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FOOD MICROBIOLOGY

Exponentially Increased Thermal Resistance of *Salmonella* spp. and *Enterococcus faecium* at Reduced Water Activity

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ABSTRACT *Salmonella* spp. exhibit prolonged survivability and high tolerance to heat in low-moisture foods. The reported thermal resistance parameters of *Salmonella* spp. in low-moisture foods appear to be unpredictable due to various unknown factors. We report here that temperature-dependent water activity ($a_{w,\ treatment\ temperature}$) plays an important role in the sharply increased thermal resistance of *Salmonella enterica* serovar Enteritidis PT 30 and its potential surrogate *Enterococcus faecium* NRRL B-2354. In our study, silicon dioxide granules, as carriers, were separately inoculated with these two microorganisms and were heated at 80°C with controlled relative humidity between 18 and 72% (resulting in corresponding $a_{w,80°C}$ values for bacteria between 0.18 and 0.72) in custom-designed test cells. The inactivation kinetics of both microorganisms fitted a log-linear model (R^2, 0.83 to 0.97). Reductions in the $a_{w,80°C}$ values of bacterial cells exponentially increased the $D_{80°C}$ (the time needed to achieve a 1-log reduction in a bacterial population at 80°C) values for *S.* Enteritidis and *E. faecium* on silicon dioxide. The log-linear relationship between the $D_{80°C}$ values for each strain in silicon dioxide and its $a_{w,80°C}$ values was also verified for organic wheat flour. *E. faecium* showed consistently higher $D_{80°C}$ values than *S.* Enteritidis over the $a_{w,80°C}$ range tested. The estimated z_{aw} (the change in $a_{w,80°C}$ needed to change $D_{80°C}$ by 1 log) values of *S.* Enteritidis and *E. faecium* were 0.31 and 0.28, respectively. This study provides insight into the interpretation of *Salmonella* thermal resistance that could guide the development and validation of thermal processing of low-moisture foods.

IMPORTANCE In this paper, we established that the thermal resistance of the pathogen *S.* Enteritidis and its surrogate *Enterococcus faecium*, as reflected by *D* values at 80°C, increases sharply with decreasing relative humidity in the environment. The log-linear relationship between the $D_{80°C}$ values of each strain in silicon dioxide and its $a_{w,80°C}$ values was also verified for organic wheat flour. The results provide new quantitative insight into the way in which the thermal resistance of microorganisms changes in low-moisture systems, and they should aid in the development of effective thermal treatment strategies for pathogen control in low-moisture foods.

KEYWORDS *Salmonella*, *Enterococcus faecium*, water activity, silicon dioxide, thermal resistance, low-moisture food

Recent *Salmonella* outbreaks in low-moisture foods (1–4) pose a serious food safety threat, with substantial economic impacts and social consequences, because many of those products are commonly consumed as ready-to-eat foods (e.g., nuts, cereals, pet foods) or are used as ingredients in a wide range of products (e.g., flours, spices, herbs). The presence of *Salmonella* bacteria in low-moisture foods is therefore identified as a potential hazard that requires preventive controls (5). Thermal processing is traditionally considered an effective method for the control of pathogens in high-moisture food products. However, *Salmonella* exhibits prolonged survivability and enhanced thermal

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resistance in a desiccated environment (6–9). A growing number of studies have investigated the effects of internal factors (e.g., *Salmonella* gene expression under desiccation stress) (10, 11) and external factors (e.g., food components, air humidity, temperature) on *Salmonella* thermal resistance in low-moisture foods. Recent findings suggest that temperature-dependent water activity ($a_{w,\ \text{treatment temperature}}$), defined as the ratio of the water vapor pressure of a food system to that of pure water at the treatment temperature, plays a key role in altering bacterial thermal resistance (12, 13).

Unlike the widely used $a_{w,20-25°C}$ (measured by an a_w meter at room temperature prior to thermal treatment), as reported in the current literature, $a_{w,\ \text{treatment temperature}}$ is the real-time a_w during thermal processing. It is well known that a_w of a food matrix is greatly influenced by food composition (12) and temperature (14, 15). At high temperatures, food components either release or absorb water molecules, effects that can be measured by changes in the relative humidity of the headspace in the food system (16–18). A previous study (12) considered that $a_{w,\ \text{treatment temperature}}$ partially contributes to the vast difference between the $D_{80°C}$ (the time needed to achieve a 1-log reduction in the bacterial population at 80°C) values for *Salmonella enterica* serovar Enteritidis PT 30 in peanut butter (17 min) and in all-purpose wheat flour (6.9 min). Prior to this study, the influence of $a_{w,\ \text{treatment temperature}}$ on the thermal resistance of microorganisms had not been reported.

Enterococcus faecium NRRL B-2354 has been widely evaluated as a potential *Salmonella* surrogate in the thermal processing of low-moisture foods; it has consistently shown greater thermal resistance than *Salmonella* in different low-moisture foods (19–21). As such, it has been utilized to validate different thermal-processing technologies (e.g., air moisture, roasting, infrared heating) (19, 22, 23). However, studies comparing the thermal resistances of *Salmonella* and *E. faecium* were limited to several selected food systems. No previous studies that support the general utility of *E. faecium* as a *Salmonella* surrogate in thermal processes have been reported (25). It is, therefore, desirable to evaluate the thermal resistance of each of these species in a low-moisture environment independently of food components.

The a_w of a food product changes with temperature (12, 14, 24). The degree of change is influenced by different factors, including moisture content, food composition, and temperature (13, 14). In a closed system, food moisture content remains constant. The a_w of the food sample at a specific temperature (e.g., 80°C in this study) is influenced mainly by food composition (25). Due to the limited temperature range of commercial a_w meters (20 to 60°C), most published studies have failed to estimate the a_w of food matrices at temperatures above 60°C (13). Recently, a thermal cell installed with high-temperature relative humidity sensors was designed (12) and modified (25) to measure the a_w of foods above 60°C. The $a_{w,80°C}$ data of peanut butter, all-purpose flour, wheat flour, almond meal, and nonfat milk powder were reported (12, 25). The Clausius-Clapeyron equation (CCE) has been applied to enable prediction of the a_w at elevated temperatures for wheat flour, almond flour, and nonfat milk powder (25).

