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Research Paper

Thermal Inactivation of *Salmonella* Agona in Low–Water Activity Foods: Predictive Models for the Combined Effect of Temperature, Water Activity, and Food Component

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ABSTRACT

Salmonella can survive in low-moisture, high-protein, and high-fat foods for several years. Despite nationwide outbreaks and recalls due to the presence of *Salmonella* in low-moisture foods, information on thermal inactivation of *Salmonella* in these products is limited. This project evaluated the impact of water activity (a_w), temperature, and food composition on thermal inactivation of *Salmonella enterica* serovar Agona in defined high-protein and high-fat model food matrices. Each matrix was inoculated with *Salmonella* Agona and adjusted to obtain a target a_w, ranging from 0.50 to 0.98. Samples were packed into aluminum test cells and heated (52 to 90°C) under isothermal conditions. Survival of *Salmonella* Agona was detected on tryptic soy agar with 0.6% yeast extract. Complex influences by food composition, a_w, and temperature resulted in significantly different ($P < 0.05$) thermal resistance of *Salmonella* for the conditions tested. It was estimated that the same point temperatures at which the *D*-values of the two matrices at each a_w (0.63, 0.73, 0.81, and 0.90) were identical were 79.48, 71.28, 69.62, and 38.42°C, respectively. Above these temperatures, the *D*-values in high-protein matrices were larger than the *D*-values in high-fat matrices at each a_w. Below these temperatures, the inverse relationship was observed. A correlation between temperature and a_w existed on the basis of the level of fat or protein in the food, showing that these compositional factors must be accounted for when predicating thermal inactivation of *Salmonella* in foods.

Key words: *D*-value; Low water activity; Pet food; *Salmonella*; Thermal inactivation

Salmonella enterica serovars are estimated to cause 1.2 million illnesses and 450 deaths per year in the United States *(8)*. Several recent recalls and outbreaks involved *Salmonella* Agona, the organism investigated in this study *(6, 7, 17, 24),* which has relatively high thermal resistance *(37, 39)*. In recent years, there have been multiple outbreaks and recalls in low–water activity (a_w) products, such as cereal, chocolate, dog food, flour, nuts, nut butters, and spices *(6, 7, 17)*. Foods are considered to be low a_w if below the a_w of 0.83. Low-a_w foods do not typically support the growth of microorganisms. However, it is well documented that *Salmonella* can survive for an extended length of time in a variety of low-a_w foods and cause illness if a low number of cells are present *(15, 22, 23, 25, 26, 33, 37)*. Also, *Salmonella* has an increased thermal resistance as a_w decreases, which poses a significant challenge to processors of low-a_w foods *(2, 3, 14, 16, 22, 27, 41,44)*. In addition, low-a_w food products, such as flour and peanut butter, may form microenvironments with increased pathogen resistance *(27, 29)*.

Commodity-specific research has been conducted on thermal inactivation of *Salmonella* in low-moisture foods, such as powdered whey protein, oregano and mint oil, wheat flour, and other food products with a wide temperature range from 56 to 140°C *(11, 36, 37, 41, 44)*. Rachon et al. *(36)* found that *Salmonella* was more resistant in a meat powder with high-protein content than in a confectionary product with high-carbohydrate content. Although studies reveal that *Salmonella* survival and inactivation in commodity-specific foods is influenced by product composition, a_w, and temperature, it does not provide insight into *Salmonella* inactivation in complex food products formulated with multiple ingredients.

Thermal inactivation models and predictive models such as log-linear and Weibull models have been developed for some *Salmonella* serovars in various foods *(5, 11, 18, 20, 32, 41)*. These models provide reliable data to predict the thermal kinetics of different *Salmonella* serovars. However, thermal inactivation models for *Salmonella* in complex food

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products with multiple ingredients examining the influence of temperature and a_w are limited.

