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Identification of a surrogate to validate irradiation processing of selected spices

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ABSTRACT

Onion powder and talc were inoculated with one of three groups of *Salmonella enterica* or a putative surrogate, *Enterococcus faecium* NRRL B-2354, and the radiation sensitivity of *S. enterica* was compared to *E. faecium*. For both inoculated onion powder and inoculated talc, D_{10}-values were greater for *E. faecium* than any of the three groups of *S. enterica*. The survival of *E. faecium* in irradiated talc was used to estimate the potential survival of *S. enterica* in irradiated spices. Onion powder, dried oregano, whole cumin seeds or peppercorns were mixed with talc inoculated with either *S. enterica* (previously associated with a foodborne disease outbreak) or *E. faecium* and irradiated. The D_{10}-values were calculated for each bacterial group and compared between *E. faecium* and *S. enterica* within each spice. For each spice, the D_{10}-value for *E. faecium* was either not statistically different from ($P < 0.05$) *S. enterica* or greater than that of *S. enterica* (onion powder). Quadratic and linear models were developed to allow the estimation of potential surviving populations, and potential decimal reductions of *S. enterica*, based on surviving populations and decimal reductions determined with *E. faecium*. The use of *E. faecium* and these mathematical models would allow a processor to validate an irradiation process by estimating the reduction in *S. enterica*, based on the population reductions of *E. faecium*.

1. Introduction

Salmonella enterica is not an uncommon contaminant of unprocessed spices (Zweifel & Stephan, 2012). Between 2000 and 2003, there were 9 recalls implicating spices for *Salmonella* contamination in the United States (Vij, Ailes, Wolyniak, Angulo, & Klontz, 2006). Following three foodborne disease outbreaks of *S. enterica* associated with spices, the Food and Drug Administration (FDA) released a draft risk profile of spices (U.S. FDA, 2013) that evaluated spices primarily at the port of entry, prior to any antimicrobial process. The risk profile was updated in 2017 (U.S. FDA, 2017a) with the evaluation of spices available to the general public at retail establishments, after antimicrobial interventions had been applied (Zhang et al., 2017). Since the incidence of *S. enterica* prevalence in the samples collected at the port of entry (before intervention) were higher than those presented for retail sale (generally after intervention), it would appear that the spice industry's microbial interventions are reasonably effective.

Microbial interventions for spices generally fall into either thermal (heat) or non-thermal (gas disinfection, irradiation) categories. Different microbial interventions are applied to different spices, as the processes may affect either the quality or labelling of the spices. As an example, heat may degrade the color, aroma or flavor of a specific spice, such that only non-thermal interventions can be used without compromising the quality of the spice. In addition, spices labelled organic are usually processed with heat, as the non-thermal intervention methods are not appropriate for organically labelled products. As with any food process designed to assure the safety of the product, the process should be validated to assure that it can in fact deliver an appropriate level of protection, generally a 5-$\log_{10}$ reduction in *S. enterica*.

Food irradiation is a well-established process for reducing microbiological contamination in a variety of foods, including spices (Farkas & Mohacsi-Farkas, 2011). It was estimated that approximately 45% of the global trade in spices was treated with irradiation in 2005 (Kume, Furuta, Todoriki, Uenoyama, & Kobayashi, 2009). The Codex Alimentarius General Standard for Irradiated Foods states that irradiation is "justified only when it fulfils a technological requirement and/or is beneficial for the protection of consumer health" (Codex Alimentarius, 2003). Although irradiation may not be suitable for all spices and all

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applications, it is effective in controlling *S. enterica* if applied in the proper doses and under the appropriate conditions.

Food processes should be validated by the evaluation of scientific and technical information to assure that the process will control the intended hazard (National Advisory Committee on Microbiological Criteria for Foods, 2006). Validation should also include in-plant demonstration that the process, as applied in the operation, can meet the previously identified criteria (Hu & Gurtler, 2017; USDA-FSIS, 2015). Since it is not practical or desirable to intentionally introduce pathogenic bacteria into the processing environment, one method of demonstrating this is through the use of surrogate bacteria, which can be used to infer the effectiveness of a process against a specific pathogen.