In the present study, we aimed to investigate the influence of $a_{w,80°C}$ on the thermal resistance parameter $D_{80°C}$ for *S.* Enteritidis and *E. faecium*. We chose *S.* Enteritidis as the target pathogen because it is primarily responsible for outbreaks in ready-to-eat nuts and nut spreads (19) and has been reported to exhibit high thermal resistance in low-moisture foods (20, 26, 27). We studied silicon dioxide (SiO_2) as a low-moisture carrier and used lithium chloride (LiCl; 0 to 18 mol/kg) to control relative humidity, keeping it between 18 and 72% (corresponding to an $a_{w,80°C}$ for bacterial cells between 0.18 and 0.72) in the headspace of a custom-designed thermal water activity cell (TAC) (28) during the isothermal inactivation tests. Finally, we compared the $D_{80°C}$ values of *S.* Enteritidis and *E. faecium* in SiO_2 (at a controlled $a_{w,80°C}$) with those in wheat flour (at low but uncontrolled $a_{w,80°C}$ levels). The specific objectives of this study were (i) to characterize SiO_2 as a carrier for *S.* Enteritidis and *E. faecium*, (ii) to evaluate the thermal inactivation of both strains at a constant $a_{w,80°C}$ in TACs, (iii) to model and compare the relationships between $\log D_{80°C}$ and $a_{w,80°C}$ values for both strains, and (iv) to verify the

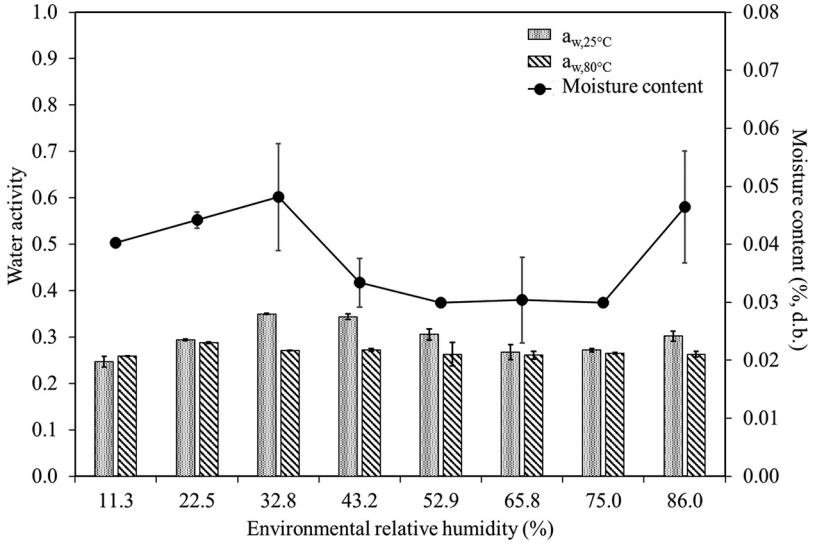

FIG 1 Water activity levels measured at 25°C (0.31 ± 0.05) and 80°C (0.27 ± 0.01) and corresponding water content (0.04% ± 0.01%, dry basis [d.b.]) of silicon dioxide preconditioned ($n = 2$) at different relative humidity levels (generated by saturated salt solutions). Results of two replicated experiments ($n = 2$) are shown.

relationships by examining the reported $D_{80°C}$ values of these strains in wheat flour and the estimated $a_{w,80°C}$ of wheat flour.

RESULTS

The water activities at 25 and 80°C of SiO_2 that was preconditioned over a wide range of relative humidity at 25°C ranged from 0.25 to 0.31 (Fig. 1). The moisture content stayed consistently low (0.03 to 0.06%, dry basis). The variation in the relative humidity level at which SiO_2 was preconditioned did not result in changes in the moisture content or the a_w of the SiO_2 samples.

The desiccation of the culture on SiO_2 caused a ~2-log CFU/g reduction in the bacterial cells. The initial inoculation level of SiO_2 for both strains was 7.8 ± 0.2 log CFU/g. Scanning electron microscopy (SEM) images of *S.* Enteritidis and *E. faecium* inoculated onto SiO_2 are shown in Fig. 2. Bacterial cells spread on SiO_2 particles that had flat surfaces. *S.* Enteritidis cells were elongated and were closely attached to SiO_2 granules, while *E. faecium* cells were clustered and maintained their coccus shape. Both strains were fully exposed to the surrounding air.

Temperature changes in the geometric center of SiO_2 in an oil bath at 80°C are shown in Fig. 3. The 1.0-g SiO_2 samples were heated to 79.5°C within 95 s and were cooled from 80°C to below 40°C within 15 s in an ice water bath. The rapid heating and

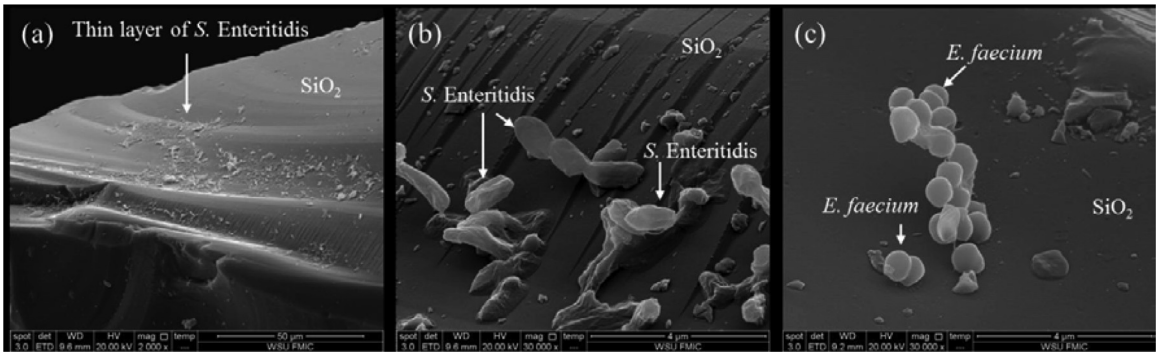

FIG 2 (a) SiO_2 serves as a vehicle for carrying *S.* Enteritidis cells on its surface. (b) *S.* Enteritidis cells shrank significantly into a flat form with many wrinkles. (c) *E. faecium* cells maintained their shapes as cocci.

