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Effect of Challenge Temperature and Solute Type on Heat Tolerance of *Salmonella* Serovars at Low Water Activity

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***Salmonella* spp. are reported to have an increased heat tolerance at low water activity (a_w; measured by relative vapor pressure [rvp]), achieved either by drying or by incorporating solutes. Much of the published data, however, cover only a narrow treatment range and have been analyzed by assuming first-order death kinetics. In this study, the death of *Salmonella enterica* serovar Typhimurium DT104 when exposed to 54 combinations of temperature (55 to 80°C) and a_w (rvp 0.65 to 0.90, reduced using glucose-fructose) was investigated. The Weibull model ($\text{LogS} = -bt^n$) was used to describe microbial inactivation, and surface response models were developed to predict death rates for serovar Typhimurium at all points within the design surface. The models were evaluated with data generated by using six different *Salmonella* strains in place of serovar Typhimurium DT104 strain 30, two different solutes in place of glucose-fructose to reduce a_w, or six low-a_w foods artificially contaminated with *Salmonella* in place of the sugar broths. The data demonstrate that, at temperatures of ≥70°C, *Salmonella* cells at low a_w were more heat tolerant than those at a higher a_w but below 65°C the reverse was true. The same patterns were generated when sucrose (rvp 0.80 compared with 0.90) or NaCl (0.75 compared with 0.90) was used to reduce a_w, but the extent of the protection afforded varied with solute type. The predictions of thermal death rates in the low-a_w foods were usually fail-safe, but the few exceptions highlight the importance of validating models with specific foods that may have additional factors affecting survival.**

Salmonella enterica is an international food-borne pathogen, which regularly causes large outbreaks of food poisoning. Salmonellosis can be fatal, in addition to the significant morbidity caused, and the cost associated with infection can be very high. A recent study estimated that each case of salmonellosis in the United Kingdom costs approximately £600 ($1,000) on average, through costs to the health sector, direct costs to patients, and lost employment (4, 61). In addition, there are important implications for the food industry through recall of products and lost prestige and income (64).

Most outbreaks of salmonellosis have resulted from the consumption of contaminated meat, eggs, or dairy products, but some large international outbreaks have been associated with foods that have a low water activity (a_w; measured by relative vapor pressure [rvp]) (2, 20, 22, 37, 55). The presence of *Salmonella* cells in low-a_w foods raises specific issues for food safety. Outbreak investigations indicate that only a very few *Salmonella* cells may be required to cause disease when consumed in low-a_w foods (22, 53). In addition, it is widely believed that cells suspended at lower a_w during thermal inactivation are more heat tolerant than those suspended at a higher a_w (6, 14, 19, 21, 38, 58). This has clear implications for food processing when heating is used to ensure the elimination of potential food pathogens including *Salmonella*.

The *Salmonella* serotypes responsible for most human infection in the United Kingdom and the United States are *Salmonella enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium (1; Public Health Laboratory Service Communicable Disease Surveillance Centre for 1990 to 1999). Outbreaks associated with low-a_w foods, however, have been caused by a number of other serovars, such as *S. enterica* serovar Napoli, *S. enterica* serovar Agona, and *S. enterica* serovar Ealing, which were associated with chocolate, a chip-type snack, and infant dried milk, respectively (2, 20, 22, 37, 53, 55). It is interesting that a large proportion of low-a_w foods are snack foods, which are a common feature of the modern diet (15, 29). It is possible that increased consumption of these food types could result in more frequent outbreaks of salmonellosis in the future, unless appropriate management steps are taken.

Historically, the heat inactivation of populations of bacteria has been described using first-order kinetics, i.e., *D* values (18, 36, 59). This makes the assumption that all bacterial cells within a population have the same heat sensitivity, but significant deviations from linearity have been reported elsewhere (5, 9, 26, 33, 40, 60). In such cases, curve fitting will give more accurate descriptions of the data than will *D* values because shoulders or tailing can be incorporated. The modeling of complete data sets and the ability to predict inactivation kinetics for given combinations of factors provide an invaluable risk assessment tool, for example, in the initial stages of a novel product formulation or in process development.

Despite most data indicating that *Salmonella* cells are more heat tolerant when in low-a_w environments, there are reports describing exceptions to the general rule. The heat tolerance of serovar Typhimurium (65.5 to 75°C with a_w reduced using sugar [19]) and *S. enterica* serovar Anatum (50 to 54°C with a_w

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reduced using glycerol [25]) increased at intermediate a_ws but decreased at rvp's below 0.75. In addition, an increase in heat tolerance might be observed only when specific carbohydrates are used to reduce a_w (47). Clearly, further research is required in order to understand fully the heat tolerance of *Salmonella* spp. at low a_w. Thermal inactivation models are published elsewhere for *Salmonella* (52 to 59°C and high a_w [17] and 55 to 65°C and 0 to 9% NaCl [8]) and for *Escherichia coli* O157 (52 to 60°C and 8% NaCl [57], 54 to 68°C with 9 or 17% NaCl [11], and 55 to 65°C and 0 to 9% NaCl [8]). There is very little available information, however, on the inactivation of these pathogens at higher temperatures (65 to 80°C) in combination with lower a_w (rvp.0.65 to 0.90), particularly when the a_w is depressed using solutes other than NaCl. In addition, much of the existing data cover only a narrow range of treatments, were analyzed assuming first-order death kinetics, or were not subsequently evaluated in foods.