Surrogate bacteria are non-pathogenic bacteria that have biological and physiological properties similar to those of the pathogen they are intended to represent. Several strains of non-pathogenic *Escherichia coli* have been established as useful surrogates for thermal processes in meat and poultry products (Keeling, Niebuhr, Acuff, & Dickson, 2009; Marshall, Niebuhr, Acuff, Lucia, & Dickson, 2005). *Enterococcus faecium* NRRL B-2354 has been used as a surrogate for *S. enterica* with thermal processes with some low moisture foods, including spices (Almond Board of California, 2007; Jeong, Marks, & Ryser, 2011; Shah, Asa, Sherwood, Graber, & Bergholz, 2017; Newkirk et al., 2018). Since *E. faecium* has already been established as a surrogate for some thermal processes in low moisture foods, it was reasonable to evaluate the suitability of this bacterium as a surrogate with irradiation processes.

The objectives of this study were to determine the relationship between the populations of inoculated *E. faecium* and *S. enterica* after irradiation processing of dried oregano, onion powder, whole cumin seeds and black peppercorns. Based on discussions with food industry representatives who were part of an advisory panel for the funding source for this project, the preferred method was to demonstrate that a non-spice substrate inoculated with a surrogate could be introduced into a spice, irradiated, and then the potential impact of the process on the hypothetical population *S. enterica* inferred from the reduction in population of the surrogate.

2. Materials and methods

2.1. Bacterial cultures

E. faecium NRRL B-2354 (obtained from Eurofins Laboratories, Minneapolis, MN) and cultures of *S. enterica*, listed in Table 1, were grown and maintained on Trypticase Soy Agar (TSA). For inoculation purposes, the *S. enterica* cultures were grown individually in Trypticase Soy Broth (TSB), and then combined into one of the three groupings listed in Table 1. The groupings were based on combining all of the cultures (Grp 01), only the two serovars associated with spice outbreaks (Grp 02) and the serovars from the ATCC MP-15 panel, excluding *S.* Typhi (Grp 03).

2.2. Spices

Four spices (dried oregano, onion powder, whole cumin seeds and black peppercorns) were obtained in an unprocessed state, prior to any antimicrobial intervention, with the assistance of the American Spice Trade Association (Washington, D.C.; http://www.astaspice.org/).

2.3. Talc powder inoculation

Fifteen ml of each *S. enterica* mixed culture or the *E. faecium* culture were added to 50 g of sterile talc powder in a Whirl-Pak® bag and homogenized by hand-massaging for 3 min. The inoculated talc powder was spread onto sterile aluminum foil in a biosafety cabinet, covered with sterile paper towels and dried at 35 °C until reaching a water activity (a_w) of 0.45 to simulate the a_w of dried oregano. The a_w of talc powder prior to mixing with the bacterial suspension was 0.28, and it was considered that drying to restore this value might unnecessarily further stress the bacteria. The target a_w was reached within 12 h, and the surviving populations of *S. enterica* and *E. faecium* in the onion powder and the talc were approximately $\log_{10}$ 5.8 colony forming units/g (cfu/g) and $\log_{10}$ 7.3 cfu/g, respectively.

Table 1
Experimental Cultures and Groupings. The groupings were based on the *S. enterica* isolates from isolated from spices (Grp 02), the ATCC MP-15 panel (Grp 03), and the combined *S. enterica cultures* (Grp 01).

Culture Designation	Serotype	Identification Number
Salmonella Grp 01	*S. enterica* subsp. *enterica* serovar Choleraesuis[a]	ATCC 13312
	serovar Enteritidis[a]	ATCC 4931
	serover Newport[a]	ATCC 6962
	serovar Typhimurium[a]	ATCC 700720
	serovar Rissen[b]	SAL 4599
	serovar Montevideo[b]	SAL 1449
Salmonella Grp 02	*S. enterica* subsp. *enterica* serovar Rissen	SAL 4599
	serovar Montevideo	SAL 1449
Salmonella Grp 03	*S. enterica* subsp. *enterica* serovar Choleraesuis	ATCC 13312
	serovar Enteritidis	ATCC 4931
	serover Newport	ATCC 6962
	serovar Typhimurium	ATCC 700720

[a] Serotypes Choleraesuis, Enteritidis, Newport and Typhimurium are part of the ATCC *Salmonella enterica* MP-15 Panel.
[b] Serotypes Rissen and Montivedeo were obtained from Dr. Monica Ponder, Department of Food Science and Technology, Virginia Polytechnic Institute and State University. These serotypes were associated with foodborne disease outbreaks from pepper.