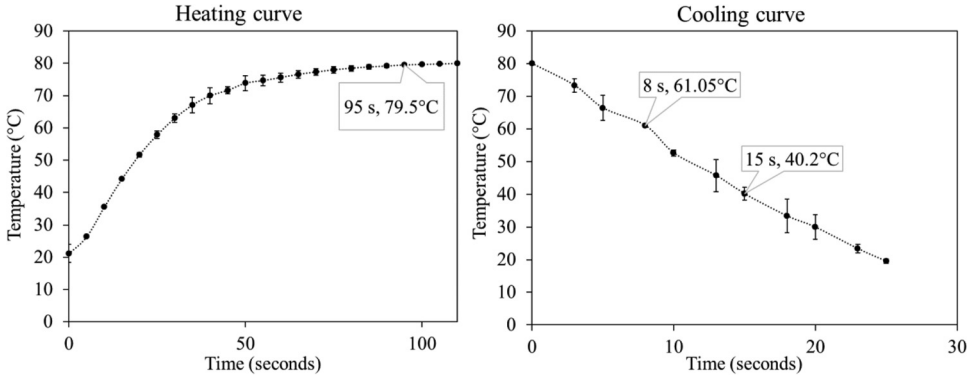

FIG 3 Heating and cooling curves of the centers of silicon dioxide samples in thermal-water activity cells. Results of two experiments ($n = 2$) are shown.

cooling (2.67°C/s) of SiO_2 samples in TACs indicate efficient heat transfer in the isothermal treatments.

The survival curves of both bacteria fitted the log-linear model well (R^2, 0.82 to 0.97 [Table 1 and Fig. 4]). Over a wide range of $a_{w,80°C}$ values, $D_{80°C}$ values in this study differed >80-fold, from 2.14 ± 0.16 min (for *S.* Enteritidis at an $a_{w,80°C}$ of 0.72) to 170.98 ± 18.54 min (for *S.* Enteritidis at an $a_{w,80°C}$ of 0.18). The log$D_{80°C}$ values of both strains as a function of $a_{w,80°C}$, from 0.18 to 0.72, fitted a linear trend (R^2, 0.97 to 0.99) (Fig. 5). Based on equation 5, the z_{aw} (the change in $a_{w,80°C}$ needed to change $D_{80°C}$ by 1 log) values of *S.* Enteritidis and *E. faecium* were 0.31 and 0.28, respectively.

The estimated $a_{w,80°C}$ values of wheat flour ranged from 0.52 to 0.85, consistently higher than the corresponding $a_{w,25°C}$ (Table 2). The log$D_{80°C}$ values for *S.* Enteritidis and *E. faecium* in wheat flour as a function of $a_{w,25°C}$ (open symbols) and $a_{w,80°C}$ (filled symbols) are shown in Fig. 6. The filled symbols overlap with linear trend lines in Fig. 6, which were previously obtained from linear least regressions of the same strains in SiO_2 (equation 5).

DISCUSSION

Characterization of SiO_2 (uninoculated and inoculated) (Fig. 1). The relatively flat curves of a_w over a wide range of environmental relative humidities indicate that SiO_2 neither absorbs nor releases moisture as the environmental humidity changes. SiO_2 has been utilized by other researchers as a carrier coated with chemicals (29) or as a foundation for biosensors used in bacterial detection (30). In addition, SiO_2 is

TABLE 1 Parameter estimates (± standard errors) for the primary linear model, root mean squared error, and R^2

Strain	$a_{w,25°C}$ ± 0.02	$a_{w,80°C}$ (predicted)	$D_{80°C}$ (min)	RMSE[a] (log CFU)	R^2
S. Enteritidis	0.11	0.18	159.31 ± 5.77	0.26	0.93
	0.20	0.27	64.04 ± 0.16	0.58	0.88
	0.31	0.37	30.69 ± 0.97	0.27	0.96
	0.42	0.47	21.33 ± 1.44	0.48	0.91
	0.50	0.54	10.37 ± 0.30	0.29	0.97
	0.61	0.64	6.80 ± 0.25	0.42	0.89
	0.70	0.72	1.80 ± 0.12	0.47	0.83
E. faecium	0.11	0.18	281.78 ± 5.78	0.32	0.97
	0.20	0.27	139.16 ± 5.07	0.57	0.90
	0.31	0.37	85.09 ± 4.75	0.49	0.82
	0.42	0.47	46.82 ± 2.36	0.21	0.97
	0.50	0.54	12.71 ± 0.32	0.53	0.88
	0.61	0.64	6.97 ± 0.06	0.29	0.95
	0.70	0.72	3.81 ± 0.11	0.41	0.89

[a]RMSE, root mean squared error.