In summary, recalls and outbreaks of *Salmonella* in low-a_w foods have identified a knowledge gap pertaining to the processing safety of these low-a_w commodities. Thermal inactivation data on *Salmonella* in complex products with multiple ingredients, such as biscuits, crackers, cookies, or other low-a_w foods, are limited. Further research on *Salmonella* thermal inactivation models during low-a_w food processing is required. In addition, evaluation of the potential use of isothermal data to predict microbial inactivation under dynamic processing conditions is necessary *(2, 30, 42)*. The objective of this project was to develop thermal inactivation models for *Salmonella,* as influenced by temperature and a_w, in high-protein and high-fat low-a_w food matrices with formulations based loosely on dog biscuits and snack crackers, respectively.

MATERIALS AND METHODS

***Salmonella* strain.** *Salmonella enterica* serovar Agona (strain 447967, associated with a puffed cereal outbreak, Arkansas Regional Laboratory, Jefferson, AR) was stored as a frozen stock. Working cultures were streaked on tryptic soy agar with 0.6% yeast extract (TSAYE; Difco, BD, Franklin Lakes, NJ) and incubated for 24 h at 37°C. Working cultures were kept at 4°C for the duration of the study and transferred monthly to fresh media.

Preparation of inoculum. Inoculum was prepared by using the methodology of Keller et al. *(22)*. An isolated colony was aseptically harvested from the working culture and inoculated into 10 mL of tryptic soy broth supplemented with 0.6% yeast extract (TSBYE; Difco, BD) and incubated for 24 h at 37°C. A 100-μL aliquot of culture was spread plated on the surface of TSAYE plates and incubated for 24 h at 37°C. Bacterial cells were harvested by adding 1 mL of buffered peptone water (BPW; Difco, BD) per plate to the agar surface, which was then gently scraped with a disposable sterile "L-shaped" spreader (Fisher Scientific, Pittsburgh, PA) to suspend cells. The cell suspension was pipetted into a sterile conical tube (Fisher Scientific, Fair Lawn, NJ). Each plate yielded approximately 0.5 mL of inoculum with a concentration of approximately 11 log CFU/mL.

Background microflora evaluation. Whole wheat flour (Pillsbury, General Mills, Minneapolis, MN), soy protein (GNC Holdings, Inc., Pittsburgh, PA), and soybean oil (Gordon Food Service, Wyoming, MI) were used as ingredients to create the model food matrices. Background levels of microflora in untreated ingredients were determined prior to formulation. Ten-gram samples of each ingredient, flour, protein, or oil, were independently weighed into 710-mL (24-oz) sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI) with 90 mL of TSBYE. Samples were soaked for 1 h at ambient conditions before being serially diluted in BPW and spread plated in duplicate on TSAYE and xylose lysine desoxycholate agar (XLD; Difco, BD) plates. A composite matrix of the three ingredients was weighed out into a sterile 710-mL (24-oz) Whirl-Pak bag and thermally treated at 85°C to evaluate spore background. After heating, 10-g samples were aseptically sampled and enumerated, as previously stated, on TSAYE and XLD.

Formulation of food matrices. Ingredients were stored in a 37% relative humidity chamber at ambient temperature conditions throughout the study to ensure a consistent starting a_w. The ingredients listed previously were used to formulate the two model food matrices. To create the high-protein matrix, 24 g of flour, 10 g of protein, and 2.4 mL of oil were combined in a 710-mL (24-oz) Whirl-Pak bag. To obtain the high-fat matrix, 24 g of flour, 2.4 g of protein, and 10 mL of oil were combined in a 710-mL (24-oz) Whirl-Pak bag. Formulations were mixed via alternately hand massaging for 1 min and stomaching (model IUL, Neutec Group Inc, Farmingdale, NY) for 30 s, three times each. A representative sample of each model food was sent out to a commercial test laboratory for proximate analysis.

Inoculation of matrices. Inoculum, diluted with additional BPW (0.4 to 20 mL total), was added to each food matrix to achieve a target a_w of 0.50, 0.60, 0.70, 0.80, 0.90, and 0.98. The amount of BPW needed to achieve the target a_w was determined in a separate experiment. The inoculated matrices were mixed by alternating, three times each, hand massaging for 1 min, and stomaching 30 s, to obtain a homogenous dough mixture. Each preparation was then allowed to equilibrate in a sealed Whirl-Pak bag at ambient conditions (23 ± 2°C) for 1 h to simulate a resting period that is common for doughs.