2.4. Onion powder inoculation

50 g of onion powder were inoculated and homogenized separately with 1 ml of the mixed *S. enterica* cultures or *E. faecium*. The resulting inoculated onion powder or talc was placed into sealed containers with a desiccant pack at 25 °C until the powder returned to the original a_w of 0.3. The target a_w was reached within 12 h, and the surviving populations of *S. enterica* and *E. faecium* in the onion powder were approximately $\log_{10}$ 6.0 cfu/g and $\log_{10}$ 7.6 cfu/g, respectively. The dried onion powder and talc was stored in Whirl-Pak® bags contained within waterproof sealed boxes at −18 °C.

2.5. Irradiation experimental design

(a) Inoculated talc and onion powder: Initial studies evaluated the effect of irradiation on the bacteria in the inoculated talc and inoculated onion powder. These studies were intended to demonstrate the relative radiation resistance of the inoculated bacteria (*S. enterica* or E. faecium) on either a spice or a substrate. The onion powder and talc were weighed (0.5 g) into 1-ml microfuge tubes and the tubes were gently tapped on the hood work surface to allow the products to settle and to eliminate any air pockets. The microfuge tubes were irradiated using a J.L. Shepard and Associates Model 81 irradiator Cesium^{137} gamma ray source (24.5 Gy/min). Samples were irradiated and removed at 0.5-kGy intervals to a maximum absorbed dose of 5 kGy.
(b) *Inoculated Spices*: Four previously unprocessed spices (dried oregano, onion powder, whole cumin seeds and black peppercorns) were mixed in a 2:1 (vol/vol) ratio with talc inoculated with *S. enterica* Grp 02 (serotypes Rissen and Montevideo) or *E. faecium*. The specific serotypes of *S. enterica* were chosen as they had previously been associated with a foodborne disease outbreak associated with spices. Volumetric measures were used because of the difference in density between the individual spices and the

inoculated talc. The individual spices and inoculated talc were measured into sterile sample bags and the bags were inverted and hand massaged for approximately 4 min to assure uniform distribution of the talc within the spice. The sample bags containing the spice and inoculated talc were repeatedly mixed during the process of measuring the inoculated spices into the microfuge tubes (approximately every other tube) to assure that the spices and talc remained uniformly mixed. Samples (0.5 g) were placed into microfuge tubes and irradiated as described above.

2.6. Microbiological analysis

Samples were homogenized, serially diluted in Buffered Peptone Water (BPW, BD-Difco) and enumerated using the thin agar layer (Kang & Fung, 2000) technique. Briefly, this method uses a selective base layer of media, overlaid with a non-selective medium. For the enumeration of *S. enterica*, the selective medium was Xylose Lysine Tergitol-4 (XLT; BD-Difco), while the non-selective layer was TSA. For *E. faecium*, both the base and overlay media were De Man, Rogosa and Sharpe (MRS; BD-Difco). Although the MRS may have enumerated some non-target bacteria, the initial inoculated population of *E. faecium* was such that any non-target bacteria would constitute < 0.1% of the enumerated population. *S. enterica* samples were incubated at 37 °C for 24–48 h, while the *E. faecium* samples were incubated at 30 °C for 72 h.