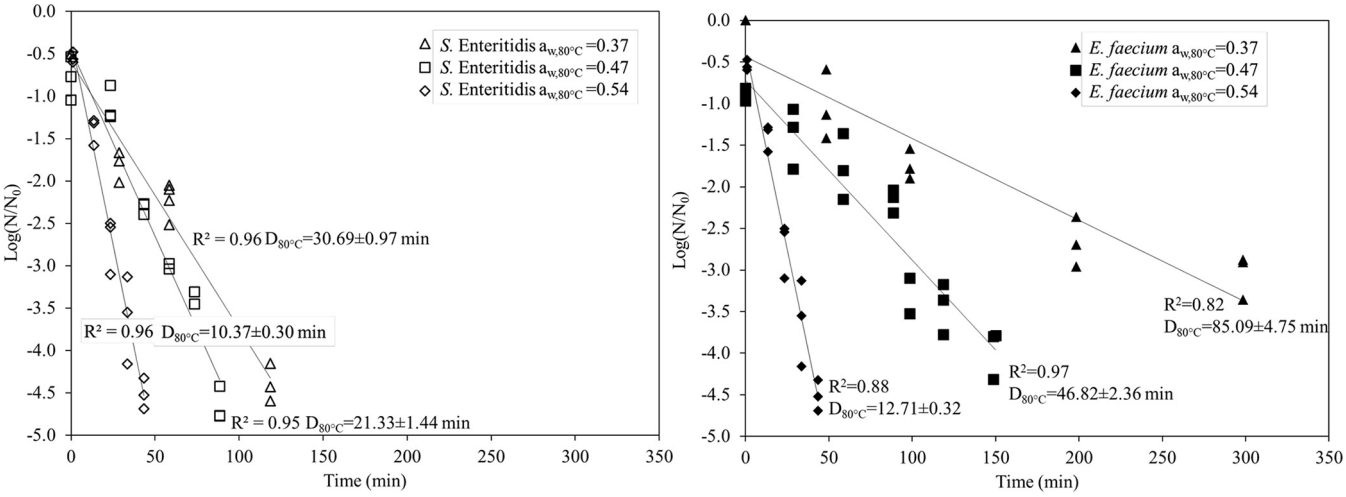

FIG 4 Representative survival curves of *Salmonella* Enteritidis and *E. faecium* in SiO_2 at 80°C with a water activity of 0.37, 0.47, or 0.54 predicted at 80°C and their trend lines, R^2, and estimated $D_{80°C}$ values. Experiments were repeated three times independently.

autoclavable and has not been reported to have any antibacterial effects. Thus, SiO_2 is a good carrier material for bacterial inactivation studies in low-moisture foods.

The thin layers of bacterial cells on SiO_2 surfaces in the SEM images support our assumption that bacterial cells in SiO_2 are directly exposed to the ambient environment. Syamaladevi et al. (31) estimated that it takes no more than a second for a bacterial cell to adjust its water content in response to humidity changes in the environment. Thus, bacterial cells were expected to be inactivated under isothermal and iso-$a_{w,80°C}$ conditions in TACs.

With low moisture contents (0.03 to 0.06%, dry basis), SiO_2 allows bacterial cells to be dehydrated naturally. The rate of dehydration depends on environmental relative humidity. *S.* Enteritidis and *E. faecium* survivors in SiO_2 are assumed to represent typical *Salmonella* and *E. faecium* cells in low-moisture environments.

Interpretation of TAC data. The purpose of using TACs was to expose the inoculated bacterial cells to controlled relative humidities at 80°C. When opening the TACs

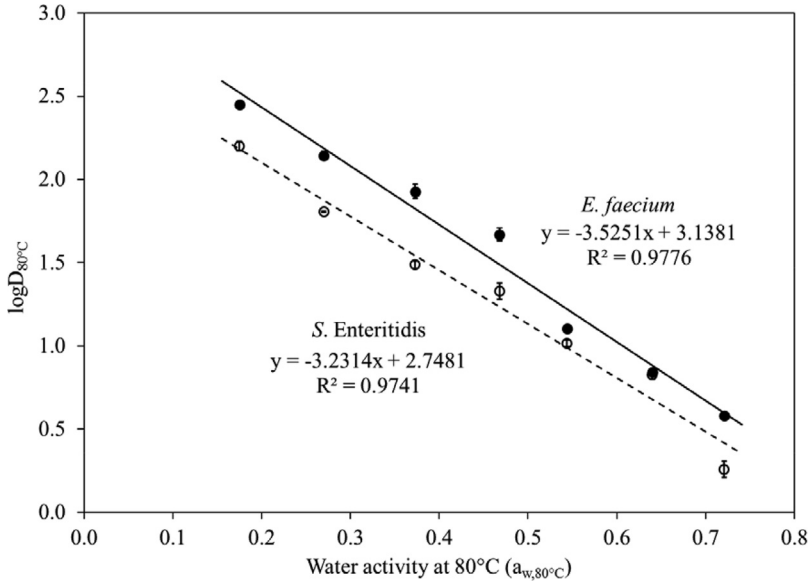

FIG 5 Log$D_{80°C}$ (decimal reduction time to achieve 90% population reduction at 80°C) values for *S.* Enteritidis and *E. faecium* in SiO_2 increased with decreasing water activity levels at 80°C (R^2 = 0.98).

TABLE 2 $D_{80°C}$ of *S.* Enteritidis and *E. faecium* in wheat flour reported for different $a_{w,25°C}$ values in the literature and the corresponding $a_{w,80°C}$ estimated in this study

Strain	$a_{w,25°C} \pm 0.02$	$a_{w,80°C} \pm 0.02$[a]	$\Delta a_w \pm 0.02$	$D_{80°C}$ (min)	Reference for $D_{80°C}$
S. Enteritidis	0.31	0.52	0.21	10.27 ± 0.65	26
	0.43	0.68	0.25	5.51 ± 0.22	
	0.58	0.81	0.23	1.27 ± 0.06	
	0.70	0.85	0.15	1.17 ± 0.06	
E. faecium	0.30	0.52	0.22	20.4 ± 0.85	45
	0.45	0.68	0.23	5.56 ± 0.49	
	0.60	0.81	0.21	2.27 ± 0.25	

[a]Predicted $a_{w,80°C}$ values of wheat flour were determined by the Clausius-Clapeyron equation (25).

at room temperature, we observed slight water condensation in some of the TACs after thermal treatments at an $a_{w,80°C}$ of ≥0.50. We were concerned that if bacterial cells absorbed water droplets at a lethal temperature (e.g., >60°C), they might be inactivated faster than if they were in low-moisture environments (13). However, based on the temperature profiles shown in Fig. 3, only 15 s was required for the SiO_2 samples in a TAC to cool from 80°C to 40°C. We anticipate that in such a short time, the water condensation in TACs would not alter inactivation results.

