

Model for the survival of *Staphylococcus aureus* in nongrowth environments¹

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Abstract

A model was developed to estimate the survival times of *Staphylococcus aureus* in nongrowth environments. A four strain mixture of *S. aureus* was inoculated into BHI broth that had a lactate buffer with various combinations of pH (3–7) and lactate (0–1%), NaCl (0.5–20%) and NaNO₂ (0–200 ppm) and stored at different temperatures (4–42°C). At appropriate times the survivors were enumerated by sampling and spreading on TSA plates. The survival curves were modeled with two forms of a logistic equation and the *D* values were determined. Polynomial regression equations were then calculated to predict the effect of the environmental factors on the *D* values. Survival times were increased with higher pH values, lower temperatures, and lower nitrite and lactate concentrations. Added salt increased survival times until the salt concentrations exceeded that of most foods.

Keywords: Temperature; pH; Salt; NaCl; Lactic acid; Nitrite; Inactivation; Foodborne pathogens

1. Introduction

Staphylococcus aureus originating from raw materials, environment/equipment and food handlers can contaminate many foods (Bergdoll, 1989). In uncooked, refrigerated and shelf stable foods, the intrinsic properties of the food (pH, salt) and temperature must interact to prevent growth or preferably promote inactivation of the pathogen. Although *S. aureus* must grow to approximately 10^5 CFU/g to produce toxin and cause illness (Bergdoll, 1989), it can survive for extended periods under conditions where growth is inhibited. Many outbreaks then result from cross contamination to a growth-permitting food or subsequent temperature abuse. Therefore, quantitative information on the factors that permit survival is needed. *S. aureus* viability was determined in cottage cheese whey (Westhoff and Engler, 1973), Domaiti cheese whey (Ahmed et al., 1983), intermediate-moisture meats (Plitman et al., 1973; Kotzekidou and Lazarides, 1991), beef jerky (Holley, 1985), fermented poultry (Raccach and Baker, 1979), pork sausage (Petchsing and Woodburn, 1990), and nonfermented snack sausage (Smith et al., 1977). However, it is desirable to have a comprehensive understanding of the influences environmental factors have on the microorganism in order to quantitatively estimate its survival without requiring extensive and long-term inoculated pack studies for each food formulation.

Models for inactivation/survival of *Listeria monocytogenes* and *Salmonella* were presented by Buchanan et al. (1993, 1994) and Whiting (1993). Under conditions of non-thermal inactivation/survival, these pathogens frequently exhibited a period of survival followed by a first-order decrease. In some conditions a long-lived subpopulation was also observed. Generally survival times were increased with decreased temperatures, increased pH, and lowered levels of lactate or nitrite anions. Salt concentrations up to 8% increased the survival times of *L. monocytogenes* and had little effect on *Salmonella* survival (Whiting, 1993). This paper reports the first survival models for *S. aureus* with temperature, pH, lactate, NaCl, and NaNO₂ being the modeled environmental factors.

2. Materials and methods

2.1. Microorganisms

Four *S. aureus* strains were obtained as follows: 196E from the Eastern Regional Research Center (Philadelphia, PA), and B-121, B-124 and B-767 from the USDA Northern Regional Research Center (Peoria, IL) stock culture collections. They were transferred monthly into Brain Heart Infusion (BHI) broths, grown overnight at 37°C and stored at 6°C. For the experimental inoculation, the strains were individually grown overnight at 37°C in BHI broth. Approximately equal numbers (ca. $10^{9.5}$ CFU/ml) were then mixed together for inoculation.

