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Development of thermal inactivation models for *Salmonella enteritidis* and *Escherichia coli* O157:H7 with temperature, pH and NaCl as controlling factors

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Abstract

The thermal inactivation of *Salmonella enteritidis* phage type 4 and *Escherichia coli* O157:H7 as affected by temperature (54.5–64.5°C), pH (4.2–9.6 with HCl or NaOH) and NaCl concentration (0.5–8.5% w/w) was studied. Cell suspensions in modified tryptone soya broth were heated in a submerged-coil heating apparatus and survivors were enumerated on tryptone soya agar incubated aerobically. For most thermal inactivation data there was a logarithmic decrease in the viable cell concentration over the initial 4–6 $\log_{10}$ reduction and *D*-values were fitted. In some cases, tailing of the survivor curves was observed with cells surviving longer than the *D*-values predicted. Models describing the effect of temperature, pH and NaCl concentration on the thermal inactivation of *S. enteritidis* and *E. coli* O157:H7 were produced. For both organisms, predicted *z*-values of 4.6–7.0 C° were obtained depending on conditions, with larger *z*-values at higher levels of NaCl. Optimum survival occurred between pH 5 and pH 7 and increasing acidity or alkalinity caused a decrease in the predicted *D*-values. At equivalent pH, acetic acid and lactic acid (at 0.5, 1 and 2% w/w) generally had a similar, or increased, lethal effect compared with HCl, whereas in most cases citric acid had a less lethal effect. For *E. coli* O157:H7, increasing NaCl concentration had a protective effect up to the maximum tested (8.5% w/w), while for *S. enteritidis* optimal survival at a NaCl concentration of 5–7% w/w was predicted. The models were validated in foods by comparing predictions with published data. Most (80%) of the predicted *D*-values from the *S. enteritidis* model were within the 95% confidence interval (within 2.45-fold of the published data) for different *Salmonella* serotypes in whole egg, egg albumen, egg yolk, beef and milk. Most (93%) of the predicted *D*-values from the *E. coli* O157:H7 model were larger than the limited published data for this organism in meat, poultry, milk and apple juice with 42% within the 95% confidence interval (within 2.05-fold of the published data). The *D*-value models were incorporated into Version 1, and subsequent versions, of the predictive microbiology software program, Food MicroModel. © 1997 Elsevier Science B.V.

Keywords: Thermal inactivation; Predictive model; *Salmonella enteritidis*; *Escherichia coli* O157:H7; Temperature; pH; NaCl; *D*-value; Log-logistic; Food MicroModel

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1. Introduction

The inactivation of infectious non-spore-forming pathogens using a heat treatment is a critical control point in the safe preparation of many foods. Insufficient processing, cooking or reheating are often contributing factors in food-poisoning outbreaks (Roberts, 1991). Both of the organisms studied in this work have been implicated with food poisoning caused by insufficient heating. A variety of foods have been implicated as vehicles of *Salmonella* infection including meat and poultry, milk, ice cream, cheese, eggs and egg products, chocolate and spices and inadequate heating has been the cause of some of the outbreaks (D'Aoust, 1989). To date, outbreaks caused by enterohaemorrhagic *Escherichia coli*, in particular serotype O157:H7, have been attributed to undercooked beef and raw milk (Padhye and Doyle, 1992), unpasteurised apple juice (McCarthy, 1996), cooked meat (Bradbury, 1996), lettuce (Anon., 1996), and unconfirmed food vehicles (Watanabe et al., 1996).

There have been many studies on the heat resistance of *Salmonella* in foods and laboratory media (D'Aoust, 1989). In some cases the relationship between the physicochemical properties of the heating menstruum, such as water activity (Baird-Parker et al., 1970; Geopfert et al., 1970) and pH (Anellis et al., 1954), and the heat resistance of *Salmonella* has been investigated. The combined effects of several factors has received much less attention and has not been quantified mathematically. Most of the thermal inactivation studies on *E. coli* O157:H7 have focused on the effect of temperature alone in different foods such as meat (Ahmed et al., 1995), ground beef (Doyle and Schoeni, 1984; Line et al., 1991; Jackson et al., 1996), milk (D'Aoust et al., 1988) and apple juice (Splittstoesser et al., 1996).

In the traditional approach to establishing a safe thermal inactivation treatment it is assumed that organisms within a population are identical and that their inactivation can be described by first order kinetics. This is achieved by plotting the logarithm of the number of survivors against exposure time and calculating the decimal reduction time (*D*-value). Calculation of *D*-values at different temperatures allows the *z*-value to be established. This is used to integrate the lethal effect of temperature within a given thermal process. This approach has served the food industry for over 50 years and, to date, forms the basis of most commercially applied thermal processes. Despite this, during the same period of time, deviations from first order inactivation kinetics have been reported frequently (Cerf, 1977; Jackson et al., 1996). Consideration of non-first order inactivation kinetics could be important in the safe application of milder heat processes or those relying on the combined effects of other factors such as pH and water activity. In this respect there is a need for models that can predict the combined effects of a number of factors on the thermal inactivation of pathogenic bacteria.

In recent years there has been increasing interest in the use of mathematical models to predict microbial growth in food (Williams et al., 1992). In the UK, a coordinated programme of research funded by the Ministry of Agriculture, Fisheries and Food has been undertaken to develop growth, survival and thermal inactivation models for foodborne pathogenic bacteria. During this research programme an approach to producing, validating and approving models was developed (McClure et al., 1994). Many of these models have now been incorporated into the commercially-developed software program, Food MicroModel (Blackburn, 1995).