The $a_{w,80°C}$ values used in this study were estimated from water vapor pressure generated from LiCl solutions of different molalities. Water vapor pressure can also be measured as relative humidity (expressed as a percentage) and converted into a_w (a_w = relative humidity/100). In the present study, $a_{w,80°C}$ refers to the equilibrium a_w of the bacterial cells when exposed to a specific relative humidity at 80°C. Our recent study (31) suggests that, through moisture diffusion, the extremely small size of bacterial cells allows them to adjust the intracellular moisture content according to changes in environmental relative humidity within seconds. The $a_{w,80°C}$ was used for interpretation and data analysis because $a_{w,80°C}$ can be easily related to $a_{w,25°C}$, which has been used to define low-moisture foods (32) and has been widely studied for its impact on thermal resistance of microorganisms in low-moisture environments. On the other hand, relative humidity (calculated as 100 × a_w) can be used as an independent parameter for process control in an open system, such as baking, roasting, or hot-air drying. The $D_{80°C}$ values in Fig. 5 and 6 can be interpreted as functions of relative humidity at high temperatures and used to develop process conditions for effective control of pathogens. On the basis of these figures, it is clear that high humidity in an open thermal treatment could facilitate pathogen control in low-moisture foods.

Thermal resistance of *S.* Enteritidis and *E. faecium*. *E. faecium* had consistently higher $D_{80°C}$ values than *S.* Enteritidis under the same test conditions. This observation matches data reported for *E. faecium* and *Salmonella* in almond kernels (20), balanced carbohydrate-protein meals (33), and wheat flour (34). The z_{aw} (as a function of $a_{w,80°C}$) of *E. faecium*, 0.28, is statistically significantly different from that of *S.* Enteritidis, 0.31 ($P < 0.05$). This observed difference lies within the accuracy range of our a_w meters (±0.02). The results presented above suggest that *E. faecium* is an appropriate surrogate for *Salmonella* in thermal processes at 80°C.

Thermal resistance of microorganisms as influenced by $a_{w,80°C}$. For both *S.* Enteritidis and *E. faecium*, a reduced $a_{w,80°C}$ increased $D_{80°C}$ exponentially ($R^2 = 0.98$).

Thermal inactivation of bacterial cells might be due to the loss of functions of subsets of heat-sensitive proteins (37), which may be denatured at elevated temperatures. Water loss from bacterial cells at low a_w results in more-stable protein structures and hinders thermal denaturation. This has been considered a major reason for the high thermal resistance of bacterial spores. For example, Sunde et al. (38) reported a low water rotational correlation time (5×10^{-11} s) in the dense core of *Bacillus subtilis* and concluded that most proteins in *B. subtilis* spores are rotationally immobilized. The similar mechanism might explain the increased thermal resistance of *S.* Enteritidis and *E. faecium* at reduced a_w.

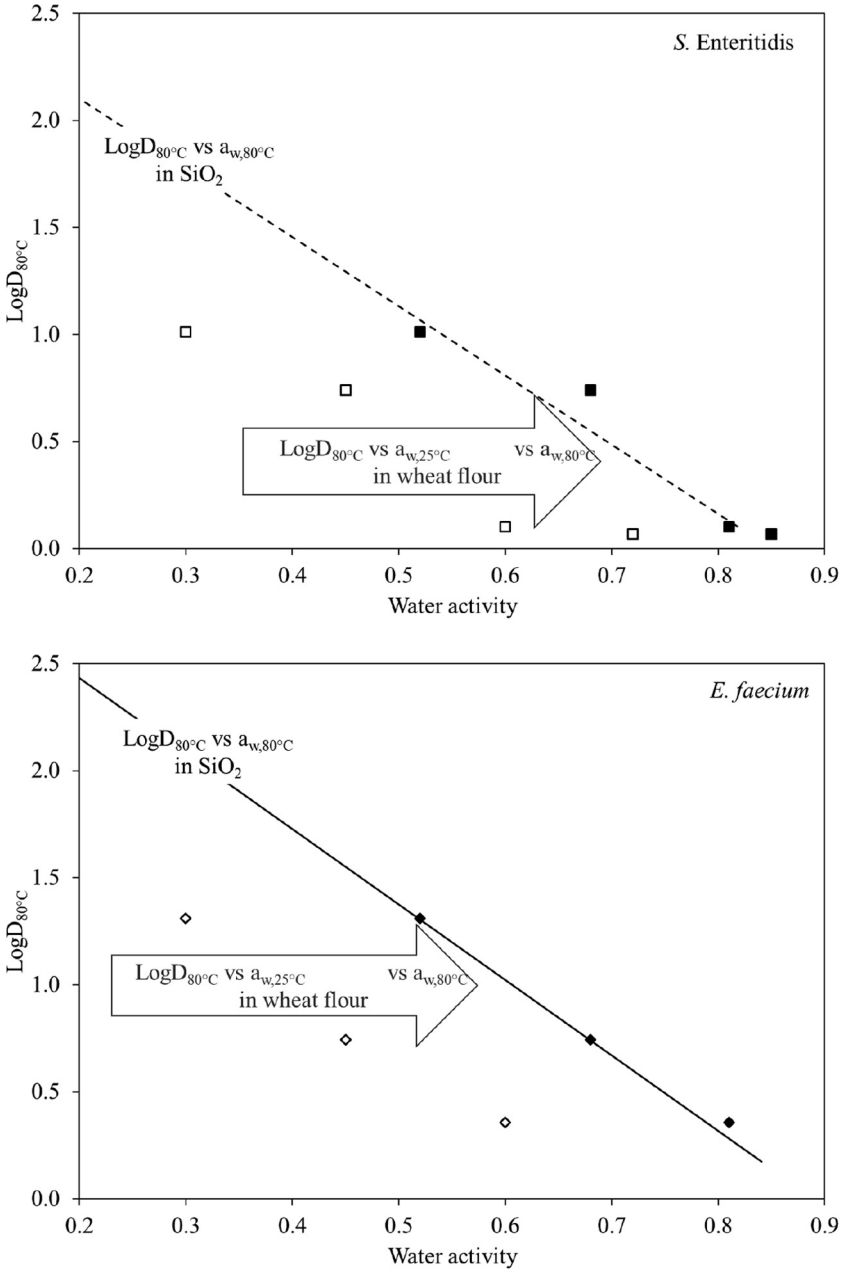

FIG 6 Log$D_{80°C}$ values for *S.* Enteritidis (top) and *E. faecium* (bottom) plotted against a_w at 25°C (open symbols) or a_w at 80°C (filled symbols) in wheat flour. In both panels, the lines show the exponential trends for *S.* Enteritidis or *E. faecium* in SiO_2 (Fig. 5).