Sample preparation and initial product measurements. To minimize sample a_w changes due to environmental relative humidity that may have differed from the target a_w, any postinoculation sample manipulation occurred in a humidity controlled glove box *(41)* preequilibrated to the target sample a_w. The a_w of the samples was determined by using the a_w meter (AquaLab 4TE Duo, Decagon Devices, Pullman, WA), which was calibrated prior to use with appropriate a_w standards. Three a_w measurements were made throughout the sample preparation period to verify that sample a_w remained at the target level. Three initial 1-g samples were serially diluted by using BPW, spread plated onto TSAYE and XLD plates, which were then incubated at 37°C for 48 h. Samples (0.7 to 1 g) for thermal analysis were packed into sterile aluminum test cells (Washington State University, Pullman) *(9)* to eliminate any headspace in the cell and sealed with antiseize lubricant (Anti-Seize Technology, Franklin Park, IL) to achieve uniform and consistent heating of inoculated samples.

Thermal treatment and microbial enumeration. Test cells were heated in an oil bath (Neslab RTE-211, Thermo Fisher Scientific, Newington, NH) at isothermal temperatures (52 to 90°C) on the basis of the a_w of the samples to obtain *D*-values. Four temperatures were evaluated at each a_w. During treatment, a Fluke 50-Series II model 52 thermometer (Fluke, Singapore), calibrated against a National Institute for Standards and Technology traceable thermometer, was used to monitor the temperature of the oil and the product using a thermocouple positioned in the oil and a thermocouple fed into the center of a filled test cell, respectively. This test cell was also used to measure the come-up time, defined as the time required for sample temperature to reach –0.5°C of the target set point temperature. Triplicate subsamples were used for each dwell time ($n \geq 6$). After treatment, samples were immediately cooled in an ice water bath for at least 1 min. A sterile spatula (Fisherbrand, Fisher Scientific, Waltham, MA) was used to aseptically transfer the sample from the test cell into a 207-mL (7-oz) sterile Whirl-Pak filter bag. The sample was diluted with BPW to create an initial 1:10 dilution and hand massaged for approximately 30 s before further serial dilutions were performed. Aliquots were spread plated on TSAYE, in duplicate, and

incubated at 37°C for 48 h. Counts were expressed as log CFU per gram.

Model regression. Prior to model regression, the come-up time was subtracted from the total processing time. Log-linear and Weibull *(43)* models were fit to *Salmonella* inactivation data sets for each temperature-a_w combination. The shape parameter of the Weibull model was tested to determine if the value was significantly different from a value of 1 (which would represent a log-linear case). Details of the methodology and results of Weibull model are presented in the supplemental material.

The log-linear primary inactivation model (equation 1) was used to describe the isothermal inactivation kinetics

$$\log\left(\frac{N}{N_0}\right) = \frac{-t}{D_{T,\,a_w}} \tag{1}$$

where N and N_0 (CFU/g) are the *Salmonella* populations at times t and 0, respectively, t (min) is the isothermal treatment time, and $D_{T,\,a_w}$ (min) is the decimal reduction time at temperature T (°C) and a_w is a_w. The survivor ratio, log N/N_0, was first normalized so that the data would have the same beginning point at time zero.

For each matrix and target a_w combination, a Bigelow secondary model (equation 2) was used to describe the effect of temperature on *Salmonella* thermal resistance

$$D_{T,\,a_w} = D_{T_{ref},\,a_w} \times 10^{\left(\frac{T_{ref}-T}{z_T}\right)} \tag{2}$$

where D_{T_{ref},a_w} (min) is the decimal reduction time at the reference temperature T_{ref} (°C), a_w is the mean a_w for the data set, and z_T (°C) is the temperature required for a log cycle change in the D_{T,a_w}-value. The reference temperature was fixed at 75°C because 75°C was frequently used as an isothermal treatment temperature throughout this study. Any variation between a_w within a target a_w was assumed to be negligible.

