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Modeling the influence of temperature, water activity and water mobility on the persistence of *Salmonella* in low-moisture foods ☆

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ABSTRACT

Salmonella can survive in low-moisture foods for long periods of time. Reduced microbial inactivation during heating is believed to be due to the interaction of cells and water, and is thought to be related to water activity (a_w). Little is known about the role of water mobility in influencing the survival of *Salmonella* in low-moisture foods. The aim of this study was to determine how the physical state of water in low-moisture foods influences the survival of *Salmonella* and to use this information to develop mathematical models that predict the behavior of *Salmonella* in these foods. Whey protein powder of differing water mobilities was produced by pH adjustment and heat denaturation, and then equilibrated to a_w levels between 0.19 ± 0.03 and 0.54 ± 0.02. Water mobility was determined by wide-line proton-NMR. Powders were inoculated with a four-strain cocktail of *Salmonella*, vacuum-sealed and stored at 21, 36, 50, 60, 70 and 80 °C. Survival data was fitted to the log-linear, the Geeraerd-tail, the Weibull, the biphasic-linear and the Baranyi models. The model with the best ability to describe the data over all temperatures, water activities and water mobilities ($f_{test} < F_{table}$) was selected for secondary modeling. The Weibull model provided the best description of survival kinetics for *Salmonella*. The influence of temperature, a_w and water mobility on the survival of *Salmonella* was evaluated using multiple linear regression. Secondary models were developed and then validated in dry non-fat dairy and grain, and low-fat peanut and cocoa products within the range of the modeled data. Water activity significantly influenced the survival of *Salmonella* at all temperatures, survival increasing with decreasing a_w. Water mobility did not significantly influence survival independent of a_w. Secondary models were useful in predicting the survival of *Salmonella* in various low-moisture foods providing a correlation of $R = 0.94$ and an acceptable prediction performance of 81%. The % bias and % discrepancy results showed that the models were more accurate in predicting survival in non-fat food systems as compared to foods containing low-fat levels (12% fat). The models developed in this study represent the first predictive models for survival of *Salmonella* in low-moisture foods. These models provide baseline information to be used for research on risk mitigation strategies for low-moisture foods.

1. Introduction

Low-moisture foods are those with water activity (a_w) levels lower than 0.70 (Blessington et al., 2012). Such foods include products which have undergone a lethality step, those that are not subjected to an inactivation step, and those in which ingredients are added after an inactivation step. A review of recall records of low-moisture foods on the Centers for Disease Control and Prevention (CDC) website showed that in the U.S., from 2007 to 2012, there were 119 recalls (5010 entries) involving pet food, powdered infant formula, peanut butter, spices, dry nuts, dry milk, seeds, etc. (CDC, 2012). From 2007 to 2012, 22 reported *Salmonella* outbreaks caused by low-moisture foods occurred globally, resulting in 2293 cases of infection and 9 deaths (CDC, 2012; EFSA, 2009; EFSA, 2010; Rodriguez-Urrego et al, 2010; SFI, 2012). The consumption of only one *Salmonella* cell in a food product may be sufficient to cause illness (D'Aoust and Maurer, 2007), and most low-moisture food products require no further cooking and have a long shelf life. Hence, the presence of *Salmonella* in low-moisture foods can cause extended outbreaks which impact large numbers of people.

Salmonella is able to survive in low-moisture foods for long periods of time. Increased heat resistance in low-moisture foods is believed to be the result of the interaction of *Salmonella* cells with food components (Podolak et al., 2010). Water, as a component of food, is considered a key factor in microbial inactivation (Podolak et al., 2010). The interaction of cells with water is often related to a_w, as it reflects the intensity with which water associates with non-aqueous components at a macroscopic level. Several studies have shown that reduced a_w protects against the inactivation of *Salmonella* in low-moisture foods (Beuchat and Scouten, 2002; Doyle and Mazzotta, 2000; Archer et al, 1998).

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However, different *D*- and *z*- values have been observed for different products under similar moisture conditions (Podolak et al., 2010). Water mobility is a measure of the translocation of water molecules in the food, with the possibility of determining the ability at which water molecules interact with the bacterial cell at a molecular level. At present, little is known about the role of water mobility in influencing the survival of *Salmonella* in low-moisture foods. The aim of this study was to determine how the physical state of water in low-moisture foods influenced the survival of *Salmonella*, and to use this information to develop mathematical models that predict the behavior of *Salmonella* in these foods.

2. Material and methods

2.1. Preparation of modified whey protein powder

The ability of whey protein (beta-lactoglobulin) to immobilize water was modified by changing the secondary and tertiary structure of the protein through pH adjustment and heat. Whey protein powder (95% protein) was obtained from Davisco Foods International (Le Sueur, MN). The pH of three 1.5 liter solutions of 40 g/l whey protein was adjusted to 2, 5 and 7, with 36.5% HCl (J.T. Baker, Phillipsburg, NJ). The protein was then denatured by heating the solution to 80 °C. The solutions were rapidly cooled under cold water and refrigerated overnight. This process stabilized the modified protein structures, but the resulting product contained sufficient bacterial spores to interfere with *Salmonella* analysis. Therefore, the protein suspensions were further pasteurized at 80 °C for 30 min after adjusting pH levels to 2.0. After cooling to room temperature, the pH of all the solutions was re-adjusted to 7 by using 10 N NaOH (J.T. Baker, Phillipsburg, NJ). The solutions were then poured into sterile aluminum pans and frozen to −40 °C overnight in a freeze drier (Freezemobile 25SL Unitop 600 l, Virtis Company, Gardiner, NY). The vacuum of the freeze drier was started once the samples reached −40 °C, and the temperature of the freeze drier was gradually increased from −40 °C to 0 °C every 24 h for a total of 96 h (−20, −10, 0). Once freeze dried, the modified whey protein powder of each structure type (denatured at pH 2, 5 and 7) was broken down to homogenous particles by crushing it with a rolling pin. The powders were stored in the dark under N_2 atmosphere with silica gel packets to avoid oxidation and moisture absorption. Protein powders denatured at pH 2, 5 and 7 are referred to as protein configuration 1, 2 and 3, respectively.

2.2. Water activity equilibration of protein powders

Protein powders were adjusted to the various a_w values in vacuum desiccators by absorption at 21 °C. Target a_w levels were: 0.11 (Lithium Chloride, Fisher scientific, Pittsburgh, PA), 0.23 (Potassium Acetate, Sigma Aldrich, St. Louis, MO), 0.33 (Magnesium Chloride Hexahydrate, Fisher scientific, Pittsburgh, PA), 0.43 (Potassium Carbonate, Anhydrous, Granular, J.T. Baker, Phillipsburg, NJ) and 0.58 (Sodium Bromide Crystal, J.T. Baker, Phillipsburg, NJ). Water activity was determined using a bench top water activity meter (AquaLab Series 4TEV, Decagon Devices Inc., Pullman, WA) of ±0.003 precision.

2.3. Water mobility determination

A Varian Inova 500 MHz spectrometer (Complex Carbohydrate Research Center, The University of Georgia, Athens, GA) was used to obtain the wide line H-NMR spectra for protein powders. Approximately 200 g of sample was packed into a 5 mm ASTM Type 1 Class B glass NMR tube (Norrell Inc., Landisville, NJ). All measurements were obtained in triplicate at 25 °C. The spectral width used was 300 kHz. The methodology used was based on that of Kou et al., 2000.

2.3.1. Effective spin-spin relaxation time (T_2^*)

A 90° 1H pulse with a pre-acquisition delay time of 2.5 s was used to obtain the H-NMR spectra of each a_w equilibrated sample. These spectra have a broad component of the peak corresponding to the immobile protons and a narrow component of the peak corresponding to the mobile protons. The spectrum of each sample was decomposed into broad and narrow components, each fitted to a Lorentzian line shape using MestRenova 7 software (Mestrelab Research, S.L., Santiago de Compostela, Spain). The areas of the broad and narrow components and the line width at half-height of each component were measured by using MestRenova 7. Effective spin–spin relaxation time (T_2^*) values were obtained using Eq. (1).

$$T_2^*\,(\mathrm{s}) = \frac{1}{\pi} \times \nu_{1/2}\ \ (\mathrm{Hz}) \tag{1}$$

where T_2^* represents the effective spin–spin relaxation time and $\nu_{1/2}$ represents the line width at half-height.

Significant differences in water mobility (T_2^*) at different water activities and for different protein configurations were analyzed by ANOVA using the General Linear Model procedure with Tukey's test at $p < 0.05$ (IBM SPSS Statistics for Windows, Version 21.0, IBM Corp. Armonk, NY). Water mobility has units of milliseconds (ms).

2.4. Sample inoculation and packaging for survival experiments

Four *Salmonella* serovars previously involved in outbreaks in dry foods were used in this study: *Salmonella* Typhimurium (peanut), *Salmonella* Tennessee (peanut), *Salmonella* Agona (dry cereal) and *Salmonella* Montevideo (pistachios and others). The cultures were stored in cryovials containing beads suspended in phosphate buffered saline, glycerol and peptone (Cryobank, Copan Diagnostics Inc., CA) and kept at −80 °C. They were prepared for use by consecutive culturing in 9 ml of Tryptic Soy Broth (TSB, Becton, Dickinson and Company, Sparks, MD) at 37 °C for 24 h. Following the second culture, a final transfer of 3 ml to 225 ml of TSB was made, followed by incubation for 24 h at 37 °C. Cells from the final culture were collected by centrifugation (3363 *g*, 30 min), the supernatant fluid was removed, and the pellet was re-suspended in 2 ml of 1% bacto-peptone (Becton, Dickinson and Company, Sparks, MD). The cell suspension was then dried in a vacuum desiccator over anhydrous calcium sulfate for a minimum of three days to obtain a_w levels below 0.1. The dried cells were pooled and manually crushed into a powder. The dried inoculum (0.05 g) was mixed with 0.95 g of moisture equilibrated test protein powder to provide a 1 g sample. This inoculation method led to starting concentrations of 10^9 CFU/g. Re-equilibration of samples to the target a_w was not necessary when using this procedure. Inoculated and control samples were packaged in retort pouches under vacuum to minimize moisture transfer to head space during survival studies. Samples were placed into standard retort pouches (Stock America, Inc., Grafton, WI). Retort pouches were then placed in FoodSaver Quart Bags, and the FoodSaver equipment (FoodSaver Silver, model FSGSSL0300-000, Sunbeam Products, Inc., Boca Raton, FL) was used for pulling a vacuum and sealing. After initial sealing of the FoodSaver bag, a second seal was applied to the retort pouch using an impulse sealer. The vacuum-sealed inoculated samples were stored at different temperatures (21 ± 0.6 °C, 36 ± 0.3 °C, 50 ± 0.5 °C, 60 ± 0.5 °C, 70 ± 0.5 °C and 80 ± 0.5 °C). For the six-month storage experiments (21 °C and 36 °C), the retort pouches were stored in desiccators at their corresponding relative humidity in controlled temperature incubators. Samples were stored in a circulating water bath (Lauda, Lauda-Konigshofen, Germany) for the high temperature experiments (50 °C, 60 °C, 70 °C and 80 °C). The water bath was equipped with custom-designed racks that kept the samples submerged and allowed for water circulation between pouches.