2.2. Survival determinations

BHI broths were prepared with added lactic acid (85%, Sigma, St. Louis, MO) and/or NaCl and adjusted to their final pH with 0.1 N HCl or NaOH. This formed a lactate buffer with various combinations of acidity and lactate concentrations. Fifty ml aliquots of the adjusted BHI broth were autoclaved and filter sterilized. NaNO_2 (Sigma) was added when needed. An inoculum of 2.5 ml of the four-strain mixture was added to give an initial level of approximately $10^{8.5}$ CFU/ml and the flasks were incubated at various temperatures. A fractional factorial design had a total of 159 flasks (151 unique combinations). The five factors ranging from pH 3 to 7, NaCl from 0.5 to 20%, total lactate (calculated as Na lactate) from 0 to 1.0%, NaNO_2 from 0 to 200 $\mu\text{g/ml}$ and temperature from 4 to 42°C were tested. Surviving *S. aureus* were enumerated at appropriate times by diluting samples in 0.1% peptone (Difco), spreading on tryptic soy agar (TSA, Difco) plates with a Spiral Plater (Spiral Systems, Inc., Cincinnati, OH) and counting after incubating for 24 h at 37°C using an automated colony counter (Model 500A, Spiral Systems, Inc.). Flasks were sampled for up to 6 months or until survivors were below the lower limit of detection (20 CFU/ml).

2.3. Modeling

Survival was modeled by fitting the data for each flask to the logistics model (Eq. (1)) (Kamau et al., 1990; Whiting, 1993; Buchanan et al., 1993, 1994).

$$Y = Y_o + \log_{10} \left[F_1 (1 + \exp(-b_1 t_1)) / (1 + \exp(b_1(t - t_1))) \right. \\ \left. + (1 - F_1) (1 + \exp(-b_2 t_1)) / (1 + \exp(b_2(t - t_1))) \right] \quad (1)$$

where Y is the log number of survivors, Y_o the log inoculum, F_1 the fraction of the original population in the major group, $F_2 = (1 - F_1)$ the fraction in the subpopulation, t_1 the lag or shoulder period, b_1 and b_2 the inactivation rates of the major and subpopulations and t the time, respectively. The respective D values can be calculated from the inactivation rates by $D = 2.3/b$. This Eq. (1) was fitted to the data using ABACUS, a nonlinear regression program that employs a Gauss-Newton iterative procedure developed at the Eastern Regional Research Center, USDA (Buchanan et al., 1993; Damert, 1994). The times required for four logs decline (T_{4D}) (99.99% inactivation) were calculated from the t_1 and b_1 values using a rearranged version of Eq. (1) without the subpopulation (Eq. (2)).

$$T_{4D} = \{ \text{Ln}[(1 + \exp(-b_1 t)) / 0.0001] - 1 \} + b_1 t_1 / b_1 \quad (2)$$

The data were also fitted to a nearly-linear logistic model by fixing the shoulder period to zero hours and setting the subpopulation parameters so they were insignificant. This estimated the overall D value (D_{ov}) for the entire survival curve. Quadratic polynomial regression equations were calculated using SAS (SAS Institute Inc., 1989) to model the effects of the environmental parameters (temperature, pH, lactate, salt and nitrite) on T_{4D} and D_{ov} . To make the variances uniform, the

\log_{10} of the T_{4D} and D_{ov} values were used when calculating the regression equations and the 95% confidence intervals. The confidence intervals for an estimated D_{ov} were calculated for specific values of the five environmental factors (Draper and Smith, 1989 p. 94; SAS Institute Inc., 1989).

3. Results and discussion

Survival times of *S. aureus* ranged from hours to months depending upon the environmental conditions (individual factor values and survival data available upon request). Fig. 1 illustrates two survival conditions, one showing a simple decline and the other a shoulder or survival period before decline. In general, survival times were longer at higher pH values, lower temperatures, and lower nitrite and lactate levels. Survival times slightly increased with increasing levels of salt.

Fitting the complete model to survival data (shoulder and subpopulation) resulted in better fits (lower residual mean squares) than did fitting the simpler form (D_{ov}). Fig. 2 shows a survival curve fitted to the simple (a) and complete (b) form of the model. The residual mean squares (RMS) were 0.842 and 0.368 for a and b, respectively, illustrating how a more complex curve can fit an individual set of data