2.7. Data analysis

Each combination of bacterial group, inoculation media (onion powder, talc) and irradiation dose was replicated independently three times, with single measurements within each replicate. The bacterial populations for each dose were transformed into $\log_{10}$ cfu/g. Decimal reduction values were calculated as the absolute value of the slope of the regression line, which plotted bacterial population (Y) versus dose (x). Statistical analysis was performed to compare the decimal reduction values between the four groups of bacteria using Winks SDA Professional 6.09 (Texasoft; http://www.texasoft.com/). Unless otherwise stated, statistical differences were determined at $P < 0.05$. For the preliminary irradiation experiments with inoculated talc, the populations of the three *S. enterica* groups were individually plotted against the population for *E. faecium* and regression analysis performed on the resulting graphs using SigmaPlot v.13 (Systat Software, Inc., https://systatsoftware.com/products/sigmaplot/). The quadratic models, model parameters, and R-squared values were part of the regression report produced by SigmaPlot.

3. Results and discussion

3.1. Inoculated onion powder and talc

The Decimal Reduction Values (D_{10}-values) for the inoculated onion powder and talc are presented in Table 2. Within inoculated medium (e.g., talc or onion powder), *E. faecium* had the highest D_{10}-value in onion powder, while the D_{10}-values of *S. enterica* Grp 01 and 02 were not statistically different ($P > 0.10$). The D_{10}-value of *S. enterica* Grp 03 was statistically lower ($P < 0.05$) than either *E. faecium* or *S. enterica* Grp 01 and 02. A minimum absorbed dose of 5 kGy would result in a theoretical reduction of approximately 4.4 $\log_{10}$ *S. enterica*/g, based on the highest radiation resistance (least population reduction) observed in onion powder for *S. enterica* Grp 01.

S. enterica Grp 01 had the highest D_{10}-value on inoculated talc, while the D_{10}-values of *S. enterica* Grp 01 and *E. faecium* were not statistically different ($P > 0.10$; Table 2). The D_{10}-values of *S. enterica* Grp 02 and Grp 03 in the inoculated talc were not statistically different, but both were statistically lower ($P < 0.05$) than either *E. faecium* or *S. enterica* Grp 01. Although it was possible to resolve the mean D_{10} values statistically, the actual differences were very small (range of 0.15 kGy).

Comparing the two inoculated media, the D_{10}-values were lower ($P < 0.05$) for *E. faecium* and *S. enterica* Grp's 01 and 02 in the inoculated talc, as compared to the inoculated onion powder. Although numerically lower in the inoculated talc, there was no statistical difference ($P > 0.10$) between the two media for *S. enterica* Grp 03. These results clearly indicate the influence of the inoculated media on the radiation sensitivity of some *S. enterica* serovars and suggests that there is a need to account for both strain and substrate variability to insure a margin of safety. Although the inoculated bacteria on talc were more sensitive to irradiation, talc did meet the requested industry criterion of an inoculated non-spice substrate (see comments in the objectives).

Using the D_{10}-values and a hypothetical initial population of both *S. enterica* and *E. faecium* arbitrarily set at 7 $\log_{10}$ CFU/g, the estimated surviving populations were calculated for irradiation doses from 0 to 5 kGy in 1-kGy increments (Table 3). With the inoculated onion powder, the estimated surviving populations of *E. faecium* were always higher than those of any of the *S. enterica* groups. With the inoculated talc, the estimated surviving populations of *E. faecium* were always higher than those of *S. enterica* groups 02 and 03, although the estimated surviving populations of *S. enterica* Grp 01 were marginally higher than those of *E. faecium*.

The populations of the three groups of *S. enterica* were plotted against the population of *E. faecium* for both the inoculated onion powder and the inoculated talc, and both linear and non-linear regressions were performed on the resulting plots. The models were selected by an iterative process, using different model types that were appropriate for the visual appearance of the graphs. The selection criteria were based on the highest R^2 value obtained by multiple analyses. For the onion powder, a linear regression model resulted in a best fit (highest R^2 value) for all three *S. enterica* groups. The intercept, slopes and regression coefficients are presented in Table 4.

Regression analysis of the surviving populations in inoculated talc

Table 2
Decimal Reduction Values (kGy) of *E. faecium* and *S. enterica* in inoculated onion powder and talc.