2.5. Experimental plan

Each survival experiment was replicated three times. Samples in six-month storage experiments at 21 °C and 36 °C were taken at: 0, 7, 14, 21, 28, 42, 56, 84,112, 140, and 168 days. Samples in one-month storage experiments at 50 °C and 60 °C were taken at: 0, 2, 6, 12, 24, 48, 96, 168, 336, 504, and 672 h. Samples in 48 h experiments at 70 °C and 80 °C were taken at: 0, 0.5, 4, 10, 30, 60, 240, 480, 960, 1440 and 2880 min. Time 0 corresponds to the time after come-up-time (the time needed to raise the temperature to reach a target level).

Uninoculated controls were analyzed for background microflora and a_w at three sampling times throughout each experiment. *Salmonella* were recovered on non-selective and selective differential media. The non-selective medium consisted of Tryptic Soy Agar (TSA, Becton, Dickinson and Company, Sparks, MD) (40.0 g/l), ferric ammonium citrate (Sigma-Aldrich Co., St Louis, MO) (0.8 g/l), yeast extract (Becton, Dickinson and Company, Sparks, MD) (3.0 g/l) and sodium thiosulfate (J.T. Baker, Phillipsburg, NJ) (6.8 g/l). The selective medium contained the same ingredients with the addition of sodium desoxycholate (Becton, Dickinson and Company, Sparks, MD) (2.5 g/l) as the selective agent. The proportion of injured cells was calculated according to Boziaris et al. (1998) and Heddleson and Doores (1994) using Eq. (2).

$$Proportion\,Injured\,Cells = \frac{A-B}{A} \tag{2}$$

where A represents the counts (CFU/g) on non-selective differential media and B represents the counts on selective differential media (CFU/g).

2.6. Development of predictive models

2.6.1. Model fitting and selection

The following inactivation models were fit to the survival data.

(1) *Log-linear model* (Bigelow and Esty, 1920)

$$N_t = N_o exp(-k_{maxB} \cdot t) \tag{3}$$

where N_t is the population at time t (CFU/g), N_o is the population at time 0 (CFU/g), k_{maxB} is the maximum specific inactivation rate (min^{-1}), t is the time (min) and $D_{value} = \frac{\ln 10}{k_{maxB}}$.

(2) *Geeraerd-tail model* (Geeraerd et al., 2000)

$$N_t = (N_o - N_{res}) \cdot exp(-k_{maxG} \cdot t) + N_{res} \tag{4}$$

where N_t, N_o and t are defined as above, N_{res} is the heat resistant population and k_{maxG} is the maximum specific inactivation rate (min^{-1}).

(3) *Weibull model* (Mafart et al., 2002)

$$\log N_t = \log N_o - \left(\frac{t}{\delta}\right)^{\beta} \tag{5}$$

where N_t, N_o and t are defined as above, δ is the time required for first decimal reduction (min) and β is a fitting parameter that defines the shape of the curve.

(4) *Biphasic-linear model* (Cerf, 1977)

$$\log N_t = \log N_o + \log\left(f \cdot \exp(-k_{max1} \cdot t) + (1-f) \cdot \exp(-k_{max2} \cdot t)\right) \tag{6}$$

where N_t, N_o and t are defined as above, f and $(1-f)$ are the heat resistant and heat sensitive fraction of the population, respectively. k_{max1} and k_{max2} (min^{-1}) are the maximum specific inactivation rates of the heat resistant and heat sensitive populations, respectively.

(5) *Baranyi growth model* as a mirror of inactivation (Baranyi and Roberts, 1994) with $m = 1$, lag time $= 0$ (min) and $\nu = \mu$

$$\log N_t = \log N_o + \frac{\mu}{ln(10)} \cdot t - \frac{1}{ln(10)} \cdot \ln\left(1 + \frac{exp\mu \cdot t - 1}{10^{\log N_f - \log N_o}}\right) \tag{7}$$

where N_t, N_o and t are defined as above, N_f is the final population ($\log_{10}$ CFU/g) and μ is the maximum specific growth rate (min^{-1}).

Data was fitted to the Baranyi model using DMFit Version 2.0 (Baranyi and Le Marc, Institute of Food Research, Norwich, UK). GInaFiT Version 1.6 (Geeraerd et al., 2005, Katholieke Universiteit Leuven, Leuven, Belgium) was used to fit data to the remaining models. To determine which of the models best described the data, the f value (f_{test}), the root mean square error ($RMSE$) and the adjusted coefficient of determination (R^2_{adj}) were calculated using Excel 2007 (Microsoft, Redmond, WA) according to the equations given below (Eqs. (8)–(14)) (den Besten et al., 2006).

$$R^2_{adj} = 1 - \frac{(n-1)\left(1-R^2\right)}{df_{model}} \tag{8}$$

$$\text{where } R^2 = \frac{\sum\left(\log N_{model} - \overline{\log N_{data}}\right)^2}{\sum\left(\log N_{model} - \overline{\log N_{data}}\right)^2 + \sum\left(\log N_{model} - \log N_{data}\right)^2}$$

$$RSS_{data} = \sum\left(average\,\log N_{data} - \log N_{data}\right)^2 \tag{9}$$

$$RSS_{model} = \sum\left(\log N_{model} - \log N_{data}\right)^2 \tag{10}$$

$$RMSE = \sqrt{\frac{RSS_{model}}{df_{model}}} \tag{11}$$

$$MSE_{model} = \frac{RSS_{model}}{df_{model}} \tag{12}$$

$$MSE_{data} = \frac{RSS_{data}}{df_{data}} \tag{13}$$

$$f = \frac{MSE_{model}}{MSE_{data}} \tag{14}$$

where RSS_{data} is the residual sum of squares of the data (sum of the squared differences between the observed values and the average values), RSS_{model} is the residual sum of squares of the model (sum of the squared differences between the observed values and the predicted values) and df is the degrees of freedom where $df_{model} = n - p$ and $df_{data} = n - m$ (n is the total number of observations at all time points, p is the number of parameters in the model and m is the number of time points).

The f_{test} value was tested against F_{table} (95% confidence). If the f_{test} value was lower than the F_{table} (df_{model}/df_{data}), the f_{test} was judged to provide an acceptable fit of the data (den Besten et al., 2006). The primary criterion used to choose the best model to describe the survival data was the capacity of the model to describe the data well for all temperature, a_w and water mobility conditions ($f_{test} < F_{table}$). If more than one model fitted the data well for all conditions, the model with best statistical parameter fits was chosen (highest R^2_{adj}, lowest $RMSE$). If these first two criteria were equally met, the number of parameters of the model and the biological meaning of the model parameters were considered (den Besten et al., 2006).

2.6.2. Secondary model development

The influence of temperature, a_w and water mobility on the survival of *Salmonella* was evaluated using Multiple Linear Regression (IBM SPSS Statistics for Windows, Version 21.0, IBM Corp.), where a_w, water mobility and temperature represent the dependant variables of the

secondary models. A t_{test} was used to assess the significance of each factor on the survival of *Salmonella*. Secondary models were developed based on parameter significance. If the significance of the test was lower than the level of confidence ($p < 0.05$), the parameter was judged to be significant and included in the secondary model. Normal probability plots were visually evaluated for a linear relationship (where linearity indicates normality). Uniform variance was verified using residual plots. If the plots of the residuals against log CFU/g values clustered around zero, variances were considered constant.

2.6.3. Validation of the secondary models

The secondary models were validated by obtaining *Salmonella* survival data (in duplicate) in whole wheat flour, low-fat peanut meal (12% fat), non-fat dry milk, whey protein and low-fat cocoa powder (12% fat) at various temperatures (from 22 °C to 80 °C), a_w levels (0.20 ± 0.03 to 0.55 ± 0.06) and storage times (from 0 to 6 months) within the range of the modeled data. The bias factor (B_f) expressed as % bias (Eq. (15)) and accuracy factor (A_f) expressed as % discrepancy (Eq. (16)) were used to measure model performance (Baranyi et al., 1999). Residuals (r) were calculated using Eq. (17) and the acceptable residual zone was established to be from −1 log CFU (fail safe) to 0.5 log CFU (fail dangerous) (Oscar, 2009). The percentage of residuals in the acceptable zone was used as a model performance measure (Oscar, 2009). A model was considered validated and the model performance acceptable with a residual percentage ≥70% (Oscar, 2009). Visual inspection of the data including the correlation coefficient values (R) (Eq. (18)) for the plots of the predicted against experimental survival data were also used for model evaluation.

$$\%\, B_f = sgn\left(Ln\, B_f\right) \times \left(exp^{\left|Ln\, B_f\right|} - 1\right) \times 100\,\% \tag{15}$$

where: $B_f = 10\hat{}\left[\dfrac{\sum_1^n log\left(logN_{model} / logN_{data}\right)}{n}\right]$

$$sgn\left(Ln\, B_f\right) = \begin{pmatrix} +1 & if & B_f > 0 \\ 0 & if & B_f = 0 \\ -1 & if & B_f < 0 \end{pmatrix}$$

$$\%\, D_f = \left(A_f - 1\right) \times 100\,\% \tag{16}$$

where: $A_f = 10\hat{}\left[\dfrac{\sum_1^n \left| log\left(logN_{model} / logN_{observed}\right)\right|}{n}\right]$

$${}_1^n r^1 = {}_1^n l^1 ogN_{observed} - {}_1^n l^1 ogN_{predicted} \tag{17}$$

$$R = Correlation\,(x,y) = \frac{\sum (x-\bar{x})(y-\bar{y})}{\sqrt{\sum (x-\bar{x})^2 \sum (y-\bar{y})^2}}. \tag{18}$$

3. Results and discussion

3.1. Water mobility of modified whey protein powder

The T_2* values for mobile and immobile protons from the H-NMR spectra analyses are presented in Table 1. The NMR spectra (not shown) for samples of different a_w indicated that sorbed water produced an increase in the relative intensity of the narrow component of the peak (representing mobilized water) and a decrease in the relative intensity of the broad component of the peak (representing immobile water). A progressive decrease in line width was observed for both the broad component and the narrow component as a_w increased. Statistical analyses indicated that the T_2* values for mobile protons (Table 1, column 3) increased with increasing a_w ($p < 0.001$). This indicated that molecular mobility successively increased with an increasing bulk water phase. Similarly, T_2* values for immobile protons (Table 1, column 4) significantly increased with increasing a_w ($p < 0.001$). Proton exchange in low-moisture conditions is slow, so the increasing mobility of immobile protons as a_w increased was not the result of proton exchange but indicated that water was causing an increase in protein mobility (Kou et al., 2000).

