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Thermal Inactivation of *Salmonella* Enteritidis PT 30 in Almond Kernels as Influenced by Water Activity

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ABSTRACT

Salmonellosis outbreaks related to consumption of raw almonds have encouraged the scientific community to study the inactivation kinetics of pathogens in this dry commodity. However, the low moisture content of the product presents a challenge for thermal control, because the time required to achieve the desired thermal inactivation of microorganisms increases sharply with reduced moisture content and water activity. In this study, we explored and modeled the heat inactivation of *Salmonella enterica* serovar Enteritidis PT 30 in almond cultivar 'Nonpareil' kernel flour at four water activity (a_w) values (0.601, 0.720, 0.888, and 0.946) using four temperatures for each a_w. The results showed that the inactivation was well fitted by both Weibull distribution (R^2 = 0.93 to 1.00) and first-order kinetics (R^2 = 0.82 to 0.96). At higher a_w values, the rate of inactivation increased and less time was needed to achieve the required population reduction. These results suggest that, to avoid deterioration of product quality, shorter process times at lower temperatures may be used to achieve desired inactivation levels of *Salmonella* Enteritidis PT 30 by simply increasing the moisture content of almonds. These goals could be achieved with the use of existing procedures already practiced by the food industry, such as washing or prewetting scalding before heat inactivation.

Pathogenic outbreaks and recalls associated with contaminated dry foods *(12, 13, 25, 27)* have brought the attention of the scientific community and industry to the increasingly important public concerns about these commodities. Salmonellosis outbreaks in the United States and Canada during 2000 to 2004 and in Sweden during 2005 to 2006 involving *Salmonella enterica* serovar Enteritidis PT 30 were associated with consumption of whole raw almonds *(7, 9),* and some outbreaks occurred thereafter. Since current sanitation practices cannot sufficiently ensure the safety of almonds, different pasteurization processing methods have been proposed, ranging from nonthermal, such as high hydrostatic pressure *(21)* and propylene oxide fumigation *(16),* to thermal, including infrared *(7),* hot water/air *(5),* steam *(28),* and radio frequency heating *(19).* Thermal processes are preferred by the food industry since they do not leave chemical residues and are relative easy to perform *(1, 6).* However, the data available in the literature on *Salmonella* thermal inactivation kinetics on low-moisture almonds are limited.

Salmonella Enteritidis PT 30 can survive on almond shells when they have been left in the field after harvest to dry at low temperatures (approximately 37°C) to achieve a hull moisture content of 8 to 12% (wet basis); they may also migrate through the nut shell to the kernel under wet conditions *(14, 40, 41),* posing health concerns over consumption. The thermal resistance of *Salmonella* bacteria in dry products varies largely with moisture content/water activity (a_w) *(3, 5, 10, 17, 37).* In fact, prewetting and a_w changes may increase spoilage and other risks, not only those associated with the presence of *Salmonella*. Thus, significantly higher temperatures and treatment times are required for control of the pathogen in products of low moisture and, consequently, low a_w. Nevertheless, high temperatures and long holding times may compromise the quality of treated products *(22).*

On the other hand, several studies have demonstrated that properly increasing product a_w to a certain level sharply reduced the thermal treatment duration needed to obtain the desired reduction of microbial population *(20, 22, 24, 29).* This may be achievable in commercial practice, for example, by soaking the almonds before heat treatment *(19).* This step can be easily integrated into washing or prewetting scalding before blanching in the almond

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packaging process *(23)*. In order to select appropriate pasteurization treatment parameters, it is important to systematically study the influence of a_w on the inactivation kinetics of *Salmonella* Enteritidis in almond kernels.

A new aluminum test cell has been developed at Washington State University (WSU) for studying thermal inactivation of food products *(11)*. This test cell allows easy loading and unloading of samples in a hermetically sealed 1.27-ml cavity. The design of the test cells allows rapid heating of dry samples in a water bath, providing close to ideal isothermal conditions. This test cell has been used to determine the decimal reduction times (*D*-values) and the changes in temperature required to change the *D*-value (*z*-values) of *Salmonella* spp. and *Escherichia coli (11, 26)*.