The aim of this study was to produce 3-factor thermal inactivation models for *S. enteritidis* and *E. coli* O157:H7 with controlling factors of temperature (54.5–64.5°C), pH (4.2–9.6 to include high-pH egg products) and NaCl (0.5–8.5% w/w) using the same approach and rigor in experimental design and validation used previously for the development of predictive models for growth.

2. Materials and methods

2.1. Organisms

Salmonella enteritidis phage type 4 (P167807) and *Escherichia coli* O157:H7 (E30138, E30228, E30480 and E32511), kindly supplied by Dr Bernard Rowe, Division of Enteric Pathogens, Central Public Health Laboratory, London, and *Salmonella senftenberg* NCTC 9959 were used. Cultures were stored at −20°C on beads (Protect, Technical Service Consultants Ltd, Heywood, Lancashire, UK).

2.2. Media

Tryptone soya broth (TSB; Oxoid Ltd, Basingstoke, UK) was prepared and NaCl added to give final concentrations up to 8.5% w/w. The pH was adjusted by the addition of 2 M HCl or 2 M NaOH to values ranging from 4.2 to 9.6, prior to autoclaving at 121°C for 15 min. In some cases the pH of the broths was altered with filter-sterilised 1M HCl or 1M NaOH after autoclaving. Some broths were prepared with the addition of acetic, citric or lactic acid at 0.5, 1 and 2% w/w.

2.3. Preparation of cell suspension

Unmodified TSB was inoculated with *S. enteritidis* or *E. coli* O157:H7 and incubated at 37°C for 24 h. The culture was diluted in maximum recovery diluent (MRD; Oxoid Ltd) to give a concentration of about 10 cfu/ml and 1 ml was used to inoculate 100 ml unmodified TSB. The broth was incubated at 37°C for 16–19 h. The culture (50 ml) was centrifuged ($6500 \times g$ for 20 min at 20°C) and resuspended in 20 ml TSB with modified pH and NaCl concentration. After equilibration for 15 min at room temperature the pH of the suspension was determined.

2.4. Thermal inactivation

The cell suspension was heated in a submerged-coil heating apparatus (Cole and Jones, 1990). Samples (0.2 ml) were removed automatically at predetermined time intervals and rapidly cooled to room temperature in TSB (5 ml). After resuscitation at room temperature for 90 min, to allow recovery of heat-injured cells, survivors were enumerated following serial decimal dilution in MRD and inoculation of duplicate tryptone soya agar (TSA, Oxoid Ltd) plates, as 1 ml pour plates, 0.1 ml spread plates or 10 μl spread on quartered plates. Plates were incubated at 37°C for 48 h. A duplicate set of plates, incubated anaerobically (Gas Generating Kit, Oxoid Ltd), were used for some experiments.

2.5. Experimental design

All combinations of conditions were selected from the following: temperature (54.5, 59.5, 62.5 and 64.5°C); pH (4.2, 4.6, 5.2, 7.0, 8.0, 8.7, 9.5 for *S. enteritidis*; and 4.4, 5.0, 6.8, 8.0 and 9.6 for *E. coli* O157:H7) and NaCl (0.5, 3.5 and 8.5% w/w). A total of 88 thermal inactivation curves for *S. enteritidis* and 67 for *E. coli* O157:H7 were used to construct two models.

2.6. Data fitting and construction of models

Linear regression was performed on the survival data for each temperature using the REG™ procedure of SAS (SAS Institute Inc., Cary, NC, USA). This produced a correlation coefficient and an estimate of the intercept and slope of the straight line for each temperature. Corresponding *D*-values were calculated from the reciprocal of the slope estimate. Only points in the straight part of the curve were included. A quadratic response surface was used to describe the effects of temperature, pH and NaCl on the $\log_{10}$ *D*-value (RSREG™ procedure of SAS). A number of data sets showed significant deviation from log-linear kinetics and so the data were also fitted using non-linear regression where the whole data set was fitted using a log-logistic function (NLIN™ procedure of SAS):

$$\log_{10}(\text{cfu ml}^{-1}) = \alpha + \frac{\omega - \alpha}{1 + \exp\frac{(4\sigma(\tau - \log_{10}\text{time}))}{\omega - \alpha}}$$

where α is the upper asymptote ($\log_{10}$ cfu/ml), ω is the lower asymptote ($\log_{10}$ cfu/ml), σ is the maximum slope of the inactivation curve ($\log_{10}$ cfu/ml against $\log_{10}$ time) and τ is the $\log_{10}$ time at which the maximum slope is reached. The only parameter significantly influenced by temperature, pH and NaCl and therefore a quadratic function was used to describe τ in response to these factors.

2.7. Validation of models with data from foods

Published data of the thermal inactivation of *Salmonella* and *E. coli* O157:H7 in food were obtained. When *D*-values were not quoted they were calculated from graphs of the data. The heating temperature of the experiment and the pH and NaCl concentration of the food was used to obtain the predicted time for a 1 $\log_{10}$ reduction from the relevant model and compared with the published

data. Where pH and/or NaCl concentration data were not provided, estimates were made.

3. Results

3.1. Screening of Salmonella and E. coli O157:H7 strains

A *Salmonella enteritidis* phage type 4 strain (P167807) was selected as being the most heat resistant of 6 strains of *S. enteritidis* phage type 4 (Davies, A.R., personal communication). This strain was 1.9–4.6 times more heat sensitive when compared with *Salmonella senftenberg* (NCTC 9959) at a NaCl concentration of 0.5% w/w (Table 1). Four strains of *Escherichia coli* O157:H7 were screened and E30228 was selected as the most heat resistant at a variety of conditions of pH and NaCl concentration (Table 2).