A modified Bigelow secondary model (equation 3), similar to a model described by Gaillard et al. *(13)*, was used to account for the effect of temperature and a_w for the high-protein and high-fat food matrices

$$D_{T,a_w} = D_{ref} \times 10^{\left[\left(\frac{T_{ref}-T}{z_T}\right)+R_{a_w}(a_{w\,ref}-a_w)\right]} \tag{3}$$

where D_{ref} (min) is the decimal reduction time at the reference temperature and a_w, T_{ref} (°C) and $a_{w\,ref}$, respectively, and R_{a_w} is total potential log cycles in the $D_{T,\,a_w}$-value contributable to a_w. The reference temperature and a_w were fixed at 75°C and 0.90, respectively. For model estimation, the measured a_w for each replication was used.

Qualitative and quantitative measurements of model fit were used to evaluate the efficacy of the model fit. The root mean square error (RMSE; equation 4) was used to estimate model error

$$\mathrm{RMSE} = \sqrt{\frac{\sum\left(\log(N/N_0)_{predicted}-\log(N/N_0)_{observed}\right)^2}{n-p}} \tag{4}$$

where n is the number of samples, N and N_0 (CFU/g) are the *Salmonella* populations at times t and 0, respectively, and p is the number of model parameters. The Durbin-Watson autocorrelation test was applied to the residuals of the primary model regressions. To evaluate the adequacies of secondary models, $D_{T,\,a_w}$ residuals were inspected for biases correlated with time, temperature, and a_w. Models with a normal distribution of residuals and low RMSE were considered to have good fit.

All model estimations were performed by using a one-step regression; that is, all primary and secondary model parameters were estimated simultaneously by using MATLAB nonlinear fitting tools (nlinfit; version 2015a; MathWorks, Natick, MA). Statistical differences between model parameters were tested by using the Welch's t test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Background levels of native microflora were lower than detectable limits in oil and protein powder (≤0.7 log CFU/g), and were 2.74 log CFU/g in flour. The background microflora in the composite high-protein matrix after the 85°C thermal treatment was 1.40 log CFU/g on TSAYE and consisted of white, dry colonies resembling typical *Bacillus* species. There were no colonies detected on XLD (≤0.7 log CFU/g). Proximate analysis results ($n = 3$) showed that the high-protein and high-fat matrices had carbohydrate:protein:fat (weight ratio) compositions of 46.8 ± 2.4:33.8 ± 1.0:9.3 ± 0.4; and 49.0 ± 0.3:16.7 ± 0.1:26.7 ± 0.3, respectively.

The temperature come-up time of the sample within an aluminum test cell was approximately 90 to 120 s. Postinoculation and postresting *Salmonella* populations in high-protein and high-fat matrices were >7 log CFU/g on both TSAYE and XLD.

In the Weibull model, more than half of the resultant estimated shape parameters for the high-fat and high-protein survival curves were not significantly different from a value of 1 ($P < 0.05$), indicating constant slope (i.e., linearity). In addition, a visual inspection of the log-linear model residuals, looking for random and normally distributed residuals, did not preclude the log-linear model. Therefore, only log-linear models were further evaluated and reported in this article. Results of Weibull model parameter estimation are presented in the supplemental material.

The isothermal inactivation data obtained in this research was fitted to the log-linear model. Figure 1 shows the thermal inactivation data fitted by the log-linear model under different a_w and temperatures in the high-protein and high-fat matrices. The RMSEs of primary model fits for the high-protein matrix ranged between 0.24 and 0.91 log CFU/g, with 20 of 24 RMSEs ≤ 0.60 log CFU/g (Supplmental Table S1). The RMSEs of primary model fits for the high-fat matrix ranged between 0.29 and 1.15 log CFU/g, with 13 of 24 RMSEs ≤ 0.60 log CFU/g (Table S2). Higher temperatures led to a shorter D-value at each a_w level in both matrices. For example, in the high-protein matrix formulated at a_w 0.90, isothermal inactivation treatments at 60, 62.5, 65, and 70°C, resulted in D-values of 59.72, 29.33, 10.86, and 2.75 min, respectively (Table S1). Similar trends were also found in other thermal studies in literature *(12, 31, 34, 36, 44)*. In addition, a correlation was found between a_w and D-value in both high-protein and high-fat matrices; that is, a higher a_w resulted in a smaller D-value under the same treatment temperature. For example, the D-values were 15.46, 3.95, and 1.07 min at 80°C in 0.50, 0.63, and 0.73 a_w high-fat matrices, respectively (Table S2). Results were consistent with the findings of others *(12, 21, 44)*, exhibiting the influence of a_w on thermal resistance.