The objective of this study was to describe the inactivation curves of *Salmonella* Enteritidis PT 30 obtained at four different temperatures and four a_w values in almond kernel flour (*Prunus dulcis* 'Nonpareil'). We used test cells and a water bath to collect experimental data and attempted mathematical models (first-order kinetics, Weibull distribution, and polynomial equation) to describe inactivation curves.

MATERIALS AND METHODS

Sample preparation. Almond kernels (*P. dulcis* 'Nonpareil') were obtained from a retailer in Puebla, Mexico, and kept in sealed glass jars under refrigeration until use. Kernels were milled with a coffee grinder (Mr. Coffee model ID557, Jarden Corporation, Rye, NY) and passed through a no. 18 mesh (16 Tyler). Samples were put into the aluminum cells to be heated in thermal baths for the inactivation of the inoculated bacteria, as will be described below.

To adjust samples to different a_w values, the initial moisture content of the almond samples was determined using the Association of Official Analytical Chemists standard (method 27.005) vacuum oven method *(4)*. Approximately 2-g amounts of samples were placed in open aluminum dishes and dried in an oven (Yamato Scientific, Inc., Santa Clara, CA) set at 100°C and 40.64 kPa for 1 h to achieve a constant weight. The moisture content of other samples was adjusted by adding calculated amounts of distilled water to 40 g of sample flour at the initial moisture content. The adjusted samples were conditioned in closed containers under refrigeration (4°C) for at least 2 days before use, allowing them to equilibrate to the predetermined moisture content and a_w. The a_w of the samples was measured in subsamples taken from different locations in the container to assure the uniformity of the moisture content throughout the sample. a_w was determined in triplicate by using an Aqualab a_w meter (CX2T, Decagon Devices, Pullman, WA) with an accuracy of ± 0.003.

During preliminary tests, we observed yellow and transparent colonies in almond samples when plated on xylose lysine deoxycholate (XLD) agar (Difco, BD, Sparks, MD). Further analyses were conducted to determine the population levels of these bacteria. Samples were plated on nutrient agar (Difco, BD) and incubated for 24 h at 37°C. Plate counts indicated the presence of significant levels of mesophilic bacteria (approximately 10^6 CFU/g). Since some of these bacteria had presented the typical yellow coloring suggestive of *E. coli* on XLD agar, several colonies were streaked onto eosin methylene blue agar plates (Difco, BD) and incubated at 37°C for 24 h. The plates presented typical dark-centered colonies with a metallic green sheen, confirming the presence of *E. coli* among the microflora. Danyluk et al. *(15)* also observed the presence of coliforms in *Salmonella*-positive almonds. We therefore decided to sterilize samples prior to inoculation so as to avoid interference in the inactivation test. Three sterilization methods were tested: autoclaving at 121°C for 15 min, autoclaving at 101°C for 10 min, and vacuum oven heating at 100°C and 40.64 kPa for 1 h. Ultimately, sterilization by autoclaving at 101°C for 10 min was chosen, since it yielded the best results with no significant changes (data not shown) in the peroxide value determined using method Cd 8b-90 of the American Oil Chemists' Society *(2)* through triplicate testing.

Preparation of cell suspension. To prepare the inoculum, a frozen culture (1:2 [vol/vol] of sterile 50% glycerol–stationary-phase broth culture) of *Salmonella* Enteritidis PT 30 (ATCC BAA1045) obtained from the School of Food Science Culture Collection at WSU was allowed to thaw at room temperature for 5 to 10 min. A loopful was then streaked onto XLD agar and incubated for 48 h at 37°C. A well-separated colony from that plate was inoculated into 9 ml of tryptic soy broth (Difco, BD) and incubated overnight at 37°C. A loopful of that broth was transferred to another 9 ml of tryptic soy broth and incubated again overnight, and then finally, a loopful was transferred to 250 ml of tryptic soy broth (pH = 7.2) and incubated overnight at 37°C (the pH determined at the end of the incubation period was 6.7). This culture was centrifuged three times for 25 min at 2,250 $\times$ *g*, and the pellet was washed with phosphate buffer (0.05 mM, pH 7). The cell population was adjusted to a level of 10^9 CFU/ml and refrigerated (4°C) for no more than 7 days before inoculation of almond kernel flour *(35, 42)*.