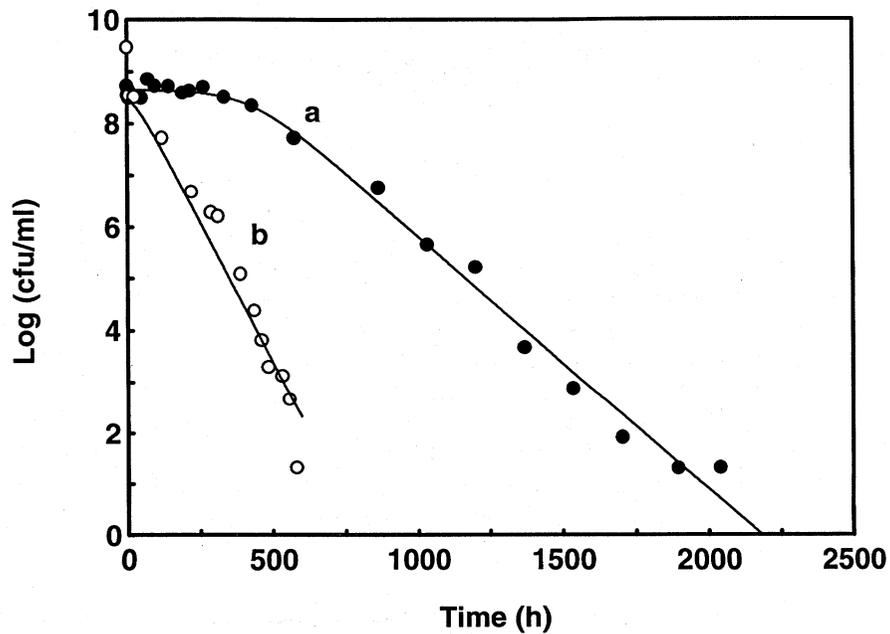


Fig. 1. Examples of model fits to *S. aureus* survival data for two environments. Conditions for curve a were 4°C, pH 5.0, 5% NaCl, 0.50% lactate and 0 ppm nitrite. Fitted parameter values were $t_1 = 424$ h and $D_1 = 204$ h. Residual mean squares (RMS) = 0.227. Conditions for curve b were 4°C, pH 5.0, 0% NaCl, 0% lactate and 200 ppm nitrite. Fitted parameter values were $t_1 = 0$ h and $D_1 = 92.6$ h. RMS = 0.528.

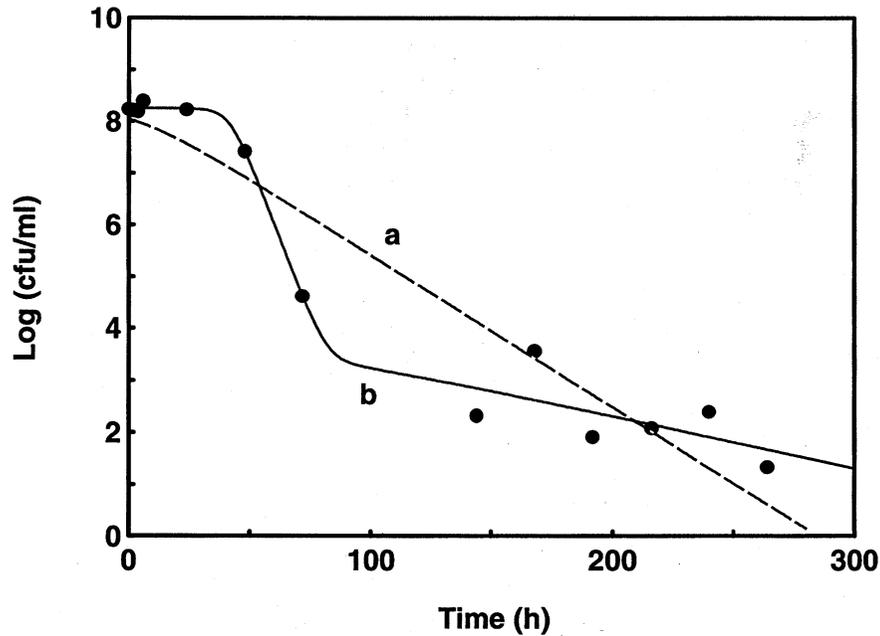


Fig. 2. Example of model fits to *S. aureus* survival data assuming a simple decline or assuming the existence of a lag period and subpopulation. Conditions were 4°C, pH 3.0, 0% NaCl, 0% lactate and 0 ppm nitrite. Fitted parameter value for curve a assumed no shoulder or subpopulation. $D_1 = 31.4$ h and RMS = 0.842. Fitted parameter values for curve b assumed a shoulder and subpopulation. $t_1 = 41.6$ h, $D_1 = 8.3$ h, $F_2 = 10^{-4.5}$, $D_2 = 98.9$ h and RMS = 0.368.