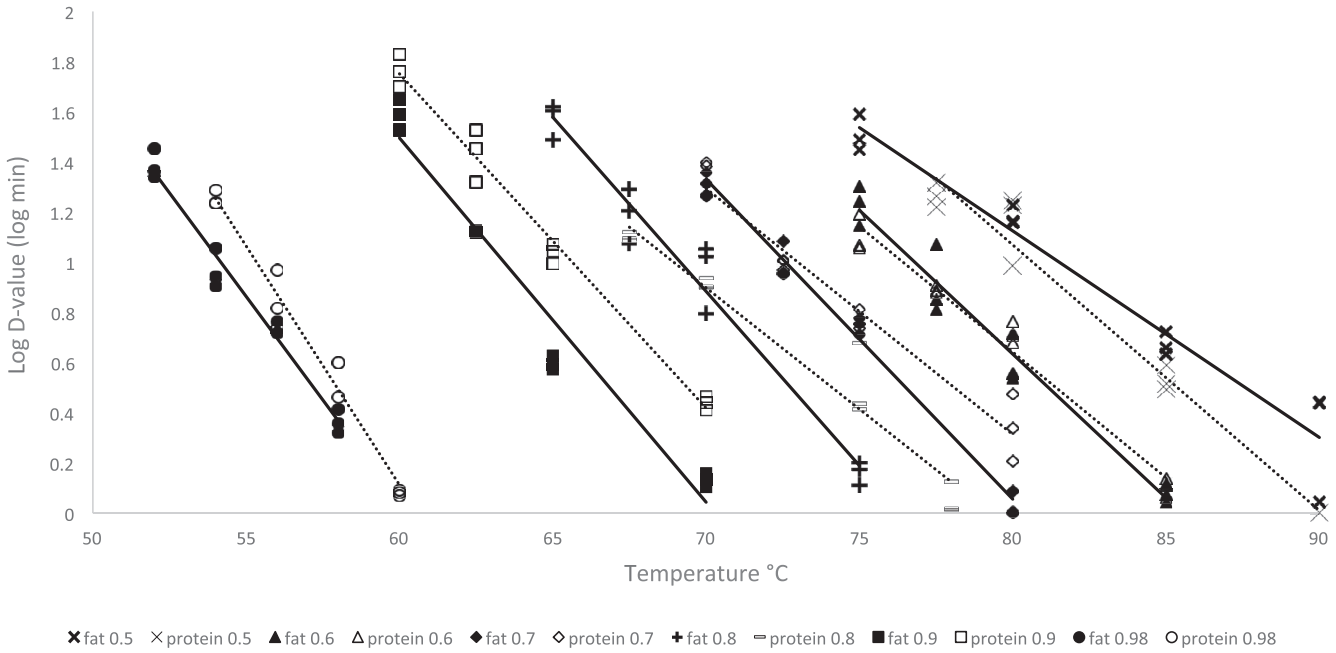

FIGURE 1. *Comparison of* Salmonella *Agona survival between high-protein and high-fat matrices at a_w 0.50, 0.63, 0.73, 0.81, 0.90, and 0.98; dotted line, high-protein matrices; solid line, high-fat matrices.*

The effect of temperature, quantified through the z_T-value, was not uniform across the tested a_w range (Table 1). The z_T-value was largest at the lowest a_w and gradually decreased with the increase of a_w in the high-fat matrix (Table 1). However, a similar clear trend was not found in the high-protein matrix (Table 1). With the exceptions of a_w 0.50 and 0.98, z_T-values in the high-protein matrix were larger than the z_T-values in the high-fat matrix at the same a_w.