Heat treatments. To obtain the thermal death curves for *Salmonella,* preconditioned almond flour samples were used at a_w values of 0.601, 0.720, 0.888, and 0.946 that corresponded to sample moisture contents of 6, 10, 14 and 18% (wet basis), respectively. The samples were heated at four different temperatures in the aluminum cells, designed and manufactured by WSU *(11),* using a water bath. The cells were fully filled with sample flour (0.5 to 0.8 g), avoiding any headspace that could leave air in the cell and influence the heat transfer in the study. The flour was inoculated with 10 μl of the *Salmonella* Enteritidis PT 30 suspension to achieve an initial population between 10^6 to 10^7 CFU/g. The cells were closed and left for 24 h at room temperature to achieve moisture equilibrium.

Five cells with inoculated samples were immersed in a hot-water bath that had been preheated to the four target temperatures for each of the four a_w values, e.g., 70, 73, 76, and 80°C at an a_w of 0.601 or 56, 60, 64, and 68°C at an a_w of 0.946. The water bath was kept in agitation using a thermal immersion circulator (PolyScience, Niles, IL) to maintain temperature homogeneity. The come-up time for the sample core to reach within 0.5°C of each set-point temperature was determined and used as time zero to provide close-to-ideal isothermal conditions. Cells were removed at five different time intervals, depending on the temperature, to achieve at least a 5-log reduction. After holding, the cells were immediately placed in an ice-water bath ($\approx$4°C, for at least 2 min) until further analysis was performed. Triplicates of each set of conditions were performed. A noninoculated cell provided with a T-type thermocouple located at the geometrical center (cold point) of the cell was used to monitor and record the time-temperature curves of each treatment using a chronometer and a data logger system (Digisense DuaLogR 991100-50, Cole-Parmer Instrument Co.). The temperatures of the hot- and ice-water baths were monitored using both a T-type thermocouple with the data logger and a mercury thermometer placed at the cold (hot-water bath) and hot (ice-water bath) spots located previously. Temperature profiles were also acquired for a_w values of 0.601 and 0.970 at 68°C.

Thermally treated almond flours were aseptically scraped into a dilution bottle with 99.5 or 99.2 ml of 0.1% peptone water, depending on the sample weight (0.5 or 0.8 g, respectively), to achieve a 100-fold dilution. Subsequent 10-fold serial dilutions were performed in 9 ml of 0.1% peptone water; 100 μl of each one was duplicate spread plated onto XLD agar and incubated at 37°C for 48 h, and cultures were counted with a colony counter (model 530 Color QCount, Spiral Biotech, Norwood, MA).

Modeling. Thermal inactivation data were fitted using both the first-order kinetic and the modified Weibull distribution models. The first-order kinetic model *(34)* was

$$\log S(t) = -t/D \tag{1}$$

where the survival ratio $S(t) = N/N_0$, in which N and N_0 are the populations at times t and 0 (CFU/g), respectively, t is the time of isothermal treatment (min), and D is the time (min) required to reduce the microbial population by 90% at a determined temperature (°C).

The equation used for the modified Weibull distribution *(34)* was

$$\log S(t) = -(t/\delta)^p \tag{2}$$

where δ reflects the overall steepness of the survival curve and p describes whether the survival curve is linear ($p = 1$) or nonlinear ($p \neq 1$) and has an upward concavity ($p < 1$) or a downward concavity ($p > 1$).

The secondary model employed to assess the influence of a_w and T on both the D and δ-values was Mafart's adapted equation of a Bigelow-type relationship *(18)*

$$\log(D/D_{\text{ref}}) = -(T - T_{\text{ref}})/z_T - (a_w - 1)/z_{a_w} - (\text{pH} - \text{pH}_{\text{ref}})^2/z_{\text{pH}}{}^2 \tag{3}$$

Since the pH was not adjusted to different levels, the influence in the D-value can be simplified into

$$\log(D/D_{\text{ref}}) = -(T - T_{\text{ref}})/z_T - (a_w - 1)/z_{a_w} \tag{4}$$

where D_{ref} is the time (min) needed to achieve 1 log reduction in the population at temperature T_{ref}, $a_w = 1$, and pH_{ref} (7); T is temperature (°C); T_{ref} is the temperature of reference (121.1°C); pH_{ref} is the reference pH; and z_{a_w}, z_T, and z_{pH} are the a_w, temperature, and pH increments needed to reduce the D-value by 90% (°C).

