductivity of copper [$k = 400$ W/(m·K)]. At each sampling time, one copper plate set, containing two duplicate bags, was rapidly withdrawn and placed into an iced water bath for 10 to 14 s. The copper plates were pried apart using a screwdriver, and the sample bags were aseptically

FIGURE 1. *Setup of duplicate sample bags between magnetic copper plates before heating (A), immersion of samples in the heated water bath (B), and release of the samples after cooling (C) and (D).*

opened with sterile scissors. Two duplicate sample bags were evaluated at each time point, and two unheated samples were also included to evaluate the initial level of inoculum in peanut paste before heat treatment (T_0). Sterile maximum recovery diluent (4.5 ml) containing 1% Tween 80 (Aeros Organics, Morris Plains, NJ) was added to each bag, and each bag was thoroughly mixed by hand for 1 min. Further decimal dilutions were made in 0.1% PW, spiral plated onto TSA, and the plates were incubated at 35°C for 24 to 48 h before enumeration.

Statistical analysis. For stability of the dry inocula on talc, the average cell counts on days 1 and 30 were determined and compared for each inoculum by using an analysis of variance test. Each data point was conducted in duplicate. For analysis, the data were converted to log CFU per gram, and the duplicates were averaged. Then, the log values from three individual replicate experiments ($n = 3$) were used to generate the inactivation curves of *Salmonella* and the surrogate in the peanut paste formulation by using log-linear regression and the Weibull model in the GInaFiT xla program *(17)*. To determine the effect of day after preparation and preparation method, the means and standard deviations of the D-values were determined and compared, and their variances were analyzed with Minitab release 14 software (analysis of variance, general linear model). The minimum times for a 5-log reduction

using the D-value concept or an equivalent of the D-values using the Weibull model were also compared. Alpha was set at $P = 0.05$.

RESULTS AND DISCUSSION

In total, four dry inocula on talc were prepared: *Salmonella* Tennessee TSA-cells, *Salmonella* Tennessee TSB-cells, *E. faecium* TSA-cells, and *E. faecium* TSB-cells. Cell concentration (CFU per gram) on the talc was evaluated right after the culture was mixed with the talc powder and after the drying step, and <1-log reduction of either *Salmonella* or *E. faecium* occurred during drying. The reductions observed were 0.21 log CFU/g for *Salmonella* TSB-cells, 0.96 log CFU/g for *Salmonella* TSA-cells, 0.55 log CFU/g for *E. faecium* TSB-cells, and 0.14 log CFU/g for *E. faecium* TSA-cells. The dry inocula were tested for changes in cell counts after 1 and 30 days of preparation (Table 2). The cell concentration of *Salmonella* Tennessee TSB-cells was 8.7 log CFU/g on day 1 and 8.6 CFU/g on day 30; for TSA-cells, cell concentration was 8.3 and 8.1 log CFU/g, respectively. Analysis of variance indicated no significant difference between *Salmonella* cell concentrations on talc at 1 or 30 days in either TSB- or TSA-cells

($P > 0.05$). Further monitoring of *Salmonella* for up to 133 days showed a 0.5-log reduction of TSB-cells and a 1.2-log reduction for TSA-cells (data not shown).

The cell concentration of TSB-cells of *E. faecium* was 9.1 log CFU/g on day 1 and 8.8 log CFU/g on 30 days after preparation; for TSA-cells cell concentration was 9.6 and 9.4 log CFU/g, respectively (Table 2). Although a slightly lower cell count was observed at 30 days, the difference was not significant ($P > 0.05$). Greater survivability over time was observed for *E. faecium* (TSA- or TSB-cells), compared with *Salmonella,* which declined only 0.6 log CFU/g after 196 days (data not shown). A greater cell concentration in the dry inocula was obtained for *E. faecium* compared with *Salmonella* Tennessee, regardless of the cell growth method used.