Additionally, the z_T-values within a target a_w, but across products, were significantly different. As a result, the secondary models are not parallel, and there is an intersecting temperature in which the *D*-values are the same (Fig. 1). The implication of this result is that the thermal resistance of *Salmonella* is not universally greater in one product over the other, and there are practical considerations when the intersecting temperature occurs during the normal thermal inactivation range. At 77.43, 79.48, 71.28, 69.62, 38.42, and 62.90°C, the predicted *D*-values of the two matrices were the same at a_w 0.50, 0.63, 0.73, 0.81, 0.90, and 0.98, respectively. At temperatures above these values, predicted *D*-values in the high-protein matrix were larger than the *D*-values in the high-fat matrix for a_w 0.63 to 0.90; however, at a_w of 0.50 and 0.98, predicted *D*-values for the high-fat matrix were larger than *D*-values for the high-protein matrix at points greater than the intersection temperatures. At a temperature below the intersection points, the opposite relationships were observed. Because the treatment temperatures in this study ranged from 52 to 90°C, caution should be observed in assuming that the *D*-values in the high-protein matrix are always larger than in the high-fat matrix at a_w from 0.50 to 0.81. However, it is justifiable to conclude that high-protein matrix had larger *D*-values than high-fat at a_w 0.9 from 52 to 90°C, because the same point temperature of 38.42°C was below any practical inactivation temperature.

The inconsistency of trends may be attributed to the formulations of the two model food matrices. As described by Li et al. *(29),* high-fat content may have a protective effect on *Salmonella* present in local microenvironments during thermal inactivation. Also, Juneja and Eblen *(19)* described that increased fat levels in beef could result in greater *D*-values at a specific temperature. Although a number of studies focused on the protective effect of fat on microorganism thermal inactivation *(1, 31, 40),* other publications have demonstrated conflicting reports with respect to the protective effect of fat *(21, 28, 32)*. The results in this study suggest that a higher fat content may affect the *D*-values and z_T-values in food matrices, but this trend was

TABLE 1. z_T-*values calculated by Bigelow model explaining temperature in high-protein and high-fat matrices*

Actual a_w (avg)	D_{75} (min)[a]	z_T-value (°C)[a]	RMSE (log CFU/g)
High protein			
0.50	35.34 (0.87)	9.78 (0.13)	0.41
0.63	15.02 (0.44)	9.46 (0.17)	0.57
0.73	6.09 (0.09)	10.4 (0.20)	0.65
0.81	2.41 (0.03)	9.85 (0.12)	0.57
0.90	0.57 (0.02)	7.42 (0.12)	0.34
0.98	0.001 (0.0002)	5.08 (0.09)	0.76
High fat			
0.50	37.7 (1.56)	11.46 (0.32)	0.59
0.63	16.16 (0.39)	8.72 (0.14)	0.77
0.73	4.77 (0.06)	7.8 (0.09)	0.55
0.81	1.47 (0.03)	7.17 (0.08)	0.65
0.91	0.24 (0.01)	7.01 (0.08)	0.49
0.98	0.004 (0.0005)	6.03 (0.10)	0.83

[a] Standard error is reported in parentheses.

TABLE 2. z_T *and* R_{a_w} *calculated by global model (Bigelow-type secondary model) in high-protein and high-fat matrices*

	High-protein matrices	High-fat matrices
T_{ref} (°C)	75	75
$a_{w\ ref}$	0.9	0.9
D_{ref} (min)	0.99	0.61
z_T (°C)	8.99	8.27
R_{a_w}	4.38	5.41
D error (min)	0.02	0.02
z_T error (°C)	0.08	0.07
R_{a_w} error	0.06	0.06
RMSE (log CFU/g)	0.65	0.76

not consistent. These conflicting reports, as well as results here, indicate that more studies should be performed to further investigate effects of high-fat and high-protein formulations on thermal inactivation parameters.