We also obtained the standard z_T value at different a_w values with the Bigelow model *(33)*

$$\log(D/D_{\text{ref}}) = -(T - T_{\text{ref}})/z_T \tag{5}$$

and the log-logistic equation for the Weibull distribution was also applied as the secondary model for the different a_w values *(32)*

$$(1/\delta)(T) = \ln\{1 + \exp[k(T - T_c)]\} \tag{6}$$

where $(1/\delta)(T)$ is a nonlinear rate that reflects the overall steepness of the survival curve in an isothermal treatment, T_c is the critical temperature (°C) at which inactivation starts to accelerate, and k is the rate at which $(1/\delta)(T)$ climbs as the temperature rises to a level well above T_c. Additionally, secondary polynomial models including T, a_w, and their interactions as variables were developed using response surface analysis in Minitab 16 software (Minitab, Inc., State College, PA). With these equations, predictions of experimental and other-than-tested conditions were made.

The goodness of fit was calculated using the R^2 coefficient, which measures the strength of the model or the proportions of the variations explained by the regression of log(S) on time *(31)*. The R^2 for the first-order kinetics was obtained using linear regression in Microsoft Excel, while the same parameter for the modified Weibull model was obtained with KaleidaGraph 3.0 (Synergic software, Reading, PA); the secondary Mafart model was fitted using Sigmaplot 12.0 (Systat Software, Inc., Chicago, IL). Significant differences ($P = 0.05$) between mean results over replicates were evaluated using analysis of variance and Tukey's test with Minitab release 16.

RESULTS AND DISCUSSION

From the temperature-time histories of almond flour samples, we can consider that the come-up time and cooling time were relatively short (come-up time, 80 to 90 s, and cooling time, 30 s) and were similar for all samples regardless of a_w.

Table 1 shows the average initial population in the inoculum used for the inactivation tests and the populations after 24 h of room temperature preconditioning in different samples. The change in population appeared to be dependent on the a_w of the sample. For example, the lowest a_w (0.601) showed a significant reduction (by ~0.8 log cycles) in the population, while the samples at a_w values of 0.720 and 0.888 had no significant difference. The sample at the highest a_w (0.946) had a significant increase in population (by ≈0.75 log cycle). Similar results for low a_w were reported in Uesugi et al. *(40),* where the population of the inoculated or ''wet'' almonds decreased after a 24-h room temperature (23 ± 3°C) drying period. These results suggest that a_w values below 0.720 have a detrimental influence on *Salmonella* populations while populations at some higher a_w values may exhibit growth at room temperature (≈25°C).

Figure 1 shows the typical nonlinear concave upwards semilogarithmic inactivation curves as influenced by temperature at a fixed a_w (0.888). These results indicate that the slope increased with increasing temperature and that lower temperatures had a more pronounced tailing effect (upward concavity). Similar tendencies were reported by Mattick et al. *(30)* for *Salmonella* Typhimurium DT104 at an a_w of 0.90 and temperatures of 55, 70, and 80°C, where at higher temperatures, the steepness of the curve was more pronounced and the tailing effect was lessened.

The D, δ, and p values were calculated from the experimental data (Table 2). The first-order kinetic model had relatively good correlation coefficients (0.82 to 0.96), which in general increased with increasing a_w. Treatment time was the main factor contributing to the reduction in bacterial population, as indicated by relatively high values of coefficients. On the other hand, the Weibull distribution model had better correlation coefficients (0.93 to 0.99) than those of the first-order kinetic model, yielding a better description of the inactivation behavior of *Salmonella*.