Bacterium	Onion Powder	Talc
E. faecium	1.70 (0.13)[1,A, 2]	0.74 (0.01)[A,3]
S. enterica Grp 01	1.14 (0.11)[B, 4]	0.77 (0.01)[A, 5]
S. enterica Grp 02	1.01 (0.06)[B, 6]	0.67 (0.06)[B, 7]
S. enterica Grp 03	0.75 (0.14)[C, 8]	0.62 (0.03)[B, 8]

1 Mean (standard deviation) in kGy. Means within columns with different letter superscripts are significantly ($P < 0.05$) different. Means within rows with different numerical superscripts are significantly ($P < 0.05$) different.

Table 3
Hypothetical populations in *S. enterica* or *E. faecium* in inoculated onion powder or talc after irradiation at specific doses (initial population $\log_{10}$ 7.0/g).

Dose (kGy)	*S. enterica* Grp 01	*S. enterica* Grp 02	*S. enterica* Grp 03	*E. faecium*
Onion Powder				
0	7.0[a]	7.0	7.0	7.0
1	6.1	6.0	5.7	6.4
2	5.3	5.0	4.3	5.8
3	4.4	4.0	3.0	5.2
4	3.5	3.0	1.7	4.6
5	2.6	2.0	0.3	4.1
Talc				
0	7.0	7.0	7.0	7.0
1	5.7	5.5	5.4	5.7
2	4.4	4.0	3.8	4.3
3	3.1	2.5	2.2	3.0
4	1.8	1.0	0.6	1.6
5	0.5	−0.5	−1.1	0.2

[a] Population in $\log_{10}$ cfu/g.

Table 4
Parameter estimates for linear equation fitted to the population data of *S. enterica* and *E. faecium* in inoculated onion powder and talc. Estimated surviving *S. enterica* population (log_{10} cfu/g) = A(x) + B, where x is the surviving population of *E. faecium* (log_{10} cfu/g).

Parameter Estimate	*S. enterica* Grp 01	*S. enterica* Grp 02	*S. enterica* Grp 03
Onion Powder			
A	−4.10	−5.38	−8.27
B	1.33	1.48	1.91
R^2	0.99	0.95	0.94
Talc			
Y_o	1.83	0.85	11.13
A	−0.69	−0.33	−3.92
B	0.13	0.12	0.38
R^2	0.89	0.96	0.92

resulted in a quadratic, nonlinear regression based on multiple regression analyses. The resulting regression equation was:

Estimated *S. enterica* population (log_{10} cfu/g in talc) = Y_0 + A(x) + B (x^2) (1)

Where x is the surviving population of *E. faecium* in log_{10} cfu/g in the talc. The parameters for the equation for each group of *S. enterica* are provided in Table 4, and plots are shown in Fig. 1. If a spice were inoculated with *E. faecium* as a surrogate and irradiated, the pre- and post-irradiation populations could be used to estimate the potential change in the populations of *S. enterica* populations by estimating a pre- and post-irradiation population of *S. enterica* and then calculating the reduction in the estimated population (Woerner et al., 2018).

3.2. Talc inoculated spices

Dried oregano, onion powder, whole cumin seeds and black peppercorns, obtained prior to any antimicrobial intervention, were inoculated with *S. enterica* Grp 02 and *E. faecium* and irradiated as previously described. Mixing the inoculated talc with the individual spices introduced a greater amount of variation into the experiments. With the exception of the talc-inoculated onion powder, the D_{10}-values were not statistically different ($P > 0.10$) between the *S. enterica* Grp 02 and *E. faecium* within spices (Table 5), with the D_{10}-value for *S. enterica* being less than that of *E. faecium* in onion powder. The D_{10}-values for *S. enterica* were not statistically different between the four inoculated spices at the pre-determined significance level of $P = 0.05$. Song et al. (2014) reported a D_{10}-value for *S.* Typhimurium in whole black pepper of 0.6 kGy, which is less than the value of 1.67 determined in this study. Differences in *S. enterica* strains and inoculation methods used in these two studies may account for the lower value reported by Song et al.