more closely. However, the calculated regression equations for the fit of the shoulder period and D_2 to the environmental factors were poor ($R^2 = 0.22$ and 0.16, respectively) indicating that the environmental factors did not exert the major control over these parameters. It is questionable whether F_2 values less than 0.001 and t_1 times less than the D_1 times indicated the existence of a subpopulation and shoulder or merely over-parameterized fitting. Therefore, the time for 4 logs of decline using the shoulder and decline of the major population was calculated for each flask and then modeled with Eq. (2). The fit of the regression equation for the $\log T_{4D}$ was good ($R^2 = 0.87$; $F = 46.2$; 20/138 df) (Table 1). However, this model is limited by being unable to estimate survival times for other than 4 log (99.99%) declines in population. As expected, fitting the data to the simpler form (D_{ov}) generally gave slightly greater residual mean squares for each survival curve (average RMS = 0.66) than the complete form of the model (average RMS = 0.54), and the calculated regression equation for the $\log D_{ov}$ was not as good a fit ($R^2 = 0.81$; $F = 30.2$; 20/138 df). However, the D_{ov} model can be used to calculate the times for any decline desired and, therefore, was judged to be the more useful of the two models.

Fig. 3 shows the calculated fits of relatively benign pH and temperature conditions on the survival times calculated with the D_{ov} model. The longest survival occurs near 20°C with neutral pH, when the times for 1 log decline are in the thousands of hours. At pH 3.0, D values were 30 h or less. When environmental conditions are more severe, as found in a fermented meat product (Fig. 4), the calculated survival times were longest at 10°C and all times were shorter than those shown on Fig. 3.

The influence of pH on the D_{ov} times for a simulated fermented meat product at ambient temperature was curvilinear (Fig. 5). Times increased from 19 h at pH 3.0 to 616 h at pH 7. The dotted lines represent the calculated 95% confidence intervals. The values for the lower and upper confidence intervals at pH 3.0 were 8 and 44 h and at pH 6.0 were 258 and 1117 h, respectively. The 95% confidence intervals were roughly from 50 to 200% of the estimated D_{ov} .

The total lactate concentration had a major effect on D_{ov} that was independent of pH (Fig. 6). Survival times decreased as the lactate concentration increased. Increasing nitrite concentrations reduced survival times, particularly at lower pH values (Fig. 7). With conditions simulating a fermented meat product, the calculated D_{ov} with no nitrite was 494 h, with 25 ppm nitrite it was 338 h, and 50 ppm nitrite it was 220 h. Nitrite levels corresponding to the added nitrite in meat products (> 100 ppm) resulted in estimated D_{ov} times of less than 50 h. Determining the appropriate nitrite concentrations to enter into these broth models that corresponds to the effective levels in a meat product needs further research. Whiting and Masana (1994) found that the residual nitrite concentration in an acidified

Table 1
Regression equations for the models which describe survival of *S. aureus*

T is temperature (°C), pH is the pH value, S is % NaCl, L is % total lactate (as sodium lactate) and N is the ppm sodium nitrite added

Regression equation for time of 4 log decline

$$\log_{10} T_4D = -3.742 + 0.03138T + 2.237pH + 0.01128S - 0.1598L - 0.01467N + 0.001674T*pH - 0.000407T*S - 0.02318T*L - 0.000020T*N + 0.002015pH*S - 0.1564pH*L + 0.001938pH*N + 0.03187S*L + 0.000629S*N - 0.01102L*N - 0.001225T^2 - 0.1629pH^2 - 0.001988S^2 + 0.6158L^2 - 0.000003N^2$$