Figure 2 shows the inactivation curves as influenced by a_w. Figures 1 and 2 further illustrate that the inactivation curves were nonlinear, with the best fit obtained with the modified Weibull model. The upward concavity curves were found again, as the p values of the modified Weibull model were considerably smaller than 1 (Table 2). These results suggest that there was a strong tailing effect or rapid destruction of heat-sensitive bacteria at the beginning of the process, while more-resistant members of the population could survive as the thermal treatment continued.

TABLE 1. Salmonella *Enteritidis PT 30 inoculum population and changes in initial population after 24 h of room temperature preconditioning in inoculated samples of almond kernel flour at different a_w values*

Salmonella population	a_w	log N_0 (mean CFU/g ± SD)[a]	No. of replicates
Initial inoculum		7.48 ± 0.61 A	11
Population after 24 h at room temp	0.601	6.73 ± 0.29 B	15
	0.720	7.40 ± 0.18 A	15
	0.888	7.78 ± 0.26 AC	12
	0.946	8.15 ± 0.68 C	15

[a] Different letters within the column indicate that means are significantly different ($P < 0.05$).

TABLE 2. *Fitted* D*-values of the first-order kinetic model and* δ *and* p *values of the reparameterized Weibull distribution for the thermal inactivation of* Salmonella *Enteritidis PT 30 inoculated into almond kernel flour at different a_w values and temperatures*

		First-order kinetics		Weibull distribution		
a_w	Temp (°C)	*D*-value (min)	R^2	δ	*p*	R^2
0.601	70	15.15	0.89	1.23	0.36	0.99
	73	8.77	0.88	0.42	0.34	0.99
	76	4.11	0.88	0.22	0.29	0.94
	80	1.63	0.82	0.15	0.33	0.94
0.720	62	24.04	0.89	7.48	0.42	0.99
	65	14.29	0.88	2.28	0.35	0.99
	68	6.97	0.89	0.86	0.33	0.93
	71	2.06	0.89	0.47	0.38	0.97
0.888	59	21.74	0.93	10.36	0.61	0.97
	62	7.21	0.92	1.36	0.48	0.97
	65	1.98	0.93	0.19	0.41	0.97
	68	0.96	0.92	0.06	0.38	0.97
0.946	56	19.34	0.94	9.19	0.65	0.98
	60	2.99	0.91	1.77	0.61	0.98
	64	0.83	0.91	0.12	0.46	0.99
	68	0.42	0.96	0.27	0.76	0.99

Also, an inverse relationship between a_w values and *D*-values was observed; that is, the higher the a_w, the shorter the time required to inactivate *Salmonella*. For example, a *D*-value of 15.15 min was obtained for a sample with a_w of 0.601 at 70°C, but the *D*-value was gradually reduced to 6.19, 0.96, and 0.42 min at 68°C for a_w values of 0.720, 0.888, and 0.946, respectively. Similar trends (i.e., decreasing *D*-values corresponding to increasing a_w or moisture content) have been reported for different foods and synthetic media by Archer et al. *(3),* Goepfert et al. *(20),* Hiramatsu et al. *(24),* McDonough and Hargrove *(31),* and Riemann *(36).* Brandl et al. *(7)* further confirm that higher a_w in almond kernels reduces the inactivation time when infrared heating is applied.

The δ value of the modified Weibull distribution confirmed the findings stated above, as it also tended to decrease at higher a_w, indicating that the rate of inactivation of *Salmonella* increased with a_w. Using the same examples of the *D*-value comparison to illustrate these changes, the δ value was 1.23 min at 70°C and a_w of 0.601 and was reduced to 0.86 and 0.06 min at 68°C when the a_w values increased to 0.720 and 0.888, respectively. These results corroborate that increasing the a_w may dramatically reduce the time needed to achieve the desired microbial inactivation. For example, using the modified Weibull model to calculate the time for a 5-log reduction (pasteurization), only 4 and 2.3 min are needed at 64 and 68°C, respectively, for heating almonds with a_w of 0.946 (18% moisture content), while 100 and 44 min would be necessary at 70 and 73°C, respectively, if the sample had a_w of 0.60 (6% moisture content).