The D_{10}-values for *E. faecium* were statistically different ($P < 0.001$) between onion powder and dried oregano and whole cumin seeds and peppercorns, with the numerically larger D_{10}-values associated with the latter two spices. This may simply be a data artifact of the greater variation associated with mixing the inoculated talc powder with the spices, or it may suggest an effect associated with specific spices. However, in every case, the D_{10}-value of *E. faecium* was either not statistically different from, or greater than (onion powder) the D_{10}-value for *S. enterica*.

The method of inoculation was compared by analyzing the D_{10}-values obtained with the directly inoculated onion powder (Table 2) and the talc-inoculated spices (Table 5). There was no significant difference ($P > 0.10$) in the D_{10}-values for *S. enterica* Grp 02, irrespective of the method of inoculation. There was no significant difference ($P > 0.10$) in the D_{10}-values for *E. faecium* between the directly inoculated onion powder and the talc-inoculated onion powder. However, the D_{10}-value for talc-inoculated peppercorns was statistically higher ($P < 0.05$) than for the directly inoculated onion powder. This indicates that in a practical application, *E. faecium* inoculated on to a substrate and mixed with a spice could be used as a suitable surrogate for *S. enterica* with irradiated spices.

Based on the experimental data, the estimated populations of both *S. enterica* and *E. faecium* were calculated in 1-kGy dose increments for all four spices, with a hypothetical initial population of 7 log_{10} per gram (Table 6). Although there were slight numerical differences between the estimated populations of *S. enterica* and *E. faecium* for dried oregano, the D_{10}-values were not statistically different and the difference in the population estimates were minimal. Farkas (1998) suggested a minimum dose range of 3–10 kGy to improve the microbiological safety of spices. The American Society for Testing and Materials (ASTM International; 2010) suggested minimum irradiation doses for selected spices and recommended 7–12 kGy (onion powder) and 6–12 kGy (oregano and black pepper). A minimum absorbed dose of 8.5 kGy would be required to achieve a 5-log_{10} reduction in population based on the largest D_{10}-value reported (1.7 kGy for *E. faecium* inoculated on to talc and mixed with peppercorns). This is well within the doses suggested by both Farkas (1998) and ASTM (2010).

The estimated surviving populations of *S. enterica* and *E. faecium* were plotted and a regression line was calculated for each spice. The models for all four spices (onion powder, oregano, cumin seed and peppercorns) were linear, following the equation:

Surviving *S. enterica* population = A + B *(surviving *E. faecium* population) (2)

The parameters for each spice are given in Table 8, and as previously stated, the equations could be used to estimate potential surviving populations of *S. enterica* based on validation data for *E. faecium*.

Some industry and regulatory standards are based on theoretical log_{10} population reductions rather than population reductions (e.g., a 3-log_{10} or 5-log_{10} reduction). For example, the FDA requires a 5-log_{10} reduction for juice processing (FDA, 2017b). Further analysis of the data was used to express the hypothetical log_{10} reductions in *S. enterica* populations, based on the actual observed log_{10} reductions for *E. faecium* populations. The populations of estimated surviving populations of *S. enterica* and *E. faecium,* based on the hypothetical populations predicted in Table 6, were plotted and a regression line was calculated for each spice. The best fit regression lines were linear using the criteria established earlier for highest R^2 value.

Estimated log_{10} reduction in *S. enterica* population = A + B *(log_{10} reduction in *E. faecium* population) (3)

The parameters for the regression lines are shown in Table 9.

To account for the observed differences in D_{10}-values between the inoculated onion powder and inoculated talc (Table 2), the regression was also calculated using the greatest D_{10}-value determined for *E. faecium* (1.7 kGy) and *S. enterica* Grp 2. Since the observed D_{10}-values were higher in the inoculated onion powder, the second regression models provide a greater margin of safety.