Table 1
Water mobility (T_2*) values for whey protein powder of 3 different configurations and equilibrated to 5 water activity (a_w) levels.

Measured a_w[a]	Configuration	T_2* Mobile Protons (ms)[b]	T_2* Immobile Protons (ms)
0.19 ± 0.03	1[c]	0.075 ± 0.01	0.0071 ± 0.0005
	2[d]	0.076 ± 0.01	0.0069 ± 0.0007
	3[e]	0.076 ± 0.009	0.0069 ± 0.0001
0.29 ± 0.03	1	0.093 ± 0.02	0.0070 ± 0.0001
	2	0.092 ± 0.009	0.0072 ± 0.0003
	3	0.098 ± 0.03	0.0072 ± 0.0004
0.36 ± 0.03	1	0.096 ± 0.001	0.0075 ± 0.0002
	2	0.121 ± 0.03	0.0079 ± 0.0008
	3	0.094 ± 0.007	0.0073 ± 0.0007
0.43 ± 0.02	1	0.101 ± 0.003	0.0075 ± 0.0003
	2	0.108 ± 0.006	0.0074 ± 0.0001
	3	0.094 ± 0.005	0.0075 ± 0.0002
0.54 ± 0.02	1	0.129 ± 0.008	0.0122 ± 0.0002
	2	0.132 ± 0.002	0.0093 ± 0.003
	3	0.106 ± 0.004	0.0095 ± 0.0007

[a] Average measured water activity ± sd of three replicates.
[b] Average measured water mobility ± sd of three replicates (where mobility is measured in milliseconds).
[c] Protein denatured at pH 2.
[d] Protein denatured at pH 5.
[e] Protein denatured at pH 7.

T_2* values for mobile protons at the lower a_w levels (0.16–0.28) did not significantly differ for the three protein configurations ($p = 0.908$), but there were significant differences in water mobility for samples at the higher a_w levels (0.37–0.59) ($p = 0.021$). Specifically, samples with configuration 2 showed greater mobility than samples of configuration 3 ($p = 0.023$) in this a_w range. No significant differences were observed in water mobility for immobile protons at the 3 protein configurations ($p > 0.05$).

3.2. Survival of Salmonella in samples held at 21 to 80 °C

Data corresponding to the survival of *Salmonella* at various temperatures in low-moisture protein powder are presented in Figs. 1 through 4. Model fit statistics for the log-linear, Baranyi, Geeraerd-tail, Weibull and biphasic-linear models for all experimental conditions under study are presented in Table 2, where the best statistical parameter fits are shown in bold. The Geeraerd-tail, Weibull and biphasic-linear models were not suitable for describing the 21 °C data because survival numbers were maintained throughout the experiment.

The *Salmonella* counts used for the data analyses, model development and model validation, were obtained using non-selective differential media. A comparison of non-selective with selective counts indicated that the proportion of injured cells (Eq. (2), data not shown) was not significantly influenced by temperature ($p = 0.228$), a_w ($p = 0.371$) or water mobility ($p = 0.411$). Storage time just significantly influenced the proportion of injured cells ($p = 0.044$), as longer storage times led to increasing proportions of injured cells. These results do not support a hypothesis that the mechanism of inactivation changed from membrane damage at lower temperatures (≤50 °C) to ribosomal degradation at higher temperatures (>50 °C) as suggested by Aljarallah and Adams (2007). Heating cells to temperatures just above their maximum growth temperature causes damage to the cytoplasmic membrane, which in

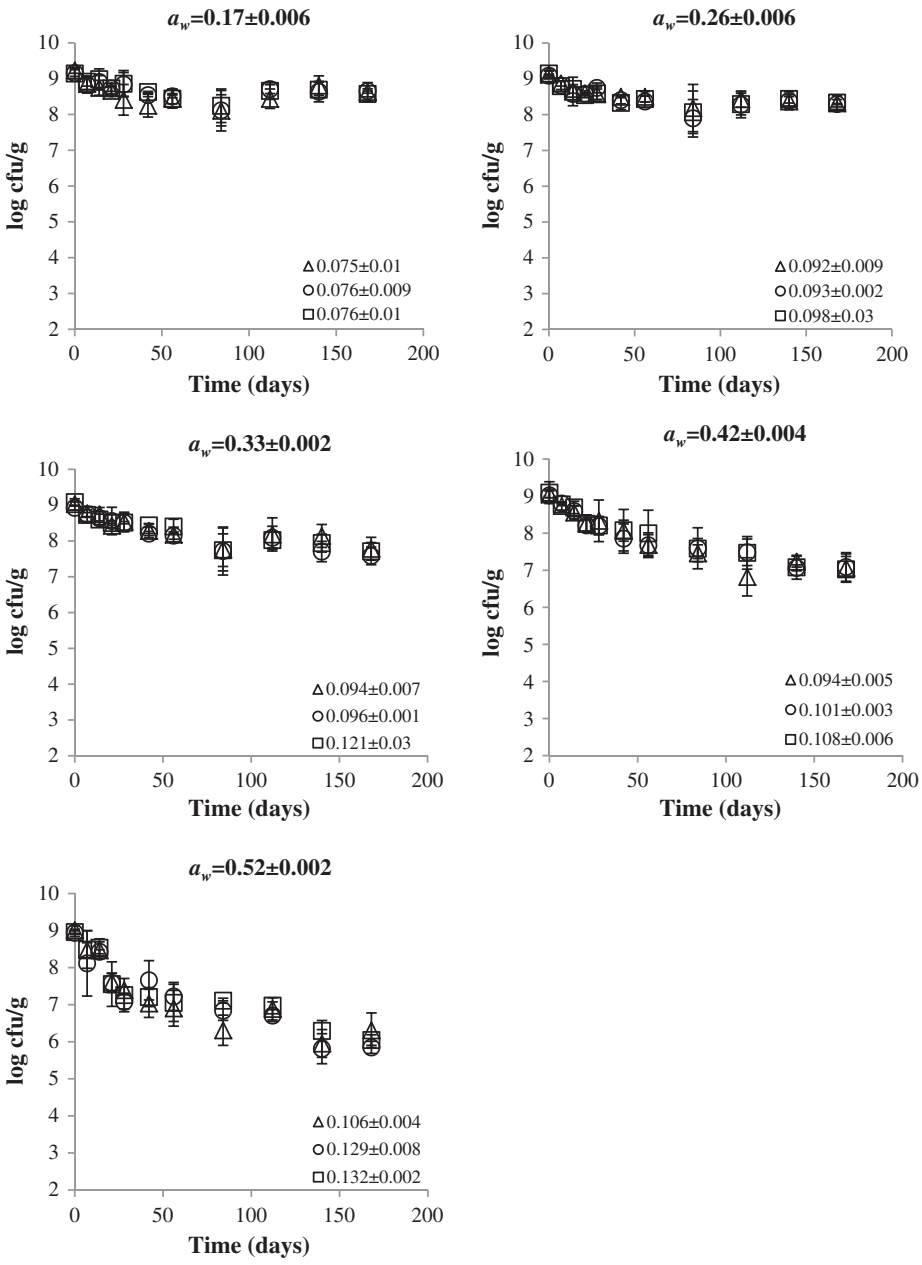

Fig. 1. Survival of *Salmonella* at 36 °C during 168 days of storage in low-moisture whey protein powder at 5 water activities (a_w) and 3 water mobilities (T_2*, where mobility is measured in milliseconds) at each a_w. Error bars represent the ± standard deviation of the average of three replicas for each "a_w-water mobility sample combination".

enteric bacteria can be detected by plating the cells on non-selective media and media containing bile salts. If cells are treated at sufficiently high temperatures, death results from ribosome degradation, and there will be a small or no difference in the ability of the survivors to grow on selective and non-selective media. Aljarallah and Adams (2007) observed these effects using *Salmonella* treated at 53 °C and 60 °C at water activities of 0.99 and 0.94. Results in the present study indicated that there were no significant differences in the proportion of injured cells among those exposed to different water activities and temperatures. However, one major difference in our study is that we investigated lower water activities (<0.6) over a wider temperature range (21 °C–80 °C).

Salmonella survival data at 21 °C during 168 days (6 months) of storage (results not shown) showed that populations were maintained under these conditions, with log reduction values of 0.001, 0.003, 0.002, 0.003 and 0.005 log CFU/day at a_w levels of 0.16 ± 0.01, 0.26 ± 0.002, 0.34 ± 0.009, 0.41 ± 0.01 and 0.53 ± 0.05, respectively. These data indicated a significantly better survival of *Salmonella* at lower a_w levels (0.16 and 0.26) as compared to higher ones (0.34 to 0.53) ($p < 0.001$). Significant differences in survival were also observed between the two highest a_w levels (0.41 and 0.53). However, no significant differences in survival were found between a_w levels of 0.16 and 0.26 ($p = 0.541$), 0.34 and 0.41 ($p = 0.730$) or 0.34 and 0.53 ($p = 0.074$). No influence of water mobility at the same a_w level was observed ($p = 0.917$). Because the survival rates were essentially linear at 21 °C, the Geeraerd-tail model, the Weibull model (with $\beta \neq 1$ in Eq. (5)) and the biphasic-linear model were not suitable for describing the data. The Baranyi and the log-linear models were appropriate in describing the data for all conditions ($f_{test} < F_{table}$) and showed similar statistical fit parameter values (Table 2).