Mattick et al. *(30)* inoculated strain 30 of *Salmonella* Typhimurium DT 104 in broth with a_w values ranging from 0.650 to 0.900, and similar tendencies were demonstrated under similar test conditions; according to their findings, the obtained *n*(*p*) values were smaller than 1, suggesting a

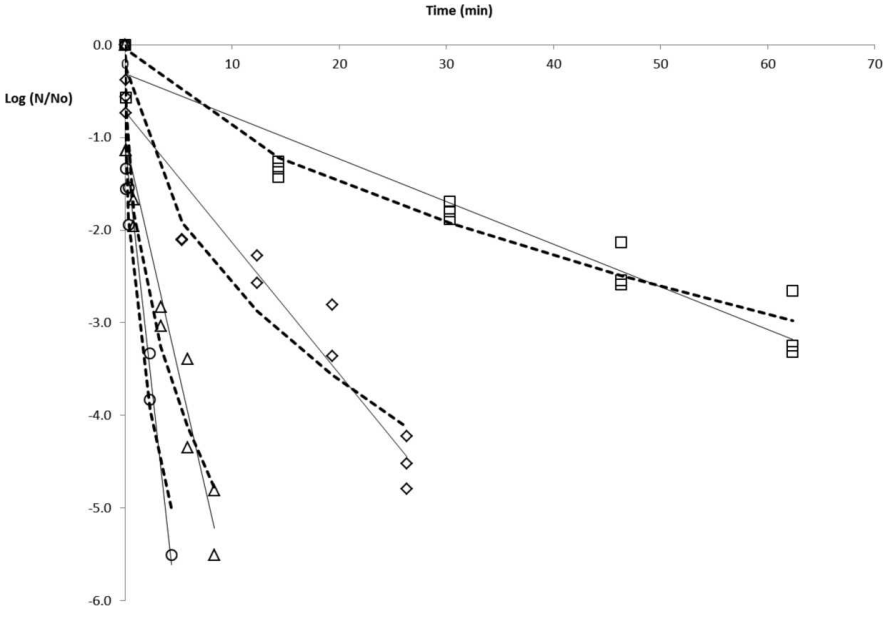

FIGURE 1. *Heat inactivation kinetics of* Salmonella *Enteritidis PT 30 inoculated in almond kernel flour with an a_w of 0.888 at different temperatures (○, 68°C; △, 65°C; ◇, 62°C; □, 59°C). — and ---- lines are the fits of the primary models: the first-order kinetic model and modified Weibull distribution, respectively.*

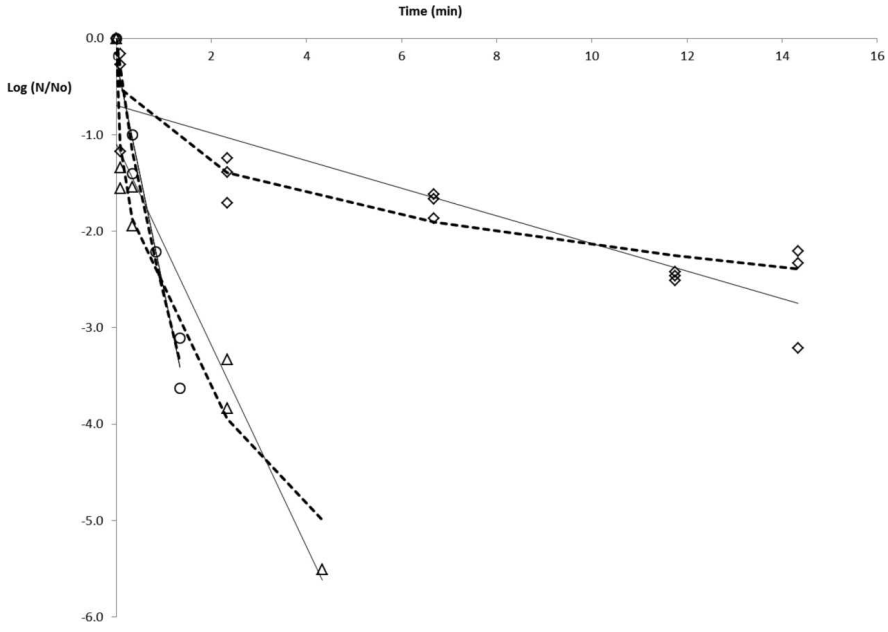

FIGURE 2. *Heat inactivation kinetics of* Salmonella *Enteritidis PT 30 inoculated in almond kernel flour at 68°C and different* a_w *values (○, 0.946; △, 0.888; ◇, 0.720). — and ---- lines are the fits of the primary models: the first-order kinetic model and Weibull distribution, respectively.*