Table 1
$D_{62.5°C}$ (s) of *S. senftenberg* and *S. enteritidis* (P167807) at 0.5% w/w NaCl

Organism	pH 7.3	pH 9.6
S. senftenberg	39.0	2.1
S. enteritidis (P167807)	8.4	1.1

3.2. Effect of aerobiosis on recovery of heat-injured cells

Anaerobic incubation of TSA plates was compared with aerobic incubation for the enumeration of heat-injured cells. Colony counts on anaerobically-incubated plates were generally greater than those incubated aerobically and this led to *D*-values up to twice as large (Table 3).

3.3. Modelling

For most thermal inactivation data there was a logarithmic decrease in the cell concentration with time over the initial 4–6 $\log_{10}$ reduction (data not shown). In some cases, however, tailing of survivor curves was seen, with low numbers of cells surviving longer than the fitted *D*-values predicted.

The *D*-value models were calculated in two stages. Linear regression analysis of thermal inactivation data gave a very good fit (*R*-square values >90%) for 84% of *S. enteritidis* curves and 83% of *E. coli* O157:H7 curves. The remaining curves showed significant shoulders and/or tails making it more difficult to define the linear portion of the curves. There was a good fit of the quadratic response surface (function of temperature, NaCl and pH) to $\log_{10}$ *D*-values, with *R*-square values of 95.5% for *S. enteritidis* and 97.4% for *E. coli* O157:H7. The

Table 2
$D_{62.5°C}$ (s) of *E. coli* O157:H7 strains

Conditions	Strain no			
	E30138	E30228	E30480	E32511
0.5% w/w NaCl, pH 4.3	19	34	15	33
3.5% w/w NaCl, pH 5.1	62	112	80	59
8.5% w/w NaCl, pH 4.3	140	158	108	114

Table 3
Effect of anaerobic incubation on the calculated *D*-values (s) of *S. enteritidis* and *E. coli* O157:H7 in 0.5% w/w NaCl

Organism	Temperature (°C)	pH	Incubation of agar plates	
			Aerobic	Anaerobic
S. enteritidis	62.5	6.9	20	25
	59.5	6.8	160	211
E. coli O157:H7	64.5	6.8	3.5	5.6
	59.5	7.0	42	82
	59.5	6.8	60	81

standard errors associated with each model were 0.194 for *S. enteritidis* and 0.156 for *E. coli* O157:H7.

Log-logistic models were determined in one step. The mean square errors of the log-logistic function fitted to *S. enteritidis* and *E. coli* O157:H7 data were 0.618 and 0.544, respectively. These values are larger than those calculated for the *D*-value models (0.038 and 0.024 for *S. enteritidis* and *E. coli* O157:H7, respectively) because they reflect the two steps of the modelling process compared with just the fitting of the quadratic response surface.

Predictions for 3 and 5 $\log_{10}$ reductions from both models were compared (Figs. 1 and 2). For a 3 $\log_{10}$ reduction, predictions from both models were similar although the *E. coli* O157:H7 log-logistic model tended to predict longer times than the *D*-value model, particularly for milder heating conditions (Fig. 1b). For a 5 $\log_{10}$ reduction, predictions from the log-logistic models were always larger than those from the *D*-value models with the most significant differences occurring for milder heating conditions (Fig. 2).

3.4. Effect of temperature

Over the temperature range studied (54.5–64.5°C) there was an approximately linear relationship between the logarithm of the predicted *D*-value and temperature for both *S. enteritidis* and *E. coli* O157:H7 (Fig. 3). The *z*-values for both organisms were approximately 4.6–7.0 C° depending on the

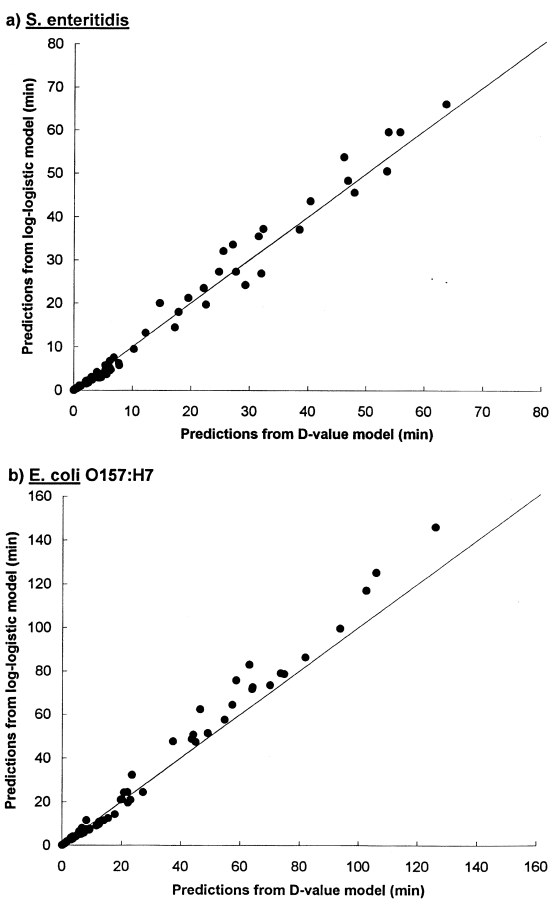

Fig. 1. Comparison of predictions for a 3 $\log_{10}$ reduction from *D*-value and log-logistic models for (a) *S. enteritidis* and (b) *E. coli* O157:H7 (line denotes equivalence).