Although *S.* Enteritidis and *E. faecium* were heat treated on SiO_2 granules in this study, the results may provide important insight into how bacteria in different food matrices would respond to heat treatments.

Verifying the log-linear model of $D_{80°C}$ and $a_{w,80°C}$. Wheat flour was chosen as the food system for verification of the log-linear relationship shown in Fig. 5. At fixed moisture contents, the $a_{w,80°C}$ values of wheat flour samples were higher than their $a_{w,25°C}$ values (Table 2). At a high moisture content (corresponding to an $a_{w,25°C}$ of 0.7), the Δa_w of wheat flour was smaller (0.15) than that at lower $a_{w,25°C}$ levels (Δa_w, 0.21 to 0.25). Thus, the log$D_{80°C}$ values of *S.* Enteritidis and *E. faecium* in wheat flour shifted to the right and matched the trend lines describing the log$D_{80°C}$ of the same strains in SiO_2 as a function of $a_{w,80°C}$ (Fig. 6). The linear relationships between the

log$D_{80°C}$ values of *S.* Enteritidis and *E. faecium* and the $a_{w,80°C}$ value of wheat flour had R^2 values of 0.95 and 0.99, respectively. This trend confirmed our observation in Fig. 5 that $a_{w,\ treatment\ temperature}$ influenced the thermal resistance of microorganisms in low-moisture foods in a log-linear fashion.

Wheat flour and SiO_2 represent different low-moisture matrices. Yet, at the same $a_{w,80°C}$, the log$D_{80°C}$ values with the two substrates overlapped both strains. Therefore, the log$D_{80°C}$ of microorganisms appears to be closely related to the $a_{w,80°C}$ of bacterial cells independent of the low-moisture systems.

Relevance of this study to food safety in low-moisture foods. (i) *E. faecium* as a *Salmonella* surrogate in thermal processes for low-moisture foods. To date, data in support of the use of *E. faecium* as a *Salmonella* surrogate in validating the thermal processing of various low-moisture foods (20, 33, 39) are insufficient. The protocols and guidelines established for the use of *E. faecium* are limited to a small number of products and specific process conditions (19). In the present study, side-by-side comparison between *E. faecium* and *S.* Enteritidis was conducted independently of food matrices (in SiO_2). The results proved that *E. faecium* is more heat resistant (higher $D_{80°C}$) than *S.* Enteritidis at any given $a_{w,80°C}$ between 0.18 and 0.72. Our study indicates that *E. faecium* can be a valid *Salmonella* surrogate in the thermal processing of low-moisture foods over a wide range of water activities.

(ii) Calibration of the surrogate *E. faecium* prior to industrial application. Our study aims to contribute to establishing standard protocols for surrogate validation and calibration and for further mathematic modeling of the thermal resistance of microorganisms in low-moisture foods during heat treatments. In practice, depending on experimental procedures and methods of culturing and collecting bacterial cells, not every batch of *E. faecium* would perform as strongly as expected. It is critical to determine the heat resistance of a surrogate prior to any thermal process validations. The guideline published by the Almond Board of California provides a "heat resistance test" procedure to characterize the resistance of *E. faecium* in almonds (19). However, the equipment (metal mesh and convection/forced-air oven) is not necessarily applicable to other food matrices, such as powdered foods. This guideline does not specify the need for monitoring the relative humidity or temperature of the food matrix, which are proven to be key factors (12). The lack of these parameters in a heat resistance test would lead to significant variance depending on the real-time relative humidity of the air. Moreover, the acceptable heat resistance range ($\leq$2.5 log reduction at 280°F for 15 min) may not apply to other low-moisture foods.

This study provides another approach for calibrating a surrogate in low-moisture foods with less uncertainty by fixing the temperature (80°C) and $a_{w,80°C}$ using TACs. For any batch of *E. faecium*, the $D_{80°C}$ values provided as a function of $a_{w,80°C}$ in Table 1 could be used as calibration references. For instance, the $D_{80°C}$ value for *E. faecium* at an $a_{w,80°C}$ value of 0.72 is 3.81 $\pm$ 0.11 min. The acceptable *Salmonella* surrogate should undergo a $\leq$2.5-log reduction at 80°C, with an $a_{w,80°C}$ of 0.72, in 9.53 min (3.81 $\times$ 2.5 = 9.53).

(iii) Mathematic modeling of thermal resistance parameters of *Salmonella* spp. and *E. faecium* in low-moisture foods under various conditions. A modified Bigelow-type model was suggested to predict *D* values by the following equation 1 (40):

$$D\left(T, a_w\right) = D_{ref} \cdot 10^{\frac{a_{w,ref} - a_w}{z_{aw}}} \cdot 10^{\frac{T_{ref} - T}{z_T}} \quad (1)$$

where D_{ref} is the *D* value (in minutes) at T_{ref} and $a_{w,ref}$; T_{ref} and $a_{w,ref}$ are a reference temperature and a_w, respectively; and z_T is the temperature needed to change the *D* value 10-fold (in degrees Celsius). Based on the present study, it is appropriate to use $a_{w,\ treatment\ temperature}$ instead of a_w at room temperature in the model presented above. The value of the $a_{w,\ treatment\ temperature}$ of a food product can be provided by mathematic estimation in a closed system (12, 25) or by the relative humidity of the environment in an open system.