strong tailing effect. The (1/δ) values increased with temperature at a given a_w. However, they found that at temperatures below 70°C, *b* values tended to be higher at lower a_w values, making *Salmonella* easier to kill at lower a_w values, while at temperatures above 70°C, the reverse result was observed. In our study, however, S*almonella* was more resistant at lower a_w.

With respect to the effect of both temperature and a_w on the parameters determined, the models obtained were

$$\begin{aligned}\log D = &-296.569+(607.617\cdot a_w)+(6.444\cdot T)\\ &-(270.392\cdot a_w{}^2)-(0.030\cdot T^2)\\ &-(11.580\cdot a_w\cdot T)+(3.894\cdot a_w{}^2\cdot T)\\ &+(0.039\cdot a_w\cdot T^2)\end{aligned} \quad (7)$$

$$\begin{aligned}(\delta)^{1/2} = &\,137.248-(54.373\cdot a_w)-(3.187\cdot T)\\ &+(0.018\cdot T^2)+(0.740\cdot a_w\cdot T)\end{aligned} \quad (8)$$

and

$$(1/p) = 2.983-(2.878\cdot a_w)+(0.025\cdot T) \quad (9)$$

Models were built with those parameters (variables and their interactions) found to be significant ($P < 0.05$). A marked effect of a_w on *D,* δ, and *p* parameters can be observed. With equations 7 to 9, predictions were calculated for the different conditions tested. Better fitting was achieved for the model based on modified Weibull distribution with respect to the first-order kinetics. Figure 3 shows an example of the predictions made for the thermal death of *Salmonella* inoculated in almond flour at an a_w of 0.720 and conducted at 71°C.

These types of models involving the different variables studied are common in the thermal inactivation of pathogenic bacteria, such as *E. coli (38, 39),* and may be useful for further predictions under other conditions.

In a study by Mattick et al. *(30),* using synthetic media (laboratory broths adjusted to selected a_w values), they calculated the time needed to obtain a 3-log reduction in foods with similar a_w values and compared them with the results obtained using real foods (pecorini cheese, pepperoni sausage, dried apricots, strawberry jam, peanut butter, and coconut cake). Although the model systems were able to predict the microbial behavior in most of the foods, they were usually fail-dangerous when used to predict the process time required to achieve a 3-log reduction in products with high sugar content, such as coconut cake. The authors attributed the failure to the extrapolation and differences in composition (high fat content). Using the values of the Weibull distribution (*n* and *b*) obtained by

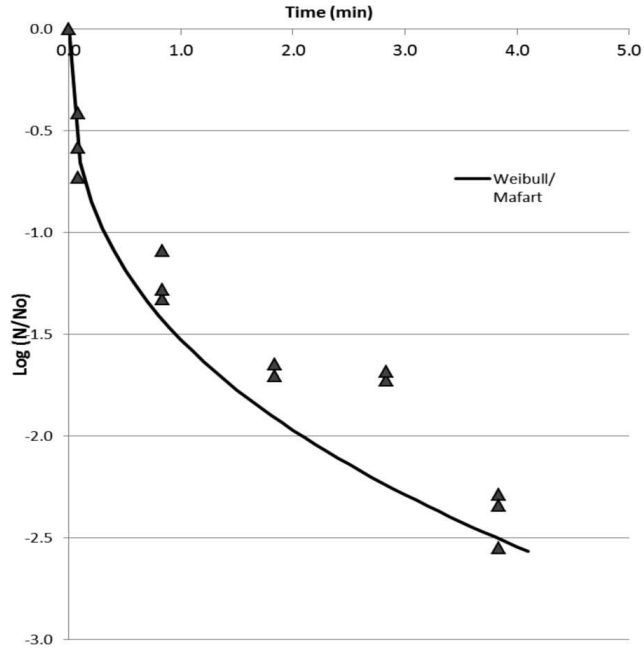

FIGURE 3. *Predictions of the thermal death of* Salmonella *Enteritidis PT 30 inoculated in almond kernel flour at an* a_w *of 0.720 and 71°C using parameters from the modified Weibull model, taking as variables both* a_w *and* T.