The global model that included parameters accounting for the effect of time, temperature, and low a_w range of 0.50 to 0.91 (Table 2 and Figs. 2 and 3), was constructed by using equation 3. The authors chose to modify a similar model by Gaillard et al. *(13)* to include a new term, R_{a_w}, in place of z_{a_w} *(13)*. R_{a_w} describes the total potential log cycles in the D_{T,a_w}-values attributable to a_w. It is more appropriate to use R_{a_w} to interpret the variable describing a_w as a total potential effect because, unlike temperature that has no upper limit, a_w is defined as a finite value ranging from 0 to 1. From Table 2, it can be seen that the total potential log cycles in D_{T,a_w}-values are 4.38 min for the high-protein matrix. Because the $D_{75°C,0.9} = 1.0$ min, the theoretical $D_{75°C,0.0} \sim 10{,}000$ min, but in no event higher, because the lower a_w boundary (zero) has been reached.

When data from the a_w of 0.98 were incorporated into the global model extending the a_w from 0.50 to 0.98, predicted D-values at 75°C were exceedingly larger (~303 times larger) than the corresponding measured D-values at 0.98 a_w for the high-protein and the high-fat matrices. This finding suggests that there is a potential transitional a_w point between a high-moisture model and a low-moisture model. Further, because the 0.98 a_w level would be considered high a_w, this result indicates that caution should be observed before applying thermal inactivation models developed for low-a_w foods to those with high a_w.

Because tertiary models describing the z_T-value as an effect of a_w would only have five data points, one for each a_w included in the global model, there were insufficient temperature-a_w combinations tested to characterize the effects of temperature and a_w (R_{a_w}-value) as anything but linear, despite the evidence that the effect of temperature (z_T-value) was nonuniform across the tested a_w range and without risking overparameterization of resultant models. Nevertheless, the fit of the current model form was satisfactory because RMSE values for the high-protein (0.65 log CFU/g) and high-fat (0.76 log CFU/g) matrices were relatively low (Table 2).

Each parameter for the global high-protein and high-fat models were significantly different. Not only was the relative scale of *Salmonella* thermal resistance different, but the models suggest that the effect of temperature and a_w on *Salmonella* thermal resistance is characteristically different. This phenomenon would be expected if two completely different low-moisture products were examined, for example, dehydrated apples and orange jam, but the two matrices examined within this experiment were composed of identical ingredients, tested at identical conditions, and only varied with the relative composition of ingredients. This suggests that differences in product formulation may result in substantially different inactivation kinetics. Although this study does not provide insight on the sensitivity of formulation changes, it does indicate that a high-protein and a high-fat characterization of a product consisting of identical ingredients should be treated as two completely