Our data support increasing $a_{w,\ treatment\ temperature}$ in the thermal processing of low-moisture foods to achieve easier kill of pathogens. However, these $D_{80°C}$ values

may not apply to open systems with changing water vapor pressures surrounding bacterial cells.

MATERIALS AND METHODS

S. Enteritidis and *E. faecium* were acquired from Linda Harris at the University of California, Davis. Both strains were kept at −80°C in tryptic soy broth (TSB) supplemented with 20% (vol/vol) glycerol. Tryptic soy agar (TSA), yeast extract, and peptone water were purchased from Becton, Dickinson and Company (Franklin Lakes, NJ); ammonium iron(III) citrate was purchased from Sigma-Aldrich Corporation (St. Louis, MO); and sodium thiosulfate 5-hydrate was made by J. T. Baker (Avantor Performance Materials, Center Valley, PA). Silicon dioxide (SiO_2; 0.2- to 0.7-mm granules) was purchased from Umicore, Brussels, Belgium. LiCl was purchased from J. T. Baker (Avantor Performance Materials, Center Valley, PA). An AquaLab vapor sorption analyzer (VSA) was purchased from METER Group Inc. (formerly Decagon Devices, Inc., Pullman, WA). A vacuum oven (Yamato Scientific America Inc., CA) was used to dry SiO_2 to an a_w of <0.1. An oil bath (GP-400; Neslab, Newington, NH) filled with ethylene glycol (VWR International, Radnor, PA) was used for isothermal treatments. We used custom-designed aluminum thermal water activity cells (TACs) (28) to assess the thermal inactivation studies (see Fig. S1 in the supplemental material).

Characterization of SiO_2. Water equilibrium isotherms of SiO_2 particles were generated in order to understand how SiO_2 exchanges moisture with the environment. In the generation of adsorption isotherms of SiO_2 at 80°C, SiO_2 samples were first vacuum-dried overnight at an absolute pressure of 10 kPa inside the vacuum oven at 50°C and then preconditioned in air-tight containers containing different saturated salt solutions at 23°C for 2 weeks. The saturated salt solutions (with the corresponding relative humidity at saturated status in parentheses) were LiCl (11.3%), CH_3COOK (22.5%), $MgCl_2$ (32.8%), K_2CO_3 (43.2%), $MgNO_3$ (52.9%), $NaNO_2$ (65.8%), NaCl (75.3%), and KCl (84.3%) at 23°C (Fisher Scientific, Houston, TX) (41). After equilibration, an aluminum cell (inner diameter, 42 mm; height, 19 mm) with a relative humidity sensor (HX15-W; Omega Engineering, Inc., Stamford, CT) was used to measure the $a_{w,80°C}$ (12). The equilibrated SiO_2 (2 to 3 g) was loaded and sealed in the aluminum cell mentioned above and was transferred to a forced-air convection oven at 80°C; $a_{w,80°C}$ was calculated and was displayed for recording. Then the sealed cell was removed from the oven and was kept at room temperature for ~30 min to reach the ambient temperature (~23°C). The moisture contents of the equilibrated samples were obtained by heating a small amount (3 to 5 g) at 80°C for 10 h in the vacuum oven under an absolute pressure of 10 kPa.

To generate desorption isotherms at 80°C, SiO_2 was mixed with distilled water to achieve an $a_{w,25°C}$ of ~1.0. The samples were then equilibrated in tightly closed jars containing saturated salt solutions. The $a_{w,80°C}$ of all preconditioned samples was measured in aluminum cells with relative humidity sensors as described above.

Preparation of inoculated SiO_2. A bacterial inoculum of *S.* Enteritidis or *E. faecium* was prepared according to the procedures described by Hildebrandt et al. (42). Briefly, frozen *S.* Enteritidis or *E. faecium* was subjected to two consecutive transfers (24 h each at 37°C) in 9 ml of tryptic soy broth supplemented with 0.6% (wt/vol) yeast extract (TSBYE), and then 1 ml was evenly spread onto a plate (150 by 15 mm) of tryptic soy agar supplemented with 0.6% (wt/vol) yeast extract (TSAYE). The bacterial lawn was harvested into 5 ml of sterile 0.1% peptone water.

One milliliter of each inoculum was mixed with 100 g SiO_2 (previously autoclaved to remove background bacteria) in a sterile stomacher bag until the pellet was visibly mixed. After mixing, the inoculated SiO_2 was placed in 150-mm-diameter petri dishes (without lids) and was dried rapidly under a biosafety hood for 12 h at the ambient temperature (~23°C) with the fan running. Ten 1-g samples were randomly selected and were analyzed to confirm the uniformity of inoculum distribution. All steps were repeated three times as independent biological replicates of both strains.

SEM imaging of microorganisms inoculated in SiO_2. A small amount of SiO_2 particles inoculated with either *S.* Enteritidis or *E. faecium* was fixed in 2% (vol/vol) paraformaldehyde–2% (vol/vol) glutaraldehyde–0.1 M phosphate buffer overnight at 4°C and was then rinsed in several changes of deionized water. The samples were rapidly frozen (~2 min) in liquid nitrogen and were then freeze-dried at −45°C overnight (Flexi-Dry freeze dryer; SP Scientific, Gardiner, NY). Freeze-dried SiO_2 granules were thinly spread onto double-coated carbon conductive tabs (Ted Pella Inc., Redding, CA) and were gold coated in a vacuum evaporator (Technics Hummer V sputter coater; Technic, San Jose, CA) to a thickness of 6 nm. An environmental field emission gun scanning electron microscope (SEM) (Quanta 200F; FEI Company, Hillsboro, OR) was used to examine samples, and the images were captured by a digital camera (Quartz Imaging Corporation, Vancouver, British Columbia, Canada).