The overall conclusion from these experiments is that *E. faecium*, inoculated onto a substrate such as talc, is a useful surrogate for *S. enterica* in spices for irradiation processing and may be used by the spice industry to evaluate the effectiveness of an irradiation process to reduce microbial contamination. *E. faecium* can be placed in spices prior to irradiation using inoculated talc or talc contained in either gelatin capsules or glassine envelopes placed at the geometric point where the minimum absorbed dose would be expected. By determining the initial and surviving populations of *E. faecium*, the estimated impact of the irradiation process on *S. enterica* can be determined within a reasonable degree of accuracy using equation (2) and the parameters in Table 7. Alternately, the overall log_{10} reduction in the population of *E. faecium* could be used to estimate the log_{10} reduction in the population of *S. enterica*, using equation (3) and the parameters in Table 8.

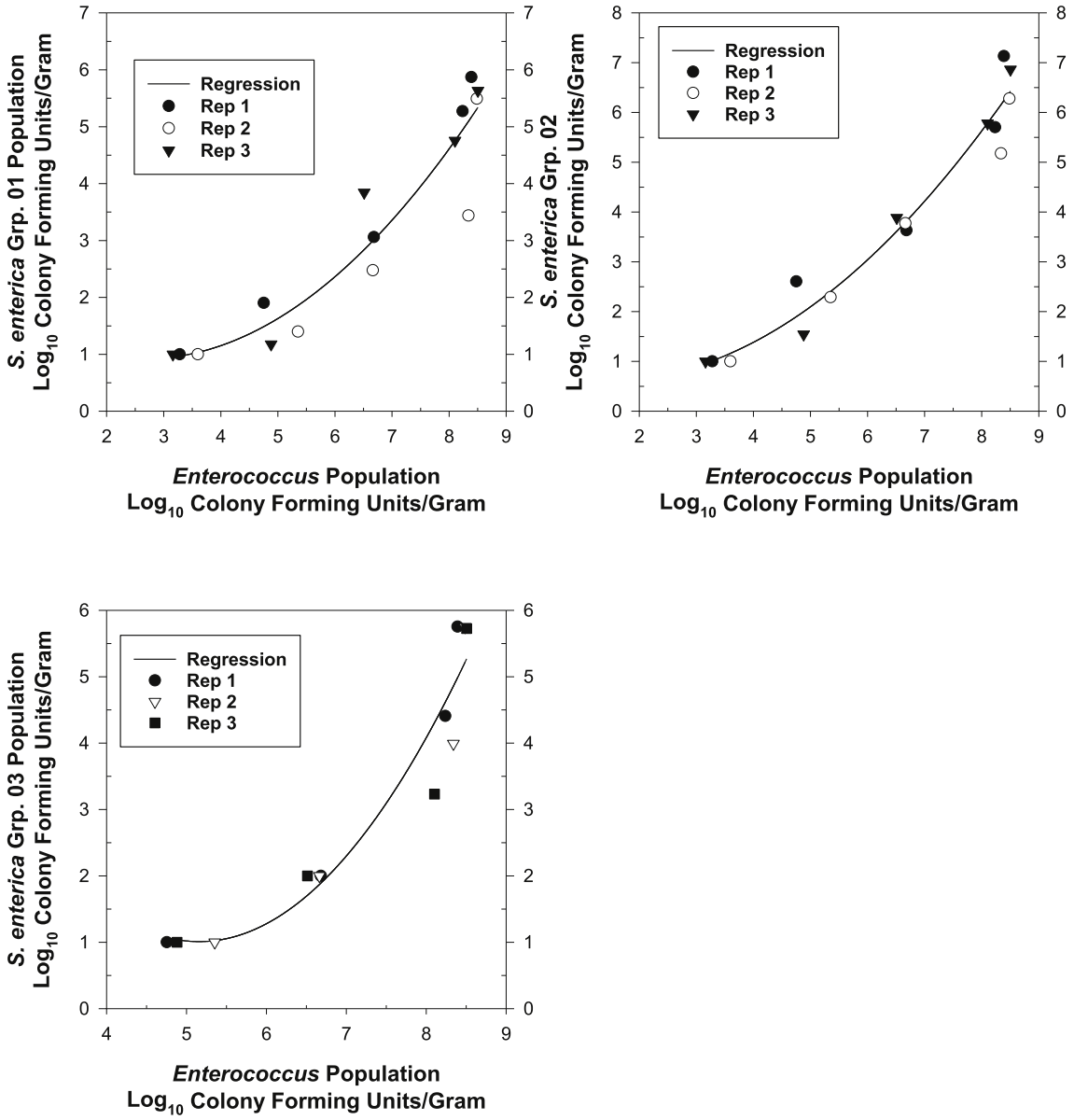

Fig. 1. Populations of *E. faecium* vs. *S. enterica* Groups in irradiated, inoculated talc powder with regression analysis.