In this study, data on the inactivation of serovar Typhimurium definitive type 104 (DT104) at 54 combinations of a_w (rvp 0.65 to 0.90, reduced using glucose-fructose) and temperature (55 to 80°C) and six further *Salmonella* strains at a subset of these conditions were generated. Secondary models were developed for serovar Typhimurium DT104 strain 30 (62) such that inactivation curves could be predicted by interpolation for conditions not tested, and this was evaluated by using intermediate-moisture foods and different solutes to reduce a_w. This paper demonstrates that, while low a_w is protective for *Salmonella* at temperatures of >70°C, it promotes more rapid death at lower temperatures.

MATERIALS AND METHODS

***Salmonella* strains and preparation of cultures.** *Salmonella* serovar Typhimurium DT104 strain 30 (62) was selected for this study on the basis of its relative tolerance of high temperature and low a_w as separate stresses (32, 44). *Salmonella* serovar Typhimurium DT104 strain 16 (41), serovar Enteritidis PT4 strain E (27), and *S. enterica* serovar Senftenberg 775W (63) were selected on the basis of their stress tolerance. Serovar Napoli (20), serovar Agona (37), and *S. enterica* serovar Java (3) were selected to represent a wide range of serovars and relevant low-a_w sources. Bacterial strains were recovered from storage at −20°C, and stationary-phase cultures were prepared in tryptone soy broth (TSB; Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) as described previously (45).

Preparation of low-a_w (high-sugar) broths. Low-a_w TSB (pH 6.5; Oxoid Ltd.) was prepared as described previously (45) using AnalaR grades of glucose and fructose (in equal proportion) and sucrose or NaCl (BDH, Poole, Dorset, United Kingdom) as the humectants. The a_w of the broth was adjusted by adding TSB (rvp 0.99: pH 6.5) to give rvp values between 0.65 and 0.90 and measured at 25°C using an Aqualab CX-3T (Labcell, Basingstoke, Hampshire, United Kingdom) water activity meter with an accuracy of ±0.003. Note that values for rvp and a_w are identical in very dilute solutions, but the term a_w is defined in terms of ideal equilibrium solutions, and the high-solute broths used in this study are too concentrated to approach ideal behavior.

Increased survival of *S. enterica* serovar Anatum during heat challenge in TSB compared with that in phosphate buffer has been reported elsewhere (47); therefore, TSB was used in order to produce as fail-safe a model as possible. There was no significant change in pH during heat challenge (data not shown).

Measurement of death rates at high temperature. One hundred fifty microliters of a stationary-phase *Salmonella* culture was inoculated into 15 ml of low-a_w TSB, giving an initial cell density of approximately 10^7 CFU ml^{-1}. To allow *Salmonella* cells to adapt to the low-a_w environment (as would occur in a low-a_w food ingredient prior to heat processing), cells were held at 21°C for 1 h prior to heat challenge. On some occasions, control cells were heat challenged immediately, in order to establish the possible effect of the 1-h pretreatment. Cultures were heat challenged as described previously (45) using a submerged heating coil (12). Strain 30 was challenged at 55, 60, 65, 70, 72, 74, 76, 78, and 80°C and rvp's of 0.65, 0.70, 0.75, 0.80, 0.85, and 0.90 (54 combinations), whereas the other *Salmonella* strains were challenged at 60, 65, and 72°C and rvp's of 0.65, 0.80, and 0.90 (9 combinations). Dilutions were made in maximal recovery diluent (Oxoid), and viable counts were performed using the method of Miles and Misra (46) with plating onto blood agar and incubation for 48 h at 37°C to ensure optimal recovery of injured cells that might have had prolonged lag periods (30).

To investigate the effect of solute used to reduce a_w, strain 30 was heat challenged at 55, 60, and 74°C at low a_w achieved using NaCl and sucrose. The lowest achievable a_w prior to saturation was rvp 0.75 for NaCl and rvp 0.80 for sucrose. These values were compared with inactivation at rvp 0.90 achieved using each solute. There was no 1-h pretreatment prior to heat challenge, since the resulting degree of survival and injury could vary considerably with solute (44).

Evaluation of the models in foods. The models were evaluated in six low- or intermediate-moisture foods (coconut cake, pecorino cheese, pepperoni sausage, strawberry jam, dried apricots, and peanut butter). These experiments were performed in New Jersey, using foods purchased in a local retail store, whereas data for the broth model were generated in the United Kingdom.

Each food was homogenized by blending. The rvp was measured using an earlier model (CX-2) of the meter described above (Decagon Devices Inc., Pullman, Wash.). The pH was measured three times using a pH meter (Corning Inc., Corning, N.Y.), and a mean value was calculated. No attempt was made to adjust the natural pH of the food. Aliquots (2 g) of the food were added to each of 10 bags which were inoculated directly into the base with 20 μl of a stationary-phase culture of DT104 strain 30, giving an initial cell density of approximately 10^7 CFU ml^{-1}. The food was mixed, spread thinly along the bottom of the bag, and allowed to stand for 1 h at 21°C. One bag was removed to a water bath at 21°C as a time zero sample, and the remainder were weighted and suspended in a preheated water bath, such that the sample was >15 cm below the water level. The required challenge temperature was achieved in less than 5 s. Bags were removed at predetermined time intervals into the 21°C water bath. Tenfold dilutions of the foods were made by adding 18 ml of diluent to the bags, and further 10-fold dilutions were made in a microtiter plate, as described above. Dilutions were plated onto blood agar and xylose lysine desoxycholate agar, with plate incubation for 48 h at 37 °C. There was minimal background flora observed from any of the foods on blood agar following incubation, and *Salmonella* bacteria were enumerated from these plates, since selective xylose lysine desoxycholate agar did not adequately recover injured cells.