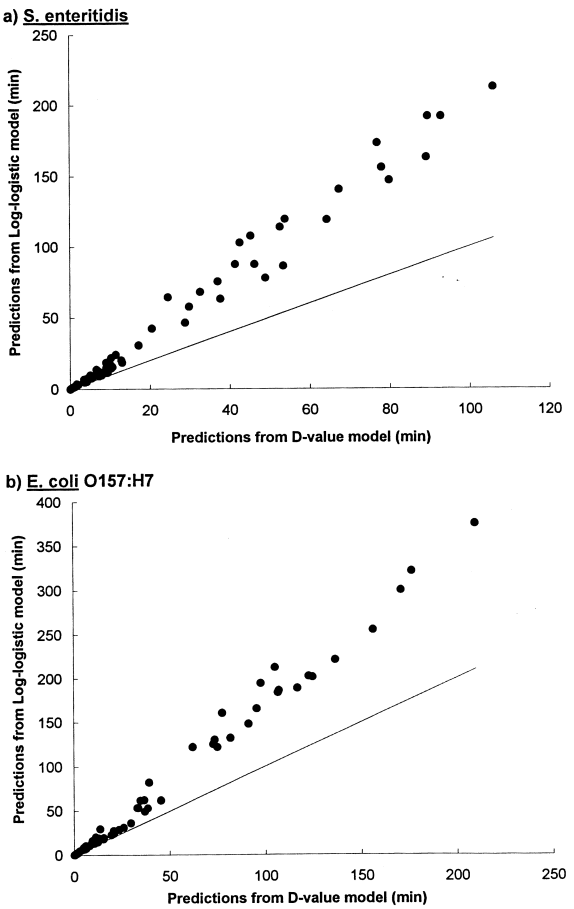

Fig. 2. Comparison of predictions for a 5 $\log_{10}$ reduction from *D*-value and log-logistic models for (a) *S. enteritidis* and (b) *E. coli* O157:H7 (line denotes equivalence).

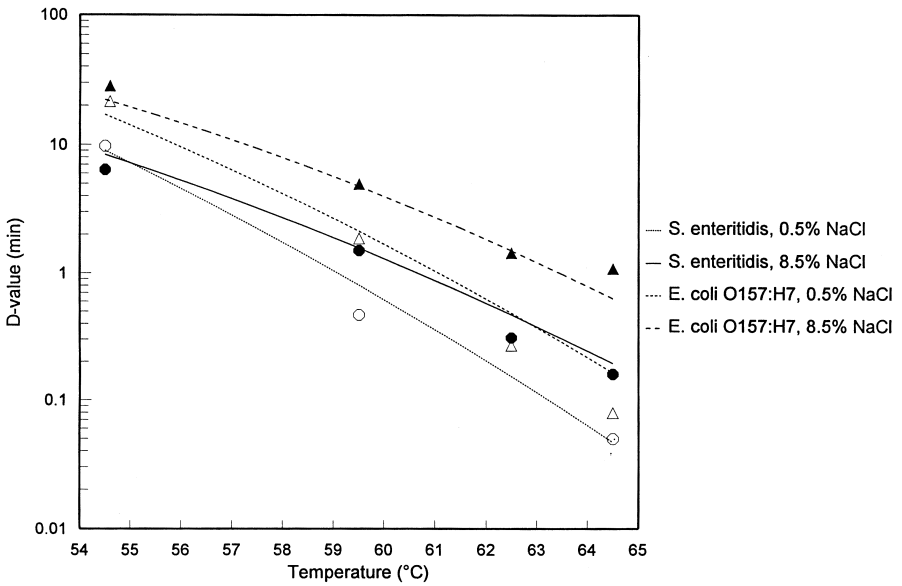

Fig. 3. Effect of temperature on the thermal inactivation of *S. enteritidis* (○,●) and *E. coli* O157:H7 (△,▲) at 0.5% w/w NaCl (○,△) and 8.5% NaCl (●,▲), pH 7.0 (lines denote predictions from models derived from the whole datasets and not just the points shown).

conditions. NaCl concentration had a more pronounced effect than pH with larger *z*-values at higher levels of NaCl. For example, *z*-values increased from 4.6–5.1 C° at 0.5% w/w NaCl to 5.8–7.0 C° at 8.5% w/w NaCl.

3.5. Effect of pH

For both *S. enteritidis* and *E. coli* O157:H7 there was an optimum pH for survival that was dependant on temperature and NaCl concentration, and increasing acidity or alkalinity increased the rate of inactivation. The optimum pH for survival was generally higher for *S. enteritidis* (pH 5.9–6.5) than for *E. coli* O157:H7 (pH 5.2–5.9). For example, at 3.5% w/w NaCl the maximum predicted $D_{64.5°C}$ for *S. enteritidis* and *E. coli* O157:H7 occurred at pH 6.4 and pH 5.6, respectively (Fig. 4).

3.6. Effect of organic acids

For *S. enteritidis* and *E. coli* O157:H7, heating in the presence of acetic or lactic acid gave similar or reduced *D*-values compared with predictions from the models, which were produced using media acidified with HCl (Tables 4 and 5). For *S. enteritidis*, 2% w/w acetic and lactic acid was considerably more lethal than 0.5 and 1% w/w (Table 4). For *E. coli* O157:H7 survival in the presence of acetic and lactic acids was greatest with 1% w/w acid compared with 0.5 and 2% w/w (Table 5). In general, citric acid was less lethal for both organisms compared with HCl.

3.7. Effect of NaCl concentration

Increasing NaCl concentration had a heat protective effect on *E. coli* O157:H7 (Fig. 5). For *S. enteritidis*, thermal inactivation was more rapid in the presence of 0.5% w/w NaCl compared with 3.5% w/w NaCl, but a concentration of 8.5% w/w NaCl frequently led to similar or more rapid inactivation than at 3.5% w/w NaCl. The resultant model predicted maximum *D*-values at about 5–7% w/w NaCl. To investigate the effect of larger NaCl concentrations, the thermal inactivation of *S. enteritidis* at 64.5°C, pH 7 and 19% w/w NaCl was determined. The resulting $D_{64.5°C}$ of 10.9 s (data not shown) was less than the maximum predicted $D_{64.5°C}$ of 13.7 s which occurred at 6.5% w/w NaCl.