Fig. 1 presents data on *Salmonella* survival at 36 °C during 168 days (6 months) of storage. Survival increased with decreasing a_w ($p < 0.001$). As with the observations at 21 °C, no influence of water mobility independent of a_w level was observed ($p = 0.507$). Average log reduction values of 0.003, 0.005, 0.008, 0.01 and 0.02

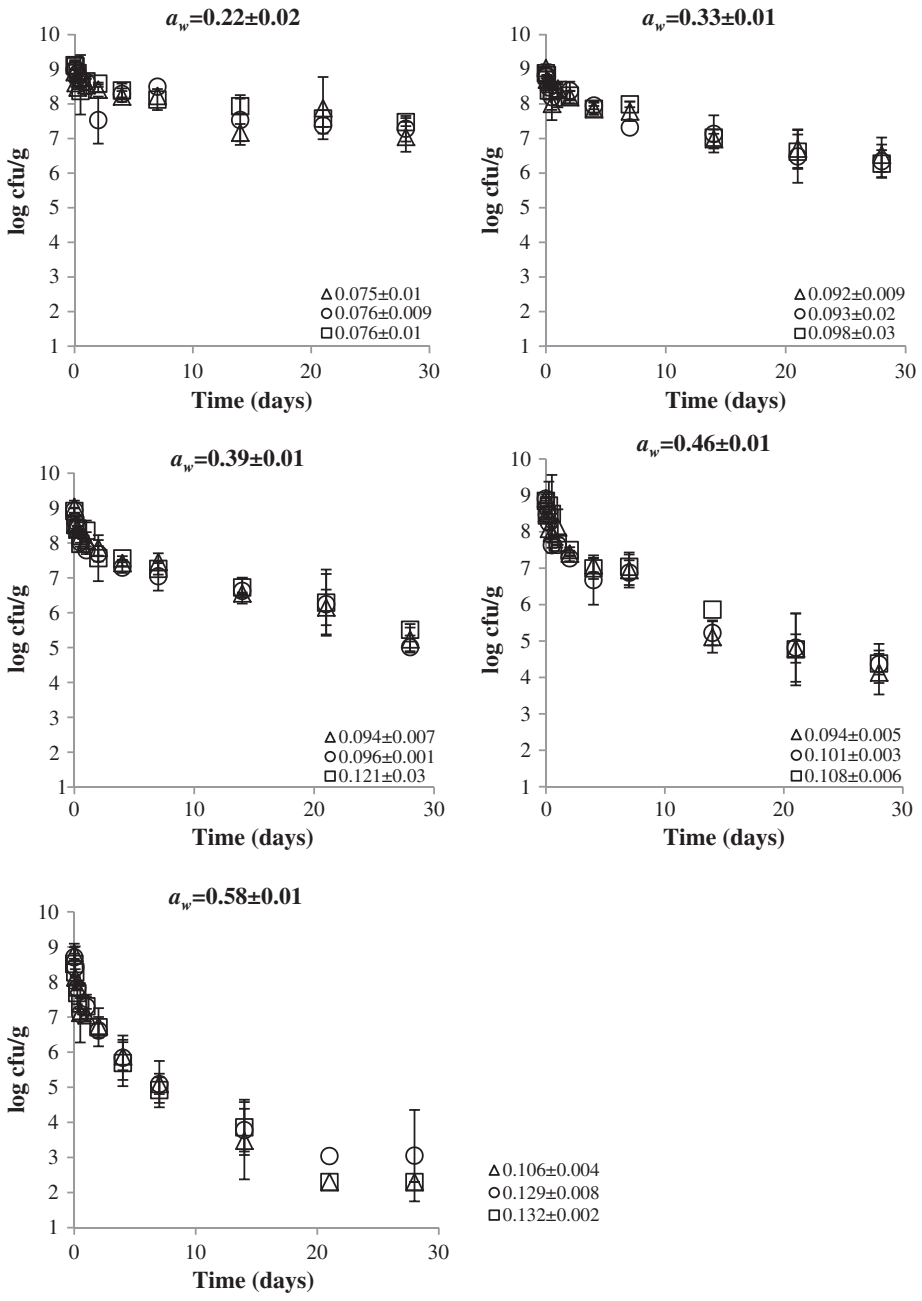

Fig. 2. Survival of *Salmonella* at 50 °C during 672 h (30 days) of storage in low-moisture whey protein powder at 5 water activities (a_w) and 3 water mobilities (T_2*, where mobility is measured in milliseconds) at each a_w. Error bars represent the ± standard deviation of the average of three replicas for each "a_w-water mobility sample combination".

log CFU/day were observed at a_w levels of 0.17, 0.26, 0.33, 0.42 and 0.52, respectively. At the lower a_w levels (0.17 and 0.26), there was a slight decline in *Salmonella* population (Fig. 1) which resembled that seen at 21 °C. Greater inactivation was seen at the higher a_w levels (0.33–0.52), with an initial decline followed by a tail starting at around 50 days of storage (Fig. 1). The model fit statistics corresponding to 36 °C survival data are presented in Table 2. Unlike the results at 21 °C, the survival data at 36 °C could be described by all models ($f_{test} < F_{table}$) with the exception of the log-linear model, which did not fit the data at the highest a_w (0.52) (Table 2). *Salmonella* survival in protein powder held at a_w level of 0.52 showed tailing after approximately 50 days of storage. The log-linear model did not describe such tailing behavior as indicated by an f_{test} which was higher than the F_{table}. The biphasic-linear model produced the best fit statistics at a_w level of 0.52 (Table 2). This model may represent samples containing two populations with differing survival rates, and therefore their fitness may be associated with using a multistrain cocktail. The highest R^2_{adj} values for survival data at 36 °C were found when fit to the Geeraerd-tail model followed by the biphasic-linear and Weibull models (Table 2).

Survival data at 50 °C showed increased heat resistance of *Salmonella* associated with decreasing a_w ($p < 0.001$) (Fig. 2). Even at temperatures as high as 50 °C, *Salmonella* continued to inactivate slowly at the lowest a_w level (0.22). Average log reduction values of 0.06, 0.09, 0.13, 0.16 and 0.22 log CFU/day were observed at a_w levels of 0.22, 0.33, 0.39, 0.46 and 0.58, respectively. No significant differences in resistance were associated with water mobilities at the same a_w level ($p = 0.418$). All models adequately described the inactivation data at the lower a_w levels (0.22 and 0.33) (Table 2). However, at the higher a_w levels (0.39–0.58),

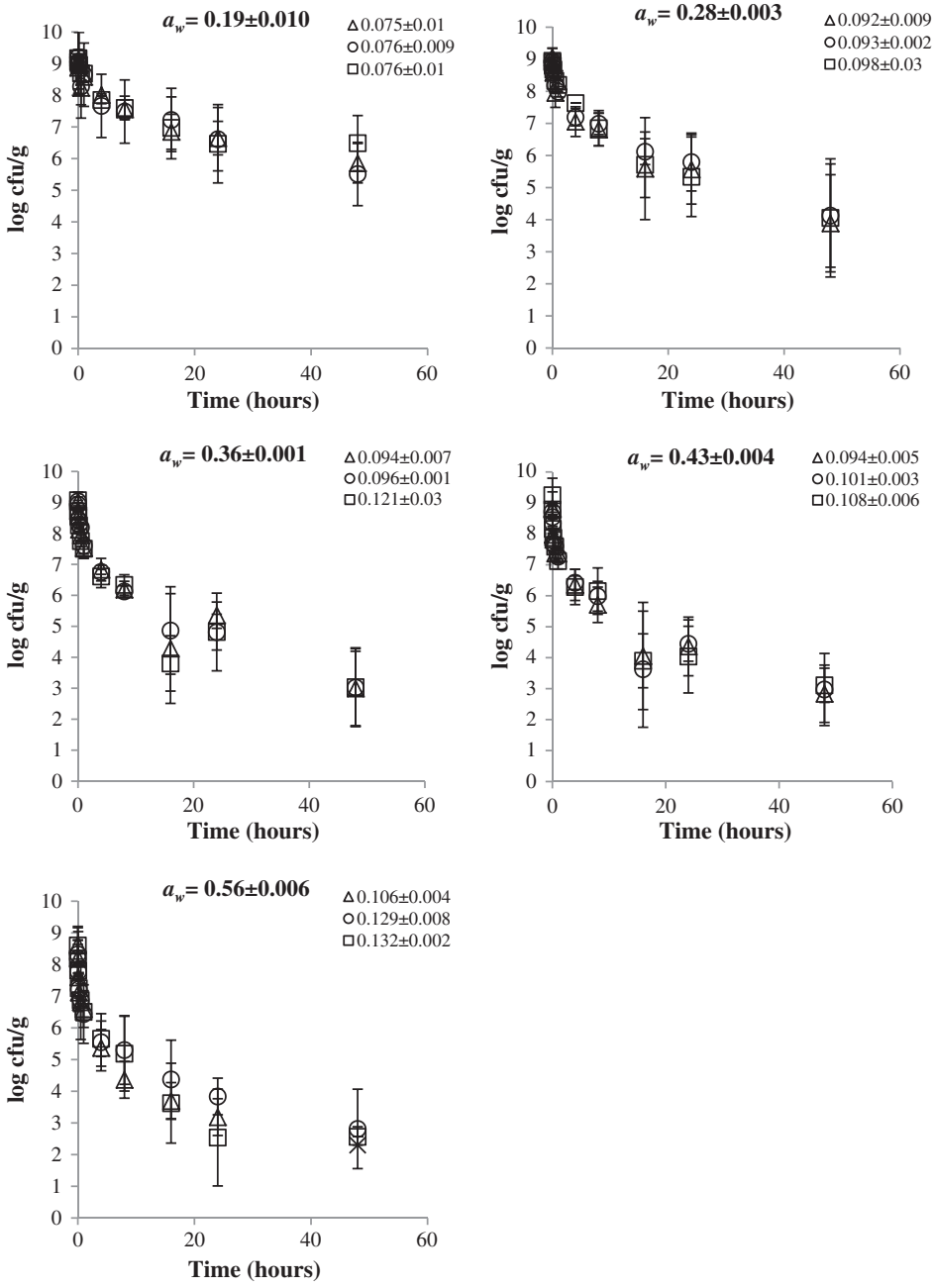

Fig. 3. Survival of *Salmonella* at 70 °C during 2880 min (48 h) of storage in low-moisture whey protein powder at 5 water activities (a_w) and 3 water mobilities (T_2*, where mobility is measured in milliseconds) at each a_w. Error bars represent the ± standard deviation of the average of three replicas for each "a_w-water mobility sample combination".

the best fits were found when using the Weibull model followed by the biphasic-linear model and the Geeraerd-tail model (Table 2). The log-linear and Baranyi models showed poorer fits at the higher a_w (0.39–0.58) because under these conditions *Salmonella* produced a non-log-linear inactivation rate (Fig. 2).