$n = 159$

Average residual mean square of fits = 0.54

$R^2 = 0.87$

$F = 46.2, 20/138$ df

Regression equation for D_o (simple model)

$$\log_{10} D_{ov} = -3.088 + 0.05338T + 1.678pH + 0.04424S - 1.0335L - 0.01924N + 0.000697T*pH - 0.000985T*S - 0.02250T*L - 0.000017T*N + 0.007831pH*S - 0.3516pH*L + 0.003608pH*N + 0.04239S*L + 0.000583S*N - 0.01252L*N - 0.0014185T^2 - 0.1061pH^2 - 0.001033S^2 + 0.4648L^2 - 0.000016N^2$$

$n = 159$

Average residual mean square of fits = 0.66

$R^2 = 0.81$

$F = 30.2, 20/138$ df

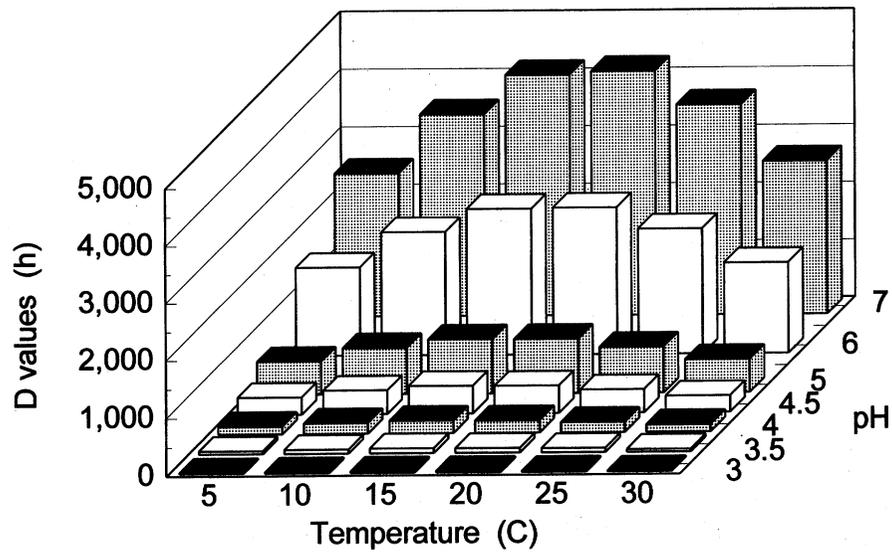


Fig. 3. Calculated interaction of pH and temperature on the survival of *S. aureus* under mild conditions. Other factors were set at 3% NaCl, 0.2% lactate and 0 ppm nitrite.

meat batter was better than the added nitrite concentration for estimating the survival of *Listeria monocytogenes* using a model developed in broths. Buchanan et al. (1993) reported a relationship between the survival times of *L. monocytogenes*

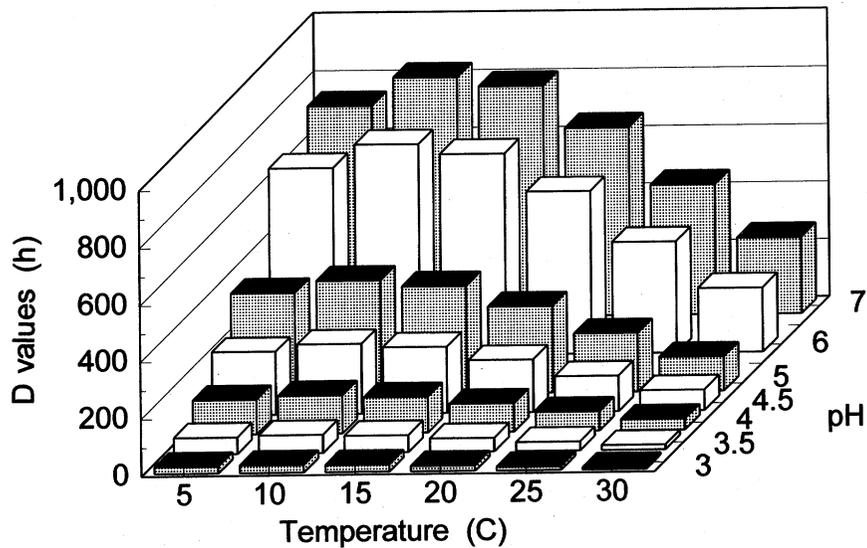


Fig. 4. Calculated interaction of pH and temperature on the survival of *S. aureus* in a simulated fermented meat product. Other factors were set at 8% NaCl, 0.8% lactate and 25 ppm nitrite.