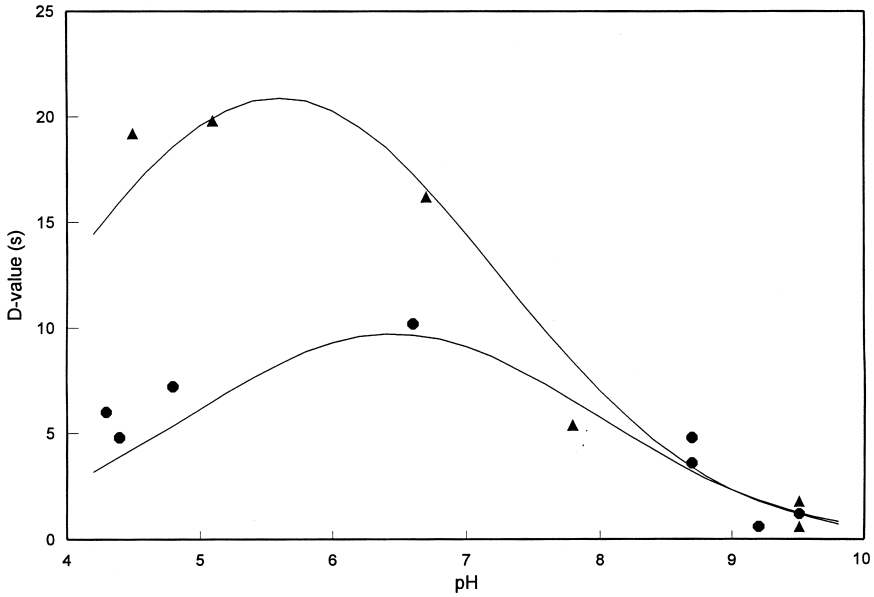

Fig. 4. Effect of pH on the thermal inactivation of *S. enteritidis* (●) and *E. coli* O157:H7 (▲) at 64.5°C, 3.5% w/w NaCl (lines denote predictions from models derived from the whole datasets and not just the points shown).

Table 4
Effect of organic acids on the $D_{62.5°C}$ values (s) of *S. enteritidis* in 3.5% w/w NaCl

Organic acid concentration (% w/w)	pH	Acid			
		Acetic	Lactic	Citric	HCl (prediction)
0.5	4.8	13.9	–[a]	27.5	17.0
	4.6	–	15.4	–	14.7
1.0	4.8	13.3	–	43.8	17.0
	4.5	–	14.8	–	13.6
2.0	4.8	4.8	–	39.4	17.0
	4.4	–	1.7	–	12.5

[a] –, not determined.

Table 5
Effect of organic acids on the $D_{62.5°C}$ values (s) of *E. coli* O157:H7 in 3.5% w/w NaCl

Organic acid concentration (% w/w)	pH	Acid			
		Acetic	Lactic	Citric	HCl (prediction)
0.5	4.9	–[a]	–	30.4	51.6
	4.8	34.8	–	–	50.2
	4.6	–	40.0	–	46.9
1.0	4.8	48.8	–	61.5	50.2
	4.5	–	47.3	–	45.1
2.0	4.8	42.0	–	148	50.2
	4.4	–	36.0	–	43.1

[a] –, not determined.

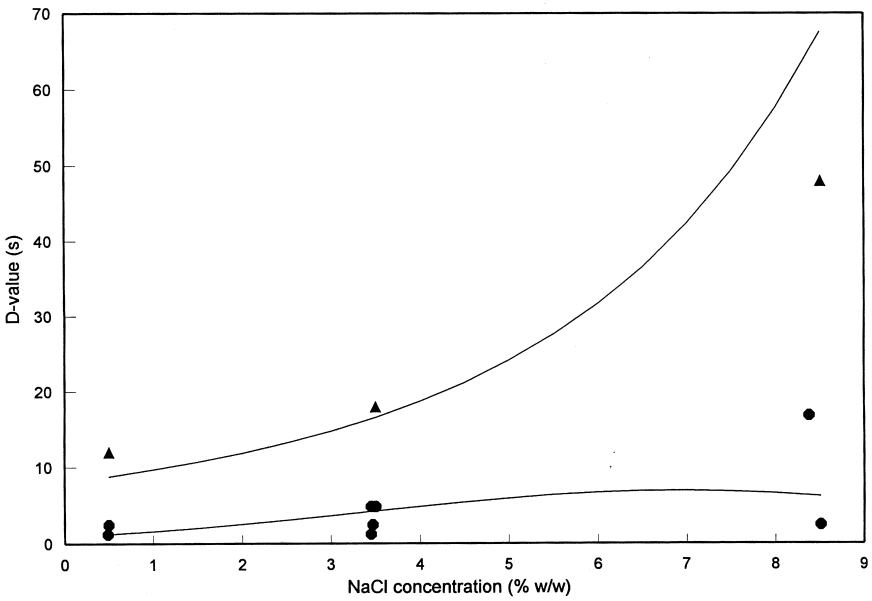

Fig. 5. Effect of NaCl concentration on the thermal inactivation of *S. enteritidis* (●) and *E. coli* O157:H7 (▲) at 64.5°C, pH 4.5 (lines denote predictions from models derived from the whole datasets and not just the points shown).

3.8. Validation of models in foods

Predictions from the thermal inactivation *D*-value models were compared with published thermal inactivation data from foods. Predictions from the *S. enteritidis* model were compared with data for 34 different *Salmonella* serotypes (excluding known heat-resistant strains) in a range of foods including whole egg, egg yolk, egg albumen, beef and milk (Fig. 6). A total of 220 comparisons were made, comprising 19 publications, and the model predicted slower inactivation ('fail safe') on 70% of occasions.