Data on survival of *Salmonella* at 60 °C showed increased survival with decreasing a_w ($p < 0.001$) (results not shown). Average log reduction values of 0.2, 0.4, 0.6, 0.6 and 0.8 log CFU/day were observed at a_w levels of 0.22 ± 0.002, 0.34 ± 0.0003, 0.39 ± 0.006, 0.46 ± 0.005 and 0.57 ± 0.002, respectively. *Salmonella* was not detected after 2 weeks (336 h) of storage at the higher a_w levels (0.46, 0.57). At the intermediate a_w levels (0.34 and 0.39) *Salmonella* was not detected in samples after 504 h (3 weeks). When *Salmonella* was held at 60 °C at a_w level of 0.22, samples contained detectable *Salmonella* even after 4 weeks of storage. No significant differences in resistance were found for survival in the different water mobilities at the same a_w level ($p = 0.880$). The survival data were well described by all the models except for the log-linear model which did not describe survival well at the highest a_w level (0.57) ($f_{test} > F_{table}$) (Table 2). The highest R^2_{adj} values were found when using the Weibull model followed by the biphasic-linear and the Geeraerd-tail models. As the storage temperature increased to 70 °C, survival kinetics became non-linear, as the inactivation curves had a non-linear mid-phase and pronounced tails (Fig. 3). Average log reduction values of 1.6, 2.5, 3.0, 3.0 and 3.0 log CFU/day were obtained at a_w levels of 0.19, 0.28, 0.36, 0.43 and 0.56, respectively. After 48 h of treatment, an average 6 log CFU reduction was observed for *Salmonella* at the higher a_w levels (0.36–0.56). Average log reduction values of 3 and 5 log CFU after 48 h of treatment were observed at a_w levels 0.19 and 0.28, respectively. Water activity significantly influenced the survival of *Salmonella* at this temperature ($p < 0.001$) while water mobility had no influence when the a_w level was constant ($p = 0.781$). The non-linear behavior of the

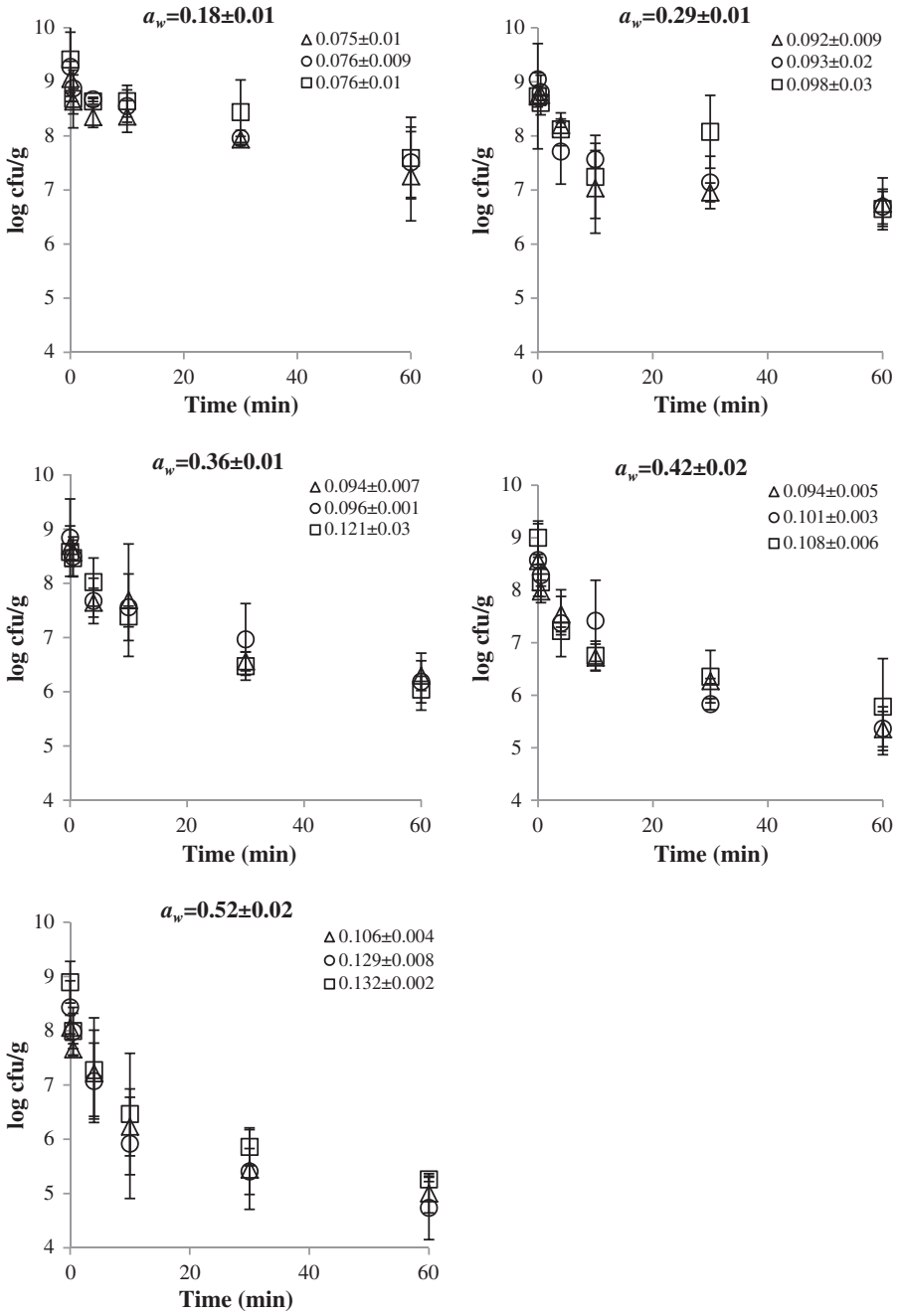

Fig. 4. Survival of *Salmonella* at 80 °C during 60 min of storage in low-moisture whey protein powder at 5 water activities (a_w) and 3 water mobilities (T_2*, where mobility is measured in milliseconds) at each a_w. Error bars represent the ± standard deviation of the average of three replicas for each "a_w-water mobility sample combination".

pathogen at this temperature (Fig. 3) made the log-linear model unsuitable for describing this data (Table 2). Similarly, the Baranyi model produced poor fit results and unacceptable f_{test} results in more than 50% of the conditions (Table 2). The best fit statistics were for the Weibull model, followed by the biphasic-linear and Geeraerd-tail models. The highest R^2_{adj} values were obtained when fitting the data to the Weibull and biphasic-linear models.

As with the results at 70 °C, survival of *Salmonella* at 80 °C (Fig. 4) produced inactivation curves with pronounced tails (tails are not shown on Fig. 4). Data during the first 60 min of storage indicated non-linear inactivation kinetics at every a_w level. Water activity significantly influenced the survival of *Salmonella* at 80 °C ($p < 0.001$). Generally 2–3 log CFU reduction numbers were observed at the lower a_w levels (0.18 and 0.29) during the first 60 min of storage followed by an additional 4–5 log CFU reduction from 60 to 1440 min (results not shown). The 80 °C treatment produced average log reduction values of 0.7, 1.3, 1.3, 1.4 and 1.5 log CFU/h at a_w levels of 0.18, 0.29, 0.36, 0.42 and 0.52, respectively. At the higher a_w levels (0.36–0.52), 2–4 log reduction values were seen after 60 min of treatment (Fig. 4). After 1440 min (24 h), *Salmonella* was only detected in the samples with the lowest a_w level (0.18). The pathogen was not detected in any samples after 24 h of treatment. Water mobility did not have a significant effect on microbial death at 80 °C independent of a_w ($p = 0.912$). When fitting the survival data at 80 °C to the models, similar statistical fit results were found with the Geeraerd-tail model, the Weibull model and the biphasic-linear model (Table 2). Model fit statistics

Table 2
Statistical parameter fit results of the log-linear, Baranyi, Weibull, biphasic-linear and Geeraerd-tail models for *Salmonella* inactivation in powders by adjusted R^2 (R^2_{adj}) and Root Mean Square Error (*RMSE*), where best values are shown in bold for experiments at 6 T (°C), 5 water activities (a_w) and 3 water mobilities (T_2^*) at each a_w.