To explore the effect of growth method and storage time on heat resistance, *D*-values at 85°C were determined. To do so, each dry inoculum was separately introduced into the model peanut paste (50% fat and 0.6 a_w) at either 1 or 30 days after dry inocula preparation. The average *D*-values for *Salmonella* TSB-cells after 1 or 30 days of preparation was 1.05 and 0.89 min, respectively. The average *D*-values for *Salmonella* TSA-cells after 1 or 30 days of preparation was 1.07 and 0.98 min, respectively (Table 2). Statistical analysis indicated that there was no difference in the *D*-value due to day (i.e. 1 or 30 days after preparing) nor cell preparation method ($P > 0.05$). The TSB-grown cells of *E. faecium* had a significantly ($P < 0.05$) greater heat resistance ($D_{85°C} = 3.4$ min at both 1 and 30 days) than TSA-grown cells ($D_{85°C} = 2.54$ min at day 1 and 2.74 at day 30) ($P < 0.05$; Table 2). The surrogate had consistently and significantly ($P < 0.05$) greater heat resistance than *Salmonella* Tennessee, regardless what cell type was used for dry inoculum preparation in all test conditions (Table 2).

The *D*-values of *Salmonella* Tennessee presented in Table 2 are approximately two times greater than in artificially contaminated peanut flour ($D_{85°C} = 0.5$ min) (data not shown), but 12 times lower than those reported by Keller et al. (25) in commercial peanut butter ($D_{85°C} = 11.95 \pm 1.55$ min). Factors contributing to a higher heat resistance of *Salmonella* Tennessee in the commercial peanut butter than in the model peanut paste formulation used in the present study include the lower a_w of the commercial peanut butter (0.3 versus 0.6), as well as additional ingredients of the peanut butter, especially sugars (20). It is well recognized that as a_w decreases, the heat resistance of *Salmonella* increases (13, 31, 34). Moreover, it has been reported that the presence of sugar in the heating menstruum may increase the heat resistance of *Salmonella* up to 100 times or even more (20). It is not clear whether these factors would similarly increase the heat resistance of a surrogate such as *E. faecium*.

The minimum time for a 5-log reduction of *Salmonella* Tennessee and *E. faecium* dry inocula on talc (TSB- and TSA-cells) was calculated using classical log-linear regression (the *D*-value concept) and the Weibull model, and both models generated comparable results for the same inoculum. For example, 5.22 min was the time needed for a 5-log reduction of *Salmonella* Tennessee TSB-cells (day 1 of

preparation) in the tested conditions when calculations were made using the log-linear regression, and 5.36 when the Weibull model was used. The calculations for a 5-log reduction of *E. faecium* TSB-cells resulted in 17.10 min when log-linear regression was used and 17.30 with the Weibull model (Table 2). The resulting correlation coefficient (R^2) for the inactivation curves from either log-linear regression or the Weibull model was in the range of 0.966 to 0.998 for all tested dry inocula on talc.

In an unpublished study, Enache et al. (14) reported more pronounced tailing (shape parameter $p < 1$) of *Salmonella* Tennessee inactivation curves in peanut paste formulations at lower temperatures (70 to 75°C) than at higher temperatures (85 to 90°C). It is noteworthy to mention that in the present study, less tailing was observed for *Salmonella* Tennessee 85°C than at 70 and 75°C (data not shown) (13). In this respect, the results are in agreement with those of Ma et al. (30) who did not observe widespread asymptotic tails in survival curves among *Salmonella* strains when tested in peanut butter ($a_w \sim 0.45$; ~53% fat) at higher temperatures (83 and 90°C), in contrast to that reported by Shachar and Yaron (37) who observed the tailing effect of survivors at higher temperatures (80 to 90°C) as well. Furthermore, tailing was not observed in the inactivation curves of *E. faecium* at 85°C, the shape parameter *p* for the surrogate was always 1 or slightly over. The trend observed in the inactivation of *E. faecium* in the tested conditions indicates that the inactivation rates may rapidly increase at temperatures higher than 85°C.