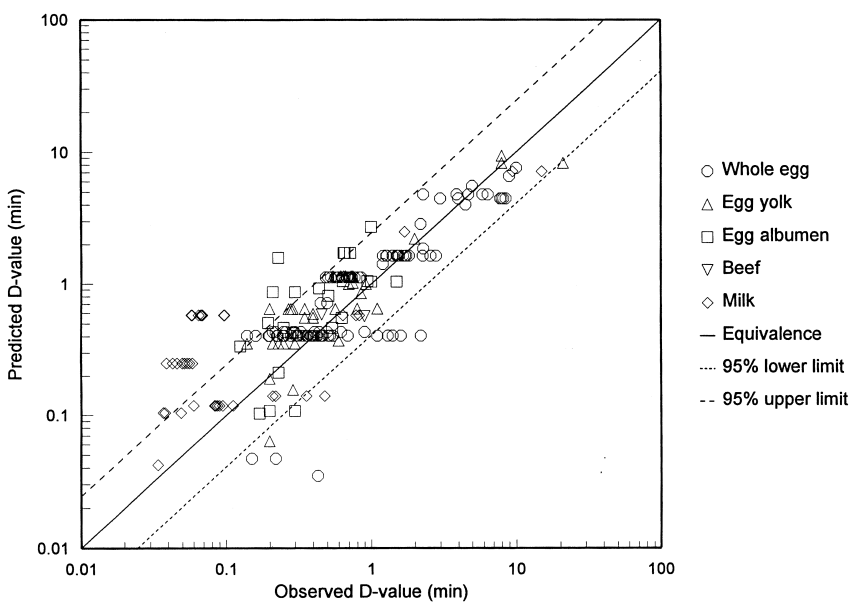

Fig. 6. Food validation of *S. enteritidis* thermal inactivation model using published data.

Table 6
Food validation of *S. enteritidis* thermal inactivation model using published data — example comparisons

No. of comparisons	Food	Strain	Temperature (°C)	pH	NaCl (w/w aqueous phase)	Observed *D*-value (min)	Predicted *D*-value (min)	Reference
4	Whole egg	*S. enteritidis*	55	(7.8)	(0.36)[a]	7.8–8.5	4.46	Humphrey (1990)
24	Whole egg	various	58	(7.8)	(0.36)	0.49–0.86	1.12	Solowey et al. (1948)
10	Whole egg	various	60	(7.8)	(0.36)	0.14–2.2	0.41	Anellis et al. (1954)
16	Whole egg	*S. enteritidis*	57.2	(7.8)	(0.36)	1.21–2.81	1.64	Shah et al. (1991)
13	Whole egg	*S. enteritidis*	60	(7.7)	(0.36)	0.21–0.62	0.43	Humphrey et al. (1990)
9	Whole egg	*S. enteritidis*	60	(7.8)	(0.36)	0.31–0.69	0.41	Baker (1990)
3	Whole egg	*S. typhimurium*	60	(7.8)	(0.36)	0.25–0.45	0.41	Garibaldi et al. (1969)
1	Whole egg	*S. typhimurium*	57.8	5.5	(0.36)	2.3	1.86	Lategan and Vaughn (1964)
4	Whole egg	various	60	(7.8)	(0.36)	0.16–0.46	0.41	Dabbah et al. (1971a)
6	Egg yolk	various	62.2	(6.5)	(0.36)	0.14–0.3	0.36	Palumbo et al. (1995)
1	Egg yolk	*S. typhimurium*	59.5	(6.5)	(0.36)	0.85	0.85	Jäckle et al. (1988)
3	Egg albumen	*S. typhimurium*	56.7	8.0	(0.34)	0.63–0.66	1.72	Corry and Barnes (1968)
1	Egg albumen	*S. typhimurium*	58.5	9.2	(0.34)	0.23	0.21	Jäckle and Schmidt-Lorenz (1989)
1	Minced beef	*S. thompson*	60	(6.1)	(0.23)	0.46	0.59	Mackey and Derrick (1987)
1	Beef	*Salmonella* spp.	60	5.9	(0.23)	0.9	0.57	Craven and Blankenship (1983)
6	Milk	various	62.8	(6.7)	(0.19)	0.06-0.1	0.12	Read et al. (1968)
1	Milk	*S. typhimurium*	62.8	(6.7)	(0.19)	0.112	0.118	Bradshaw et al. (1987)
8	Milk	various	61.5	(6.7)	(0.19)	0.039-0.059	0.25	D'Aoust et al. (1987)
4	Milk	various	62.5	(6.7)	(0.19)	0.21-0.48	0.14	Dabbah et al. (1971b)