Water activity[a]	Water mobility[b] (ms)	T °C	Log-linear[c]	Baranyi[d]	Weibull[e]	Biphasic[f]	Geeraerd[g]	Log-linear	Baranyi	Weibull	Biphasic	Geeraerd
			R^2_{adj} Values					*RMSE* values				
0.19 ± 0.03	0.075 ± 0.011	21	**−0.02**	–0.11	–[h]	–	–	**0.26**	0.27	–	–	–
		36	0.01	0.29	0.26	0.33	**0.36**	0.39	**0.19**	0.33	0.32	0.31
		50	0.60	0.61	**0.64**	0.63	**0.64**	0.46	0.44	**0.43**	0.44	**0.43**
		60	0.94	0.94	0.94	0.94	**0.95**	0.45	0.43	0.44	0.43	**0.42**
		70	0.78	0.83	**0.89**	0.88	0.86	0.53	0.44	**0.37**	0.39	0.43
		80	–	0.82	**0.91**	0.90	0.84	–	0.56	**0.41**	0.44	0.55
	0.076 ± 0.009	21	**0.04**	−0.07	–	–	–	**0.19**	0.20	–	–	–
		36	0.13	0.32	0.31	0.36	**0.38**	0.29	**0.16**	0.26	0.25	0.25
		50	0.73	0.75	0.76	0.76	**0.77**	0.38	**0.35**	**0.35**	**0.35**	**0.35**
		60	**0.94**	0.93	**0.94**	**0.94**	**0.94**	**0.39**	0.41	**0.39**	**0.39**	**0.39**
		70	–	0.75	**0.84**	**0.84**	0.77	–	0.60	**0.50**	**0.50**	0.59
		80	–	–	**0.94**	0.93	0.90	–	–	**0.45**	0.47	0.56
	0.076 ± 0.010	21	**−0.03**	−0.11	–	–	–	**0.34**	0.35	–	–	–
		36	0.19	0.35	0.32	0.38	**0.40**	0.29	**0.13**	0.27	0.25	0.25
		50	0.70	0.69	0.79	**0.80**	0.73	0.32	0.32	**0.27**	**0.27**	0.30
		60	0.84	0.83	0.84	0.79	**0.85**	0.70	0.73	0.71	0.82	**0.68**
		70	0.61	0.76	**0.79**	**0.79**	**0.79**	0.71	0.53	**0.52**	**0.52**	**0.52**
		80	0.71	–	**0.87**	0.86	0.79	0.75	–	**0.50**	0.52	0.63
0.29 ± 0.03	0.092 ± 0.009	21	**0.01**	−0.09	–	–	–	**0.47**	0.48	–	–	–
		36	0.33	0.43	0.50	0.50	**0.51**	0.28	**0.10**	0.24	0.24	0.24
		50	0.78	0.80	**0.86**	0.85	0.82	0.41	0.38	**0.32**	0.33	0.37
		60	0.66	0.73	**0.83**	0.82	0.77	0.56	0.48	0.39	**0.41**	0.46
		70	0.70	–	**0.87**	0.86	0.82	0.82	–	**0.53**	0.57	0.64
		80	0.75	0.76	0.87	**0.91**	0.79	0.71	0.67	0.52	**0.42**	0.65
	0.093 ± 0.021	21	**0.06**	−0.04	–	–	–	**0.20**	0.21	–	–	–
		36	0.32	0.50	0.49	0.53	**0.54**	0.31	**0.16**	0.26	0.26	0.25
		50	0.81	0.81	**0.85**	0.84	0.83	0.39	0.38	**0.35**	0.37	0.37
		60	0.78	0.74	0.83	**0.85**	0.77	0.60	0.63	0.53	**0.49**	0.61
		70	–	–	**0.95**	0.94	0.88	–	–	**0.29**	0.32	0.45
		80	–	0.82	0.92	**0.94**	0.84	–	0.56	0.39	**0.32**	0.55
	0.098 ± 0.028	21	**0.30**	0.22	–	–	–	**0.17**	0.18	–	–	–
		36	0.27	0.50	0.51	0.54	**0.56**	0.29	0.24	0.24	**0.23**	**0.23**
		50	0.88	0.88	**0.92**	**0.92**	0.90	0.31	0.29	**0.26**	**0.26**	0.28
		60	0.77	0.73	0.78	**0.79**	0.76	0.77	0.81	0.76	**0.74**	0.79
		70	0.69	0.84	0.85	**0.86**	0.85	0.87	0.61	**0.60**	**0.60**	**0.60**
		80	0.58	0.57	0.70	**0.72**	0.64	0.74	0.70	0.62	**0.60**	0.68
0.36 ± 0.03	0.094 ± 0.007	21	**0.07**	−0.02	–	–	–	**0.25**	**0.25**	–	–	–
		36	0.49	0.59	0.60	0.62	**0.63**	0.33	**0.29**	0.30	**0.29**	**0.29**
		50	–	–	**0.82**	0.78	NA	–	–	**0.47**	0.53	–
		60	0.57	0.88	0.94	**0.95**	0.89	0.73	0.62	**0.47**	0.53	0.59
		70	–	–	**0.96**	0.93	–	–	–	**0.28**	0.36	–
		80	0.67	0.72	**0.78**	0.77	0.77	0.58	0.50	**0.47**	0.48	0.48
	0.096 ± 0.001	21	**0.14**	0.05	–	–	–	**0.26**	0.27	–	–	–
		36	0.67	0.71	0.72	0.73	**0.74**	0.29	0.27	0.27	0.27	**0.26**
		50	0.81	0.78	0.89	**0.91**	0.80	0.49	0.51	0.37	**0.34**	0.50
		60	0.69	0.65	**0.84**	0.83	0.70	0.77	0.81	**0.56**	0.58	0.77
		70	–	–	**0.95**	0.93	0.90	–	–	**0.34**	0.41	0.50
		80	0.78	0.79	**0.89**	**0.89**	0.83	0.68	0.63	**0.49**	**0.49**	0.61
	0.121 ± 0.027	21	**0.13**	0.04	–	–	–	**0.23**	0.24	–	–	–
		36	0.68	0.74	**0.76**	0.75	0.75	0.26	**0.23**	**0.23**	0.24	**0.23**
		50	0.81	0.78	**0.86**	**0.86**	0.80	0.49	0.49	**0.40**	**0.40**	0.48
		60	0.81	0.78	0.83	**0.88**	0.80	0.64	0.67	0.60	**0.51**	0.66
		70	–	–	–	–	–	–	–	–	–	–
		80	–	0.84	**0.93**	0.73	0.87	–	0.48	0.94	0.94	**0.47**
0.43 ± 0.02	0.094 ± 0.005	21	**0.49**	0.43	–	–	–	**0.21**	0.22	–	–	–
		36	0.75	0.82	0.83	0.84	**0.85**	0.36	**0.29**	0.30	**0.29**	**0.29**
		50	–	0.91	**0.95**	0.93	0.92	–	0.46	**0.37**	0.42	0.45
		60	0.53	0.77	0.76	0.80	**0.81**	1.10	0.72	0.78	0.71	**0.69**
		70	–	0.79	**0.91**	0.86	0.82	–	0.69	**0.47**	0.57	0.65
		80	–	0.79	**0.93**	**0.93**	0.83	–	0.45	0.29	**0.27**	0.36
	0.101 ± 0.003	21	**0.40**	0.34	–	–	–	**0.16**	0.17	–	–	–
		36	0.75	0.81	0.84	**0.85**	0.84	0.36	0.30	0.29	**0.28**	0.29
		50	0.83	0.86	**0.93**	0.92	0.87	0.65	0.58	**0.43**	0.45	0.57
		60	0.54	0.71	**0.83**	0.77	0.76	0.79	0.59	**0.48**	0.55	0.57
		70	–	–	**0.94**	0.90	0.88	–	–	**0.35**	0.46	0.51
		80	–	0.82	0.89	**0.91**	0.85	–	0.63	0.52	**0.49**	0.61
	0.108 ± 0.006	21	**0.12**	0.03	–	–	–	**0.26**	0.27	–	–	–
		36	0.76	0.78	**0.81**	0.80	**0.81**	0.37	0.34	**0.33**	0.34	**0.33**
		50	0.83	0.83	**0.89**	**0.89**	0.85	0.64	0.62	0.53	**0.52**	0.61
		60	0.45	0.69	**0.76**	0.72	0.74	0.91	0.65	**0.60**	0.65	0.63
		70	–	–	**0.93**	0.88	0.81	–	–	**0.35**	0.46	0.59
		80	–	0.82	0.84	**0.85**	**0.85**	–	0.43	0.43	**0.41**	0.42
0.54 ± 0.02	0.106 ± 0.004	21	**0.29**	0.22	–	–	–	**0.31**	0.32	–	–	–

Table 2 (*continued*)

Water activity[a]	Water mobility[b] (ms)	T °C	Log-linear[c]	Baranyi[d]	Weibull[e]	Biphasic[f]	Geeraerd[g]	Log-linear	Baranyi	Weibull	Biphasic	Geeraerd
			R^2_{adj} Values					*RMSE* values				
		36	–	0.87	0.85	**0.89**	**0.89**	–	0.37	0.42	**0.36**	**0.36**
		50	–	0.72	0.89	**0.90**	0.77	–	0.58	0.38	**0.37**	0.56
		60	0.39	0.69	**0.80**	0.76	0.76	1.32	0.87	**0.76**	0.83	0.83
		70	–	–	**0.94**	–	–	–	–	**0.33**	–	–
		80	0.68	0.62	**0.81**	**0.81**	0.73	0.49	**0.26**	0.41	0.43	0.49
	0.129 ± 0.008	21	**0.34**	0.27	–	–	–	**0.31**	0.32	–	–	–
		36	0.78	0.79	**0.83**	0.82	0.81	0.51	0.48	**0.45**	0.46	0.47
		50	–	–	**0.89**	–	–	–	–	**0.57**	–	–
		60	–	0.73	**0.92**	0.82	0.78	–	0.71	**0.41**	0.62	0.68
		70	–	0.78	**0.91**	0.88	0.84	–	0.63	**0.43**	0.48	0.55
		80	0.74	0.84	0.87	**0.89**	**0.89**	0.55	0.38	0.39	**0.36**	**0.36**
	0.132 ± 0.002	21	**0.51**	0.46	–	–	–	**0.31**	0.32	–	–	–
		36	–	0.76	0.84	**0.86**	0.80	–	0.48	0.41	**0.38**	0.45
		50	–	0.78	**0.86**	0.85	0.82	–	0.46	**0.38**	0.40	0.44
		60	0.35	0.74	0.74	0.76	**0.79**	1.56	0.91	0.98	0.94	**0.88**
		70	–	–	**0.93**	**0.93**	–	–	–	**0.38**	**0.38**	–
		80	–	0.72	**0.87**	0.83	0.77	–	0.76	**0.56**	0.63	0.73

[a] Average measured water activity ± sd of 3 replicates at all *T*.
[b] Average measured water mobility ± sd of 3 replicates at all *T* (milliseconds).
[c] Bigelow and Esty (1920).
[d] Baranyi and Roberts (1994).
[e] Mafart et al. (2002).
[f] Cerf (1977).
[g] Geeraerd et al. (2005).
[h] $f_{test} > F_{table}$ thus model does not describe data well.

showed (Table 2) that the log-linear model did not describe the survival behavior of *Salmonella* in half of the conditions. The Baranyi model provided a better fit as compared to the log-linear model, but did not adequately describe the data at the lowest a_w (0.18). The highest R^2_{adj} values at 80 °C were found when using the biphasic-linear and the Weibull models, which is in line with the results seen at 50°, 60° and 70 °C. Consequently, the best description of *Salmonella* inactivation in low-moisture foods at high temperatures ($T > 50$ °C) requires a model that includes a non-constant inactivation rate at the mid-phase and the ability to incorporate tailing.