Statistical analysis, curve fitting, and data modeling. A minimum of three replicate trials for each combination of temperature, rvp, serovar, and solute was performed. The data were analyzed in Excel. The raw data (CFU $milliliter^{-1}$) were converted into the $\log_{10}$ of the surviving fraction of bacteria (LogS) at a given time, *t*.

(i) Curve fitting. Curves were fitted using the Weibull model, $\text{LogS} = -bt^n$, where LogS is the $\log_{10}$ of survival ratio at time *t*, and *b* and *n* are the scale and shape parameters, respectively (49). The Weibull model can describe linear inactivations or curves with shoulders or tailing and is mathematically simple. Values for *b* and *n* were derived for each inactivation curve in the Solver function of Excel, aiming to minimize the sum of the squares of LogS (observed) minus LogS (predicted). Curves were fitted by instructing Solver to minimize the residual sum of squares by iteratively changing *b* and *n*. The convergence criterion was set to 1×10^{-9}, and the nonnegative option was selected. Default settings for other options were used. Note that Solver will occasionally fail to converge and an error message will occur to this effect. This can be overcome by substituting some small positive value (e.g., 0.000001) for the first (i.e., zero) time point. The derived values for *b* and *n* were used to predict the time required to obtain a 3-$\log_{10}$ or 5-$\log_{10}$ reduction in cell concentration.

When the death curves dropped below the detection threshold of the experiment (20 CFU ml^{-1}), a value of 5 was used for the first censored observation only, with further censored data being excluded. This value was chosen since there was no significant difference between the fitted *b* and *n* values when using censored observations of 5 or 20 (data not shown) and because the square root of the threshold value (in this case close to 5) has been used previously (Keith Jewell, personal communication).

To validate the use of Excel Solver for curve fitting, 10 individual data sets were fitted using both Solver and the nonlinear regression module of Statistica (a statistical analysis package; Statsoft, Tulsa, Okla.), and these gave reasonable agreement ($P < 0.05$).

(ii) Response surface modeling. To simplify the numerical computation and make the regression coefficients more comparable, the experimental variables rvp and temperature were first normalized as follows:

$$\begin{aligned} \text{rvpn} &= [\text{rvp} - \text{mean (rvp)}]/\text{standard deviation} \\ &= [\text{rvp} - 0.775]/0.094 \end{aligned}$$

$$\begin{aligned} \text{tn} &= [\text{temperature} - \text{mean (temperature)}]/\text{standard deviation} \\ &= [\text{temperature} - 70.0]/8.441 \end{aligned}$$

TABLE 1. Regression coefficient estimates for b_{trans} ($R^2 = 0.9672$) and n ($R^2 = 0.5219$)

Variable	b_{trans}		n	
	Coefficient	P value	Coefficient	P value
Intercept	1.0423	<0.0001	0.7337	<0.0001
tn	0.4519	<0.0001	−0.1518	<0.0001
rvpn	0.0391	0.0018	−0.0038	0.8785
tn × rvpn	0.1057	<0.0001	−0.1294	<0.0001
tn^2	0.0691	<0.0001	−0.0662	0.0261
$rvpn^2$	−0.0014	0.9257	−0.0054	0.8666

where rvpn and tn are normalized rvp and normalized temperature, respectively.

Inspection of frequency plots of b and n (data not shown) showed that the distribution of n was approximately normal but that of b was highly skewed. A high degree of skewness indicates that the variable should be transformed before regression analysis, in order to improve the goodness of fit. A Box-Cox transformation was used, in this case a power transformation such that $b_{trans} = b^{0.3}$.

Multiple regression analysis was performed using SAS software (SAS Institute, Cary, N.C.) to estimate the response of b_{trans} and n to rvpn and tn. All main effects, interactions, and quadratic terms were included, and a table of regression coefficient estimates and P values was prepared (Table 1). The relative importance of each factor can be judged by the P value, with factors with small P values being most influential and predictive of the response variable.

RESULTS

Inactivation of DT104 strain 30 at high temperature and low a_w (achieved using glucose-fructose). The semilogarithmic inactivation curves were nonlinear but could be described by the Weibull model. The inactivation curve for each set of conditions can be derived by substituting the values for b and n from Tables 2 and 3 into the Weibull equation. Values for n were usually <1, indicating that the curvature was concave upwards, with distinct tailing during inactivation (Table 2). Values for b also showed clear trends (Table 3). For any given a_w, b increased from 55 to 80°C. That is, the slope of the curve became steeper, indicating that there was faster death at the higher temperatures (as expected). At 55 and 60°C, b tended to be higher at the lower a_w tested, indicating that cells died faster when exposed to the dual stress of temperature and low a_w. At temperatures of ≥70°C, however, the b value was greater at rvp 0.90 than at rvp 0.65, and this was very pronounced at high temperature. At 80°C, for example, the b value was 7.11 at rvp 0.90 whereas it was 3.63 at rvp 0.65 (Table 3). This indicates that, at high temperature, low a_w protected the cell against thermal death (Fig. 1) with a longer time to obtain a 3-$\log_{10}$ reduction (Fig. 2). The resulting models for b and n were