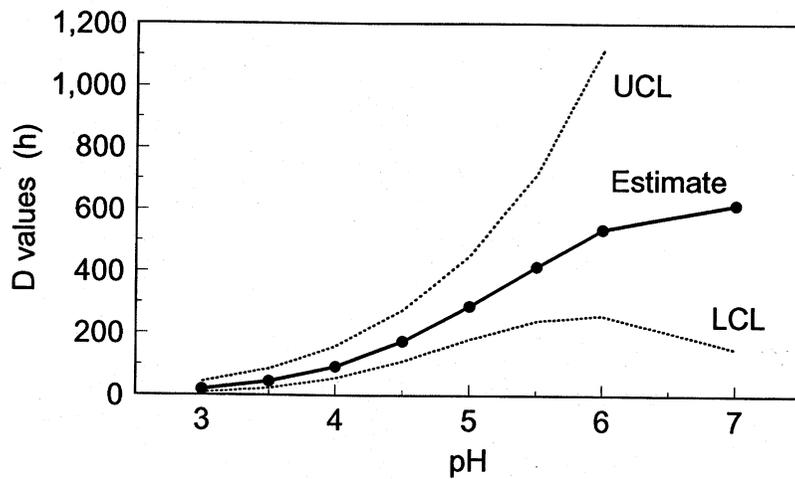


Fig. 5. Calculated D values at varying pH values. Other factors were set at 21°C, 8% NaCl, 0.8% lactate and 25 ppm nitrite. Dotted lines represent the upper (UCL) and lower (LCL) 95% confidence limits.

and the amount of undissociated lactic acid. The logarithm of the times for 4 logs of decline was inversely related to the square root of the undissociated lactic acid concentration. Our data did not give regression equations having a better fit using the undissociated lactic acid and nitric acid on $\log D_{ov}$ ($R^2 = 0.70$) or $\sqrt{D_{ov}}$ ($R^2 = 0.51$) than using lactate and nitrite.

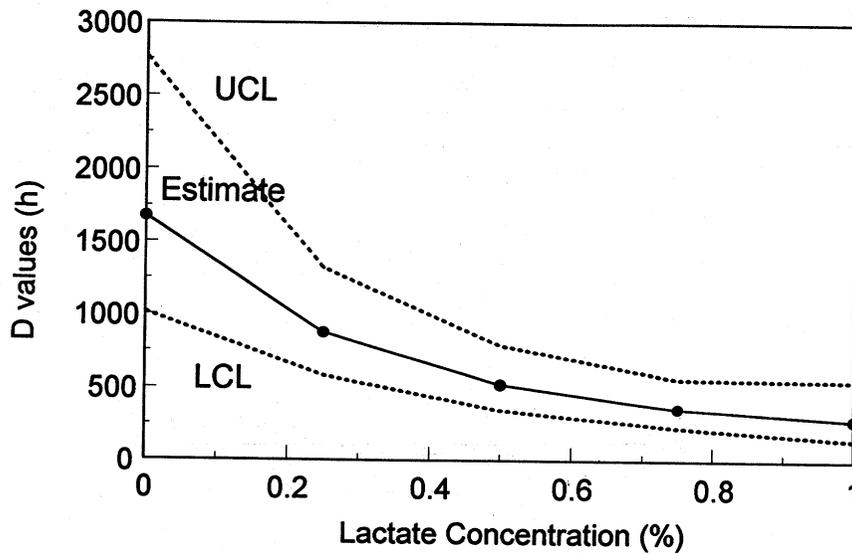


Fig. 6. Calculated D values at varying lactate concentrations. Other factors were set at 21°C, pH 5.2, 8% NaCl and 25 ppm nitrite.