Survival data at temperatures ranging from 21 to 80 °C demonstrate the highly adaptive capacity of *Salmonella* to survive in low-moisture foods for long periods of time, even when subject to high heat. Results also indicate that a_w significantly influences the survival of *Salmonella* at all temperatures, with survival increasing with decreasing a_w. These results are consistent with previous studies showing the protective effect of a_w against the inactivation of *Salmonella* in low-moisture foods (Beuchat and Scouten, 2002; Archer et al., 1998; Mattick et al., 2001). In contrast to that found by Hills et al. (1997), water mobility has shown to have no effect on survival of *Salmonella* independent of a_w.

Increased tailing was associated with increased inactivation temperature for any given a_w-water mobility condition (Figs. 1–4). Similarly, at the same inactivation temperature, increasing a_w (and thus water mobility) led to curves with a more pronounced downward concavity while different water mobilities at the same a_w showed no effect on curve shape (Figs. 1–4). The F_{test} results indicated that the log-linear and Baranyi models did not describe the data well for several storage conditions ($f_{test} > F_{table}$), except as previously noted for survival at 21 °C. Therefore, these models were not selected for further analyses. The statistical parameters presented in Table 2 indicated that the Weibull model was the best of those under study for describing the survival of *Salmonella* at five temperatures from 36 °C to 80 °C, five a_w and three water mobility levels at each a_w. The Weibull model provided suitable fits for the inactivation data under all experimental conditions except one ($T = 70$ °C, $a_w = 0.36$, water mobility = 0.121 milliseconds), and generally gave the highest statistical fit parameters (Table 2). The biphasic-linear model was the second best model under study as it provided suitable data fits under almost all conditions and had statistical fit parameters which approximated those of the Weibull model (Table 2). The Geeraerd-tail model had lower goodness of fit as compared to the Weibull and biphasic-linear models (Table 2). The Geeraerd-tail model described the data well in only 69 of the 75 conditions and generally showed poorer statistical fits (Table 2). These results are consistent with previous studies showing non-linear models, particularly the Weibull model, describe the thermal resistance of *Salmonella* in low-moisture foods more accurately as compared to log-linear ones (Podolak et al., 2010).

Based on the previous analysis, the Weibull model was selected for secondary modeling. The Weibull model satisfactorily described the greatest number of conditions and statistical parameters indicated the best fit. Moreover, the Weibull model could also produce linear fits (with $\beta = 1$ in Eq. (5)) and thus also described linear inactivation kinetics as obtained at 21 °C. Table 3 presents δ and β parameter values (Eq. (5)) for the fits of the Weibull model for all conditions under study. Because δ values for data at distinct temperatures differed by several orders of magnitude, these values were transformed to log scale. The log δ (log min) are presented in Table 3.

3.3. Secondary inactivation models

Linear models relating the time required for first decimal reduction (log δ) and shape factor values (log β) to temperature, a_w and water mobility were fit using multiple linear regression. The β values were log transformed to normalize the data. The analysis indicated that temperature was a significant factor influencing the time required for first decimal reduction and the shape of the inactivation curve ($p < 0.001$). Water activity was also a significant factor in the model that related the time required for first decimal reduction to temperature ($p < 0.001$). Water activity did not significantly influence the shape of the inactivation curve ($p = 0.279$). Water mobility did not significantly influence the time required for first decimal reduction or the shape of the inactivation curve ($p > 0.05$). The secondary models developed

Table 3
δ and β values of the Weibull model fit for *Salmonella* inactivation experiments at 6 T (°C), 5 water activities (a_w) and 3 water mobilities (T_2^*) at each a_w.

Water activity[a]	Water mobility (ms)[b]	T °C	log δ[c](log min)	log se δ[d]	β[e]	se β[f]
0.19 ± 0.03	0.075 ± 0.011	21	6.40	7.36	1.00	3.92
		36	6.48	7.09	0.09	0.09
		50	4.11	3.88	0.47	0.18
		60	3.68	3.07	0.81	0.09
		70	2.24	1.92	0.42	0.07
		80	1.38	1.14	0.38	0.05
	0.076 ± 0.009	21	–[g]	–	–	–
		36	6.17	6.34	0.21	0.12
		50	4.12	3.74	0.55	0.16
		60	3.75	3.08	0.89	0.10
		70	2.11	1.92	0.41	0.08
		80	1.08	0.83	0.39	0.05
	0.076 ± 0.010	21	–	–	–	–
		36	5.94	5.95	0.28	0.15
		50	4.03	3.72	0.37	0.09
		60	3.79	3.36	0.88	0.18
		70	2.02	1.95	0.36	0.09
		80	1.39	1.20	0.41	0.07
0.29 ± 0.03	0.092 ± 0.009	21	6.29	7.30	0.04	0.09
		36	5.57	5.38	0.26	0.10
		50	3.60	3.33	0.39	0.07
		60	2.72	2.56	0.35	0.07
		70	1.59	1.47	0.35	0.06
		80	0.81	0.68	0.43	0.08
	0.093 ± 0.021	21	6.03	6.35	1.08	1.57
		36	5.43	5.22	0.28	0.10
		50	3.81	3.48	0.49	0.11
		60	3.29	2.98	0.63	0.14
		70	1.68	1.33	0.35	0.04
		80	0.76	0.47	0.41	0.05
	0.098 ± 0.028	21	5.95	5.87	0.78	0.51
		36	5.52	5.40	0.22	0.08
		50	3.88	3.34	0.55	0.08
		60	3.26	3.03	0.69	0.18
		70	1.73	1.64	0.38	0.08
		80	0.83	0.87	0.38	0.11
0.36 ± 0.03	0.094 ± 0.007	21	7.88	8.69	0.18	0.20
		36	5.12	4.80	0.39	0.12
		50	3.49	3.04	0.47	0.06
		60	2.22	2.24	0.29	0.07
		70	1.15	0.85	0.30	0.03
		80	0.74	0.64	0.40	0.11
	0.096 ± 0.001	21	6.35	6.59	0.35	0.26
		36	5.08	4.59	0.53	0.13
		50	3.30	3.05	0.40	0.07
		60	2.45	2.37	0.36	0.07
		70	1.27	1.01	0.32	0.03
		80	0.71	0.60	0.40	0.07
	0.121 ± 0.027	21	6.11	6.33	0.71	0.74
		36	5.06	4.57	0.45	0.10
		50	3.49	3.25	0.43	0.08
		60	3.26	2.93	0.67	0.14
		70	0.99	0.85	0.29	0.04
		80	0.76	0.44	0.43	0.05
0.43 ± 0.02	0.094 ± 0.005	21	5.51	4.76	1.59	0.74
		36	4.69	4.28	0.50	0.09
		50	3.64	3.15	0.67	0.09
		60	2.29	2.38	0.37	0.11
		70	0.89	0.83	0.28	0.04
		80	0.52	0.24	0.40	0.06
	0.101 ± 0.003	21	5.98	5.80	0.59	0.29
		36	4.82	4.35	0.56	0.11
		50	3.38	3.07	0.53	0.08
		60	2.04	2.02	0.29	0.06
		70	1.13	0.91	0.30	0.03
		80	0.39	0.35	0.35	0.06
	0.108 ± 0.006	21	6.18	6.41	0.56	0.56
		36	4.72	4.32	0.53	0.10
		50	3.63	3.31	0.65	0.13
		60	1.63	1.83	0.25	0.06
		70	0.31	0.23	0.22	0.02
		80	−0.21	−0.16	0.25	0.05
0.54 ± 0.02	0.106 ± 0.004	21	–	–	–	–
		36	4.14	3.89	0.43	0.07
		50	2.47	2.26	0.36	0.06
		60	1.37	1.60	0.26	0.07
		70	0.19	0.09	0.22	0.02
		80	0.76	0.64	0.43	0.13
	0.129 ± 0.008	21	5.53	5.02	1.00	0.62
		36	4.41	4.10	0.52	0.10
		50	2.39	2.31	0.34	0.05
		60	1.53	1.46	0.28	0.04
		70	0.71	0.66	0.26	0.04
		80	0.61	0.43	0.44	0.10
	0.132 ± 0.002	21	5.38	4.35	1.81	0.74
		36	4.29	4.01	0.45	0.08
		50	2.87	2.57	0.48	0.09
		60	1.28	1.53	0.28	0.08
		70	0.79	0.62	0.30	0.04
		80	0.07	0.05	0.32	0.06

[a] Average measured water activity ± sd of 3 replicates at all T.
[b] Average measured water mobility ± sd of 3 replicates at all T (measured in milliseconds).
[c] Time required for first decimal reduction (measured in minutes).
[d] Standard error of δ parameter value.
[e] Fitting parameter that defines the shape of the curve.
[f] Standard error of β parameter value.
[g] log-linear regression gives a positive slope.

for *Salmonella* spp. survival in low-moisture foods are presented in Eqs. (19) and (20).

$$\log \delta = -0.10 \times T - 4.34 \times a_w + 9.91 \quad R^2 = 0.96 \tag{19}$$

$$\log \beta = -0.006 \times T \quad R^2 = 0.74 \tag{20}$$

In Eq. (19), the standard error (s.e.) of log δ was 0.35, that of the temperature parameter (T) was 0.003, that of the water activity parameter (a_w) was 0.52 and that of the constant was 0.26. In Eq. (20), the s.e. of log β was 0.22 and that of the T parameter was 0.001.