TABLE 2. Mean values of n (representing curvature in the Weibull model) derived from experimental data observations for the inactivation of serovar Typhimurium DT104 at 54 combinations of a_w and temperature

a_w (rvp)	Temp (°C)								
	55	60	65	70	72	74	76	78	80
0.65	0.72	0.55	0.82	0.71	0.76	0.85	0.76	0.35	0.50
0.70	0.60	0.69	0.70	0.64	0.83	0.70	0.81	0.57	0.73
0.75	0.61	0.56	0.78	0.67	0.65	1.06	0.80	0.45	0.24
0.80	0.59	0.72	0.73	0.63	0.85	0.76	0.55	0.45	0.49
0.85	1.16	0.89	0.72	0.62	1.07	0.67	0.56	0.42	0.53
0.90	1.26	1.30	0.79	0.50	0.63	0.35	0.34	0.27	0.24

TABLE 3. Mean values of b (representing rate of inactivation in the Weibull model) derived from experimental data observations for serovar Typhimurium DT104 at 54 combinations of a_w and temperature

a_w (rvp)	Temp (°C)								
	55	60	65	70	72	74	76	78	80
0.65	0.15	0.38	0.36	0.94	1.26	1.60	1.87	2.77	3.63
0.70	0.20	0.21	0.47	1.03	1.31	2.03	2.23	3.89	5.78
0.75	0.18	0.40	0.42	1.06	1.82	1.45	2.56	3.96	4.97
0.80	0.15	0.19	0.39	1.22	1.80	2.47	3.52	4.57	5.36
0.85	0.01	0.07	0.41	1.20	1.44	2.61	3.51	4.41	5.22
0.90	0.01	0.02	0.45	1.77	3.17	4.00	4.82	7.21	7.11

$$b = (1.0412 + 0.452\text{tn} + 0.039\text{rvpn} + 0.106\text{tn} \times \text{rvpn} + 0.069\text{tn}^2 - 0.001\text{rvpn}^2)^{(1/0.3)}$$

$$n = 0.734 - 0.152\text{tn} - 0.004\text{rvpn} - 0.129\text{tn} \times \text{rvpn} - 0.066\text{tn}^2 - 0.005\text{rvpn}^2$$

These equations can be used to derive b and n for any given conditions of (normalized) temperature and rvp in the model range. Three-dimensional representations of these models are given in Fig. 3. For both models, normal probability plots of residuals were inspected and no significant deviations from normality were observed (data not shown). The models were used to predict b and n values for all conditions in the experimental design matrix. The predicted values of b and n were substituted into the Weibull equation and used to predict survival curves for all treatments. Observed values were compared

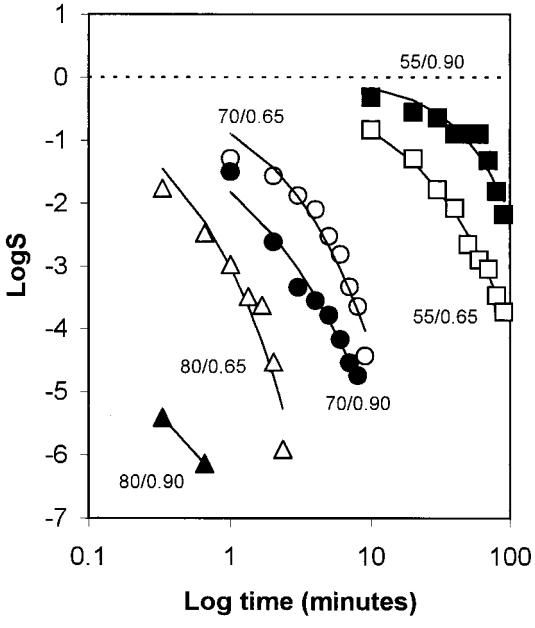

FIG. 1. Graph of the log surviving serovar Typhimurium DT104 at 55°C (squares), 70°C (circles), and 80°C (triangles) and rvp 0.65 (open symbols) or 0.90 (closed symbols) plotted against log time in minutes, demonstrating the effect of the two water activities on the heat tolerance at three temperatures. The dashed line represents the initial CFU of *Salmonella* $milliliter^{-1}$.

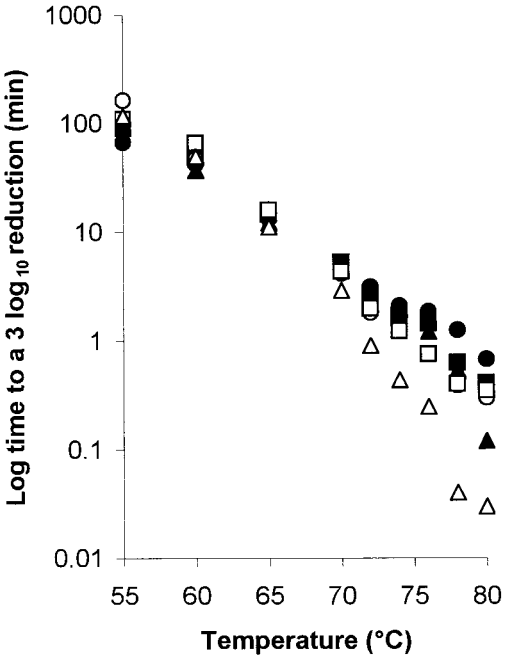

FIG. 2. Graph of the $\log_{10}$ time to obtain a 3-$\log_{10}$ reduction in the concentration of serovar Typhimurium DT104 for each a_w (rvp 0.65 [closed circle], 0.70 [closed square], 0.75 [closed triangle], 0.80 [open circle], 0.85 [open square], and 0.90 [open triangle]) against the challenge temperature, demonstrating that the protective effect of low water activity is apparent only at temperatures of ≥70°C.