TABLE 3. *Calculated* D_{ref}, z_{a_w}, *and* z_T *values from the first-order kinetic and Weibull distribution models for the thermal inactivation of* Salmonella *Enteritidis PT 30 inoculated into almond kernel flour at different* a_w *values*

Parameter	Value derived from model	
	First-order kinetics	Weibull distribution
D_{ref} or $\delta_{121.1°C}$ (min)	8.92×10^{-8}	6.36×10^{-10}
z_T (°C)	8.28	6.72
z_{a_w}	0.187	0.218
R^2	0.927	0.818

TABLE 4. *Calculated* z_T *values from the first-order kinetics and* T_c *and* k *values for the Weibull distribution models on the thermal inactivation of* Salmonella *Enteritidis PT 30 inoculated into almond kernel flour at different* a_w *values*

	Bigelow model		Log-logistic model		
a_w	z_T (°C)	R^2	T_c (°C)	k	R^2
0.601	10.4	0.99	65.1	0.12	0.95
0.720	8.4	0.98	64.7	0.17	0.97
0.888	6.6	0.99	61.1	0.42	0.99
0.946	7.2	0.96	58.1	0.30	0.84

Mattick et al. *(30)* at a_w values and T values similar to those used in our study to achieve a 3-log reduction (data not shown), it was demonstrated that their model was fail-dangerous at the lower a_w while being fail-safe at a_w values of 0.888 and 0.946, suggesting that differences in composition of the food as compared with a model system may play an important role in the inhibition of *Salmonella,* especially at very low a_w values.

Mafart's modified Bigelow model showed a good fit for both D and δ values ($R^2 = 0.927$ and 0.818, respectively). The overall z-values or thermosensitivity and a_w sensitivity of *Salmonella* under the conditions studied are shown in Table 3, where the z_T and z_{a_w} values obtained for both D and δ suggest a resistance closer to that observed in some spores ($z_T = 9.28°C$ and $z_{a_w} = 0.164$ for *Bacillus cereus* spores *(18)*). The z_T values at different a_w values (Table 4) showed that an increase in a_w resulted in more sensitive bacteria, with lower z-values, a lower critical temperature (T_c), and a higher rate of inactivation (k), which render *Salmonella* easier to kill at relatively low temperatures and high a_w. These suggest that when the cell is in a stressful environment such as a low-moisture food, some defense mechanisms get triggered and the microorganism becomes more resistant (higher D-values) and less sensitive (higher z-values).

According to the results obtained, to comply with the USDA pasteurization standard, a minimum 4-log reduction, a treatment of 12 min at 60°C and a_w of 0.946 could be used. The natural contamination level of *Salmonella* Enteritidis PT 30 in almonds is less than 10^2 CFU/g *(15)*. Another proposed treatment was reported by Buransompob et al. *(8),* who demonstrated that almonds treated with hot air for 10 min at 60°C and stored at 35°C for 60 days preserved their quality attributes.

In summary, thermal inactivation of *Salmonella* Enteritidis PT 30 in 'Nonpareil' almond kernel flour was nonlinear and could be described by the modified Weibull distribution. The study also showed that small increases in a_w (directly related to increases in moisture content) dramatically reduced the inactivation time and treatment temperature for almonds. These findings may allow the use of relatively low temperatures and short thermal treatment times, which may inflict fewer losses in sensory attributes of the almonds. The short treatment time would also lead to less energy use. The recommended a_w value (0.946) may be easily achieved in commercial operations by adding a quick prewashing of kernels, which can be readily incorporated into current industrial processing procedures.

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