Control of relative humidity ($a_{w,80°C}$ of bacterial cells) in TACs. An LiCl solution was selected to control the relative humidity of the headspace in TACs because LiCl generates specific water vapor pressure depending on its concentration and temperature. The molalities of LiCl solutions and the corresponding water vapor pressures at temperatures between 20 and 100°C have been reported by Gibbard and Scatchard (43). A user-friendly chart relating relative humidity and LiCl solutions in an article by Tadapaneni et al. (28) was used to select the appropriate LiCl molality for the desired relative humidity at 25 and 80°C in TACs. In our study, we used 6.1- to 18-mol/kg LiCl solutions to provide relative humidities of 18 to 72% at 10% intervals. Since the relative humidity (expressed as a percentage) in the headspace of the TAC is directly related to the $a_{w,80°C}$ (calculated as relative humidity/100) of the LiCl solution, we use only $a_{w,80°C}$ values in most of the discussion.

The estimated $a_{w,80°C}$ values during the isothermal inactivation of microorganisms on SiO_2 were 0.18, 0.27, 0.37, 0.47, 0.55, 0.63, and 0.72 (see Table S1 in the supplemental material). The $a_{w,80°C}$ levels of 0.8 and 0.9 were not tested in this study because both microorganisms were inactivated by more than 3 log

units after the come-up time (CUT) at these two a_w levels, and the $D_{80°C}$ values of both strains were too low to be determined.

Inactivation of microorganisms in TACs. With prepared LiCl salt solutions, three portions of 1 g inoculated SiO_2 were placed in thin aluminum cups (Fig. S1b in the supplemental material). The cups (~0.2 g; thickness, 0.02 mm) were handmade in the shape of TAC sample wells from heavy-duty aluminum foil (Western Family Foods, Tigard, OR). Three cups were placed in one TAC base with 3 ml of the respective LiCl solution in the central liquid-holding well. The TACs were then sealed and were stabilized at room temperature (~23°C) for >2 h to let the water vapor pressure from the LiCl solution reach an equilibrium inside the TACs. Isothermal inactivation tests were performed at 80°C for seven $a_{w,80°C}$ levels (0.18 to 0.72, with intervals of 0.1).

To obtain thermal death curves for *S.* Enteritidis and *E. faecium*, TACs containing inoculated samples at selected $a_{w,80°C}$ values (controlled by the LiCl solution in the central well) were subjected to isothermal treatments at 80°C. Only two TACs at one time were placed in the oil bath containing the ethylene glycol heating medium so as to avoid an excessive temperature drop. The CUT for the geometric center of the sample to reach 79.5°C was 1.58 min. Thermal treatments at uniform time intervals were performed, starting with time zero (at the CUT). Once removed from the oil bath, TACs were immediately placed in an ice water bath to stop thermal inactivation. Sample cups filled with 1 g treated SiO_2 were transferred gently from the TACs for the recovery and enumeration of survivors (Fig. S1c in the supplemental material).

Thermally treated SiO_2 samples were transferred to sterile stomacher bags, diluted 1:9 with 0.1% peptone water, and homogenized for 3 min at 260 rpm in a Seward stomacher (Seward, London, UK) (44). Appropriate serial dilutions were plated in duplicate onto modified TSAYE with 0.03% sodium thiosulfate and 0.05% ammonium ferric citrate (for *S.* Enteritidis) or TSA (for *E. faecium*). Colonies were enumerated after incubation at 37°C for 48 h. All of the tests were conducted one time per independent biological replicate.

Model verification using reported and estimated data. To verify the relationships between the $\log D_{80°C}$ and $a_{w,80°C}$ values of *S.* Enteritidis and *E. faecium* in SiO_2, data reported on thermal resistance parameters and water sorption isotherms in wheat flour (Eden Foods, Clinton, MI) were analyzed. The $D_{80°C}$ values of *S.* Enteritidis and *E. faecium* in wheat flour were reported by Smith et al. (26) and Liu et al. (45). In those studies, though, sample water activities at 25°C were reported.

The $a_{w,80°C}$ values of the wheat flour samples were estimated from the reported $a_{w,25°C}$ values by CCEs according to reference 25, as follows:

$$a_{w2} = a_{w1} \exp\left[\left(\frac{q_{st}}{R}\right)\left(\frac{1}{T_1} - \frac{1}{T_2}\right)\right] \quad (2)$$

where a_{w1} and a_{w2} are the a_w values of a sample with a fixed moisture content at temperatures T_1 and T_2, respectively; q_{st} is the net isosteric heat of sorption (in joules per mole) that describes the difference between the total enthalpy change for the sorption process and the latent heat of water vaporization; and R is the universal gas constant (8.314 J mol^{-1} K^{-1}).

For wheat flour, q_{st} was calculated (25). The q_{st} is dependent on the moisture content of the food matrix and the relationship as shown in equation 3:

$$q_{st} = 87.8 \exp(-0.26 \cdot \text{moisture content}) \quad (3)$$

The differences between the estimated $a_{w,80°C}$ and corresponding $a_{w,25°C}$ values were obtained as Δa_w values.

Data analysis. The first-order kinetic, or log-linear, model has been primarily used to characterize bacterial inactivation (46) as follows:

$$\log(N/N_0) = -t/D \quad (4)$$

where log reduction, $\log(N/N_0)$, was calculated by dividing survivor counts (N) by the population at time zero (N_0) for the respective replicate; t is the time of the isothermal treatment (in minutes) after the CUT, and D is the time (in minutes) required to reduce the microbial population by 90% at a specified temperature (in degrees Celsius).

The relationships between the $\log D_{80°C}$ values of *S.* Enteritidis and *E. faecium* and the $a_{w,80°C}$ values in this study were modeled according to reference 47 as follows:

$$\log D_{80°C} = -\frac{1}{z_{aw}} \cdot a_{w,80°C} + A \quad (5)$$

where A is constant and z_{aw} describes the water activity change necessary to alter the thermal death time by 1 log-cycle.

All water activities were estimated at 80°C (Table S1 in the supplemental material). The graphs were drawn with Excel or PowerPoint 2016, and tables were made with Excel 2016. All differences were considered significant if the probability was <0.05. Analysis of variance (ANOVA) and *t* tests for the experimental data were performed using Minitab 14.1.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.02742-17.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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