3.4. Model validation

Thirteen δ (time required for first decimal reduction) and β (shape factor) values for *Salmonella* survival were obtained from 151 CFU measurements. These correspond to survival in low-fat cocoa powder at 22 °C for 168 days ($a_w = 0.35$), 35 °C for 168 days ($a_w = 0.32$ and $a_w = 0.34$) and 70 °C for 24 h ($a_w = 0.33$ and $a_w = 0.35$), low-fat peanut meal at 60 °C for 672 h ($a_w = 0.21$ and $a_w = 0.35$), non-fat dry milk at 50 °C for 96 h ($a_w = 0.28$ and $a_w = 0.41$), wheat flour at 36 °C for 84 days ($a_w = 0.20$ and $a_w = 0.55$) and whey protein at 80 °C for 60 min ($a_w = 0.21$ and $a_w = 0.42$). Because δ values for data at distinct temperatures differed by several orders of magnitude, log δ were calculated. A plot of observed versus predicted log δ values (Fig. 5a) as well as a plot of observed versus predicted β values (Fig. 5b) including their corresponding correlation coefficients is presented in Fig. 5. Observed versus predicted *Salmonella* count values for all data are presented in Fig. 6. Additionally, Table 4 shows the correlation (R), % discrepancy (% D_f) and % bias (% B_f) values for predicted versus observed time required for first decimal reduction (δ), shape factor values (β) and *Salmonella* counts in the different food products used.

Data presented in Fig. 5a and the results in Table 4 (all data) indicate that the secondary model (Eq. (19)) provides a high correlation between observed versus predicted times required for first decimal reductions ($R = 0.97$, $p < 0.001$). The correlation of observed versus predicted shape factor values was not as satisfactory ($R = 0.03$, $p = 0.915$), with Eq. (20) both over and under predicting β values (Fig. 5b). Still, as seen

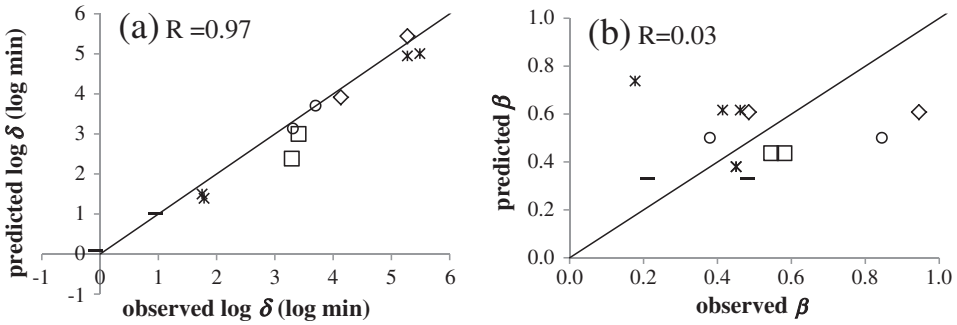

Fig. 5. *Salmonella* inactivation experiments at 6 T (°C), 5 water activities (a_w), 3 water mobilities (T_2^*, where mobility is measured in milliseconds) at each a_w, in 5 food products: (∗) low-fat cocoa powder, (□) low-fat peanut meal, (○) non-fat dry milk, (◊) wheat flour, (-) whey protein; (a) observed versus predicted time required for first decimal reduction (log δ); (b) observed versus predicted shape factor values (β).

in Fig. 6 and Table 4, a significant correlation ($R = 0.94$, $p < 0.001$) of observed versus predicted CFU values was obtained when using the developed secondary models to predict the survival of *Salmonella* in all tested food types. The degrees of discrepancy and bias found between the secondary predictive models and the data used to develop these models was found to be 16% discrepancy and −2% bias. A negative percent bias is indicative of a tendency of the models to underestimate survival numbers (even when using the data that derived the model). This underestimation followed from the degree to which the shape parameter (in Eq. (20)) deviated from the observed values and was more prominent at the lower CFU values. The extent to which the models underestimated the survival of *Salmonella* in the validation data is illustrated in Fig. 6. Data points which appear below the equivalence line are CFU values that have been underestimated and are consistent with the shape factor results in Fig. 5b. As seen in Table 4, the % bias and % accuracy factors showed a discrepancy of 41% and a bias of −7% for all validation data collected. These discrepancy and bias values differ from those inherent to the models (16% and −2%). However, the data collected in non-fat products including wheat flour, non-fat dry milk and whey protein powder (Table 4) gave 12% discrepancy and −3% bias. The bias and accuracy percentage results in non-fat food are within the error margin inherent to the models, and are an example of the consistency of the models in predicting survival data in non-fat foods. The higher discrepancy and bias percentages obtained for the whole dataset are the result of the higher discrepancy and bias percentages found for data in low-fat food products (which contain 12% fat). Table 4 shows low-fat products to have 50% discrepancy and −9% bias. These increased discrepancy and bias values seen in food containing low levels of fat are most probably due to the greater resistance of *Salmonella* in the presence of fat (Podolak et al., 2010). Despite the higher discrepancy and bias percentages seen when predicting *Salmonella* survival in products containing fat, the models still showed an overall acceptable prediction performance of 81% (for both non-fat and low-fat food). The prediction performance of the models when only data from non-fat food products was included increased by 8%. Both prediction performances (81% for all data and 88% for only non-fat data) showed that a high percentage of the residuals were within the acceptable fail safe and dangerous zone (−1 to 0.5 log CFU). In fact, even the prediction performance for low-fat food products showed an acceptable prediction rate of 79%.

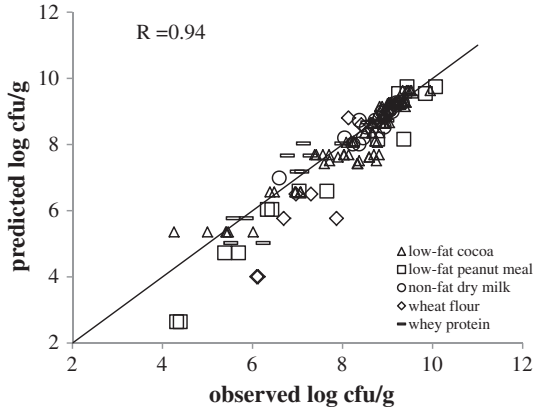

Fig. 6. Observed versus predicted *Salmonella* counts (log CFU/g) for validation experiments carried at 6 T (°C) and 5 water activities (a_w) in 5 food products.

The previously discussed results demonstrate the validity of the secondary models developed in this study to predict the survival of *Salmonella* in low-moisture foods at any given temperature and a_w within the data range evaluated. To the authors' knowledge, previously developed models for survival of *Salmonella* in low-moisture foods are those by Lambertini et al. (2012) and Danyluk et al. (2006) for use in risk assessment of *Salmonella* in almonds. These are models that assumed log-linear declines of *Salmonella* in almonds at three temperatures (−20, 4 and 24 °C). The models developed in this study represent the first predictive models developed for survival of *Salmonella* in low-moisture foods that are validated for temperatures 21–80 °C and $a_w < 0.6$. Because the data used to derive the models were collected by simulating how food may be contaminated and stored, the models are useful and credible for use in a wide range of products (Jaykus et al., 2006). The models will be useful for providing quantitative support for a hazard analysis and critical control point system (HACCP) (Zwietering and Nauta, 2007). The models can also be used in quantitative microbiological risk assessment to provide a more accurate risk quantification of *Salmonella* in low-moisture foods (Jaykus et al., 2006; Zwietering and Nauta, 2007). This will aid in developing policies for protecting the safety of consumers (Jaykus et al., 2006). It will also serve for confirmation of product adherence to a food safety objective (FSO) (Zwietering and Nauta, 2007). However, model predictions are not absolute, and decisions should not be based only on modeling (Zwietering and Nauta, 2007). In addition to quantitative data, qualitative and knowledge based information should be considered for an optimal risk management decision support system (McMeekin et al., 2006). The predictive models developed in this study will aid in the selection of appropriate strategies to decrease the risk of *Salmonella* in low-moisture foods.

4. Concluding remarks

Water activity significantly influenced the survival of *Salmonella* in low-moisture foods ($a_w < 0.6$) at temperatures ranging from 21 to 80 °C, while water mobility had no effect independent of a_w. The

Table 4
Correlation, discrepancy and bias between predicted and observed: time required for first decimal reduction values (δ), shape factor values (β) and *Salmonella* cfu validation counts according to product type.

Food product		R[a]	p-Value[b]	% D_f[c]	% B_f[d]
All	Pred vs obs[e]	0.94	<0.001	41	−7
(peanut meal, cocoa powder, wheat flour, whey protein, non-fat dry milk)	δ[f]	0.97	<0.001	–[g]	–
	β[h]	0.03	0.915	–	–
Low-fat[i]	Pred vs obs[e]	0.95	<0.001	50	−9
(peanut meal, cocoa powder)	δ	0.98	<0.001	–	–
	β	−0.74	0.058	–	–
Non-fat	Pred vs obs[e]	0.91	<0.001	12	−3
(wheat flour, whey protein, non-fat dry milk)	δ	1.00	<0.001	–	–
	β	0.60	0.208	–	–

[a] Calculated correlation statistic.
[b] Significance of the correlation test.
[c] Percent discrepancy.
[d] Percent bias.
[e] Predicted versus observed bacterial count values.
[f] Time required for first decimal reduction (Eq. (19)).
[g] Not applicable.
[h] Shape factor (Eq. (20)).
[i] The product contains 12% fat.

Weibull model provided the best description of survival kinetics for *Salmonella* survival in low-moisture foods. Secondary models were developed which predicted the time required for first decimal reduction (δ) and shape factor values (β) as influenced by temperature and a_w. These models were useful in predicting the survival of *Salmonella* in several tested low-moisture foods providing acceptable prediction performances. The models were more accurate in predicting the survival of *Salmonella* in non-fat food systems as compared to foods containing low-fat levels. These models provide baseline information to be used for research on risk mitigation strategies for low-moisture foods.

In future research, the models developed will be expanded to include fat content and other food components that may affect *Salmonella* survival. Available literature data on *Salmonella* survival studies in low-moisture foods will be incorporated into future validation studies. Future research will also include survival studies using different initial inoculum levels, different inoculation preparation methods and experiments to determine the effects of salt and sugar on survival kinetics.

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