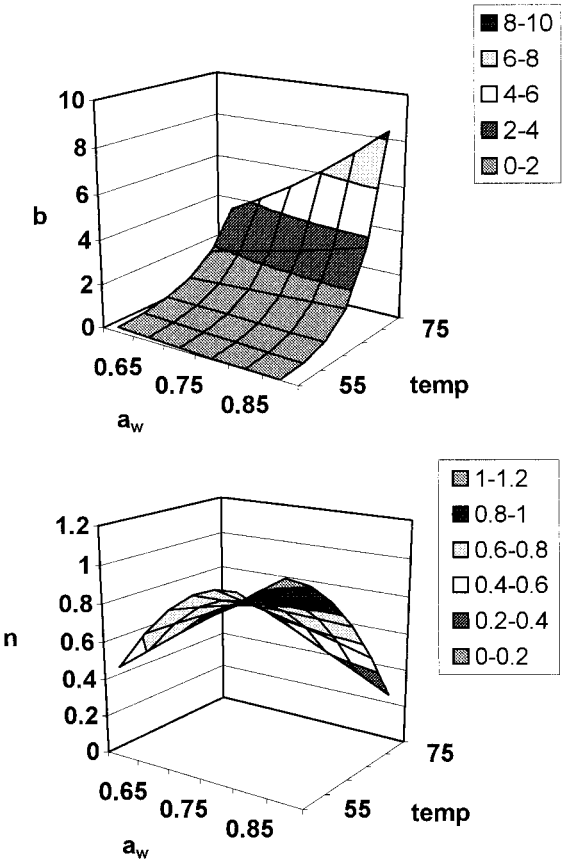

FIG. 3. Three-dimensional representation of the b-a_w-temperature relationship (top) and the n-a_w-temperature relationship (bottom), demonstrating the effect of a_w on the survival of high temperature by serovar Typhimurium DT104.

with these predicted survival curves and compared favorably, and a plot of observed and predicted time to obtain a 3-$\log_{10}$ reduction in cell concentration showed good agreement (Fig. 4).

Incubation at low a_w (achieved using glucose-fructose) for 1 h at 21°C had no effect on the thermal death of serovar Typhimurium DT104 strain 30 (data not shown), and therefore data for the effect of solute type can be compared directly.

Heat tolerance of different *Salmonella* strains at low a_w (achieved using glucose-fructose). The inactivation curve for each set of conditions can be derived by substituting the values for b and n from Tables 4 and 5 into the Weibull equation. With the other *Salmonella* strains tested, the time to obtain a 3-$\log_{10}$ reduction at 60°C was always lower at rvp 0.65 than at rvp 0.90 but at 72°C the opposite was observed. No clear trends in heat tolerance at 65°C were observed, presumably because this is the approximate temperature at which the reversal in effect of low a_w occurs. For example, the slowest death was at rvp 0.65 for serovar Typhimurium strain 16; rvp 0.80 for serovar Typhimurium strain 30, serovar Agona, serovar Java, and serovar Senftenberg 775W; and rvp 0.90 for serovar Enteritidis strain E.

Overall, serovar Senftenberg 775W, serovar Java, and serovar Agona were the least heat-tolerant isolates for the a_w range tested. Of the *Salmonella* strains from outbreaks, only serovar Napoli showed heat tolerance similar to that of serovar Typhimurium and serovar Enteritidis. *Salmonella* serovar Enteritidis was always relatively heat sensitive at rvp 0.65 but was one of the most heat-tolerant strains at rvp 0.90. For each inactivation with $n < 1$, an increase in time to obtain a 3-$\log_{10}$ reduction was usually associated with an increase in n value, such that it was closer to 1 (Table 8). In other words, increased heat tolerance of *Salmonella* was usually associated with more linear death kinetics. For all treatments, higher n values were usually

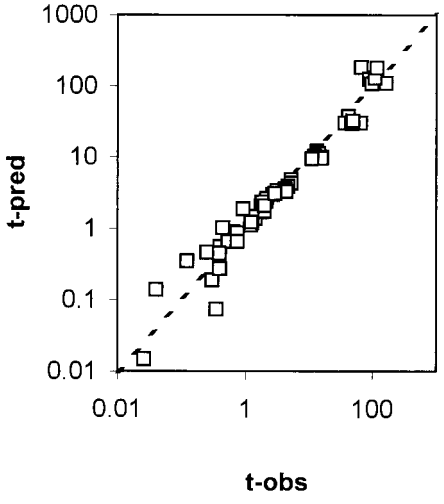

FIG. 4. Plot of observed (from experimental data) and predicted (using the models generated using the experimental data) time to obtain a 3-$\log_{10}$ reduction in cell concentration.