As the use of pathogens is not acceptable in food processing facilities, the identification of an appropriate surrogate for challenge studies for process validation would be very helpful. It is desirable for a surrogate to have similar thermal inactivation parameters as *Salmonella* in the food, in this case low-moisture foods. Although *E. faecium* may not be an universal surrogate to model inactivation of all scenarios of *Salmonella* in low-moisture foods, it has been successfully used in the past. *E. faecium* NRRL B-2354 has been established as a surrogate for *Salmonella* for the validation of thermal processes to ensure a 4- to 5-log reduction of *Salmonella* in almonds (1, 5, 27); this organism is also regarded as a suitable surrogate for foodborne pathogens in thermal processing of other nuts, dairy products, juices, and meat, and it has been extensively used in validation studies for different low-moisture food products, including almonds, walnuts, peanut butter, extruded products (i.e. carbohydrate-protein meal), and others (1, 3, 4, 6, 29, 32, 34). Ma et al. (29) demonstrated that the heat resistance of *E. faecium* NRRL B-2354 was consistently and significantly greater (17.7 times) than that of *Salmonella* in ground beef, raising the concern that the surrogate may overly exceed the heat resistance of the target pathogen; therefore, its use in thermal process validation may result in overprocessing and higher energy costs (29). In contrast, two *Pediococcus* isolates used in the same study demonstrated a much closer heat resistance (2.5 to 4.1 times) to that of *Salmonella*; consequently, the authors recommended the two *Pediococcus* isolates as alternate surrogates for thermal inactivation of *Salmonella* in ground

beef. The present study indicates *E. faecium* as a conservative, but suitable, surrogate for *Salmonella* in the tested conditions, being 2.5 to 3 times more heat resistant than the target organism, and not overly high (~18 times) as described by Ma et al. *(29)*. It should be taken into consideration that heat resistance observed in high-moisture products is not applicable to low-moisture products. It is also noteworthy that Kopit et al. *(27)* demonstrated that *E. faecium* NRRL B-2354 lacks the phenotypic and genomic characteristics found in other strains of the same species responsible for nosocomial infections; therefore, the use of this specific strain as a surrogate for validation of the thermal processes is safe.

Current methods for the inoculation of low-moisture foods often involve inoculating the food with a liquid culture and allowing the product to dry before conducting the challenge (such as thermal inactivation). An example of this is the Almond Board of California method. It was desired to develop a method for validation studies using a dry carrier to inoculate the food product and to show that the same method was suitable for the preparation of the surrogate.

Dry inocula using talc as a carrier has been reported in studies of rhizobacteria *(26), Pseudomonas fluorescens (38), Pseudomonas putida (2, 7),* and *Bacillus subtilis (2).* In 1993, Hoffmans and Fung *(21)* developed a dry inoculation method for *Salmonella* Typhimurium by using chalk as a carrier to inoculate dry poultry feed. They stated that the inoculated chalk was a superior way of inoculating dry particles, compared with a wet inoculum, as it created a more homogenous mixture with the feed without altering any properties of the feed itself. Although the use of a dry inoculum is not new, the use of dry inocula in challenge studies for process validation in low-moisture foods was rather limited. A study by Blessington et al. *(6)* described a dry inoculation method for almonds and walnut kernels, using sand uniformly coated with *Salmonella* cells, to eliminate the need for the postinoculation drying step in the wet inoculation methods. They concluded that the dry inoculation method is a useful, viable alternative for survival challenge studies. Although the wet inoculation was successfully used for nuts, it was recognized that factors such as temperature and relative humidity during drying may affect the length of postinoculation drying and may also modify the properties of the kernel surface *(6).* Moreover, similar cell counts in the dry inoculum before and after inoculation in two commercial products (peanut butter, 56% fat and 0.13 a_w,; peanut spread, 33% fat and 0.15 a_w) and a model peanut paste formulation (56% fat and 0.6 a_w) were observed, indicating 100% recovery of *Salmonella* cells from each inoculated paste. When the products were inoculated through the wet inoculation method, using *Salmonella* culture broth, the cell recovery from the inoculated peanut butter or the model peanut paste formulation ranged from 67 to 71%, and 100% from peanut spread.

In conclusion, the dry inocula on talc of *Salmonella* Tennessee and *E. faecium* were stable in terms of survival and heat resistance for at least 30 days. Therefore, the dry inocula could be prepared and reused several times or prepared centrally and sent to different locations to be used. The cell growth method (lawn versus broth) did not affect the stability or heat resistance of *Salmonella* and *E. faecium*. Furthermore, *E. faecium* proved to be an appropriate surrogate for *Salmonella* in low-moisture food matrices consistently showing better survival and greater heat resistance than that of *Salmonella,* without being overly conservative. Previous reports *(13, 24)* also indicated *E. faecium* was a suitable surrogate for long-term survival challenge studies for *Salmonella* in peanut butter products. More research on the characteristics of the dry inocula is warranted, for example, by investigating stability over longer periods of time and thermal resistance over a wider temperature range.

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