[a] Parentheses indicate estimated values

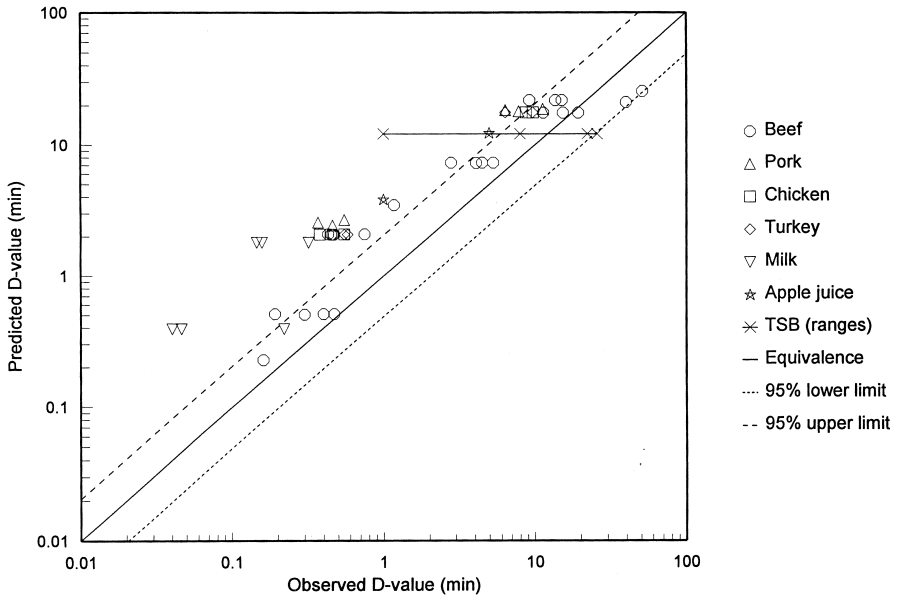

Fig. 7. Food validation of *E. coli* O157:H7 thermal inactivation model using published data.

Table 7
Food validation of *E. coli* O157:H7 thermal inactivation model using published data — example comparisons

No. of comparisons	Food	Temperature (°C)	pH	NaCl (w/w aqueous phase)	Observed *D*-value (min)	Predicted *D*-value (min)	Reference
1	Ground beef	54.5	(5.7)[a]	(0.25)	39.8	21.2	Doyle and Schoeni (1984)
1	Ground beef	64.3	(5.7)	(0.25)	0.16	0.23	Doyle and Schoeni (1984)
1	Ground beef	62.8	(5.7)	(0.31)	0.47	0.51	Line et al. (1991)
1	Ground beef	60	(5.7)	(0.26)	0.47	2.07	Ahmed et al. (1995)
1	Ground beef	57.2	(5.7)	(0.25)	2.8	7.31	Shipp et al. (1991)
3	Ground beef (acidified)	54[b]	5.0	(0.25)	9.2-15.0	21.9	Abdul-Raouf et al. (1993)
3	Milk	63	(6.8)	(0.2)	0.05–0.22	0.39	D'Aoust et al. (1988)
1	Chicken	55	(5.8)	(0.16)	9.74	17.7	Ahmed et al. (1995)
1	Turkey	60	(5.9)	(0.1)	0.58	2.07	Ahmed et al. (1995)
1	Pork sausage	55	(6.1)	3.09	11.3	18.8	Ahmed et al. (1995)
1	Apple juice	55	4.4	1.4[c]	5.0	12.4	Splittstoesser et al. (1996)
1	Apple juice	58	4.4	1.4[c]	1.0	3.84	Splittstoesser et al. (1996)
87[d]	Tryptic soy broth	55	7.3	0.5	1.0–25.6	12.2	Jackson et al. (1996)
4[e]	Tryptic soy broth	55	7.3	0.5	8.0–22.2	12.2	Murano and Pierson (1993)

[a] Parentheses indicate estimated values.
[b] Prediction outside temperature range of model.
[c] Equivalent a_w to 12° brix.
[d] Different incubation temperatures, holding times and growth phases prior to heating.
[e] Different heat-shock treatments and atmospheres for incubation of agar plates.

For egg products and beef, 87% of the *D*-values were within the 95% confidence interval of predictions from the model ($\pm 2 \times$ SE of $\log_{10}$ *D*-value) i.e. there was a difference of less than 2.45-fold between predicted and observed values. The difference was greater than four-fold in only 2.7% of comparisons. For milk, however, there was greater variation, with only 45% of the 38 comparisons within the 95% confidence interval and 45% showing a greater than four-fold difference between predicted and observed *D*-values (Table 6).

A total of 45 comparisons, comprising seven publications, were made for the *E. coli* O157:H7 model and on 93% of occasions the model gave fail safe predictions (Fig. 7). The published data were within the 95% confidence limits of the *E. coli* O157:H7 model for 42% of comparisons i.e. a difference of less than 2.05-fold compared with the prediction. Predicted *D*-values were larger than observed *D*-values by more than four-fold for 29% of comparisons (Table 7).

4. Discussion

Due to its prevalence in cases of salmonellosis, a strain of *S. enteritidis* phage type 4 was used to develop a *Salmonella* thermal inactivation model. Some strains of *S. senftenberg* and *S. bedford* can be more heat resistant, but this is not typical of salmonellas in general (Baird-Parker et al., 1970). With the strains used in this study, *E. coli* O157:H7 was more heat resistant than *S. enteritidis* but the converse has also been reported (Doyle and Schoeni, 1984).

The calculated *z*-values (4.6–7.0 C°) for both organisms are similar to the range of values (4–6 C°) that have been reported (D'Aoust, 1989; Line et al., 1991; Ahmed et al., 1995). However, results from the present study showed that the *z*-value could vary depending on the conditions, particularly NaCl. The *z*-value of *Salmonella typhimurium* in milk has been shown to be affected by a_W, increasing from 4°C to 6.8 C° with an increase in solids from 10% to 50% (Dega et al., 1972). This highlights the potential inaccuracies of determining a *z*-value under one set of conditions and applying it to other conditions.

In general, the heat resistance of bacteria increases with decreasing water activity. In the present study, heat resistance of *E. coli* O157:H7 increased with increasing NaCl up to 8.5% w/w. A similar effect has been seen for *Listeria monocytogenes* at NaCl concentrations up to 4.5% (Cole et al., 1993). For *S. enteritidis*, however, there was a predicted optimum NaCl concentration for survival of 5–7% w/w and thermal inactivation data at 19% w/w NaCl provided further evidence for a predicted optimum. An optimal level of NaCl for heat protection of other *Salmonella* serotypes (6–9%) (Baird-Parker et al., 1970) and *Aeromonas hydrophila* (2%) (Stecchini et al., 1993) has also been obtained.