TABLE 4. Values for n in the equation $\text{LogS} = -b \times t^n$, when used to describe inactivation curves for *Salmonella* serovars exposed to high temperature and low a_w[a]

Serovar and/or strain	Value for n at heat challenge temp and water activity (rvp)								
	60°C			65°C			72°C		
	0.65	0.80	0.90	0.65	0.80	0.90	0.65	0.80	0.90
Typhimurium DT104 strain 30	0.55	0.72	1.30	0.82	0.73	0.79	0.76	0.85	0.63
Typhimurium DT104 strain 16	0.91	0.68	1.42	1.01	0.95	0.88	0.49	0.72	0.42
Enteritidis PT4 strain E	0.37	0.56	0.82	0.48	0.83	1.12	0.62	0.59	0.45
Napoli	0.85	0.87	1.44	1.19	0.76	1.05	1.07	1.01	0.44
Agona	0.68	0.46	0.59	0.60	0.72	0.51	0.90	0.64	0.33
Java	0.60	0.67	0.56	0.51	0.66	0.52	0.90	0.68	0.45
Senftenberg 775W	0.47	0.63	0.54	0.43	0.68	0.65	0.53	0.74	0.46

[a] If $n = 1$, then the inactivation is a straight line (when plotted as LogS versus t); when $n < 1$, then tailing is observed; when $n > 1$, the curve has a shoulder.

associated with the more heat-tolerant *Salmonella* serovars (serovar Typhimurium, serovar Enteritidis, and serovar Napoli).

Effect of solute type on heat tolerance of serovar Typhimurium DT104 strain 30. Use of sucrose and NaCl to reduce the a_w of the challenge broth revealed that the temperature-dependent effects of low a_w were still observed when using these solutes in place of glucose-fructose but that the extent of the effects varied with solute type (Table 6).

At 55 and 60°C, the presence of NaCl in the challenge broth with an a_w close to saturation (rvp 0.75) was detrimental compared with the effect at a higher a_w (rvp 0.90; $P = 0.03$ and 0.05, respectively). At 68°C, there was no difference in death rate at the two a_ws tested ($P = 0.79$). At 70 and 72°C, the lower a_w gave marginal protection ($P = 0.20$ and 0.47, respectively), and at 74°C, cell death was too rapid for accurate measurements to be taken. At 55°C, a broth containing sucrose (rvp 0.80) was detrimental to heat tolerance compared with the effect of a higher a_w (rvp 0.90; $P = 0.04$); at 60°C, there was no difference in effect; and at 74°C, significant protection was observed ($P = 0.003$). Sucrose was more protective at all temperatures than was glucose-fructose, which in turn was more protective than NaCl (Table 6). The difference in observed death rates at 55°C and rvp 0.90 between use of sucrose and use of NaCl to reduce a_w was nearly 20-fold.

Evaluation of the thermal inactivation models using food products. With pecorino cheese, pepperoni sausage, strawberry jam, and dried apricots, death occurred at the rate predicted by the models or higher at each temperature (Table 7), indicating that the models gave conservative (fail-safe) predictions for these foods. The discrepancies between the observed and predicted times to a 3-$\log_{10}$ reduction are probably the result of variations in pH, fat content, and other factors between the foods and the sugar broths.

In coconut cake and peanut butter, however, *Salmonella* sometimes survived for longer than predicted (Table 7). The predicted and observed times to achieve a 3-$\log_{10}$ reduction were <2-fold different at each temperature in coconut cake but >100-fold different at 55°C in peanut butter. The peanut butter had an rvp that was significantly below the intended range of the model, and these data confirm that extrapolating a model far beyond its intended range should be avoided.

The a_w and pH of the six low- and intermediate-moisture foods are given in Table 7. In strawberry jam, a 1- to 2-$\log_{10}$ decrease in cell concentration was observed during the 1-h pretreatment at 21°C ($P = 0.00001$), presumably due to the very low pH, but in all other foods there was no significant change in cell concentration during pretreatment.

DISCUSSION

Much of the thermal inactivation data generated in this study showed significant tailing, particularly at the higher temperatures tested, and this is consistent with published data for other bacteria under similar conditions (60). Since linear descriptions of cellular death would not accurately describe the data, curves were fitted to the inactivation data using the Weibull model (49). Other investigators have used the logistic (13) and Gompertz (10, 43) models and other models (52).

A polynomial function derived by multiple regression anal-

TABLE 5. Values for b in the equation $\text{LogS} = -b \times t^n$, when used to describe inactivation curves for *Salmonella* serovars exposed to high temperature and low a_w

Serovar and/or strain	Value for b at heat challenge temp and water activity (rvp)								
	60°C			65°C			72°C		
	0.65	0.80	0.90	0.65	0.80	0.90	0.65	0.80	0.90
Typhimurium DT104 strain 30	0.38	0.19	0.02	0.36	0.39	0.45	1.26	1.80	3.17
Typhimurium DT104 strain 16	0.09	0.23	0.01	0.19	0.23	0.29	0.17	0.10	0.54
Enteritidis PT4 strain E	0.99	0.41	0.13	1.24	0.33	0.14	0.16	0.21	0.52
Napoli	0.15	0.10	0.01	0.15	0.55	0.22	0.01	0.02	0.44
Agona	0.32	0.70	0.40	0.95	0.58	1.18	0.03	0.20	0.95
Java	0.42	0.23	0.45	1.20	0.55	1.04	0.02	0.14	0.47
Senftenberg 775W	0.68	0.32	0.52	1.46	0.54	0.78	0.27	0.11	0.52

TABLE 6. Effect of inactivation of serovar Typhimurium DT104 strain 30 at low a_w (achieved using sucrose and NaCl), measured as the time in minutes to achieve a 3-$\log_{10}$ reduction in cell concentration (standard error)