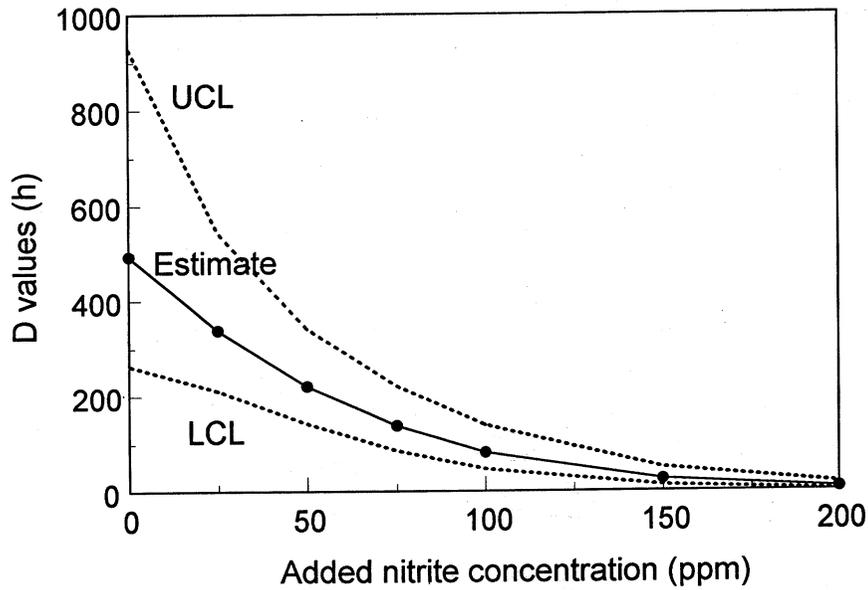


Fig. 7. Calculated D values at varying nitrite concentrations. Other factors were set at 21°C, pH 5.2, 8% NaCl and 0.8% lactate.

The sodium chloride concentration had a smaller effect on survival times than the other factors (Fig. 8). The survival time increased from 229 h with 0.5% to 332 h with 7.5% NaCl for conditions simulating fermented meat products. Addition of NaCl is also known to increase the resistance of *S. aureus* to thermal treatments (Bergdoll, 1989).

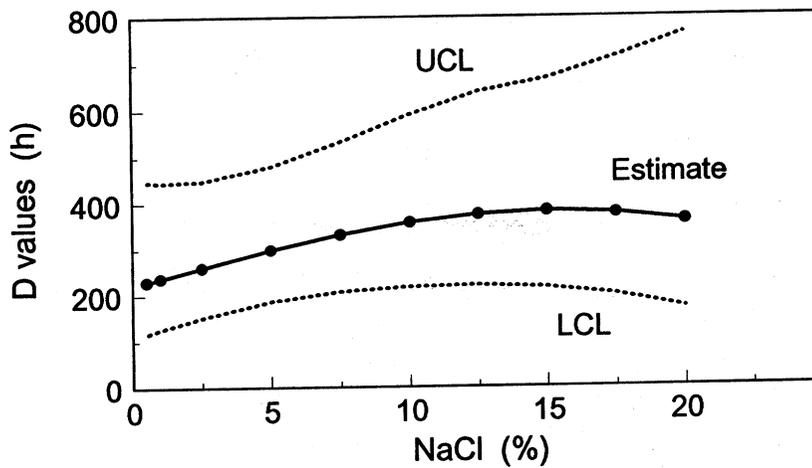


Fig. 8. Calculated D values at varying NaCl concentrations. Other factors were set at 21°C, pH 5.2, 0.8% lactate and 25 ppm nitrite.