The pH of the heating menstruum also affected thermal inactivation, with low and high pH values generally decreasing heat resistance. A similar effect has been observed with the inactivation of *Salmonella* in liquid whole egg (Anellis et al., 1954) and *E. coli* O157 and *S. enteritidis* in buffer (Teo et al., 1996). It has been shown that *E. coli* is more tolerant of low pH at non-lethal temperatures than *Salmonella* (Gauthier and Clément, 1994) and the present study provides evidence for a similar effect at lethal temperatures. The thermal inactivation models were produced using hydrochloric acid as the acidulant and acetic and lactic acids had either no effect or an increased lethal effect compared with predictions from the models. There was evidence, however, that under certain conditions citric acid had a less lethal effect. Similar observations have been made for the differential effect of organic acids on the thermal inactivation of bacterial spores (McClure, unpublished observations). Citric acid was shown to increase the heat resistance of spoilage yeasts in concentrated orange juices (Juven et al., 1978) and Abdul-Raouf et al. (1993) demonstrated that the rate of thermal inactivation of *E. coli* O157:H7 in acidified beef slurry was dependent on the acidulant and increased in the order: citric acid, lactic acid and acetic acid. Citric acid has also been shown to cause less sub-lethal injury to *S. enteritidis* at non-lethal temperatures than acetic and lactic acids (Alexandrou et al., 1995).

The kinetics of the thermal inactivation of bacteria has been a topic of much discussion over the years. It has been generally accepted that inactivation follows a log-linear relationship, although deviations, which are not explained easily by experimental factors, have been observed frequently (Cerf, 1977; Jackson et al., 1996). Fitting the logistic function to

thermal inactivation data plotted against log time has been shown to account for some of the deviations from log-linear kinetics of *L. monocytogenes* (Cole et al., 1993). The log-logistic modelling approach has also been applied to the survival of *Yersinia enterocolitica* at sub-optimal pH and temperature (Little et al., 1994).

The method of enumerating survivors of heat inactivation experiments can affect the percentage recovery of heat-injured cells and hence has a bearing on the calculated heat resistance of the organism. Anaerobic incubation of agar plates led to *D*-values up to twice as great as those from aerobically incubated plates and similar results have been obtained for *Salmonella* (Xavier and Ingham, 1993) and *E. coli* O157:H7 (Murano and Pierson, 1993). Xavier and Ingham (1993) stated that it is unlikely that anaerobic heating or storage of food after heating reflect most practical situations, but it may be important in evaluating the microbiological safety of lightly heated foods packaged under low-oxygen conditions.

Published thermal inactivation data for *Salmonella* and *E. coli* O157:H7 in a range of foods were compared with predictions from the *D*-value models. For the *S. enteritidis* model, 70% of comparisons showed faster inactivation than the model predicted ('fail safe'), which contrasted with 93% for the *E. coli* O157:H7 model. This difference may reflect the use of a relatively more heat-resistant strain of *E. coli* O157:H7 and the fact that the *S. enteritidis* model was compared with more data comprising a wide range of *Salmonella* serotypes and strains, which can have differences in heat resistance of up to 16-fold (Anellis et al., 1954). The heat resistance of the *E. coli* O157:H7 strain together with the smaller standard error of the model may reflect the smaller number of comparisons (42%) within the 95% confidence interval of the model compared with the *S. enteritidis* model (80%). It would be expected that the errors associated with published data would be no smaller, and probably considerably larger, than the errors associated with the models because of the additional factors that introduce variability. For most of the published data no pH, NaCl concentration or a_W measurements of the food were given and estimates had to be made. There is difficulty in reproducing experimental conditions accurately and *D*-values for *E. coli* O157:H7 differing by up to 3 fold were obtained for replicate analyses (Splittstoesser et al., 1996). The physiological condition of the cells can affect their heat resistance and different temperatures and holding times prior to heating caused variation in the *D*-value of *E. coli* O157:H7 of 26-fold (Jackson et al., 1996).

Comparing the *S. enteritidis* model with thermal inactivation data in eggs and beef, only 13% of comparisons were outside the 95% confidence limits. Some of these can be explained by the 16-fold variation in heat-resistance of different strains in liquid whole egg (Anellis et al., 1954) compared with the 6-fold variation in *D*-value that the 95% confidence interval reflects. Published thermal inactivation data for *Salmonella* and *E. coli* O157:H7 in milk compared less well. Many of the data were generated using a pilot-scale plate pasteuriser unit and the minimum process temperature and time were used to obtain predictions from the *S. enteritidis* and *E. coli* O157:H7 models (D'Aoust et al., 1987). Exposure to temperatures and times in excess of these values and any lethality during heating and cooling, which was slower than commercial units (D'Aoust et al., 1987), would not be predicted. The use of models that can take into account the heat delivered during heating and cooling would reflect more accurately data generated in process equipment.

Heat resistance data for microorganisms is established traditionally in microbiological media at neutral pH and high a_W. In practice, the pH and a_W of the food will often significantly influence the heat resistance. The models developed in this study enable the combined effect of these factors to be quantified. Other factors, such as strain to strain variability and differences in cell physiology and biochemistry, may also influence heat resistance but, at present, these effects are not easily quantified. Nevertheless, models that describe the interactions of factors that are controlled easily would be very useful in determining the extent to which an existing heat process should be modified for different foods or formulations. The use of *D*-values to describe thermal inactivation data is widely accepted and easily applied in practical situations. Non-linear kinetics are applied less easily and would significantly increase the heat process required for larger reductions in cell numbers. The deviations from log-linear kinetics in this study were described better

using the log-logistic approach, but more knowledge about the apparently more heat resistant fractions of bacterial populations is required before these data can be used and applied to heat processes.

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