Humectant	Temp (°C)	Value at rvp:			Effect of low a_w	*P*
		0.75	0.80	0.90		
NaCl	55	23.5 (2.9)		35.5 (2.0)	Detrimental	0.03
	60	11.1 (3.2)		13.9 (2.2)	Detrimental	0.05
	68	0.74 (0.05)[a]		0.76 (0.08)[a]	No effect	0.79
	70	0.40 (0.02)[a]		0.32 (0.05)[a]	Protective	0.20
	72	0.38 (0.05)[a]		0.22 (0.00)[a]	Protective	0.47
Sucrose	55		198 (20)	682 (170)	Detrimental	0.04
	60		56.4 (16)	56.3 (4.0)	No effect	1.00
	74		1.91 (0.04)	0.97 (0.06)	Protective	0.003
Glucose-fructose	55	92.3 (21)	198 (67)	144 (28)	Detrimental	
	60	38.9 (7.1)	48.0 (5.4)	52.4 (5.3)	Detrimental	
	74	2.01 (0.17)	1.43 (0.37)	0.71 (0.03)	Protective	

[a] Time to a 5-log decrease given due to rapid death.

ysis was used for the secondary inactivation models, in common with previous researchers (13, 34), whereas others have used Arrhenius-Eyring (51) and linear Arrhenius-Davey (16) models. By modeling a response surface, reliable predictions of thermal inactivation under conditions that have not been tested but are within the range of the experimental matrix can be generated by interpolation. The secondary models were produced with data generated using serovar Typhimurium DT104 strain 30, and to confirm the main observations of the models, six further strains of *Salmonella* were analyzed at a subset of conditions. These were serovar Typhimurium DT104 strain 16, serovar Enteritidis (reported elsewhere to be relatively tolerant to low a_w [44]), serovar Senftenberg 775W (reported to be more heat tolerant than other *Salmonella* strains at high a_w [23, 40, 48]), and three outbreak strains (serovars Napoli, Agona, and Java).

Existing inactivation data for *Salmonella* and related organisms at high temperature and low a_w were compared to the data from this study. Gibson (19) presented *D* values for heat tolerance (55 to 70°C) of serovar Typhimurium and serovar Senftenberg at rvp 0.71 to 0.99 (reduced using sucrose, adding glucose for rvp's of <0.85). Gibson's work gave similar results for five comparable combinations of temperature and rvp, but at rvp 0.90 and 60°C our data indicated approximately fourfold-higher heat tolerance, using glucose-fructose in place of sucrose. Sumner et al. (58) looked at sucrose solutions (rvp 0.83 to 0.98) with 3-h osmotic equilibration prior to heat challenge at 66 to 77°C. Results were comparable at 74°C and 0.90, but at rvp <0.90 or a temperature of <74°C our data indicated a lower level of heat tolerance, using glucose-fructose in place of sucrose.

All *Salmonella* strains tested demonstrated that low a_w (rvp 0.65 compared with 0.90) was detrimental to survival at 55 or 60°C, whereas at ≥70°C the lower a_w was always protective. The most heat-tolerant serovars over the range of conditions tested were serovar Typhimurium DT104, serovar Enteritidis

TABLE 7. Survival of serovar Typhimurium DT104 in sugar solutions and in low a_w foods at 55 to 74°C, expressed as the time to obtain a 3-$\log_{10}$ decrease in cell concentration

Food	Challenge temp (°C)	Death rate predicted in sugar solution			Death rate observed in foods		
		a_w	pH	Time to obtain a 3-log decrease (min)	a_w	pH	Time to obtain a 3-log decrease (min)
Pecorino cheese	55	0.87	6.5	143	0.87	5.7	10
	65			9.5			0.7
	74			1.1			0.8
Coconut cake	55	0.86	6.5	67[a]	0.86	6.2	111[a]
	65			9.5			18
	74			1.2			0.7
Pepperoni sausage	55	0.84	6.5	123	0.84	5.2	13
	65			9.6			1
	74			1.3			0.7
Strawberry jam	55	0.82	6.5	114	0.82	3.0	15
	65			9.7			3
	74			1.4			0.3
Dried apricots	55	0.64	6.5	206	0.64	4.0	44
	65			12			6
	74			2.5			1
Peanut butter	55	0.50[b]	6.5	13,679[a]	0.50[b]	6.1	98[a]
	65			5.6[a]			24[a]
	74			1.7[a]			6[a]

[a] Where the fall in bacterial concentration was less than 3 $\log_{10}$, the time to decrease the population by 1.5 $\log_{10}$ is given.
[b] Prediction for a food with an a_w well outside the range of the model.

TABLE 8. Effect of reduced water activity on the heat tolerance of *Salmonella* strains, measured as the time in minutes to obtain a 3-$\log_{10}$ reduction (standard error)

Serovar and/or strain	Value at heat challenge temp (°C) and water activity (rvp)								
	60°C			65°C			72°C		
	0.65	0.80	0.90	0.65	0.80	0.90	0.65	0.80	0.90
Typhimurium DT104 strain 30	45 (6)	48 (6)	49 (3)	13 (1)	16 (2)	11 (1)	3.5 (0.6)	1.9 (0.3)	0.93 (0.1)
Typhimurium DT104 strain 16	47 (6)	43 (5)	49 (1)	15 (1)	13 (2)	13 (1)	4.0 (0.5)	1.8 (0.1)	1.0 (0.1)
Enteritidis PT4 strain E	20 (5)	33 (4)	44 (0.8)	6.4 (0.4)	14 (2)	15 (1)	1.9 (0.2)	1.5 (0.3)	0.78 (0.0)
Napoli	35 (1)	50 (4)	50 (1)	12 (0.7)	9.2 (0.5)	12 (0.1)	3.9 (0.2)	2.3 (0.0)	1.4 (0.1)
Agona	28 (1)	23 (7)	32 (5)	7.0 (0.4)	9.7 (0.3)	6.1 (0.7)	2.5 (0.1)	1.2 (0.2)	0.52 (0.0)
Java	27 (1)	47 (2)	28 (0.8)	5.6 (0.2)	13 (0.4)	7.7 (0.3)	3.5 (0.3)	1.6 (0.2)	1.0 (0.3)
Senftenberg 775W	24 (1)	35 (2)	27 (0.9)	5.4 (0.4)	12 (0.3)	8.2 (0.3)	1.5 (0.3)	1.3 (0.0)	0.77 (0.0)