Table 5
Comparison of Decimal Reduction Values between spices inoculated with *S. enterica* Grp 02 and *E. faecium* on talc, in kGy.

Spice	*S. enterica* Grp 02	*E. faecium*
Onion Powder	0.84 (0.07)[1,A]	1.10 (0.03)[B]
Dried Oregano	1.16 (0.30)[A]	1.10 (0.15)[A]
Whole Cumin Seeds	1.57 (0.54)[A]	1.62 (0.16)[A]
Peppercorns	1.67 (0.40)[A]	1.70 (0.08)[A]

1 Least squares mean (standard deviation) in kGy; Means within rows (spices) with different superscripts are significantly different ($P < 0.05$).

4. Conclusions

Based on the Decimal reduction values on both the inoculated talc and the talc-inoculated spices, *E. faecium* is a useful surrogate for *S. enterica* during the irradiation processing of spices. The mathematical models presented in this study allow for both the estimation of surviving populations of *S. enterica*, as well as an estimation of the $\log_{10}$ reductions. The use of *E. faecium* would allow processors to validate a specific process for the reduction of *S. enterica*. Although no surrogate or model can accurately predict every possible outcome, the procedures and equations presented in this study would provide an additional level of assurance that could not be achieved by finished product testing alone. The basic premise of using surrogates to validate food process interventions is valid, and the fundamental approach used in this study may well be applicable to other commodities and processes.

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Table 6
Estimated surviving populations of *S. enterica* Grp 02 and *E. faecium*, calculated in 1-kGy dose increments, based on the Decimal Reduction Values.

Dose (kGy)	Onion Powder	Dried Oregano	Whole Cumin Seeds	Peppercorns
0				
S. enterica	7.0	7.0	7.0	7.0
E. faecium	7.0	7.0	7.0	7.0
1				
S. enterica	5.8	6.1	6.4	6.4
E. faecium	6.1	6.1	6.4	6.4
2				
S. enterica	4.6	5.3	5.7	5.8
E. faecium	5.2	5.2	5.8	5.8
3				
S. enterica	3.4	4.4	5.1	5.2
E. faecium	4.3	4.3	5.1	5.2
4				
S. enterica	2.2	3.6	4.5	4.6
E. faecium	3.4	3.4	4.5	4.7
5				
S. enterica	1.0	2.7	3.8	4.0
E. faecium	2.5	2.5	3.9	4.1

Table 7
Estimated $\log_{10}$ reduction in the population of *S. enterica* Grp 02, based on the $\log_{10}$ reduction in the population of *E. faecium*, in $\log_{10}$ cfu/g., Surviving *S. enteric* population = A + B *(surviving *E. faecium* population).

Spice	A	B	R^2
Onion Powder	$-6.81*10^{-8}$	1.31	0.94
Dried Oregano	$-1.43*10^{-3}$	0.95	0.96
Whole Cumin Seed	$-1.94*10^{-3}$	1.03	0.97
Peppercorns	$-4.61*10^{-4}$	1.02	0.95
D_{10}-value of Inoculated Onion Powder (Table 2)	$2.39*10^{-3}$	1.68	0.98

Table 8
Estimated surviving population of *S. enterica* Grp 02, based on the surviving population of *E. faecium*, in $\log_{10}$ cfu/g, with spices mixed with inoculated talc. Surviving *S. enteric* population = A + B *(surviving *E. faecium* population).

Spice	A	B	R^2
Onion Powder	−2.19	1.31	0.96
Dried Oregano	0.362	0.948	0.98
Whole Cumin Seed	−0.196	1.028	0.98
Peppercorns	−0.138	1.019	0.97

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2018.12.018.

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