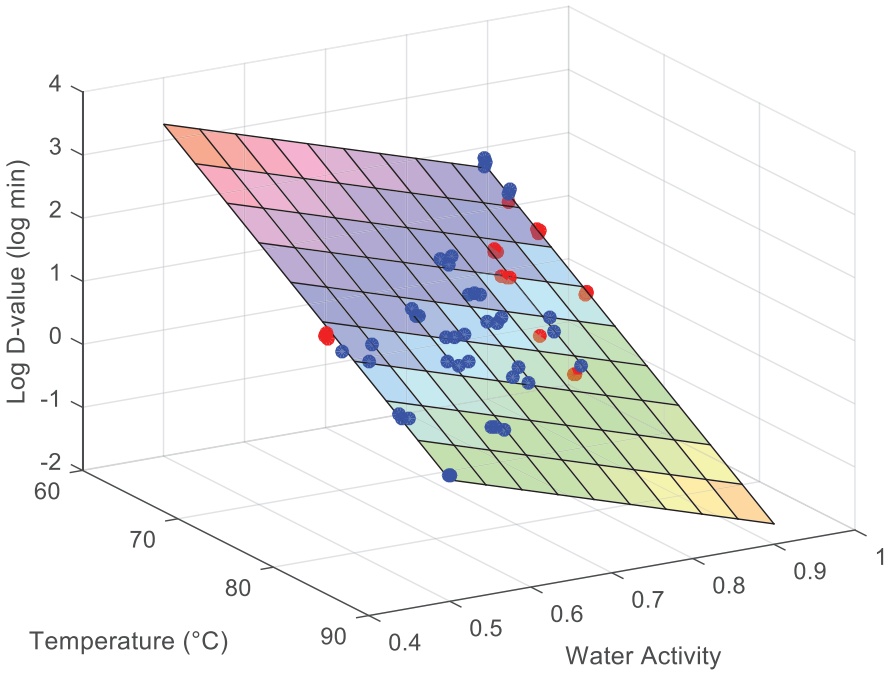

FIGURE 2. *Predicted* D*-values with respect to temperature and* a_w *in high-protein matrices (residuals =* $log\ D_{measured} - log\ D_{predicted}$*). Blue circles, positive log* D *residuals; red circles, negative log* D *residuals.*

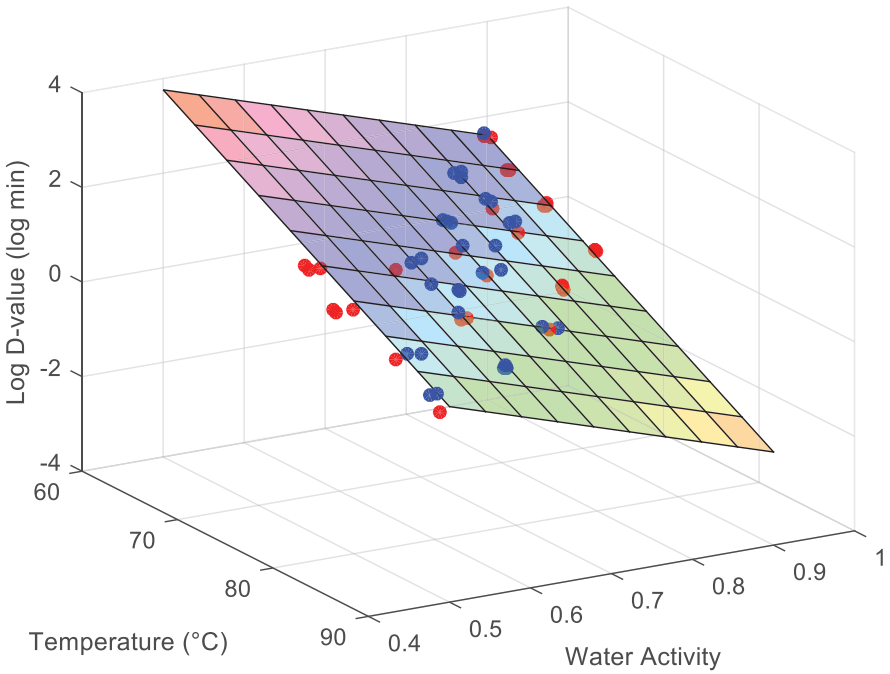

FIGURE 3. *Predicted* D*-values with respect to temperature and* a_w *in high-fat matrices (residuals =* $\log D_{measured} - \log D_{predicted}$*). Blue circles, positive log* D *residuals; red circles, negative log* D *residuals.*

different food matrices when applying thermal inactivation models.

This research shows that both temperature and a_w have significant effects on the thermal inactivation rate of *Salmonella*. Determination and control of a_w in the production of high-protein and high-fat foods is needed to obtain microbiologically safe products. The relative component of a food matrix has a complex role in thermal resistance of *Salmonella* as *D*-values obtained in the high-fat and high-protein matrices were not universally larger in one product over the other and also depended on temperature and a_w of the product. To the authors' knowledge, this is the first time such a complex relationship related to food components, temperature and a_w has been described in the literature. The isothermal models generated in this project may be used to provide the *D*-value of any combination of a_w and temperature in the ranges of foods tested. The comprehensive *D*-values and *z*-values data may be of use in estimating the thermal inactivation of *Salmonella* in high-protein or high-fat foods during dynamic thermal processes. Further work should be performed to validate the efficacy of the isothermal models on foods processed in pilot- and commercial-scale fields prior to implementation. Further work is needed to develop thermal inactivation models in foods of variable product formulations.

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: https://doi.org/10.4315/0362-028X.JFP-18-041.s1.

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