PT4, and serovar Napoli. Strains isolated from outbreaks associated with low-a_w foods did not appear to be more heat tolerant at low a_w than did other strains. This indicates that *Salmonella* strains from outbreaks associated with low-a_w foods may not have particular characteristics promoting their survival during heat processing and subsequent storage in low-a_w foods but that their characteristics may instead relate to the contamination source. The temperature-dependent effect of low a_w on heat tolerance was independent of the solute type used to reduce a_w, although the extent of protection afforded did vary. Sucrose was generally more protective than was glucose-fructose, which in turn resulted in significantly lower thermal death rates than with NaCl at all temperatures tested.

Most published reports indicate that the heat tolerance of *Salmonella* increases as the a_w decreases (14, 21, 38, 58), but a small number of reports indicate that heat tolerance of *Salmonella* may increase or decrease at low a_w (6, 19, 25). We propose that the temperature-dependent effects of low a_w on the heat tolerance of *Salmonella* reflect different targets for death at low temperatures than at high temperatures. The high-temperature target(s) appears to be protected by low a_w, perhaps through improved stability of proteins, reduced mobility of water, or the direct effects of solutes, whereas the lower-temperature target(s) is clearly not protected by low a_w. For example, the high-temperature targets could be the ribosomes, since it is reported that their heat stability is increased at low a_w (56). Air-dried cells, as well as those suspended at low a_w, exhibit increased tolerance (38); therefore, general dehydration probably gives rise to the observed increased heat tolerance at the higher temperatures tested. Gibson stated that proteins and other macromolecules are more stable in the dry state (19). Corry studied the turbidity of serovar Typhimurium as a measure of the degree of plasmolysis, and this correlated with the degree of protection afforded by the high concentration of solutes during heating at 65°C (14).

Published data indicate that serovar Senftenberg strain 775W is significantly more heat tolerant than are most other *Salmonella* strains at optimal a_w (rvp 0.995) but not at low a_w (6, 19, 21). Our data confirm that serovar Senftenberg strain 775W was relatively heat sensitive over the rvp range 0.65 to 0.90. A disparity between the behavior of strain 775W and that of serovar Typhimurium was reported previously, with the heat tolerance of 775W being virtually unaffected by reducing the a_w (rvp 0.94 to 0.997 [24]). These data indicate that there is an unusual interaction between heat tolerance and a_w in this strain. They also show clearly that serovar Senftenberg strain 775W is not an appropriate strain to estimate the efficacy of thermal processes for low-a_w foods.

Validation of the models was performed with six retail foods, selected to represent a wide range of low- and intermediate-moisture food types. In addition, some of the foods had particular properties that could increase the heat tolerance of *Salmonella*. In other words, the foods were selected to challenge the ability of the model to produce fail-safe predictions of thermal inactivation. Pepperoni sausage was used since it has been demonstrated that *Salmonella* strains attached to muscle tissue may be more resistant to heat than strains that are not (28). Coconut cake was chosen since desiccated coconut has previously been associated with outbreaks of *Salmonella* (3, 7). Peanut butter has a high fat content, which has been reported elsewhere to protect microorganisms against high temperature (33, 42, 54), although other reports are less conclusive (35, 39).

Salmonella died more quickly in pecorino cheese, pepperoni sausage, strawberry jam, and dried apricot than would be predicted by the broth models. This indicates that predictions are fail-safe, as is required in order to design safe processes. The more rapid death observed is likely to be due partly to the additional stress of low pH in some of the foods, compared to the model predictions based on data generated at pH 6.5. Unfortunately there is currently no model available to generate predictions for relevant combinations of high temperature, low a_w, and low pH. Predicted inactivations for coconut cake (55 and 65°C) and peanut butter (65 and 74°C), however, were more rapid than those actually observed in the foods. The predicted and observed times to achieve a 3-$\log_{10}$ reduction differed by approximately twofold in coconut cake. With peanut butter (rvp 0.50), however, death was far slower than predicted by the model, and this highlights the dangers associated with extrapolating a predictive model beyond its intended range. The peanut butter had a relatively neutral pH and a very high fat content (53% [wt/wt]), and these factors may account for the differences seen.

It is clearly important to evaluate laboratory-based models with real foods, since the individual properties of foods will have a great effect on the survival of microorganisms within foods. Other researchers have demonstrated increased heat tolerance of microorganisms in foods compared with that predicted in broth models (31, 50). In addition, the food validation studies reported here indicate that pH is an important factor

influencing the survival of *Salmonella* at low a_w when exposed to heat, and this requires further investigation.

In conclusion, the greater heat sensitivity at low a_w (rvp 0.65 compared with 0.90) at the lower inactivation temperatures (55 and 60°C) could have implications for food process design, development of new food formulations, and risk assessment. It is clearly important that thermal processes for low-a_w foods are designed using thermal inactivation data generated in low-a_w systems. Therefore, it is hoped that these data will make a positive contribution to food safety for manufacturers of low-a_w foods whose process involves a heat treatment step.

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