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 (aw) values (0.601, 0.720, 0.888, and 0.946) using four temperatures for each aw. 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 aw 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.

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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 (aw) (3, 5, 10, 17, 37). In fact, prewetting and aw 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 aw. 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 aw 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
In order to select appropriate pasteurization treatment parameters, it is important to systematically study the influence of aw 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 aw 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 aw 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 aw. The aw 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. aw was determined in triplicate by using an Aqualab aw 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 × 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 aw 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 aw values, e.g., 70, 73, 76, and 80°C at an aw of 0.601 or 56, 60, 64, and 68°C at an aw 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 (≈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 DualLogR 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 aw 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

\[ \log S(t) = -t/D \]

where the survival ratio \( S(t) = N/N_0 \), in which \( N \) and \( N_0 \) are the populations at time \( t \) 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

\[ \log S(t) = -(t/\delta)^p \]

where \( \delta \) 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 \( \delta \)-values was Mafart’s adapted equation of a Bigelow-type relationship (18)

\[ \log(D/D_{ref}) = -(T - T_{ref})/z_T = -(a_w - 1)/z_{a_w} \]

\[ -(pH - pH_{ref})^2/z_{pH}^2 \]

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

\[ \log(D/D_{ref}) = -(T - T_{ref})/z_T = -(a_w - 1)/z_{a_w} \]

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)

\[ \log(D/D_{ref}) = -(T - T_{ref})/z_T \]

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

\[ (1/\delta)(T) = \ln[1 + \exp(k(T - T_c))] \]

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 (Synergy 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 \( D, \delta, \) 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

<table>
<thead>
<tr>
<th>Salmonella population</th>
<th>(a_w)</th>
<th>log (N_0) (mean CFU/g ± SD)</th>
<th>No. of replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial inoculum</td>
<td>0.601</td>
<td>7.48 ± 0.61 (A)</td>
<td>11</td>
</tr>
<tr>
<td>Population after 24 h at room temp</td>
<td>0.720</td>
<td>6.73 ± 0.29 (B)</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>0.888</td>
<td>7.78 ± 0.26 (AC)</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.946</td>
<td>8.15 ± 0.68 (C)</td>
<td>15</td>
</tr>
</tbody>
</table>

\(A\) Different letters within the column indicate that means are significantly different (\(P < 0.05\)).

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 \(\delta\) 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 \(\delta\) 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

TABLE 2. Fitted \(D\)-values of the first-order kinetic model and \(\delta\) 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

<table>
<thead>
<tr>
<th>(a_w)</th>
<th>Temp (°C)</th>
<th>First-order kinetics</th>
<th>Weibull distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(D)-value (min)</td>
<td>(\delta)</td>
</tr>
<tr>
<td>0.601</td>
<td>70</td>
<td>15.15</td>
<td>1.23</td>
</tr>
<tr>
<td>0.720</td>
<td>62</td>
<td>24.04</td>
<td>7.48</td>
</tr>
<tr>
<td>0.946</td>
<td>59</td>
<td>21.74</td>
<td>10.36</td>
</tr>
</tbody>
</table>

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.
strong tailing effect. The \(1/\delta\) 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, Salmonella 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:

\[
\log D = -296.569 + (607.617 \cdot a_w) + (6.444 \cdot T) \\
- (270.392 \cdot a_w^2) - (0.030 \cdot T^2) \\
- (11.580 \cdot a_w \cdot T) + (3.894 \cdot a_w^2 \cdot T) \\
+ (0.039 \cdot a_w \cdot T^2)
\]

\(\delta^{1/2} = 137.248 - (54.373 \cdot a_w) - (3.187 \cdot T)\)

\(+ (0.018 \cdot T^2) + (0.740 \cdot a_w \cdot T)\)

and

\[
(1/p) = 2.983 - (2.878 \cdot a_w) + (0.025 \cdot T)
\]

Models were built with those parameters (variables and their interactions) found to be significant \((P < 0.05)\). A marked effect of \(a_w\) on \(D\), \(\delta\), 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

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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.

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 $D_{0.05}$, $z_{aw}$, and $z_T$ values from the first-order kinetics and Weibull distribution models for the thermal inactivation of Salmonella Enteritidis PT 30 inoculated into almond kernel flour at different $a_w$ values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value derived from model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First-order kinetics</td>
</tr>
<tr>
<td>$D_{ref}$ or $\delta_{121.1 \text{C}}$ (min)</td>
<td>$8.92 \times 10^{-8}$</td>
</tr>
<tr>
<td>$z_T$ (°C)</td>
<td>8.28</td>
</tr>
<tr>
<td>$z_{aw}$</td>
<td>0.187</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.927</td>
</tr>
</tbody>
</table>

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.

Mafar’s modified Bigelow model showed a good fit for both $D$ and $\delta$ 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 $z_{aw}$ values obtained for both $D$ and $\delta$ suggest a resistance closer to that observed in some spores ($z_T = 9.28$°C and $z_{aw} = 0.164$ 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.

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

<table>
<thead>
<tr>
<th>$a_w$</th>
<th>$z_T$ (°C)</th>
<th>$R^2$</th>
<th>$T_c$ (°C)</th>
<th>$k$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.601</td>
<td>10.4</td>
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<td>65.1</td>
<td>0.12</td>
<td>0.95</td>
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<tr>
<td>0.720</td>
<td>8.4</td>
<td>0.98</td>
<td>64.7</td>
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<td>0.97</td>
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<td>0.99</td>
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