Table 2
Comparison of reported *S. aureus* survival with the model predictions

Reference	Product	Temp (°C)	pH	NaCl (%)	Lactic acid (%)	Nitrite (ppm)	D value from literature (Days)	D_{ov} value from model (Days)	LCL of model D_{ov} value (Days)	UCL of Model D_{ov} value (Days)
Minor and Marth, 1972	Acidified media (HCl)	37	6	0.5	(3)	(0)	No decline	89	14	— ^a
		37	6	7.5	(0)	(0)	No decline	42	8	—
		37	4.2	0.5	(0)	(0)	1	7	3	8
Westhoff and Engler, 1973	Cottage cheese whey	35	4.5	(0.2)	(0.5)	(0)	1	4	2	10
		5	4.5	(0.2)	(0.5)	(0)	5	9	6	16
Ahmed et al., 1983	Dommati cheese whey	30	5.3	15	(0.3)	(0)	6	11	5	25
		30	5.3	0	(0.3)	(0)	Growth	30	11	78
Abdalla et al., 1993	Pickled cheese (starter culture) (no starter culture)	4	4.6	4	(0.5)	(0)	10	12	7	19
		4	7.1	4	(0)	(0)	Growth	171	32	—

Table 2 (continued)

Holley, 1985	Jerkey	20	5.6	18 ^a	(0)	(0)	<26	32	12	74
Daly et al., 1973	Fermented sausage	37	5.5	3	(0.2)	(10)	50	20	6	66
		37	3	4.4	(0.5)	(10)	1	2	1	5
Lee et al., 1977	Genoa sausage (drying stage)	12	6.2	12 ^a	(0.5)	(10)	35	50	26	97
Niskanen and Nurmi, 1976	Dry sausage	17	5.1	15 ^a	(0.6)	(10)	13	22	13	36
Pitman et al., 1973	IM pork	25	6.4	15.7 ^a	(0)	(0)	44	57	17	194
		6	5.3	15 ^a	(0.1)	(10)	200	32	20	50
Kotzekidou and Lazarides, 1991	IM beef	6	5.3	15 ^a	(0.1)	(10)	200	32	20	50

() Data not given in paper, estimates used.

— Exceeds 180 day range of model.

^aNaCl equivalent to measured a_w .

Comparisons of estimates from the model to published *S. aureus* survival data (Table 2) generally showed good agreement between *D* values. The *D* values from the literature frequently are approximate because they were inferred from figures, often covered conditions of changing pH or a_w , and did not specify all of the factors required by the model. Growth was observed in two situations; this shows the necessity to also consult a growth model when making an estimate of expected microbial behavior. Whiting and Masana (1994) found that estimates for *Listeria* were not always accurate with either growth or survival models at certain combinations of environmental factors that permitted slow growth and lengthy survival. With fermented foods there is also the potential for microbial competition, bacteriocins and acid injury to affect survival, factors which are not included in these models.

Only in papers by Daly et al. (1973) and Kotzekidou and Lazarides (1991) were the predicted D_{ov} values smaller than those observed, which resulted in fail-dangerous predictions. The *D* in the former paper was within the confidence interval, while the latter paper reported very slow declines in *Staphylococcal* numbers. Lee et al. (1977) also reported variable survival rates depending on inoculum size and location. The *D* values for the most rapid decline in the core of the salami was calculated for Table 2. Little or no declines were observed on the surface, but conditions there may not be appropriate for models derived from broth cultures. Overall, the D_{ov} model reported in this paper was fail-safe when confidence intervals of two standard deviations (95%) were allowed.

From the model presented in this paper, the time for a one log inactivation of *S. aureus* in a fermented meat product was calculated to be about 400 h (17 days). A decline of 90% of the *S. aureus* in refrigerated (7°C) fresh meats with a higher pH (5.8) and no added inhibitors was calculated to be 106 days (confidence range 42 to 269 days). This is a wide ranging model with calculated D_{ov} ranging from under 10 h to over 4000 h (167 days), and thus the confidence intervals are not extremely tight. The model should be used for an initial estimate of survival times and for relative comparisons between different combinations of environmental factors. A limited number of inoculated trials should be made in an actual product to validate the model for that product and confirm that the product does not contain additional factors that affect survival. Some situations may also permit growth by *S. aureus*, but our model does not attempt to predict the outcome of the simultaneous growth/death processes. Checking for the possibility of growth by reference to a growth model when temperatures exceed 12°C and pH values are greater than 4.5 is recommended.

This model is contained in the USDA Pathogen Modeling Program (contact R.C. Whiting).

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